Endocrine Facets of Ageing.
Novartis 242

Print ISBN 0-471-48636-1 Online ISBN 0-470-84654-2

ENDOCRINE FACETS OF AGEING

Endocrine Facets of Ageing.
Novartis 242

Print ISBN 0-471-48636-1 Online ISBN 0-470-84654-2

The Novartis Foundation is an international scientific and educational charity (UK Registered Charity No. 313574). Known until September 1997 as the Ciba Foundation, it was established in 1947 by the CIBA company of Basle, which merged with Sandoz in 1996, to form Novartis. The Foundation operates independently in London under English trust law. It was formally opened on 22 June 1949.

The Foundation promotes the study and general knowledge of science and in particular encourages international co-operation in scientific research. To this end, it organizes internationally acclaimed meetings (typically eight symposia and allied open meetings and 15–20 discussion meetings each year) and publishes eight books per year featuring the presented papers and discussions from the symposia. Although primarily an operational rather than a grant-making foundation, it awards bursaries to young scientists to attend the symposia and afterwards work with one of the other participants.

The Foundation's headquarters at 41 Portland Place, London W1N 4BN, provide library facilities, open to graduates in science and allied disciplines. Media relations are fostered by regular press conferences and by articles prepared by the Foundation's Science Writer in Residence. The Foundation offers accommodation and meeting facilities to visiting scientists and their societies.

Information on all Foundation activities can be found at
http://www.novartisfound.org.uk

Endocrine Facets of Ageing.
Novartis 242

Print ISBN 0-471-48636-1 Online ISBN 0-470-84654-2

Novartis Foundation Symposium 242

ENDOCRINE FACETS OF AGEING

2002

JOHN WILEY & SONS, LTD

Endocrine Facets of Ageing.
Novartis 242

Print ISBN 0-471-48636-1 Online ISBN 0-470-84654-2

Published in 2002 by John Wiley & Sons Ltd,
Baffins Lane, Chichester,
West Sussex PO19 1UD, England

National 01243 779777
International (+44) 1243 779777
e-mail (for orders and customer service enquiries): cs-books@wiley.co.uk
Visit our Home Page on http://www.wiley.co.uk
or http://www.wiley.com

Other Wiley Editorial Offices

John Wiley & Sons, Inc., 605 Third Avenue,
New York, NY 10158-0012, USA

WILEY-VCH Verlag GmbH, Pappelallee 3,
D-69469 Weinheim, Germany

Jacaranda Wiley Ltd, 33 Park Road, Milton,
Queensland 4064, Australia

John Wiley & Sons (Asia) Pte Ltd, 2 Clementi Loop #02-01,
Jin Xing Distripark, Singapore 129809

John Wiley & Sons (Canada) Ltd, 22 Worcester Road,
Rexdale, Ontario M9W 1L1, Canada

Novartis Foundation Symposium 242
viii+290 pages, 46 figures, 12 tables

Library of Congress Cataloging-in-Publication Data

Chadwick, Derek.
Endocrine facets of ageing / Derek J. Chadwick, Jamie A. Goode.
p.cm. – (Novartis Foundation sympoisum ; 242) (Ciba Foundation symposium)
Includes bibliographical references and index.
ISBN 0-471-48636-1 (alk. paper)
1. Aging–Endocrine aspects–Congresses. I. Goode, Jamie. II. Title. III. Series. IV. Series: Ciba Foundation symposium
QP187.3.A34 C475 2002
612.6′7–dc21 2001046746

British Library Cataloguing in Publication Data

A catalogue record for this book is available from the British Library

ISBN 0 471 48636 1

Typeset in 10½ on 12½ pt Garamond by Dobbie Typesetting Limited, Tavistock, Devon.
Printed and bound in Great Britain by Biddles Ltd, Guildford and King's Lynn.
This book is printed on acid-free paper responsibly manufactured from sustainable forestry, in which at least two trees are planted for each one used for paper production.

Endocrine Facets of Ageing.
Novartis 242

Print ISBN 0-471-48636-1 Online ISBN 0-470-84654-2

Contents

Novartis Foundation symposium on Endocrine facets of ageing, held at the Novartis Foundation, London, 30 January–1 February 2001

Editors: Derek J. Chadwick (Organizer) and Jamie A. Goode

This symposium is based on a proposal made by Johannes D. Véldhuis and Zvi Laron

Endocrine Facets of Ageing.
Novartis 242

Print ISBN 0-471-48636-1 Online ISBN 0-470-84654-2

Participants

Per Björntorp Department of Heart and Lung Diseases, University of Göteborg, Sahlgrenska University Hospital, S-413 45 Göteborg, Sweden

Cyril Y. Bowers Department of Endocrinology, Tulane Medical School, 1430 Tulane Avenue, SL53, New Orleans, Louisiana 70112, USA

Georg Brabant Abteilung Klinische Endokrinologie, Medizinische Hochschule Hannover, Carl-Neubergstr 1, Hanover, D-30623, Germany

Henry G. Burger Prince Henry's Institute of Medical Research, Monash Medical Centre, Post Office Box 5152, Clayton, VIC 3168, Australia

Bernard Carroll Pacific Behavioral Research Foundation, 26386 Carmel Rancho Lane, Suite 202, PO Box 223040, Carmel, CA 93922-3040, USA

Dariush Elahi Geriatric Research Laboratory, GRBSB-0015, Massachusetts General Hospital, 55 Fruit Street, Boston, MA 02114, USA

Andrea Giustina Department of Internal Medicine, 2A Medicina - Spedali Civili, P Le Spedali Civili I, Brescia, Italy

David J. Handelsman ANZAC Research Institute, Department of Andrology, Concord Hospital, University of Sydney, Sydney, NSW 2139, Australia

Erhard Haus Department of Laboratory Medicine and Pathology, University of Minnesota, Department of Anatomic and Clinical Pathology, Regious Hospital, 640 Jackson Street, St Paul, MN 55101-2595, USA

Zvi Laron Endocrinology and Diabetes Research Unit, Schneider Children's Medical Center of Israel (SCMCI), Tel Aviv University, 14 Kaplan Street, 49202, Petach Tiqva, Israel

John Monson Department of Medicine and Endocrinology, St Bartholomew's and the Royal London School of Medicine, West Smithfield, London EC1A 7BE, UK

John E. Morley Division of Geriatric Medicine, St Louis University School of Medicine, 1402 S Grand Boulevard, Room M238, St Louis, MO 63104-1083, USA

Eugenio E. Müller University of Milan, Department of Pharmacology, Via Vanvitelli 32, 10129 Milan, Italy

Jerilynn C. Prior The University of British Columbia, Department of Endocrinology, 380-575 W 8th Avenue, Vancouver, Canada BC V5Z 1C6

B. Lawrence Riggs Division of Endocrinology/Metabolism, Mayo Clinic and Foundation, 200 1st Street SW, Rochester, MI 80220-3706, USA

David Robertson Departments of Medicine, Pharmacology, and Neurology, Vanderbilt University School of Medicine, AA 3228 MCN, Nashville, TN 37232-2195, USA

Antonio Ruiz-Torres Instituto Universitario de Investigación, Gerontologica y Metabolica, Hopital de la Princesa, Diego de Leon 62, 28006 Madrid, Spain

Stephen M. Shalet Department of Endocrinology, Christie Hospital, Wilmslow Road, Manchester M20 4BX, UK

Annewieke van den Beld Department of Internal Medicine, University Hospital Dijkzigt, 40 Dr Molewaterplein, 3015 GD, Rotterdam, The Netherlands

Johannes D. Veldhuis (*Chair*) Division of Endocrinology, Department of Internal Medicine, University of Virginia School of Medicine, PO Box 800202, Charlottesville, VA 22908-0202, USA

Christina Wang General Clinical Research Center (Box 16), Harbor-UCLA Medical Center, 1000 W Carson Street, Torrance, CA 90509, USA

Endocrine Facets of Ageing.
Novartis 242

Print ISBN 0-471-48636-1 Online ISBN 0-470-84654-2

Chair's introduction

Johannes Veldhuis

University of Virginia Health System, NIH General Clinical Research Center, Department of Internal Medicine, Division of Endocrinology and Metabolism, PO Box 800202, Charlottesville, Virginia 22908-0202, USA

The idea from this symposium arose from some conversations that Professor Zvi Laron and I had about a year and a half ago, when we considered meeting in Europe to discuss some of the issues in the ageing field that were puzzling us. From these notions grew a more formalized structure, which I am delighted to see hosted today by the Novartis Foundation. The glory of the format for this type of small meeting is that it is very open to query and enquiry.

I think that it is always productive first to try to focus on some of the unresolved issues in a field. One aspect of research philosophy that I like is that one can identify areas of ignorance honestly: it is considered a point of brilliance to be aware of ignorance, because one can then address the corresponding issue. Thus, this conference will examine some unresolved issues, some of which perhaps were thought to be *fait accompli*, but in fact are not clearly understood. This foundation of fact building is very important in generating new avenues for research. Smaller discussion groups spawn an interchange of techniques and occasionally stimulate emergence of a new technique: this is something that has happened for me in the past, and it has been a privilege to go away, find a mathematician or physiologist as a collaborator and then perhaps come up with a new method to answer a question that previously couldn't be addressed directly. Thus a corollary aim of this conference is to define possible areas wherein techniques are deficient in ageing research to explore important queries that remain unresolved.

A second intent of this symposium is to represent themes from many branches of endocrinology and selected non-endocrine facets of ageing research. Ecumenism in science promotes interdisciplinary collaboration. One of the joys of my career so far has been viewing research areas that initially appear disparate and trying to catalyse some interaction. This often results in synergistic outcomes and novel, exciting insights. The present symposial format accomplishes this objective.

A third challenge in contemporary ageing research is to coalesce 'among-axes uniformities' in ageing, as distinguished from the between-axes differences. In a fundamental way, clarifying how different neuroendocrine axes behave according

to some similar template as they age is going to be important. Then, identifying their distinctions may teach us something vital.

Lastly, modern work is concerned with how neuroendocrine control systems interact. A number of recent papers show that researchers are beginning to examine expressly how the gonadotrophic axis in ageing is interconnected at several levels with the somatotrophic axis. This is a somewhat obvious connection, but I think that we all know from our neuroscience experience that other axes interface in subtle and basic ways. In the broadest sense, axes collaborate via common and parallel neurotransmitter pathways that drive feedforward and feedback signalling. Interactions emerge at the target tissue, too, and this is where one of the big challenges remains: how does one define ageing effects across axes at the distal effector-site level? Most of us study just a single axis, and this is complicated enough. Thus, to begin to integrate results across different axes is one aim of this symposium.

Endocrine Facets of Ageing.
Novartis 242

Print ISBN 0-471-48636-1 Online ISBN 0-470-84654-2

Endocrine aspects of healthy ageing in men

Annewieke W. van den Beld and Steven W. J. Lamberts

Department of Internal Medicine, Erasmus University Hospital, Rotterdam, The Netherlands

Abstract. Frailty is characterized by generalized weakness, impaired mobility and balance and poor endurance. Loss of muscle strength is an important factor in the process of frailty, and is the limiting factor for an individual's chances of living an independent life until death. In men, several hormonal systems show a decline in activity during ageing. Serum bioavailable testosterone and oestradiol, dehydroepiandrosterone and its sulfate, and growth hormone and insulin-like growth factor 1 concentrations all decrease during ageing in men. Physical changes during ageing have been considered physiologic, but there is evidence that some of these changes are related to this decline in hormonal activity. Studies on hormone administration in the elderly appear to be promising. However, until now, hormone replacement has not yet been proven to be beneficial and safe.

2002 Endocrine facets of ageing. Wiley, Chichester (Novartis Foundation Symposium 242) p 3–25

The concept of frailty and successful ageing

Age-related disability is characterized by generalized weakness, impaired mobility, impaired balance and poor endurance. This state is also called 'frailty' and is defined as a syndrome of multi-system reduction in physiological capacity as a result of which an older person's function may be severely compromised by minor environmental challenges, giving rise to the condition 'unstable disability' (Campbell & Buchner 1997). The increase in heterogeneity with age makes research findings more difficult to generalize, therefore it might be better for certain research to focus on the least frail and 'non-diseased', which implies the successfully aged. Older persons with minimal physiological loss, or none at all, when compared to the average of their younger counterparts, can be regarded in physiological terms as having aged more broadly successfully (Rowe & Kahn 1987). The concept of frailty focuses mainly on the physical or physiological aspects of ageing, while the concept of successful ageing comprises a broader range of aspects. Both concepts are not easy to define in a single measure.

Although definitions of successful ageing in gerontology are numerous, there is still no generally accepted definition. Rowe and colleagues defined it as including three main components: low probability of disease and disease-related disability, high cognitive and physical functional capacity, and active engagement with life (Rowe & Kahn 1997). Fries, amongst others, defined successful ageing as optimizing life expectancy while simultaneously minimizing physical, psychological and social morbidity (Fries 1988). Vaillant argued that in addition to physical health, there are three further dimensions, or outcomes, of successful ageing: mental health, psychosocial efficiency and life satisfaction (Vaillant & Vaillant 1990).

Since many of the predictors of the physical functional status appear to be potentially modifiable, research must be done to refine diagnostic criteria and elucidate practical methods of measurement of key physiological capacities in order to identify proper targets for interventions with 'normal' elderly and thus to enhance the proportion of the older population that ages successfully.

In a research we performed among 403 independently living elderly men (aged 73–94 years) from a blue-collar suburb of Rotterdam, The Netherlands, we have measured several physical characteristics. We assessed subjective and objective functional ability using the modified health assessment questionnaire and a physical performance test, respectively. Furthermore, we measured bone mineral density, body composition, muscle strength and cognition. Muscle strength was independently, positively related to lean body mass, bone mineral density and physical performance, and inversely related to the number of problems in activities of daily living. Muscle strength and functional ability can be considered the key characteristics of physical functional status in independently living elderly men. These findings confirm previous studies in which it was demonstrated that loss of muscle strength is a strong predictor of physical functional problems (Fiatarone et al 1994, Guralnik et al 1994). The clinical correlates of sarcopenia, the age-related loss in skeletal muscle, include falls, fractures, loss of mobility, and development of independence in basic activities of daily living.

Successful ageing also encompasses terms like psychological well-being, quality of life (QoL) and life satisfaction, which are all used interchangeably. QoL is measured by subjective indicators only, while successful ageing can be measured by both objective and subjective indicators. There are numerous general and non-specific QoL questionnaires. In our study among elderly men, we used a QoL questionnaire recently developed by Henrich and Herschbach, which includes a weighting for the relative importance of each dimension for the individual concerned (Herschbach 1995, Huber et al 1988).

Part of the ageing process affecting body composition (loss of muscle size and strength, loss of bone, and increase in fat mass) might well be related to changes in the endocrine system (Korenman et al 1990, Rudman et al 1990).

Endocrinology of ageing

In men, several hormonal systems show a gradual decline in activity during ageing, represented by a decrease in their bioactive hormone concentrations. The 'andropause' is characterized by a gradual decline in serum total and bioavailable testosterone, due to a decrease in testicular Leydig cell numbers and in their secretory capacity, as well as by an age-related decrease in episodic and stimulated gonadotropin secretion (Vermeulen 1991). Both cross-sectional (Vermeulen 1991) and longitudinal (Morley et al 1997) studies have shown that in healthy males mean serum total testosterone (T) levels decrease by about 30% between age 25 and 75, whereas mean serum free T levels decrease by as much as 50% over the same period. The steeper decline of free T levels is explained by an age-associated increase in sex-hormone binding globulin (SHBG) binding capacity (Vermeulen & Verdonck 1972). Conflicting results have been reported concerning the question of whether luteinizing hormone (LH) increases with age or remains relatively stable (Morley et al 1997, Ongphiphadhanakul et al 1995, van den Beld et al 1999). One reason may be that the ageing-induced decrease in T is primarily testicular in some men, mainly due to hypothalamo-pituitary insufficiency in others, and of mixed origin in a third group. In our study we found a significant increase of LH with age and an inverse relationship between serum LH and testosterone concentrations.

It has recently become clear that not only T decreases with age, but that serum oestradiol (E2) also significantly decreases in ageing males (Ferrini & Barrett-Connor 1998, Khosla et al 1998). In our population of elderly men, a significant decrease in serum oestradiol levels was also observed, while serum oestrone (E1) decreased to an even greater extent. In normal men small amounts of oestradiol are derived from direct secretion by the testes and indirectly from adrenal androgens. Most E2, however, is formed from testicular androgens by peripheral aromatization of T to E2 (MacDonald et al 1979).

The second hormonal system demonstrating age-related changes is the circulating levels of dehydroepiandrosterone (DHEA) and its sulfate (DHEAS), which gradually decline resulting in 'adrenopause' (Ravaglia et al 1996, Herbert 1995). At age 30, DHEAS levels are approximately five times higher than at age 85. The decline in DHEA(S) levels contrasts with the maintenance of plasma cortisol concentrations at the same level, and seems to be caused by a selective decrease in the number of functional zona reticularis cells in the adrenal cortex rather than regulated by a central (hypothalamic) pacemaker of ageing (Herbert 1995).

The third endocrine system that gradually declines in activity with ageing is the growth hormone (GH)/insulin-like growth factor 1 (IGF1) axis (Corpas et al 1993). Mean pulse amplitude, duration, and free fraction of GH secreted, but not pulse frequency, gradually decrease during ageing. In parallel, there is a progressive fall in circulating IGF1 levels (Corpas et al 1993). The IGF1

reduction probably results from reduced stimulation of the liver to produce IGF1 rather than an age-related insensitivity or inability of the liver to respond to circulating GH. The predominant IGF-binding protein (IGFBP) concentration in the blood, IGFBP3, reaches maximum levels at puberty and decreases between 18 and 79 years (Corpas et al 1993). The second most abundant IGFBP, IGFBP2, decreases after birth until puberty, after which it gradually increases again, especially after the age of 60 (Clemmons 1997). At age 80 concentrations are nearly twice as high as in young adults. In agreement with this, our study in subjects aged between 73 and 94 years showed a decrease in serum IGF1 and IGFBP3 levels and an increase in both serum IGFBP1 and IGFBP2 levels.

Clinical consequences of the decline in activity of the hormonal systems

Andropause

Testosterone has long been known for its anabolic effects (Brodsky et al 1996). Muscle weakness, anaemia, lowered bone mass, and mood disturbances rapidly normalize in mid-adult hypogonadal men during T replacement therapy. Since the decrease in serum T concentrations occurs in parallel with the decrease in muscle mass, strength and bone mass, it has been suggested that these are causatively related. Several cross-sectional and longitudinal studies have demonstrated relationships in older men between serum T levels and muscle strength, changes in body composition and bone mineral density (Rudman & Shetty 1994, Murphy et al 1993, Ongphiphadhanakul et al 1995). In agreement, in a cross-sectional study of 403 elderly men, we found positive relationships between non-SHBG-bound T and muscle strength and bone mineral density, and an inverse relationship with fat mass. A cross-sectional study among 856 elderly men demonstrated that bioavailable T levels were significantly lower for the 25 men with categorically defined depression than levels observed in all other men (Barrett-Connor et al 1999). This suggests that T treatment might improve depressed mood in older men who have low levels of bioavailable testosterone. On the other hand, in the population of elderly men we studied, no significant relation was observed between general life satisfaction and T levels. However, non-SHBG-bound T was positively related to some separate questions of the questionnaire, like the satisfaction related to mobility, physical performance and independency.

It is not known whether T therapy in older men has beneficial effects on muscle function, sexual function, sense of well-being and quality of life, and whether this therapy can be done safely. Studies involving physiological replacement therapy of testosterone in older men are limited. However, the results of these studies are consistent; reported are treatment-related declines in body fat mass ranging from

6.4–14%, and increases in lean mass ranging from 3.2–5.0% are both reported (Tenover 1998, Snyder et al 1999a). Several studies evaluating muscle strength in older men during testosterone replacement have demonstrated a statistically significant increase in strength with therapy (Tenover 1998, Sih et al 1997). However, Snyder et al (1999a) evaluated lower extremity strength in a double-blind, placebo-controlled study involving 108 men, and did not demonstrate a significant change compared to the placebo group, nor did they observe an increase in physical performance in response to testosterone (Snyder et al 1999a). In the same study, testosterone replacement did not increase lumbar spine bone density in the overall group, but it did in men with low pretreatment serum testosterone concentrations (Snyder et al 1999b). It has to be mentioned, however, that the men in this study did not have very low T concentrations.

Although it has been thought for years that T administration adversely affects serum lipid concentrations, recent research shows that T probably has a favourable effect on total and LDL cholesterol concentration, and probably also on triglycerides concentration (Tenover 1992). So far it is unclear whether T replacement in older men can be considered safe with regard to cardiovascular risk. In young men, T administration generally decreases HDL cholesterol levels, while LDL and triglycerides levels remain unchanged. Physiological doses of androgens in elderly men reduced total and LDL cholesterol and had no effect on HDL cholesterol (Zgliczynski et al 1996).

Additionally, the effect of T supplementation on the prostate remains unclear. It has been claimed that about 50% of men aged over 50 years have a subclinical prostate carcinoma, which can be activated by T administration (Jackson et al 1989). However, Snyder et al (1999b) reported that T treatment was associated with a small increase in mean serum prostate-specific antigen concentrations, but not with increases in other parameters that reflect prostate disease (Snyder et al 1999b). Furthermore, it is known that T increases haematocrit within the normal range (Sih et al 1997), while a negative role in the sleep apnoea syndrome has been reported in a few individuals (Matsumoto et al 1985). Overall, the beneficial effects of T replacement in older men seem to be promising, and T administration can probably be given safely to elderly men, provided they are monitored frequently. However, since only few double-blind placebo-controlled studies have been done on T replacement in the elderly, more research is necessary to define dose, form and subjects for T therapy.

In our study of 403 elderly men, serum LH levels were inversely related to muscle strength and lean mass, and positively to self-reported disability (van den Beld et al 1999). All relationships were independent of T, suggesting that LH reflects the serum androgen activity in a different manner than T, possibly more closely reflecting the combined feedback effect of T, dihydrotestosterone and oestrogen.

In most women, the period of decline in oestrogens during menopause is accompanied by vasomotor reactions, depressed mood, and changes in skin and body composition (increase in body fat and decrease in muscle mass). In the subsequent years, the loss of oestrogen is followed by a high incidence of cardiovascular disease, loss of bone mass and cognitive impairment (Lindsay et al 1996). Only recently has it become evident that oestrogens may not only play an important role in regulating bone turnover in women, but also in men. Smith et al (1994) described a male with a homozygous mutation in the oestradiol receptor gene who, even in the presence of normal T levels, had unfused epiphysis and marked osteopenia, along with elevated indexes of bone turnover. A few studies now have demonstrated significant relations between serum (bioavailable) oestradiol levels and bone mineral density in elderly men (Khosla et al 1998).

In normal men small amounts of oestradiol (15% of the total daily production) are derived from direct secretion from the testis; about equal amounts of oestradiol are synthesized in adipose tissue, muscle, skin, breast and liver, as well as indirectly from adrenal androgens via the conversion of androstenedione to oestrone to oestradiol or by peripheral aromatization of testosterone to oestradiol (MacDonald et al 1979). Bioavailable oestradiol decreases dramatically with age in community-dwelling men, independent of body size, health and chronic disease (Ferrini & Barrett-Connor 1998). In our population of elderly men, we also found strong positive relations between serum oestradiol and bone mineral density. In addition, however, we observed a strong positive relation between serum oestradiol concentrations and life satisfaction. It remains to be examined whether oestradiol has a central action on the brain, or whether this relationship represents an indirect effect of oestradiol on physical characteristics, which in turn lead to a better quality of life. Only a few studies have examined the relation between oestrogens and atherosclerosis in men (Price et al 1997). Oestrogens may offer some degree of protection against cardiovascular disease by influencing the lipid profile (Bagatell et al 1994, Giri et al 1998). The relationship between this decline in endogenous oestradiol levels and fragility, impaired functioning and chronic diseases (such as osteoporosis, diabetes, cancer, prostate and heart disease) should be the focus of future research.

Adrenopause

DHEA in its free, sulfated and lipoidal forms is the most abundant steroid secreted by the zona reticularis of the adult human adrenal. Circulating DHEAS levels in healthy adults are more than 10 times higher than those of cortisol (Ravaglia et al 1996). It is well known that ageing is associated with a marked decrease of the adrenal androgens DHEA and its sulfate (Orentreich et al 1984, Labrie et al 1997). DHEA is a universal precursor for androgenic and oestrogenic steroid

formation in peripheral tissues, which contain a number of DHEA-metabolizing enzymes (Herbert 1995). A variety of factors influence cortisol and DHEA levels: obesity, meals, insulin, stress, alcohol and smoking all alter adrenal steroid levels.

DHEA has been called 'the Fountain of Youth'. However, despite the abundance of DHEA and DHEAS in human serum, it remains unclear whether they play a functional role in the ageing process, either directly or through conversion into other steroids. Our knowledge of the functions of adrenal hormones is mainly derived from animal studies. Studies in rodents, which have very low circulating DHEAS levels, suggest that DHEA administration prevents obesity, diabetes mellitus, cancer and heart disease, while it enhances immune function. Supportive data in humans are limited, highly controversial and as yet unresolved.

Functional parameters of daily living in males over 90 years old were lowest in those with the lowest DHEAS levels (Ravaglia et al 1996). In a group of independent community-dwelling older men, men in the highest quartile of serum DHEAS concentrations were leaner, more fit and had a favourable lipid profile compared with those in the lowest quartile (Abbasi et al 1998). In our population of independently living men, a significant relationship between serum DHEAS and bone mineral density became non-significant after adjustment for oestradiol or testosterone, suggesting that any potential effect on bone mineral density is indirect through the conversion of DHEAS into androgens and oestrogens. Serum DHEA(S) levels in the same population were not related to self-reported disability, physical performance, muscle strength or body composition, nor to quality of life.

Administration of 50 mg DHEA to elderly men leads to serum DHEAS concentrations similar to those in healthy adults, while circulating serum oestrogens significantly increase (Arlt et al 1999). This DHEA-induced increase in oestrogenic activity may contribute to the beneficial effects of DHEA in men. Two randomized placebo-controlled studies support the concept that oral administration of DHEA has beneficial effects (Baulieu 1995). Three months of daily treatment with 50 mg of DHEA in 20 adults, most of whom were non-elderly individuals (40–70 years old), increased DHEA(S) levels to young adult levels, increased plasma androgen and IGF1 concentrations, and induced a remarkable increase in perceived physical and psychological well-being in both sexes without an effect on libido. In a subsequent study, 100 mg of DHEA given daily for 6 months to 9 men and 10 women increased lean body mass in both sexes but increased muscle strength in men only (Morales et al 1998). In a randomized, double-blind, placebo-controlled trial among 140 elderly men and 140 elderly women (Baulieu et al 2000) it appeared that 50 mg DHEA a day for one year led to changes in bone mineral density in women but not in men. In addition, no effect of DHEA administration on vascular properties in elderly men was observed. A

nine month cross-over study in 39 elderly men, did not confirm the effects of the drugs publicized by others, such as on the sense of well-being (Flynn et al 1999). DHEA might influence CNS activity (Wolf et al 1998), although short-term DHEA replacement does not appear to improve cognition, memory, mood or well-being (Wolf et al 1997, 1998).

Higher DHEAS levels are accompanied by a modestly reduced risk of death from cardiovascular disease in males (Barrett-Connor & Goodman-Gruen 1995, Feldman et al 1998). Data with regard to a protective effect of DHEA on cardiovascular disease in humans are conflicting and unresolved; studies using animal models, however, are quite promising. Animal studies also show that DHEA reverses the immuno-incompetence of aged animals. One study in humans indeed shows an activation of the immune system in elderly men. DHEA administration increased the number of monocytes and natural killer cells, and the functional activation of T cells (Khorram et al 1997).

Although neither Flynn et al (1999) or Baulieu et al (2000) demonstrated a change in prostate specific antigen concentration after DHEA replacement, it is so far unknown whether the increase in sex steroid levels induced by DHEA is safe with regard to the development of prostate, or other types of cancer.

DHEA has received great attention from the general population as potentially being able to increase the sense of well-being and sexual function, and to partially reverse the ageing process. So far, however, results are neither clear nor consistent. It seems prudent to await more trials, in order to be certain that exogenous DHEA is beneficial and can be used safely.

Somatopause

Growth hormone (GH) is an important anabolic hormone with stimulatory effects on protein synthesis and on lipolysis. In man both ageing and GH deficiency are associated with reduced protein synthesis, decreases in lean body mass and bone mass, and increases in body fat (Corpas et al 1993). In several studies of healthy individuals of a broad age range, an association was observed between the maximum aerobic capacity and circulating IGF1 levels (Papadakis et al 1995). Within the elderly population, however, cross-sectional studies have demonstrated weak or no correlations between measures of body composition and bone mass on the one hand, and serum IGF1 or GH levels on the other (Goodman-Gruen & Barrett-Connor 1997, Rudman & Shetty 1994). Similarly, largely negative findings have been reported concerning the association between IGF1 and muscle strength, and measures of functional ability (Papadakis et al 1995, Welle et al 1996). In our study in elderly men, we also failed to demonstrate a significant relationship between IGF1 and IGFBP3, and measures of physical functional status. We did, however, demonstrate strong inverse relationships

between serum IGFBP2 concentrations and physical performance, muscle strength, lean body mass, fat mass and bone mineral density. A positive relationship was observed between IGFBP2 and self-reported disability. Serum IGFBP2 levels in an individual represent an integrative response to the nutritional state, 24 h GH-secretion, serum IGF1 and IGF2 concentration and, to a lesser extent, serum insulin concentrations (Clemmons 1997). Therefore low IGFBP2 levels might serve as an indicator of better overall functional physical status. As mentioned above, we did not find an association between the physical characteristics of ageing and serum IGFBP3 levels, which are mainly regulated by GH and therefore serve as an indicator of serum GH levels. We did, however, find a positive relationship between serum IGFBP3 levels and quality of life, measured with the questionnaire developed by Herschbach and Huber (Herschbach 1995, Huber et al 1988). In a study of 52 elderly men and 80 elderly women, positive correlations were observed between HDL cholesterol and IGFBP3 concentrations, which served as an index of GH secretion (Ceda et al 1998).

The expectation that somatopause contributes to the decline of functional capacity in the elderly is mainly derived from studies in which GH replacement therapy of GH-deficient adults was shown to increase muscle mass, muscle strength, bone mass and quality of life. A beneficial effect on the lipid profile and an important decrease in fat mass were also observed in such patients (Salomon et al 1989). The GH/IGF1 axis may also play a role in the age-related decline of certain cognitive functions (Aleman et al 1999).

GH administration for 3 to 6 months to healthy elderly individuals increased IGF1 levels to those observed in control individuals half their age, while muscle strength, muscle mass, skin thickness and bone mineral content significantly increased and fat mass decreased (Rudman et al 1990, Welle et al 1996). Unfortunately, GH replacement in 52 healthy older men did not improve muscle strength and functional ability (Papadakis et al 1996). If GH was administered in combination with resistance exercise training, however, a significant positive effect on muscle mass, muscle strength and bone mineral density was recorded but did not differ from that seen with placebo treatment, which suggests that GH does not add to the beneficial effects of exercise (Yarasheski et al 1995, 1997). Preliminary results of a randomized placebo-controlled trial of 6 weeks GH administration in elderly individuals with an acute hip fracture indicate that in individuals over 75 years old, GH administration causes a statistically significant earlier return to independent living after the fracture. Finally, it has to be mentioned that side effects of GH therapy are common, namely carpal tunnel syndrome, gynaecomastia and hyperglycaemia.

Other components in the regulation of the GH/IGF1 axis are effective in activating GH and IGF1 secretion. Long-acting derivates of the hypothalamic peptide growth hormone-releasing hormone (GHRH), given twice daily

subcutaneously for 14 days to 70 year old healthy men, increased GH and IGF1 levels to those encountered in 35 year olds (Corpas et al 1992). These studies support the concept that somatopause is primarily hypothalamically driven and that pituitary somatotrops retain their capacity to synthesize and secrete high levels of GH. GH-releasing peptides (GHRPs) are oligopeptides with even more powerful GH-releasing effects. Originally developed by design, it has recently been suggested that they mediate their GH-secretory effects through endogenous specific receptors (Howard et al 1996). Non-peptide analogues such as MK-677 and L-692,429 have powerful GH-releasing effects, restoring IGF1 secretion in the elderly to levels encountered in young adults (Chapman et al 1996). Long-term oral administration of MK-677 to healthy elderly individuals increased lean body mass but not muscle strength. If proven to be GH-specific, these orally active GHRP derivates might be important alternatives to subcutaneously administered GH in the reversal of somatopause, the prevention of frailty, and the reversal of acute catabolism. The long-term safety of the activation of GH/IGF1 levels remains uncertain with regard to tumour growth, as most human solid cancers express IGF1 receptors (Chapman et al 1996).

Andro-, adreno- and somatopause

Some of the changes in hormones concentrations of the different hormonal axes which take place during ageing occur in parallel. However, it is not known whether interrelations exist between the hormones of the different axes, or whether they independently influence the physiological changes during ageing. Interactions between these hormonal systems may play a physiologically relevant role in the elderly population in the management of age-associated alterations in physical performance, muscle strength and body composition. Compared with healthy young men, 80% of healthy young men are hyposomatomedinaemic (lowered IGF1 levels) and hypogonadal (lowered testosterone levels); compared with healthy old men, 30% of chronically institutionalized old men are both hyposomatomedinaemic and hypogonadal. In institutionalized old men, hyposomatomedinia and hypogonadism are usually of central origin, but their occurrences are not significantly associated (Abbasi et al 1993). Although few relationships were present between hormones of the different axes in the study of 403 independently living elderly men, the associations between hormone concentrations and physical characteristics were all independent. This implies that subjects with low IGF1/GH levels do not necessarily have low androgens or adrenal hormones. This suggests that changes in hormones concentrations of the different axes influence the ageing process independently, which is extremely important for the selection of subjects for the respective replacement therapies.

Conclusions

In elderly men, ageing is accompanied by a decrease in objective and subjective physical function, as well as changes in body composition and bone mass. In parallel, important changes in the endocrine system occur. Testosterone administration seems to influence bone mass and perhaps muscle strength, although insufficient evidence exists to confirm that testosterone substitution in the elderly is indicated. Also, oestradiol seems to influence bone mineral density in elderly men. Although DHEA and its sulfate have been regarded as the hormone of youth, studies performed so far do not yet indicate a role for these adrenal hormones in maintaining physical functional status. DHEA, nevertheless, might have beneficial effects on cardiovascular and immunological processes. It remains unclear how hormones of the somatotropic axis contribute to the ageing process. Traditionally, the ageing process has been considered physiological and unavoidable. In recent years, however, it has become evident that it might not be necessary to accept the grim stereotype of ageing as an unalterable process of decline and loss (Fiatarone et al 1994, Rowe & Kahn 1987). When sufficient research has been done on hormonal replacement therapy in elderly men, hormonal substitution might be used to delay the ageing process and to allow us to live for a longer period in a (relatively) independent state of successful ageing.

References

Abbasi AA, Drinka PJ, Mattson DE, Rudman D 1993 Low circulating levels of insulin-like growth factors and testosterone in chronically institutionalized elderly men. J Am Geriatr Soc 41:975–982

Abbasi A, Duthie EH Jr, Sheldahl L et al 1998 Association of dehydroepiandrosterone sulfate, body composition, and physical fitness in independent community-dwelling older men and women. J Am Geriatr Soc 46:263–273

Aleman A, Verhaar HJ, De Haan EH et al 1999 Insulin-like growth factor-I and cognitive function in healthy older men. J Clin Endocrinol Metab 84:471–475

Arlt W, Haas J, Callies F et al 1999 Biotransformation of oral dehydroepiandrosterone in elderly men: significant increase in circulating estrogens. J Clin Endocrinol Metab 84:2170–2176

Bagatell CJ, Knopp RH, Rivier JE, Bremner WJ 1994 Physiological levels of estradiol stimulate plasma high density lipoprotein2 cholesterol levels in normal men. J Clin Endocrinol Metab 78:855–861

Barrett-Connor E, Goodman-Gruen D 1995 The epidemiology of DHEAS and cardiovascular disease. Ann N Y Acad Sci 774:259–270

Barrett-Connor E, von Mühlen DG, Kritz-Silverstein D 1999 Bioavailable testosterone and depressed mood in older men: the Rancho Bernardo Study. J Clin Endocrinol Metab 84:573–577

Baulieu EE 1995 Studies on dehydroepiandrosterone (DHEA) and its sulphate during aging. C R Acad Sci III 318:7–11

Baulieu EE, Thomas G, Legrain S et al 2000 Dehydroepiandrosterone (DHEA), DHEA sulfate, and aging: contribution of the DHEAge study to a sociobiomedical issue. Proc Natl Acad Sci USA 97:4279–4284

Brodsky IG, Balagopal P, Nair KS 1996 Effects of testosterone replacement on muscle mass and muscle protein synthesis in hypogonadal men—a clinical research center study. J Clin Endocrinol Metab 81:3469–3475

Campbell AJ, Buchner DM 1997 Unstable disability and the fluctuations of frailty. Age Ageing 26:315–318

Ceda GP, Dall'Aglio E, Magnacavallo A et al 1998 The insulin-like growth factor axis and plasma lipid levels in the elderly. J Clin Endocrinol Metab 83:499–502

Chapman IM, Bach MA, Van Cauter E et al 1996 Stimulation of the growth hormone (GH)-insulin-like growth factor I axis by daily oral administration of a GH secretogogue (MK-677) in healthy elderly subjects. J Clin Endocrinol Metab 81:4249–4257

Clemmons DR 1997 Insulin-like growth factor binding proteins and their role in controlling IGF actions. Cytokine Growth Factor Rev 8:45–62

Corpas E, Harman SM, Pineyro MA, Roberson R, Blackman MR 1992 Growth factor (GH)-releasing hormone-(1-29) twice daily reserves the decreased GH and insulin-like growth factor-I levels in old men. J Clin Endocrinol Metab 75:530–535

Corpas E, Harman SM, Blackman MR 1993 Human growth hormone and human aging. Endocr Rev 14:20–39

Feldman HA, Johannes CB, McKinlay JB, Longcope C 1998 Low dehydroepiandrosterone sulfate and heart disease in middle-aged men: cross-sectional results from the Massachusetts Male Aging Study. Ann Epidemiol 8:217–228

Ferrini RL, Barrett-Connor E 1998 Sex hormones and age: a cross-sectional study of testosterone and estradiol and their bioavailable fractions in community-dwelling men. Am J Epidemiol 147:750–754

Fiatarone MA, O'Neill, Ryan ND et al 1994 Exercise training and nutritional supplementation for physical frailty in very elderly people. N Engl J Med 330:1769–1775

Flynn MA, Weaver-Osterholtz D, Sharpe-Timms KL, Allen S, Krause G 1999 Dehydroepiandrosterone replacement in aging humans. J Clin Endocrinol Metab 84:1527–1533

Fries JF 1988 Aging, illness, and health policy: implications of the compression of morbidity. Perspect Biol Med 31:407–428

Giri S, Thompson PD, Taxel P et al 1998 Oral estrogen improves serum lipids, homocysteine and fibrinolysis in elderly men. Atherosclerosis 137:359–366

Goodman-Gruen D, Barrett-Connor E 1997 Epidemiology of insulin-like growth factor-I in elderly men and women. The Rancho Bernardo Study. Am J Epidemiol 145:970–976 (erratum: 1997 Am J Epidemiol 146:357)

Guralnik JM, Simonsick EM, Ferrucci L et al 1994 A short physical performance battery assessing lower extremity function: association with self-reported disability and prediction of mortality and nursing home admission. J Gerontol 49:M85–M94

Herbert J 1995 The age of dehydroepiandrosterone. Lancet 345:1193–1194

Herschbach P 1995 The difference between ill persons and patients (translated from German). Psychother Psychosom Med Psychol 45:83–89

Howard AD, Feighner SD, Cully DF et al 1996 A receptor in pituitary and hypothalamus that functions in growth hormone release. Science 273:974–977

Huber D, Henrich G, Herschbach P 1988 Measuring the quality of life: a comparison between physically and mentally chronically ill patients and healthy persons. Pharmacopsychiatry 21:453–455

Jackson JA, Waxman J, Spiekerman AM 1989 Prostatic complications of testosterone replacement therapy. Arch Intern Med 149:2365–2366

Khorram O, Vu L, Yen SS 1997 Activation of immune function by dehydroepiandrosterone (DHEA) in age-advanced men. J Gerontol A Biol Sci Med Sci 52:M1–M7

Khosla S, Melton LJ III, Atkinson EJ, O'Fallan WM, Klee GG, Riggs BL 1998 Relationship of sex steroid levels and bone turnover markers with bone mineral density in men and women: a key role for bioavailable estrogen. J Clin Endocrinol Metab 83:2266–2274

Korenman SG, Morley JE, Mooradian AD et al 1990 Secondary hypogonadism in older men: its relation to impotence. J Clin Endocrinol Metab 71:963–969

Labrie F, Bélanger A, Cusan L, Candas B 1997 Physiological changes in dehydroepiandrosterone are not reflected by serum levels of active androgens and estrogens but of their metabolites: intracrinology. J Clin Endocrinol Metab 82:2403–2409

Lindsay R, Bush TL, Grady D, Speroff L, Lobo RA 1996 Therapeutic controversy: estrogen replacement in menopause. J Clin Endocrinol Metab 81:3829–3838

MacDonald PC, Madden JD, Brenner PF, Wilson JD, Siiteri PK 1979 Origin of estrogen in normal men and in women with testicular feminization. J Clin Endocrinol Metab 49:905–916

Matsumoto AM, Sandblom RE, Schoene RB et al 1985 Testosterone replacement in hypogonadal men: effects on obstructive sleep apnoea, respiratory drives, and sleep. Clin Endocrinol 22:713–721

Morales AJ, Haubrich RH, Hwang JY, Asakura H, Yen SS 1998 The effect of six months treatment with 100 mg daily dose of dehydroepiandrosterone (DHEA) on circulating sex steroids, body composition and muscle strength in age-advanced men and women. Clin Endocrinol 49:421–432

Morley JE, Kaiser F, Raum WJ et al 1997 Potentially predicative and manipulative blood serum correlates of aging in the healthy human male: progressive decreases in bioavailable testosterone dehydroepiandrosterone sulfate, and the ratio of insulin-like growth factor 1 to growth hormone. Proc Natl Acad Sci USA 94:7537–7542

Murphy S, Khaw KT, Cassidy A, Compston JE 1993 Sex hormones and bone mineral density in elderly men. Bone Miner 20:133–140

Ongphiphadhanakul B, Rajatanavin R, Chailurkit L et al 1995 Serum testosterone and its relation to bone mineral density and body composition in normal males. Clin Endocrinol 43:727–733

Orentreich N, Brind JL, Rizer RL, Vogelman JH 1984 Are changes and sex differences in serum dehydroepiandrosterone sulfate concentrations throughout adulthood. J Clin Endocrinol Metab 59:551–555

Papadakis MA, Grady D, Tierney MJ, Black D, Wells L, Grunfeld C 1995 Insulin-like growth factor 1 and functional status in healthy older men. J Am Geriatr Soc 43:1350–1355

Papadakis MA, Grady D, Black D et al 1996 Growth hormone replacement in healthy older men improves body composition but not functional ability. Ann Intern Med 124:708–716

Price JF, Lee AJ, Fowkes FG 1997 Steroid sex hormones and peripheral arterial disease in the Edinburgh Artery Study. Steroids 62:789–794

Ravaglia G, Forti P, Maioli F et al 1996 The relationship of dehydroepiandrosterone slufate (DHEAS) to endocrine-metabolic parameters and functional status in the oldest-old. Results from an Italian study on healthy free-living over-ninety-year-olds. J Clin Endocrinol Metab 81:1173–1178

Rowe JW, Kahn RL 1987 Human aging: usual and successful. Science 237:143–149

Rowe JW, Kahn RL 1997 Successful aging. Gerontologist 37:433–440

Rudman D, Shetty KR 1994 Unanswered questions concerning the treatment of hyposomatotrophism and hypogonadism in elderly men. J Am Geriatr Soc 42:522–527

Rudman D, Feller AG, Nagraj HS et al 1990 Effects of growth hormone in men over 60 years old. N Engl J Med 323:1–6

Salomon F, Cuneo RC, Hesp R, Sonksen PH 1989 The effects of treatment with recombinant human growth hormone on body composition and metabolism in adults with growth hormone deficiency. N Engl J Med 321:1797–1803

Sih R, Morley JE, Kaiser FE, Perry HM III, Patrick P, Ross C 1997 Testosterone replacement in older hypogonadal men: a 12-month randomized controlled trial. J Clin Endocrinol Metab 82:1661–1667

Smith EP, Boyd J, Frank GR et al 1994 Estrogen resistance caused by a mutation in the estrogen-receptor gene in man. N Engl J Med 331:1056–1061

Snyder PJ, Peachey H, Hannoush P et al 1999a Effect of testosterone treatment on body composition and muscle strength in men over 65 years of age. J Clin Endocrinol Metab 84:2647–2653

Snyder PJ, Peachey H, Hannoush P et al 1999b Effect of testosterone treatment on bone mineral density in men over 65 years of age. J Clin Endocrinol Metab 84:1966–1972

Tenover JS 1992 Effects of testosterone supplementation in the aging male. J Clin Endocrinol Metab 75:1092–1098

Tenover JS 1998 Androgen replacement therapy to reverse and/or prevent age-associated sarcopenia in men. Baillière's Clin Endocrinol Metab 12:419–425

Vaillant GE, Vaillant CO 1990 Natural history of male psychological health, XII: a 45-year study of predictors of successful aging at age 65. Am J Psychiatry 147:31–37

van den Beld A, Huhtaniemi I T, Pettersson KS et al 1999 Luteinizing hormone and different genetic variants, as indicators of frailty in healthy elderly men. J Clin Endocrinol Metab 84:1334–1339

Vermeulen A 1991 Clinical review 24: androgens in the aging male. J Clin Endocrinol Metab 73:221–224

Vermeulen A, Verdonck L 1972 Some studies on the biological significance of free testosterone. J Steroid Biochem 3:421–426

Welle S, Thornton C, Statt M, McHenry B 1996 Growth hormone increases muscle mass and strength but does not rejuvenate myofibrillar protein synthesis in healthy subjects over 60 years old. J Clin Endocrinol Metab 81:3239–3243

Wolf OT, Neumann O, Hellhammer DH et al 1997 Effects of a two-week physiological dehydroepiandrosterone subsitiution on cognitive performance and well-being in healthy elderly women and men. J Clin Endocrinol Metab 82:2363–2367

Wolf OT, Naumann E, Hellhammer DH, Kirschbaum C 1998 Effects of dehydro-epiandrosterone replacement in elderly men on event-related potentials, memory, and well-being. J Gerontol A Biol Sci Med Sci 53:M385–M390

Yarasheski KE, Zachwieja JJ, Campbell JA, Bier DM 1995 Effect of growth hormone and resistance exercise on muscle growth and strength in older men. Am J Physiol 268:E268–E276

Yarasheski KE, Campbell JA, Kohrt WM 1997 Effect of resistance exercise and growth hormone on bone density in older men. Clin Endocrinol 47:223–239

Zgliczynski S, Ossowski M, Slowinska-Srzednicka J et al 1996 Effect of testosterone replacement therapy on lipids and lipoproteins in hypogonadal and elderly men. Atherosclerosis 121:35–43

DISCUSSION

Veldhuis: When you use the term 'unselected', surely these are patients who are self-selected; they agreed to come in. In this sense it is not fully random sampling. One of the long-standing problems in this area is getting full population

representation, but your numbers are extraordinary and the relationships are excellent.

This is sort of an elementary statistician's nightmare, because one has multiple possibilities for correlation. What I suspect you've probably done is to use some of the modern principal-component or cluster-variable-type statistical approaches, which lead to the question: can I select two or three variables which (in unique combination) explain one of several critical outcomes? There are statisticians who make their livelihood just out of this sort of principal component analysis. Can you tell us anything about that?

van den Beld: In comparing all the different axes, we tried to see which components are dependent on each other. It might not be just one hormone that is an indicator for an overall good physical functional status. These relationships are all independent of each other. The relationship of IGFBP2 with all the physical characteristics was very strong. For example, testosterone remains significantly related to muscle strength as well as bone mineral density after adjustment for IGFBP2. With regard to just the physical characteristics, I think the physical performance test that we measured by a method described by Guralnik et al (1994) was a very good one, as was the muscle strength test. These are probably the best indicators: if you measure them you know whether somebody is ageing healthily or not.

Veldhuis: There are three technical terms that I would suggest you take to your statistician. These are 'cluster' (not the cluster that we invented, this is a pre-existing cluster), 'principal component', and 'pathway' or 'critical component'. There are only a few statisticians who work with this, but they're looking at *n*-dimensional correlations. These are mind boggling to even visualize. You are asking yourself, 'Is there anyway in this constellation of features to find little nebulae that control the behaviour of the constellation?' You presented extraordinary data that a statistician of that ilk would be extremely enthusiastic about.

Laron: What is the effect of testosterone on visceral adipose tissue? It seems that the increase of adipose tissue with advancing age is as visceral fat. Are there any data on this? It is said that GH affects mainly visceral fat. As there are data showing that testosterone reduces adipose tissue, I wonder whether it has a differential effect on general versus visceral adipose tissue.

van den Beld: I could not determine that in my study because we measured only total fat mass and could not discriminate the visceral fat. I understand from the literature that testosterone does indeed also have an effect on the visceral fat.

Björntorp: Some years ago we carried out a 9 month study looking at testosterone effects in which we measured adipose tissue volumes by CT scan. We found a significant decrease only in visceral fat, which seemed to be a specific effect on visceral tissue. This might be because the density of androgen receptors is higher

in visceral fat than other adipose tissue. On this theme, I missed the Snyder report that you referred to in your paper (Snyder et al 1999a,b), but there are problems associated with performing studies like this. One of them is how to administer the testosterone: if you inject it you get peaks and non-optimal concentration curves. We tried various preparations in our studies and found that the transdermal administration was quite good because this generates a reasonably stable level within normal levels.

How were these men screened? When we screened our population we were careful to screen potential subjects with prostate-specific antigen (PSA) levels and ultrasound of prostate size: if there was any suspicion of something abnormal then we didn't include them. We found, for example, two men with elevated PSA, which would not have been good had they been included.

van den Beld: The subjects were strictly selected for this study.

Björntorp: As Professor Laron mentioned, there are similar studies of GH. I think it would be extremely interesting to try a combination of testosterone and GH treatment because there is evidence that these hormones amplify each other's effect in the periphery. We are planning to do a study like this, but we can't get the funding.

Veldhuis: We're very interested in the concept of low-dose GH treatment bringing an older individual to a mid-adult level. Targeted IGF1 combined with targeted testosterone would have low toxicity and may exert anabolic synergy. We performed a one month study in 10 men, each of whom was studied at baseline and then for one month on blinded transdermal 5 mg T-patch which brought the testosterone to about 20 nM (600 ng/decilitre). IGF1 was stimulated through 6.25 μg/kg GH per day. This was only a one-month study, but the muscle IGF1 peptide gene expression was increased in nine out of 10 men. We picked such biochemical endpoints, because it was a single-month study and we didn't expect functional, bone mineral or body fat changes. I think this needs to be explored further.

Björntorp: To add more to this from our studies, we looked at the risk factor pattern. Blood lipids went down (but the HDL cholesterol fraction did not change as far as I remember), and blood pressure decreased.

van den Beld: In our study we also measured the intima/medial thickness of the carotid artery. We also found a beneficial relationship between testosterone and the intima/medial thickness: the higher the testosterone levels the lower the intima/medial thickness.

Prior: The concern I have is that we are talking here about relationships at one point in time. What I find much more physiologically important and valuable are changes within people over time. I hope that you are carrying on these studies.

van den Beld: Last summer I performed a four year follow-up of all the elderly men, but I'm still waiting for the hormone results.

Carroll: I have a couple of questions about your sample. First of all, were these men medically screened at all?

van den Beld: Yes.

Carroll: They came from a working class suburb of Rotterdam. Did you screen for alcoholism, for example? Or diabetes?

van den Beld: Yes.

Carroll: Were these all eliminated?

van den Beld: There were five people with non-insulin dependent diabetes, and about 60 people taking medication for hypertension. We adjusted for medication.

Carroll: The correlations that you showed us are statistically significant by virtue of the large sample size, but in truth they don't account for more than about 10% of the variance in any one case. A lot of other things are going on here.

van den Beld: Yes, that's a problem.

Carroll: If you do a path analysis can you get to the directional links among these variables because the correlations themselves are not striking.

van den Beld: Exactly. This is still quite a selected group: the age range is narrow and there is an element of having to deal with survival of the fittest as well. They were all independently living men and they could all walk to the centre themselves. Secondly, I think a lot of the associations are determined genetically, so we are working in a restricted range.

Carroll: With regard to the QoL instrument, it was disappointing that this did not to seem to relate to very much except IGFBP2. What is in that instrument? Is there a specific mood score?

van den Beld: I haven't shown all the data. For example, questions about the patients' health, financial status and living conditions. The health part contains questions about how well they can see, or whether they have problems with their mobility or hearing. Within the first part they are asked how important they think it is, and in the second part they are asked how satisfied they are about this same question. Then the hormone-related questions are about sleep, anxiety, and this sort of thing.

Veldhuis: Can you summarize the main correlates of quality of living? I thought oestradiol was a strong endocrine correlate of quality of life. Was IGFBP3 a positive or negative correlate?

van den Beld: Positive. IGFBP1 was negative, as was BP2, but I didn't mention them because when we adjusted for muscle strength and physical performance, they were also strongly related to these QoL scores and there was no relationship. The relationship between IGFBP2 and QoL was probably explained by the good physical functional status of the subjects.

Veldhuis: So oestradiol and BP3 were both positive.

van den Beld: Yes.

Veldhuis: Here you have a possible linkage that is potentially very interesting. You could find these clusters and then look for some theoretical explanation that you could test further to try to explain successful living.

van den Beld: Testosterone was also positively related to quality of life, but this relationship also disappeared after adjustment for muscle strength and physical performance. Thus the effect of testosterone on quality of life is probably through the physical status of these men.

Carroll: Do you do any better if you look subscores on that instrument?

van den Beld: Yes, and then there are many more relationships. For example, testosterone was strongly positive with the question about mobility. There was also a question about sexual function and testosterone was positive with this.

Veldhuis: You might expect testosterone to have no real direct relationship with QoL within the normal range.

Laron: You show that mobility is vitally important for the well-being of the elderly males. When we take into consideration that increased exercise builds up the muscles, increases GH secretion and reduces adipose tissue, how much can we achieve by postponing the negative effects of ageing by increasing exercise, thus preventing the undesirable effects of the changes in hormone secretion?

van den Beld: Quite a lot. If you can try to get them to exercise it is much better than giving them hormone replacement therapy.

Laron: Have you tried to do this?

van den Beld: No.

Björntorp: I heard Steve Blair recently describing his phenomenal studies in which they followed 10 000 people performing exercise tests for 10–20 years. He found it was worse to be lean and untrained than fat and trained.

Morley: A meta analysis in the *Journal of Gerontology* clearly shows that exercise has no outcome or long-term effect on disability (Keysor & Jette 2001). It has outcomes on strength, but when you look at all the studies that have been done that look at function, beyond a couple of months you cannot see this. I was surprised, but this is a very good meta analysis. People tend to look at the short-term effects and say they're wonderful, but in studies in which exercise is maintained long-term in controlled situations the results are disappointing.

Following up on Zvi's point about mobility, there is absolutely no evidence that mobility is important for function. All the function tests have been built around mobility, but if you look at basic activities of daily living, while we are focused on lower limb strength it is in fact upper limb strength and cognition that decide whether you can function or not. We focus on legs, but legs get exercised all the time, so hormones are much less likely to make a major difference there than they are in the upper limbs. If you look at the literature for some of the hormones, upper limb strength tends to improve more than you will find with lower limb strength.

Handelsman: We have recently completed two placebo-controlled trials using alternative androgen delivery. There was no effect at all on upper limb strength over three months in either study.

Morley: I think we have overlooked a couple of important general points. Firstly, there are a number of longitudinal studies out there. We have got to pay attention to these. For instance, a study in Wisconsin showed short-term, in healthy elderly people, DHEA tends to go up a little (E. Duthie, E. Burns, unpublished observations). Over a four year period in healthy older people the changes are different than if you take big groups of people where clearly it goes down. The longitudinal studies in addition have to take into account seasonal effects. It is clear that most hormones have not only daily effects but they also have circannual effects. Franz Halberg has recently pointed out that if you look at 10 year effects, you can plot myocardial infarction very nicely against sunspots which are about a quasi 10–11 year period, so when we look at a single period of time we are probably looking at nothing in an ageing process that takes place over 70 or 80 years (Halberg et al 2001). Until we start to pay a little more attention to this we are going to get into trouble. At least in our cross-sectional study, the New Mexico Process Study, we showed that not only testosterone but also IGF1 in males did correlate with exercise, physical activity and food intake (Baumgartner et al 1999). There are actually very few data on females, and these are mostly on oestrogen. In fact, oestrogen has nothing to do with muscle strength. At the time of the menopause, although there is a rapid loss of muscle strength, it is not related to oestrogen.

Handelsman: I wanted to pick up the issue of the nature of the sample. You described them as unselected, but while they may not have been selected by you, they were selected by themselves. What was the population from which you drew the 400 and why did these 400 participate?

van den Beld: I did not refer to them as unselected. They are only unselected with regard to hormone replacement studies. We sent invitations to all men aged above 73 in the town. 25% participated but of the 75% who did not participate some of them already lived in nursing homes, so it's difficult to define the potential group that could have participated.

Handelsman: Were they paid?

van den Beld: No.

Riggs: It seems to me that the term 'healthy ageing' is a bit of an oxymoron. In ageing research, if you just study healthy people you are going to have a rather severe bias. I also have an issue with epidemiological studies in general. I think they're very good for generating hypotheses, but at the end of the day you are going to have to intervene: this is the only way you can be absolutely sure. The pathway analysis that you showed is so complex that it is going to take quite a while for all of us working together to be able to back this up with interventional studies. I think this is the only way we will learn.

Veldhuis: How would you design an ideal intervention study? What points would you tend to focus on given the analysis you have so far of these Rotterdam data?

van den Beld: We have already performed an intervention study, with DHEA replacement therapy. We took hundreds of really healthy men, not being treated for hypertension, and we selected them on muscle strength. We tried to get subjects with low muscle strength because four years ago we thought that this would make a good endpoint to look at, since it was so highly correlated with all the other physical characteristics and it is easy to measure. We also measured all the other physical characteristics to see what would make the best endpoint. However we could not find any effect of DHEA replacement therapy for 9 months.

Wang: Was this with 50 mg DHEA?

van den Beld: Yes.

Morley: Baulieu et al (2000) showed the same thing in a huge study. It is very difficult to support the DHEA concept, at least at 50 mg. The studies that showed any positive effects were performed at 100 mg, except for mood. They had a problem with their placebo which will have affected mood.

Veldhuis: I have a comment on the converse strategy. Picking a group at high risk for catabolism, such as the perioperative hip study that you did with GH which appeared to show a subgroup response that was dramatic as an interventional strategy, is interesting. However, one of the tough issues for all of us is which group to pick for an intervention study. Does the choice risk a type II error, risk confounds and risk a short lifetime on our part to ever publish the data?

van den Beld: We gave subjects GH therapy or placebo within 24 h of hip fracture, continuing for 6 weeks. I'm not sure about the dose we gave but the subjects over the age of 80 who received GH returned home significantly earlier compared to the placebo-treated subjects.

Wang: Are these all men?

van den Beld: Men and women.

Wang: Bill Bremner's group has data from Seattle, where they treated men after knee surgery with high doses of testosterone (600 mg i.m. weekly for around 6 weeks) (abstract presented at the Endocrine Society Annual Meeting, June 2000, Toronto, Canada). Their stay in hospital was remarkably decreased.

Morley: The best testosterone study so far published is the one by Bakhshi et al (2000), where they looked at a rehabilitation group and clearly showed improvement of the rate of rehabilitation with an adequate but not massive replacement dose. To take frail people and give massive doses of anything is going to get you into trouble if you continue for long enough, we shouldn't be doing it. One of the major problems with this field is that people have taken non-hypogonadal, non-GH deficient people and given them huge pharmacological doses. We should remember that the Snyder et al (1999a,b) study was actually in

non-hypogonadal men on the whole. I would never show the Snyder study as a study for testosterone replacement because it didn't look at real people in whom you would be replacing testosterone.

van den Beld: Snyder also showed that there might be a threshold. Indeed, this is a problem: if you have healthy men you don't have the threshold.

Haus: We have carried out a study, which in some aspects was similar to Dr Van den Beld's, in cooperation with the C. I. Parhon Institute of Endocrinology of the Romanian Academy of Medical Sciences in Bucharest, Romania. We studied the circadian time organization in about 300 elderly subjects of both sexes, of ages ranging from the 6th to the 10th decade (mean age 77 11 SEM). The subjects were regarded as clinically healthy by their physicians and were not suffering any acute, active and debilitating disease, although partially age-dependent conditions like atherosclerosis, osteoarthritis and mild hypertension were present as to be expected in any average population of this age. We studied some 15 hormonal variables and about 30 other biochemical variables at six time points over one 24 h span, and in 32 subjects of this group four times over a 24 h span, each time during a different season (Nicolau et al 1984, Haus et al 1988).

We found that circadian periodicity is well maintained in clinically healthy elderly and old subjects (Haus et al 1988, 1989). However, there are some characteristic differences. If the temporal order of 39 endocrine and biochemical variables is analysed by cluster (pattern) analysis, the timing of the rhythms varies predominantly with gender, while the amplitude (expressed as percentage of the circadian mean value) varies with age (Ticher et al 1994). It is of interest that in women of different age groups, the epidemiologically determined risk to develop breast cancer was characterized by clustering of the rhythm's timing (the peak times or acrophases) and not according to the amplitude, although age is regarded as one of the prominent risk factors for the development of breast cancer (Ticher et al 1994, 1996). The majority of endocrine variables showed a decrease in circadian amplitude with ageing, which was pronounced in melatonin, growth hormone, aldosterone, adrenal androgens, catecholamines, TSH, serum iron, and in others in contrast to LH and insulin, which showed an increase of level and amplitude with advancing age (Haus et al 1989, Haus & Touitou 1997).

We explored the relation of rhythm parameters to the functional state of the elderly subjects, as measured by the activities of daily living (ADL) index and a mental status index developed by psychiatrists of the C. I. Parhon Institute. We found a better functional state in subjects with higher (although not hypertensive) blood pressure, higher (although not obese) body weight, lower aldosterone (with higher salt excretion) and lower GH. Elderly men with higher circadian mean testosterone concentrations did better than the ones with lower testosterone, while the women with higher testosterone did worse.

Unfortunately, we did not have sex hormone binding protein determinations available at that time.

In the elderly, we found absence of circannual rhythms or seasonal variations in several variables as a group phenomenon, for example, in catecholamines and some thyroid functions. If we assume that these seasonal variations are due to external factors (e.g. cold), their absence may indicate a defect of adaptation in the elderly, or if we assume that these variations are the expression of endogenous circannual rhythms, their absence may indicate a desynchronization within the group, e.g. with free running circannual rhythms in some of the subjects as we could observe in the elderly, e.g. in blood pressure.

A word of warning has to be said about the comparison of populations of different ethnic/geographic background. We had the opportunity to study under comparable conditions groups of subjects in Japan, the USA and Romania. Using cluster analysis, there was a similar acrophase clustering in the Americans and Romanians, while the Japanese appeared to be different in their temporal organization (Ticher et al 1996). Also the ageing patterns in the pulsatile secretion of prolactin and of cortisol were different in American women as compared with Japanese. Any generalizations concerning ageing changes in ethnically/geographically different populations have to be treated with caution.

Laron: There may be a resistance to aldosterone/GH hormone developing with age as there is with insulin.

Haus: Unfortunately we did not have IGF1 levels. This could tell us whether this may be a resistance to GH function at the receptor level.

Morley: The Paris study (Maison et al 1998) showed that high GH levels were associated with death in Parisian men. I think that's the problem with high GH: none of the studies, including the animal studies, have shown that it does anything but kill you as you get older.

Veldhuis: It does raise the question of stress confounds, one of which is nutrition, lowering IGF1 and stimulating some GH elevation. Do you have cortisol or interleukin/cytokine data?

van den Beld: That's something we are working on at the moment. We did measure cortisol but the only correlation we found was that it was inversely correlated with quality of life.

References

Bakhshi V, Elliott M, Gentili A, Godschalk M, Mulligan T 2000 Testosterone improves rehabilitation outcomes in ill older men. J Am Geriatr Soc 48:550–553

Baulieu EE, Thomas G, Legrain S et al 2000 Dehydroepiandrosterone (DHEA), DHEA sulfate, and aging: contribution of the DHEAge Study to a sociobiomedical issue. Proc Natl Acad Sci USA 97:4279–4284

Baumgartner RN, Waters DL, Gallagher D, Morley JE, Garry PJ 1999 Predictors of skeletal muscle mass in elderly men and women. Mech Ageing Dev 107:123–136

Guralnik JM, Simonsick EM, Ferrucci L et al 1994 A short physical performance battery assessing lower extremity function: association with self-reported disability and prediction of mortality and nursing home admission. J Gerontol 49:M85–M94

Halberg F, Cornelissen G, Watanabe Y et al 2001 Near 10-year and longer periods modulate circadians: intersecting anti-aging and chronoastrobiological research. J Gerontol 56:M304–M324

Haus E, Touitou Y 1997 Chronobiology of development and aging. In: Redfern PH, Lemmer B (eds) Handbook of experimental pharmacology: physiology and pharmacology of biologic rhythms. Springer-Verlag, Heidelberg, p 95–134

Haus E, Nicolau GY, Lakatua D, Sackett-Lundeen L 1988 Reference values for chronopharmacology. Annu Rev Chronopharmacol 4:333–424

Haus E, Nicolau G, Lakatua DJ, Sackett-Lundeen L, Petrescu E 1989 Circadian rhythm parameters of endocrine functions in elderly subjects during the seventh to the ninth decade of life. Chronobiologia 16:331–352

Keysor JJ, Jette AM 2001 Have we oversold the benefit of late-life exercise? J Gerontol 56:M412–M423

Maison P, Balkau B, Simon D, Chanson P, Rosselin G, Eschwege E 1998 Growth hormone as a risk for premature mortality in healthy subjects: data from the Paris prospective study. Br Med J 316:1132–1133

Nicolau GY, Lakatua DJ, Sackett-Lundeen L, Haus E 1984 Circadian and circannual rhythms of hormonal variables in elderly men and women. Chronobiol Int 1:301–319

Snyder PJ, Peachey H, Hannoush P et al 1999a Effect of testosterone treatment on body composition and muscle strength in men over 65 years of age. J Clin Endocrinol Metab 84:2647–2653

Snyder PJ, Peachey H, Hannoush P et al 1999b Effect of testosterone treatment on bone mineral density in men over 65 years of age. J Clin Endocrinol Metab 84:1966–1972

Ticher A, Sackett-Lundeen L, Ashkenazi IE, Haus E 1994 Human circadian time structure in subjects of different gender and age. Chronobiol Int 11:349–355

Ticher A, Haus E, Ron IG, Sackett-Lundeen L, Ashkenazi IE 1996 The pattern of hormonal circadian time structure (acrophase) as an assessor of breast-cancer risk. Int J Cancer 65: 591–593

Endocrine Facets of Ageing.
Novartis 242

Print ISBN 0-471-48636-1 Online ISBN 0-470-84654-2

Ageing, stress and the brain

Bernard J. Carroll

Pacific Behavioral Research Foundation, 26386 Carmel Rancho Lane, Suite 202, PO Box 223040, Carmel, CA 93922-3040, USA

Abstract. Ageing of the brain is an important factor in overall ageing and mortality, and new insights have clarified the relationship between neuroregulation and ageing. First, neuronal loss in normal ageing is now known to be a minor change. Loss of synapses through dystrophic neuronal change is the hallmark of normal ageing. Second, similar dystrophic changes occur in the brain with chronic stress. In both instances, forebrain sites experience loss of synaptic input from brainstem regulatory nuclei. Third, functional ageing is attributed in part to lifetime stress, under the concept of 'allostatic load'. Being inseparable from the functions of appraising and responding to stress, the brain is an ultimate mediator of stress-related mortality, through hormonal changes that lead to proximate pathologies like hypertension, glucose intolerance, cardiovascular disease and immunological impairment. In chronic stress the brain shows clear allostatic compensations that lead to pathology. Two subtle and chronic mechanisms that may mediate brain pathology and accelerated ageing in chronic stress are proposed. These are abnormal glucocorticoid receptor (GR) occupancy over the 24 h cycle, and elevated body temperature. These factors lead to GR-mediated tissue changes and to acceleration of general cellular ageing mechanisms. Human depression is discussed as an exemplary demonstration of these principles.

2002 Endocrine facets of ageing. Wiley, Chichester (Novartis Foundation Symposium 242) p 26–45

In 1988 Joseph Meites described a neuroregulatory theory of ageing, emphasizing the integrative role of the nervous system for neuroendocrine axes and circadian rhythms (Meites 1988). As the brain aged, Meites proposed, so would the hypothalamus age, leading to menopause, andropause, somatopause and dysregulated circadian rhythms. Meites tested pharmacological strategies to augment hypothalamic neurotransmitter function, which he found could reverse these 'biomarkers of ageing', a result that comports with the age-associated decline of hypothalamic monoamine neurotransmitter systems (Rodríguez-Gómez et al 1995). Though it can be modified by recent insights, the general perspective of Meites that we endocrinologists are also neuroscientists remains valid. Ageing of the brain is an important factor in overall ageing and mortality.

Since that time, four new insights have changed our understanding of neuroregulation and ageing. First, neuronal loss in normal ageing is minor; loss

of synapses through dystrophic neuronal change is the hallmark of normal ageing. Second, similar dystrophic changes occur in the brain with chronic stress. Third, functional ageing is attributed in part to lifetime stress, under the concept of 'allostatic load'. Being inseparable from the functions of appraising and responding to stress, the brain is an ultimate mediator of stress-related mortality, through hormonal changes that lead to proximate pathologies like hypertension, glucose intolerance, cardiovascular disease and immunological impairment. Fourth, neurogenesis does occur in the adult mammalian brain, which opens new possibilities for treatments. The topic of neurogenesis is beyond the scope of this chapter, though we may say with Pasko Rakic (1998) that, as a result of this discovery, 'the word impossible is not in the vocabulary of contemporary neuroscience'.

Ageing of the normal brain

Until recently, ageing and senility were considered firm partners. We now know, however, that normal ageing of the brain is a process distinct from the degenerative dementias. Early neuropathology studies led to the dogma that up to 40% of neurons inexorably died over the lifespan. That belief was overturned by improved methodology using unbiased stereological techniques (Morrison & Hof 1997). In ageing primates and ageing rodents, rather than a loss of cortical pyramidal cells there is decreased neuronal size and loss of synaptic arborization (Masliah et al 1993, Peters et al 1994). Gómez-Isla et al (1996) found no age-related loss of cells in the entorhinal cortex of the temporal lobe in normal subjects aged from 60 to 90, in contrast to losses up to 65% in early Alzheimer's disease (AD).

A modest loss of brain volume occurs in normal ageing. In men, total brain volume at 60 is 10% less than at 25 years (Murphy et al 1992). This change affects cortical and subcortical white matter rather than grey matter (Guttmann et al 1998, Peters et al 1994) and is associated with myelin pathology in vertical fibres traversing deeper layers of the cortex. This myelin pathology will slow nerve conduction in association pathways, and may lead to functional deficits in reaction time and working memory (Peters 1996, Peters et al 1994, 2000). In the rhesus monkey, neurons of prefrontal cortex and substantia nigra show severe dendritic pathology, with loss of organelles, vacuolation of cytoplasm, membranous whorls and dense inclusion bodies (Siddiqi & Peters 1999, Peters et al 1998). There is a reduction of 30–60% in the density of apical synapses on pyramidal cells in prefrontal cortex. These changes correlate with age-related cognitive impairment (Peters et al 1996, 1998).

It was also accepted wisdom that age-related neuronal loss occurs in subcortical nuclei of the human brainstem, such as locus ceruleus (LC), which project

widely to the cerebral cortex and to subcortical limbic system sites, including the hypothalamus (Chan-Palay & Asan 1989). Recent results with unbiased stereological methods are conflicting, with predominantly negative studies (Ohm et al 1997, Kubis et al 2000), though some continue to report substantial cell loss in LC with age (Manaye et al 1995). Whether LC cells do or do not die with age, their forebrain and hypothalamic projections certainly are dystrophic, with reduced terminal arborization, loss of synaptic contacts and abnormal axonal branching (Ishida et al 2000). A similar pattern of preserved neuronal number but aberrant axonal formations with ageing occurs in the serotonin-containing rostral projections of the dorsal raphe nucleus (Nishimura et al 1998, van Luijtelaar et al 1992).

A particular focus of recent work has been the trisynaptic perforant pathway (PP) circuit that projects from layer II of the entorhinal cortex (EC) to granule cells in the dentate gyrus of the hippocampus. These neurons in turn send mossy fibre projections to the CA1 and CA3 regions of Ammon's horn, with further relays to the hippocampal outflow paths via the subiculum and the fimbria-fornix (see Morrison & Hof 1997). This circuit is critical for associative memory, as the EC receives highly processed information from heteromodal cortical association areas, which it funnels into the hippocampus. Layer II of the EC is affected by neurofibrillary tangles (NFTs) very early in AD, but it is minimally affected in normal ageing. Likewise, there is up to a 50% neuronal loss in the EC in early AD but not in normal ageing.

Nevertheless, there is evidence of functional impairment of the perforant pathway in normal ageing. Smith et al (2000) reported that spatial learning deficits in aged rats correlated with reduced synaptophysin staining in the PP entry zone, as well as in the CA3 region, which suggests loss of synaptic integrity between EC and hippocampus via the PP. There is also a significant decrease of *N*-methyl-D-aspartate (NMDA) receptor subunit 1 (NMDAR1) in the distal dendrites of the dentate gyrus granule cells that receive the PP input (Gazzaley et al 1996) — the inference is that distal dendritic pruning has occurred in the granule cells. It is now clear that glucocorticoid excess and chronic stress produce similar dendritic changes (McEwen 2000). The role of the PP and NMDA receptors in long-term potentiation in the hippocampus is well known, and the changes just described have been suggested as a basis of benign, age-related memory decline (Morrison & Hof 1997).

In the hypothalamus itself, ageing and AD also are quite distinct with respect to NFTs (Swaab et al 1992) and cell survival. Age-related changes of neuronal number in human hypothalamic nuclei are quite variable (Swaab 1995, Hofman 1997). The sexually dimorphic interstitial nucleus of the anterior hypothalamus displays a greater than 80% decrease of cell number in both sexes. In contrast, the vasopressin- and oxytocin-producing cells of the supraoptic nucleus (SON) and

the paraventricular nucleus (PVN) remain intact in old age. In the infundibular nucleus there is increased cell number and activity, with hypertrophic neurokinin B neurons in postmenopausal women.

Age-related neuronal dystrophic changes occur in the hypothalamus, similar to those described in EC, hippocampus and cerebral cortex. For example, in the arcuate nucleus the number of dendritic segments, total dendritic length, branching and spine densities are reduced (Leal et al 1998). Likewise, in the SON of the rat, marked dendritic regression is seen even though there is no neuronal loss. The dendritic regression is thought to result from deafferentation due to the preceding age-related loss of the noradrenergic input to the SON from the brainstem (Flood & Coleman 1993). A related contribution is the decrease of a key growth factor, brain-derived neurotrophic factor (BDNF) in ageing (Croll et al 1998). These dystrophic changes in the ageing hypothalamus resemble those seen in the hippocampus and the frontal cortex with stress (see below). The morphologic similarities are accompanied by activation of hypothalamic nitric oxide synthase (NOS) through NMDA receptor activation in both stress and ageing (Kishimoto et al 1996, Vernet et al 1998). This process has been identified as responsible for corticosterone-produced dystrophic dendritic changes in CA3 hippocampal pyramidal cells (Reagan et al 1999), and may be a general mechanism by which stress and glucocorticoids cause neuronal dystrophy. Likewise, both stress and ageing are associated with a decrease of BDNF in hippocampus, hypothalamus and cortex. Furthermore, in ageing rats the dynamic responses of BDNF and its receptor, TrkB, to stress are impaired (Smith et al 1995, Smith & Cizza 1996), a dysregulation that appears to be glucocorticoid-mediated (Cosi et al 1993).

In summary, age-associated changes in the brain are not like the pathology of AD. They more closely resemble the changes caused by stress. There is no marked loss of cortical neurons and only minimal appearance of NFTs in the PP–hippocampal circuit or the hypothalamus. There is significant pathology of myelin and of glial cells, which may slow nerve conduction velocities, reduce the formation of synapses, and impair normal associative functioning. Most striking is the dystrophy of forebrain projections from key brainstem nuclei. Axons display reduced terminal arborization and abnormal branching, which is followed by dendritic atrophy in the terminal fields. This deafferentation and the loss of synapses result in functional disconnection and consequent dysregulation of hypothalamic functions, including the gonadal, growth hormone and hypothalamus–pituitary–adrenal (HPA) axes. Candidate mechanisms for the functional disconnection of the forebrain and hypothalamus in ageing are NMDA receptor–NOS activation, compounded by impaired responsiveness of mRNA for BDNF and TrkB, which would normally counter-regulate the dendritic and axonal dystrophy in brainstem and forebrain sites. Both these

neurochemical changes are glucocorticoid-mediated and are seen in chronic stress.

Allostasis, allostatic load and ageing

Another shift in the neuroregulatory perspective is the proposal that ageing is related to lifetime stress, under the concepts of allostasis and allostatic load. Sterling & Eyer (1988) introduced the term allostasis to explain morbidity and mortality associated with chronic stress, especially social stress in human populations. They defined allostasis as 'maintaining stability through change'. The boundaries between allostasis and related constructs such as homeostasis, adaptation and pathology are sometimes unclear. It is a heuristic construct that is still being refined (for example by Koob & Le Moal 2001). Speaking generally, allostasis is distinct from homeostasis in maintaining a compensated equilibrium rather than a physiological equilibrium: stability is maintained at a price. The allostatic set point is abnormal relative to the homeostatic set point, the system is inherently less stable, and it has a relatively narrow dynamic range. Finally, a system in allostasis leads to pathology whereas a system in homeostasis does not.

The term allostatic load was introduced by McEwen & Stellar (1993) to denote the cost of responding repeatedly or chronically to stress. McEwen (1998) emphasized four types of responses that produce this 'wear and tear': (i) repeated acute stress challenges; (ii) failure to adapt (i.e. to extinguish) the acute (or chronic) stress responses normally with repeated (or chronic) exposure; (iii) excessively prolonged acute stress responses; and (iv) inadequate acute stress responses leading to elevated activity of other, normally counter-regulated allostatic systems after stress (for which the Lewis rat is a proposed model). Both developmental and genetic dimensions have been proposed as modifiers of allostatic load that may determine vulnerability and risk of pathology (McEwen 2000).

When we view the brain's response to stress, all the features of allostasis are discernible (McEwen 2000). Chronic stress produces changes in brain function and structure consistent with a compensated equilibrium and altered set points. These include increased activity of the HPA axis; impaired fast and delayed glucocorticoid feedbacks; and elevated circulating glucocorticoids. There is a restricted dynamic range, with diminished circadian amplitude and elevated nadir values. The resultant pathology includes decreased neurogenesis in the dentate gyrus; loss of terminal arborization in the forebrain projections of the LC and the raphe nuclei (Kitayama et al 1994, Duman et al 1997); dendritic regression in hippocampus; and loss of synapses in hypothalamus and cortex. Antidepressant treatments that induce mRNA for BDNF and TrkB can reverse these changes (Kitayama et al 1997, Nibuya et al 1995). The functional result of these changes is

a feed-forward cascade of disinhibited HPA activity, and other dysregulations such as loss of oestrous cycling, impaired hedonic function (decreased spontaneous motor activity, caloric intake and sweet food preference, and intracranial self-stimulation), and decreased adaptive behaviours (Hatotani et al 1977, 1979, Katz 1982). These effects of chronic stress resemble those seen in ageing. Consistent with this similarity is the decreased longevity of inbred rat strains that are hyperreactive to stress. Mean lifespan is 15 months in the spontaneously hypertensive rat (SHR), and 21.5 months in the Wistar–Kyoto (WKY) rat, compared with 31 months for the Brown Norway rat (Gilad & Gilad 1987, Brandle et al 1997).

In summary, the neuropathological and neuroregulatory changes of normal ageing resemble those associated with chronic stress. These findings are consistent with the proposal that longevity is affected by allostatic load, and give new meaning to Selye's famous phrase, 'the stress of life'. The glucocorticoid-mediated allostatic effect on lifespan must be mediated through relatively subtle and chronic processes. Comparisons often invoked, for example by Sapolsky (2000), between chronic stress and grossly pathological hypercortisolaemic states like Cushing's disease are not appropriate or informative. The exposure of tissues to glucocorticoids in human and animal chronic stress paradigms is well below the cushingoid range. Two more relevant candidate mediators of allostatic pathology in chronic stress are proposed below.

First, the critical allostatic link between chronic stress, brain dystrophic change and longevity is not likely to be the exposure of tissues to pathologically raised concentrations of glucocorticoids. A chronobiological explanation is more likely, namely, an altered HPA circadian rhythm that leads to the continuous occupancy of glucocorticoid receptors (GRs) in target tissues. An important consideration here is that normally GRs are essentially unoccupied during 25–30% of the day (Dallman & Akana 1991). With the allostatic resetting of the circadian HPA programme by stress, elevated nadir values of cortisol or corticosterone are observed, which are sufficient to produce continuous occupancy of GRs over 24 h (e.g. López et al 1998). Continuous GR occupancy is abnormal, as evidenced by tissue change such as thymic atrophy, even though the mean 24 h cortisol value is held normal (Akana et al 1991). GR-containing target sites in the brain affected by this process will include the LC, raphe nuclei and hippocampus, where stress-related dystrophic change occurs. Other GR-responsive tissues such as skin, bone and liver will also be affected, with consequent changes such as hair loss, skin ulceration, osteoporosis, decreased muscle mass, increased susceptibility to infections and glucose intolerance that are characteristic of chronically stressed rats.

A second candidate allostatic mechanism leading to pathology is the stress-associated phenomenon of elevated body temperature, which is most marked during the quiet phase of the activity and HPA circadian rhythms (Meerlo et al

1997). The persistent, subtle hyperthermia results in a hypermetabolic state that will accelerate general cellular ageing mechanisms. These include mitochondrial failure and mitochondrial DNA damage, accumulation of oxygen free radicals and protein conformational changes (Drachman 1997). The combination of chronic hyperthermia with abnormal GR occupancy may be additive for pathology. The significance of the allostatic load of nocturnal hyperthermia as a link between stress and ageing is apparent from studies that find body temperature differences associated with differential longevity (Hunter et al 1999). The SHR rat strain provides an especially good example of stress-associated hyperthermia, end organ pathology and shortened lifespan (Berkey et al 1990, Morley et al 1990). Parenthetically, calorie restriction, which dramatically increases longevity, leads to a persistent reduction of body temperature (Lane et al 1996). Thus, these two factors, abnormal GR occupancy and hyperthermia, which occur together during the quiet phase of the HPA circadian cycle, are candidate mechanisms for the subtle and chronic effects of stress on the brain and on longevity. Both factors operate also in major depression, a clear instance of an allostatic disorder.

Depression: an allostatic disorder with premature mortality

Human depression is a clear example of the allostatic link between chronic stress and reduced longevity. The importance of psychosocial stress for provocation of depressive episodes, for vulnerability to, and for risk of depression has been reviewed by Checkley (1996). Depression is an established outcome of stress, and the ongoing depressive episode itself constitutes a chronic stress. In large, prospective studies of adverse medical outcomes in depression, the customary psychiatric distinction between depressive symptoms and a depressive syndrome appears not to be important. Stress is a risk factor for both major depression (syndrome) and minor depression (symptoms). Minor depression is both a prodrome of major depression and an outcome of major depression; and major depression is a major outcome of minor depression.

The neuroendocrinology of human depression closely resembles that of chronic stress models in the laboratory (Checkley 1996, López et al 1998). One sees increased central HPA activity, reduced feedback, and adrenocortical hypertrophy. Mean 24 h plasma cortisol concentrations are normal to slightly elevated, as are urinary free cortisol excretion, plasma and cerebrospinal fluid free cortisol concentrations, and salivary cortisol. Nocturnal plasma cortisol concentrations are significantly raised, whereas daytime plasma cortisol concentrations usually are not. Moreover, body temperatures, both nocturnal and diurnal, are significantly elevated in depression by 0.4–0.6 °C (Szuba et al 1997, Rausch et al 2000). These are classical allostatic changes.

Recent studies suggest a linear relationship between the number of depressive symptoms and premature mortality. Koenig et al (1999) found in a nine-year prospective study of 1001 male general medical inpatients that the adjusted mortality hazard increased 10% for each one-point increase in baseline depression score. In a prospective study of 7518 elderly community-resident women followed for six years, Whooley & Browner (1998) found 7% mortality in those with no depressive symptoms, 17% in those with three to five symptoms, and 24% in those with six or more depressive symptoms. The excess mortality was associated with both cardiovascular and non-cardiovascular deaths, but not with cancer. Depression is associated with many cardiovascular risk factors. These include hypertension, elevated plasma noradrenaline concentrations, decreased heart rate variability (an index of cardiac vagal tone), increased platelet reactivity, increased plasma fibrinogen, myocardial ischaemia and myocardial infarction. Non-cardiac morbidity associated with depression includes Type II diabetes mellitus, osteoporosis, increased risk of falls (a correlate of decreased muscle mass), increased intra-abdominal fat (a corticosteroid-related risk factor for cardiovascular disease), impaired wound healing and immune system suppression with increased incidence of influenza in the elderly (see Whooley & Browner 1998).

Several of these depression-associated pathologies can be related to abnormal GR occupancy (for example, osteoporosis, increased intra-abdominal fat, immune system impairment and impaired glucose tolerance). Others may reflect accelerated ageing through the interaction of allostatic nocturnal hyperthermia with general cellular degenerative mechanisms, as was discussed above in relation to chronic stress. Thus, this human disorder well illustrates the connections among ageing, stress and the brain. Human depression provides an excellent model for refining the concept of allostasis and for advancing our understanding of the long-term effects of stress on lifespan.

References

Akana SF, Bradbury MJ, Cascio S et al 1991 Low levels of exogenous corticosterone (B) impair regulation of ACTH and B in intact rats. Endocrinology 128A:41

Berkey DL, Meeuwsen KW, Barney CC 1990 Measurements of core temperature in spontaneously hypertensive rats by radiotelemetry. Am J Physiol 258:R753–R759

Brandle M, al Makdessi S, Weber RK, Dietz K, Jacob R 1997 Prolongation of life span in hypertensive rats by dietary interventions. Effects of garlic and linseed oil. Basic Res Cardiol 92:223–232

Chan-Palay V, Asan E 1989 Quantitation of catecholamine neurons in the locus ceruleus in human brains of normal young and older adults and in depression. J Comp Neurol 287:357–372

Checkley S 1996 The neuroendocrinology of depression and chronic stress. Br Med Bull 52:597–617

Cosi C, Spoerri PE, Comelli MC, Guidolin D, Skaper SD 1993 Glucocorticoids depress activity-dependent expression of BDNF mRNA in hippocampal neurons. Neuroreport 4:527–530

Croll SD, Ip NY, Lindsay RM, Wiegand SJ 1998 Expression of BDNF and trkB as a function of age and cognitive performance. Brain Res 812:200–208

Dallman MF, Akana SF 1991 Negative feedback of glucocorticoids on the brain and pituitary: role of feedback and its interactions with circadian and stress signals. In: Costa E, Paul SM (eds) Neurosteroids and brain function. Thieme Verlag, Stuttgart (Fidia Research Found Symp 8) p 25–29

Drachman D 1997 Aging and the brain: a new frontier. Annals of Neurology 42:819–828

Duman RS, Heninger GR, Nestler EJ 1997 A molecular and cellular theory of depression. Arch Gen Psychiatry 54:597–606

Flood DG, Coleman PD 1993 Dendritic regression dissociated from neuronal death but associated with partial deafferentation in aging rat supraoptic nucleus. Neurobiol Aging 14:575–587

Gazzaley AH, Siegel SJ, Kordower JH, Mufson EJ, Morrison JH 1996 Circuit-specific alterations of N-methyl-D-aspartate receptor subunit 1 in the dentate gyrus of aged monkeys. Proc Natl Acad Sci USA 93:3121–3125

Gilad GM, Gilad VH 1987 Age-related reductions in brain cholinergic and dopaminergic indices in two rat strains differing in longevity. Brain Res 408:247–250

Gómez-Isla T, Price JL, McKeel DW Jr, Morris JC, Growdon JH, Hyman BT 1996 Profound loss of layer II entorhinal cortex neurons occurs in very mild Alzheimer's disease. J Neurosci 16:4491–4500

Guttmann CR, Jolesz FA, Kikinis R et al 1998 White matter changes with normal aging. Neurology 50:972–978

Hatotani N, Nomura J, Yamaguchi T, Kitayama I 1977 Clinical and experimental studies on the pathogenesis of depression. Psychoneuroendocrinology 2:115–130

Hatotani N, Nomura J, Inoue K, Kitayama I 1979 Psychoendocrine model of depression. Psychoneuroendocrinology 4:155–172

Hofman MA 1997 Lifespan changes in the human hypothalamus. Exp Gerontol 32:559–575

Hunter WS, Croson WB, Bartke A, Gentry MV, Meliska CJ 1999 Low body temperature in long-lived Ames dwarf mice at rest and during stress. Physiol Behav 67:433–437

Ishida Y, Shirokawa T, Miyaishi O, Komatsu Y, Isobe K 2000 Age-dependent changes in projections from locus coeruleus to hippocampus dentate gyrus and frontal cortex. Eur J Neurosci 12:1263–1270

Katz RJ 1982 Animal model of depression: pharmacological sensitivity of a hedonic defect. Pharmacol Biochem Behav 16:965–968

Kitayama I, Nakamura S, Yaga T et al 1994 Degeneration of locus ceruleus neurons in stress-induced depression model. Brain Res Bull 35:573–580

Kitayama I, Yaga T, Kayahara T et al 1997 Long-term stress degenerates, but imipramine regenerates, noradrenergic axons in the rat cerebral cortex. Biol Psychiatry 42:687–696

Kishimoto J, Tsuchiya T, Emson PC, Nakayama Y 1996 Immobilization-induced stress activates neuronal nitric oxide synthase (nNOS) mRNA and protein in hypothalamic-pituitary-adrenal axis in rats. Brain Res 720:159–171

Koenig HG, George LK, Larson DB, McCullough ME, Branch PS, Kuchibhatla M 1999 Depressive symptoms and nine-year survival of 1,001 male veterans hospitalized with medical illness. Am J Geriatr Psychiatry 7:124–131

Koob GF, Le Moal M 2001 Drug addiction, dysregulation of reward, and allostasis. Neuropsychopharmacology 24:97–129

Kubis N, Faucheux BA, Ransmayr G et al 2000 Preservation of midbrain catecholaminergic neurons in very old human subjects. Brain 123:366–373

Lane MA, Baer DJ, Rumpler WV et al 1996 Calorie restriction lowers body temperature in rhesus monkeys, consistent with a postulated anti-aging mechanism in rodents. Proc Natl Acad Sci USA 93:4159–4164

Leal S, Andrade JP, Paula-Barbosa MM, Madeira MD 1998 Arcuate nucleus of the hypothalamus: effects of age and sex. J Comp Neurol 401:65–88

López JF, Chalmers DT, Little KY, Watson SJ 1998 Regulation of serotonin 1A, glucocorticoid, and mineralocorticoid receptors in rat and human hippocampus: implications for the neurobiology of depression. Biol Psychiatry 43:547–573

Manaye KF, McIntire DD, Mann DM, German DC 1995 Locus ceruleus cell loss in the aging human brain: a non-random process. J Comp Neurol 358:79–87

Masliah E, Mallory M, Hansen L, DeTeresa R, Terry RD 1993 Quantitative synaptic alterations in the human neocortex during normal aging. Neurology 43:192–197

McEwen BS 1998 Protective and damaging effects of stress mediators. N Eng J Med 338: 171–179

McEwen BS 2000 Allostasis and allostatic load: implications for neuropsychopharmacology. Neuropsychopharmacology 22:108–124

McEwen BS, Stellar E 1993 Stress and the individual. Mechanisms leading to disease. Arch Intern Med 153:2093–2101

Meerlo P, Overkamp GJ, Koolhaas JM 1997 Behavioural and physiological consequences of a single social defeat in Roman high- and low-avoidance rats. Psychoneuroendocrinology 22:155–168 (erratum: 1997 Psychoneuroendocrinology 22:385)

Meites J 1988 Neuroendocrine biomarkers of aging in the rat. Exp Gerontol 23:349–358

Morley RM, Conn CA, Kluger MJ, Vander AJ 1990 Temperature regulation in biotelemetered spontaneously hypertensive rats. Am J Physiol 258:R1064–R1069

Morrison JH, Hof PR 1997 Life and death of neurons in the aging brain. Science 278: 412–419

Murphy DG, DeCarli C, Schapiro MB, Rapoport SI, Horwitz B 1992 Age-related differences in volumes of subcortical nuclei, brain matter, and cerebrospinal fluid in healthy men as measured with magnetic resonance imaging. Arch Neurol 49:839–845 (erratum: 1994 Arch Neurol 51:60)

Nibuya M, Morinobu S, Duman RS 1995 Regulation of BDNF and trkB mRNA in rat brain by chronic electroconvulsive seizure and antidepressant drug treatments. J Neurosci 15:7539–7547

Nishimura A, Ueda S, Takeuchi Y, Matsushita H, Sawada T, Kawata M 1998 Vulnerability to aging in the rat serotonergic system. Acta Neuropathol 96:581–595

Ohm TG, Busch C, Bohl J 1997 Unbiased estimation of neuronal numbers in the human nucleus coeruleus during aging. Neurobiol Aging 18:393–399

Peters A 1996 Age-related changes in oligodendrocytes in monkey cerebral cortex. J Comp Neurol 371:153–163

Peters A, Leahu D, Moss MB, McNally KJ 1994 The effects of aging on area 46 of the frontal cortex of the rhesus monkey. Cereb Cortex 4:621–635

Peters A, Rosene DL, Moss MB et al 1996 Neurobiological bases of age-related cognitive decline in the rhesus monkey. J Neuropathol Exp Neurol 55:861–874

Peters A, Sethares C, Moss MB 1998 The effects of aging on layer 1 in area 46 of prefrontal cortex in the rhesus monkey. Cereb Cortex 8:671–684

Peters A, Moss MB, Sethares C 2000 Effects of aging on myelinated nerve fibers in monkey primary visual cortex. J Comp Neurol 419:364–376

Rakic P 1998 Young neurons for old brains? Nat Neurosci 1:645–647

Rausch JL, Johnson M, Fei Y et al 2000 Depressed outpatients have elevated temperatures. Biol Psychiatry 47:93S

Reagan LP, McKittrick CR, McEwen BS 1999 Corticosterone and phenytoin reduce neuronal nitric oxide synthase messenger RNA expression in rat hippocampus. Neuroscience 91: 211–219

Rodríguez-Gómez JA, de la Roza C, Machado A, Cano J 1995 The effect of age on the monoamines of the hypothalamus. Mech Ageing Dev 77:185–195

Sapolsky RM 2000 Glucocorticoids and hippocampal atrophy in neuropsychiatric disorders. Arch Gen Psychiatry 57:925–935

Siddiqi ZA, Peters A 1999 The effect of aging on pars compacta of the substantia nigra in rhesus monkey. J Neuropathol Exp Neurol 58:903–920

Smith MA, Cizza G 1996 Stress-induced changes in brain-derived neurotrophic factor expression are attenuated in aged Fischer 344/N rats. Neurobiology of Aging 17: 859–864

Smith MA, Makino S, Kim SY, Kvetnansky R 1995 Stress increases brain-derived neurotrophic factor messenger ribonucleic acid in the hypothalamus and pituitary. Endocrinology 136:3743–3750

Smith TD, Adams MM, Gallagher M, Morrison JH, Rapp PR 2000 Circuit-specific alterations in hippocampal synaptophysin immunoreactivity predict spatial learning impairment in aged rats. J Neurosci 20:6587–6593

Sterling P, Eyer J 1988 Allostasis: a new paradigm to explain arousal pathology. In: Fisher S, Reason J (eds) Handbook of life stress, cognition and health. Wiley, Chichester, p 629–649

Swaab DF 1995 Ageing of the human hypothalamus. Horm Res 43:8–11

Swaab DF, Grundke-Iqbal I, Kremer HP, Ravid R, van de Nes JA 1992 Tau and ubiquitin in the human hypothalamus in aging and Alzheimer's disease. Brain Res 590:239–249

Szuba MP, Guze BH, Baxter LR Jr 1997 Electroconvulsive therapy increases circadian amplitude and lowers core body temperature in depressed subjects. Biol Psychiatry 42:1130–1137

van Luijtelaar MG, Tonnaer JA, Steinbusch HW 1992 Aging of the serotonergic system of the rat forebrain: an immunocytochemical and neurochemical study. Neurobiol Aging 13:201–215

Vernet D, Bonavera JJ, Swerdloff RS, Gonzalez-Cadavid NF, Wang C 1998 Spontaneous expression of inducible nitric oxide synthase in the hypothalamus and other brain regions of aging rats. Endocrinology 139:3254–3261

Whooley MA, Browner WS 1998 Association between depressive symptoms and mortality in older women. Arch Intern Med 158:2129–2135

DISCUSSION

Veldhuis: One of the things I am struggling with is this relationship between stress and depression: which comes first? These both aggravate each other. Can you separate this a little bit further for us? In a sense, just being alive and producing cortisol must be a chronic stressor.

Carroll: That's what Selye meant when he talked about the 'stress of life'. As far as the relationship with depression is concerned it doesn't matter whether it's the chicken or the egg — there are probably several paths to it. One of these paths is genetic. A small number of individuals have the genes for bipolar disorder or familial unipolar depression. They often become depressed in the first instance

through some kind of precipitating life stress, but after that the illness seems to run a course of its own and the recurrences often come independently of stress. By the time we get to study them they've been depressed for a month or two and, as I said, that in itself constitutes a new chronic stress for the individual. The state of being depressed is about the most painful existential condition that humans can endure.

Veldhuis: Are there data on glucocorticoid receptor-deficient mice that would help in establishing this hypothesis? Right now the challenge I see, although it is extremely attractive from an associative viewpoint, is causal connectivity. Maybe you could demonstrate that a mouse with a constitutively activated glucocorticoid receptor system, independently of peripheral steroid levels, tended to develop all these diseases and die early and that the resistant receptor genotype had the converse experience. Are people gathering such information?

Carroll: It's a very good idea; I don't know of any data on this.

Veldhuis: In humans we don't think of the type I receptor as connected to the metabolic features of cortisol activity except at the level of sodium retention and potassium excretion. However, the ratio of these receptors changes in ageing. What is your assessment of the role of the type I mineralocorticoid receptor (MR) in the rodent during the ageing process? This is sort of a black box to me as an endocrinologist.

Carroll: The best answer I can give to that is to say that MRs are concerned with the amplitude of the rhythms, there is down regulation of MRs as I pointed out in the stressed animal and in the depressed patient and that may well be the mechanism by which the nadir cortisol values are elevated. It's the MR that raises the nadir of the values, but the elevated occupancy of glucocorticoid receptor round the clock is what causes the tissue damage.

Burger: I wondered if you could say a little bit more about the direction of the changes you've described in the male reproductive axis. You mentioned in the rat the loss of cycling, which is probably also true of the human female. What about the effects of chronic stress and depression on the male reproductive axis and on testosterone levels?

Carroll: Acute stress clearly leads to a decrease in testosterone production. In chronic stress this may come back a little, but it still remains suppressed. We have to be careful about which chronic stresses we are discussing, because if it's a social stress where a male rat is defeated by a stronger male, in that context the testosterone stays very low. That's the way the animal hierarchies of dominance work.

Burger: Do they also have a shorter lifespan?

Carroll: Yes, if you put these animals in the visible burrow system where you can observe their interactions, you can see that the ones that are pushed to the periphery get less of everything: less food, less shelter and less access to the opposite sex. They develop stress-related pathologies.

Burger: Has any attempt been made to actually replace or increase the testosterone levels of animals in that situation?

Carroll: There have been a few experiments along these lines. The studies that I am aware of look at male animals that have been through a social defeat. Their testosterone is greatly reduced. If they are put back into a group of rats they already knew, their stress parameters come back pretty rapidly, but if they are kept in isolation then their stress parameters stay low. There are social interactions with the rate of recovery from a severe stress.

Burger: Is that true for exogenous replacement as well?

Carroll: I am not aware that anyone has looked at exogenous replacement.

Veldhuis: That would be very interesting.

Handelsman: I've been quite struck by the number of depressed people on selective serotonin reuptake inhibitors (SSRIs) who have relatively low testosterone levels. Against that, in placebo-controlled studies there seems little change in healthy young men in testosterone. The depression in testosterone seems unrelated to whether they get better or not.

Laron: Do SSRIs reduce growth hormone (GH) as well?

Veldhuis: No, they stimulate GH slightly.

Handelsman: This may be a naïve question, but what is the effect on life expectancy of adrenalectomy, with or without maintenance of glucocorticoids?

Carroll: I don't know.

Veldhuis: This could be interesting. Giving glucocorticoids back according to the rhythmic hypothesis would be necessary, or giving a modern anti-glucocorticoid that's fairly selective to an intact animal could work as well.

Müller: There are data showing that adrenalectomy reduces hippocampal damage (Stein & Sapolsky 1988). In referring to the senile brain, you mentioned impairment in the monoaminergic systems (Gottfries 1992). What about impairment of acetylcholine neurotransmission (Sherman & Friedman 1990, Pepeu et al 1993)? As far as depression is concerned we know that there is a cholinergic theory of depression: typical antidepressant drugs are endowed with anticholinergic activity (Baldessarini 1996).

Carroll: That idea has been around for a long time. In the cholinergic system there are similar changes as in the monoaminergic system in normal ageing, but these changes are very different from the loss of cholinergic cells in the nucleus basalis that occurs in AD. Ageing is not like AD.

Björntorp: You are the pioneer in dexamethasone suppression testing. This is useful for Cushing's disease and depression. When we are looking at a functional increase of cortisol in patients with chronic stress, which is the best way to assay the feedback loop and the receptor loop? We have tried a lower dose than the conventional one (0.5 mg rather than 1 mg) but I'm not sure that we really know

what we are measuring. It might be useful to look at the escape of the inhibition; you might see something there.

Carroll: Even though I did introduce the dexamethasone suppression test for depression, I would never use dexamethasone again, because a major confound in all of the dexamethasone work in psychiatry has been accelerated metabolism of the steroid. I think we know why that happens now, because we give dexamethasone at 11 p.m. and we sample blood the next day. With the hyperthermia there is increased pharmacokinetic clearance, and we know that these escapers very often have lower plasma dexamethasone concentrations when the cortisol samples are drawn. I would use some other paradigm, such as a hydrocortisone infusion in the human, and look for indices of adrenocorticotropic hormone (ACTH) suppression.

Björntorp: You say that the metabolism of dexamethasone explains all this, but are you sure that the circulating dexamethasone is mirroring what's happening? Is this a measurement of bound dexamethasone?

Carroll: It depends who you ask. If you ask Ron de Kloet he would say that with dexamethasone the brain is like an adrenalectomized brain because the synthetic dexamethasone doesn't get to the brain, and that the site of action of dexamethasone suppression of the HPA axis is the anterior pituitary. I don't think everyone agrees with that. If you put dexamethasone into the cerebral ventricles it will certainly act on glucocorticoid receptors. Don't forget about the pituitary: it is sitting there, between the brain and the adrenal gland and makes human experiments on direct central nervous system (CNS) feedback difficult because whatever you think you are doing to the brain, at the same time you are also doing it to the anterior pituitary.

Prior: The experience I have as a clinician is that many women who present with ovulatory disturbances of the menstrual cycle often give a story of childhood sexual, psychological or physical abuse. It seems that this sets their hyper-responsiveness to other life stresses for a long time. One of the common threads besides disturbances of ovulation is sleep disturbance. Again, when we talk about sleep we are talking about higher cortisol during the night when it should be at its nadir.

Carroll: That is an emerging story; it certainly happens in a significant number of depressed women. Be careful not to over-generalize from that sample because there are many women with depression where this clearly does not apply. Then there are all the depressed men and it doesn't apply to them either.

Prior: To reflect on the idea that those who have fewer children have less allostatic load, I'll agree with that in terms of the outcomes after pregnancy, but also there is an amplification effect of oestradiol on corticotropin-releasing hormone (CRH). It is further amplified by social stress, as shown by Kirschbaum's group in young men (Kirschbaum et al 1996). The question is one

of homeostatic balance. Until you understand why women have fewer children I wouldn't necessarily jump to the conclusion it was related to long life and homeostasis!

Carroll: Go back and read Westendorp & Kirkwood (1998) and I think you will get the point. Look at those telling data from the English aristocracy. You mostly won't see it in modern times because very few families have 8–12 children, but they used to.

Veldhuis: Perinatal imprinting is another subtlety that could confound retrospective and even prospective studies. The Plotsky data published on maternal–infant separation producing delayed adult differences in stress responses, tell me there are sparingly plastic changes in CNS feedback that occur early in life. These are a bit frightening to us in clinical research, because we don't know what they all are. They are not always acknowledged even for the elements we know, such as sexual abuse in childhood. They can confound and add heterogeneity to these cross-sectional studies and perhaps add some strange autocorrelation to the prospective studies. The oestrogen issue is complicated — I'd love to have a separate symposium on sex steroid interactions with the stress axis.

Handelsman: I did some work with David Phillips looking at two separate birth cohorts. In these, low birth weight (which is a well known predictor of cardiovascular disease), was also a strong predictor of lifelong non-married state. This suggests there are a lot of complexities that are not necessarily related to traumatic experiences.

Morley: Clearly when we talk about cortisol we have to look at plasma clearance. In ageing there is a decrease in plasma cortisol clearance time which makes measurements of cortisol very difficult to interpret. The Kirkwood data (Westendorp & Kirkwood 1998) were strongly contested at a meeting in Holland about 14 months ago by data that looked exactly the opposite. You can look at the two sets of data and take whichever set you like: women who have lots of kids learn to adapt and function well if they live in Holland, but if they live in England they can't adapt. The thing that really interested me is that you alluded to dietary restriction as everybody does at an ageing meeting. The real problem with dietary restriction is what you are really doing is taking animals who are living the worlds most gluttonous, although reasonably stressful, life in unusually small cages. We've recently completed a study looking at baboons in Ethiopia and compared them to the average American baboon kept in relatively poor conditions in a farm. Using leptin as a surrogate for fat, it turned out that leptin is almost unmeasurable in the average baboon living in a normal environment. With regard to dietary restriction, nobody will ever get fat levels down to that level: it would be considered too cruel and unacceptable by any animal review board so we have to recognize that dietary restriction isn't going to be valid for

humans. Also, in mice and rats restriction really produces premature ageing if you want to look at the hormones. Almost all the hormone changes in rodents look just like the hormone changes you see in old animals and the possible interpretation of this, which makes a lot of sense, is that if you slow down life so that almost nothing happens, you can live a long time. These data also hint that we shouldn't replace hormones because replacing hormones may hurry life up: we may have two great years, but on the other hand we might die 10 years earlier.

Carroll: It depends what you call 'ageing'. This takes us back to Meites, who talked about menopause and somatopause as biomarkers of ageing. It all depends on the biologic context in which it occurs. Your point about food restriction illustrates the same thing: animals in the wild who are existing under high foraging demand for food are very stressed and calorie restricted at the same time. Animals in the lab have it easy, when we cut back their calories by 30% they've still got much more than the high-foraging-demand animals in the wild, so they are not stressed even though they have equivalent calorie intake.

Veldhuis: Are you saying that over-feeding is stressful and conducive to shorter life?

Handelsman: Remember to keep in perspective that people have never lived longer or been better fed than now.

Morley: If you look at the epidemiological studies that show that low cholesterol is good for the heart, the only way they could ever do those is to take out the amount of food eaten because the studies clearly showed that the more food you ate the less likely you were to have a heart attack. In fact the conundrum is easily explainable, because the very heavy eaters are the people who exercise a lot, so the only way they could get rid of the high food intake was to take out the exercise. These things are never easy and the pitfalls we all have to deal with demonstrate that we shouldn't accept that just because we overeat that it is bad for us. Gross overeating is clearly bad, somewhere in between is most probably OK.

Björntorp: Having gone through some of the literature on cortisol in ageing, I found a study looking at cortisol turnover with ageing. If you are fat then the adipose tissue transforms cortisol to cortisone by 11β-hydroxysteroid dehydrogenase.

Veldhuis: I agreed with that review. Our deconvolution methods depend upon an assumption of a stable distribution. I haven't found consistent data reporting altered distributions in ageing.

Riggs: I was particularly interested in your comments that normal ageing is associated with a disproportionate decrease in synaptic connections, with a smaller decrease in the number of neurons. I was reminded of the data in rodents showing that synaptic connections can be related to the degree of stimulus the rodents are exposed to. Is there any evidence in humans that those elderly people

that stay busy, that read, and remain active and stimulated have less affected synaptic connections?

Carroll: The only data I know that touch tangentially on this are those on AD. The risk of AD is clearly related in Snowdon et al's (1996) study of nuns to the complexity of their mental operations at age 18, when they wrote essays about why they wanted to enter the convent. They were later followed through to death and autopsy, and those who developed AD late in life had less complex mental operations in their teenage years. You can interpret that several ways, you can say AD begins at birth, or you can say that the ones who were better educated and stimulated intellectually somehow managed to mitigate their independent risk of AD.

Morley: The problem with this is that the more active and higher your education level, the less likely you are to develop AD or dementia quickly because the diagnosis takes time. There was a recent paper that actually looked at plaques in people who did and didn't have AD and there was no difference. You can come to the conclusion that there might be very little difference between normal ageing and AD. Our animal model in the SAMP8 mice clearly suggest that it is just an exaggeration: if everybody and every animal overproduces β-amyloid it's how much you over produce and for how long — if you excessively overproduce you will get AD (Morley et al 2000, Kumar et al 2000). But in bright people the diagnosis will not be made as early. All the educational epidemiological studies show the higher your education the less likely you are to get AD. I don't think this is because your synapses are necessarily better, its just that its harder to diagnose someone who is very bright.

Carroll: I think you have overstated the case here; the data from autopsy confirm the *in vivo* clinical diagnoses with about 80% agreement

Müller: Although there are data showing that education level is inversely related to the development of AD (Geerlings et al 1999), this may be due to the improved lifestyle of better educated people. Several studies have shown that oestrogen therapy can improve cognitive function or prevent AD in elderly women (Paganini-Hill & Henderson 1994, Jacobs et al 1998, Tang et al 1996), whereas other studies have not found an association (Brenner et al 1994, Shaywitz & Shaywitz 2000). Anyway, women with high serum concentrations of non-protein bound and bioavailable oestradiol are less likely to develop cognitive impairment than women with low concentrations (Yaffe et al 2000). Reportedly, oestrogens induce synapsis formation in the hippocampal pyramidal neurons (McEwen & Alves 1999). I think that we have to consider all these aspects together.

Robertson: In human epidemiology, I wonder whether there are data from agents that affect body temperature, for example non-steroidal anti-inflammatory agents and barbiturates. Is there any evidence that these might be having a favourable impact on ageing?

Carroll: There are data showing that NSAIDs reduce the risk of AD. I do not know of any similar data for ageing in general.

Robertson: You gave the impression that you wished the glucocorticoid receptor in human subjects was a little less active. Do you think a slightly less effective receptor or a slightly less effective adrenal cortex might be beneficial for ageing in human beings?

Carroll: I would rather say let's eliminate that nocturnal secretion of cortisol. There are ways this can be done. For example, one of the current experimental approaches of treating depression is to give metyrapone, or ketoconazole, but this is mostly done with daytime administration. I would prefer to go with a midnight or 10 p.m. administration; there you might have a chance of success because this would truly uncouple the GR from circulating steroids for a certain length of time at the right period of the circadian cycle.

Björntorp: I heard that there is now a CRH inhibitor available for treating depression.

Carroll: Every pharmaceutical corporation that I know is trying to develop CRH antagonists, and to get them into clinical trials as rapidly as possible. The potential indications include depression, but also anxiety states, for example. Any data currently available are very preliminary and so we just have to wait.

Handelsman: Can you comment on the point that Dr Müller made about oestradiol and/or other oestrogens preventing AD? Are there are any studies of testosterone in men having similar effects?

Carroll: The data show that oestrogen is worthless as a treatment of already diagnosed AD patients, but the epidemiologic studies suggest that women who are maintained on oestrogen after menopause have a reduced incidence of AD.

Prior: However, it is wise to remember that these studies are highly biased by the differences between oestrogen-taking women and non-oestrogen taking women (Barrett-Connor 1991).

Handelsman: This may be like the cardiovascular story all over again.

Prior: Are the temperature differences that you are talking about measurable in a practical way? Can you detect the difference between depressed and non-depressed patients?

Carroll: Yes. There have even been studies measuring daytime temperatures in depressed patients when they come in for an outpatient visit. There are clear 0.3–0.4 °C differences between depressed and control subjects even in the daytime.

Prior: What would be the best time to measure it?

Carroll: I would go for the night time, when the differences will be greatest.

Prior: Could it be measured at the patient's usual bedtime? Or do you have to wake them in the middle of the night?

Carroll: We could give them a capsule to swallow and get telemetric data from it.

Prior: We're not talking epidemiology here! I guess we could get the equipment for small studies.

Veldhuis: Those capsules are about US$80 a piece and the monitoring equipment is a couple of hundred dollars.

Carroll: But the data they produce are wonderful.

Prior: They have been used in the past to show that core temperature rises prior to vasomotor episodes (Freedman & Woodward 1996).

References

Baldessarini RJ 1996 Drugs and the treatment of psychiatric disorders: depression and mania. In: Hardman G, Linmbird LE, Molinoff PB, Ruddon RY, Goodman Gilman A (eds) Goodman & Gilman's the pharmacological basis of therapeutics, 9th edition, MacGraw-Hill, New York, p 431–459

Barrett-Connor E 1991 Postmenopausal estrogen and prevention bias. Ann Intern Med 115:455–456

Brenner DE, Kukull WA, Stergachis A et al 1994 Postmenopausal estrogen replacement therapy and the risk of Alzheimer's disease: a population-based case-control study. Am J Epidemiol 140:262–267

Freedman RR, Woodward S 1996 Core body temperature during menopausal hot flushes. Fertil Steril 65:1141–1144

Geerlings MI, Schmand B, Jonker C, Lindeboom J, Bouter LM 1999 Education and incident Alzheimer's disease: a biased association due to selective attrition induce of a two-step diagnostic procedure? Int J Epidemiol 28:492–497

Gottfries CG 1992 Clinical and Neurochemical aspects of diseases with cognitive impairment. Rev Neurosci 3:191–205

Jacobs DM, Tang MX, Stern Y et al 1998 Cognitive function in nondemented older women who took estrogen after menopause. Neurology 50:368–373

Kirschbaum C, Schommer N, Federenko I et al 1996 Short-term estradiol treatment enhances pituitary–adrenal axis and sympathetic responses to psychosocial stress in healthy young men. J Clin Endocrinol Metab 81:3639–3643

Kumar VB, Farr SA, Flood JF et al 2000 Site-directed antisense oligonucleotide decreases the expression of amyloid precursor protein and reverses deficits in learning and memory in aged SAMP8 mice. Peptides 21:1769–1775

McEwen BS, Alves SE 1999 Estrogen actions in the central nervous system. Endocr Rev 20:279–307

Morley JE, Kumar VB, Bernardo AE et al 2000 β-amyloid precursor polypeptide in SAMP8 mice affects learning and memory. Peptides 21:1761–1767

Paganini-Hill A, Henderson VW 1994 Estrogen deficiency and risk of Alzheimer's disease in women. Am J Epidemiol 140:256–261

Pepeu G, Casamenti F, Pepeu IM, Scali C 1993 The brain cholinergic system in ageing mammals. J Reprod Fertil Suppl 46:155–162

Shaywitz BA, Shaywitz SE 2000 Estrogen and Alzheimer's disease: plausible theory, negative clinical trial. JAMA 283:1055–1056

Sherman KA, Friedman E 1990 Pre-and post-synaptic cholinergic dysfunction in aged rodent brain regions: new findings and an interpretative review. Int J Dev Neurosci 8:689–708

Snowdon DA, Kemper SJ, Mortimer JA, Greiner LH, Wekstein DR, Markesbery WR 1996 Linguistic ability in early life and cognitive function and Alzheimer's disease in late life. Findings from the Nun Study. JAMA 275:528–532

Stein BA, Sapolsky RM 1988 Chemical adrenalectomy reduce hippocampal damage induced by kainic acid. Brain Res 473:175–180

Tang MX, Jacobs D, Stern Y et al 1996 Effect of oestrogen during menopause on risk and age at onset of Alzheimer's disease. Lancet 348:429–432

Westendorp RG, Kirkwood TB 1998 Human longevity at the cost of reproductive success. Nature 396:743–746

Yaffe K, Lui L-Y, Grady D, Cauley J, Kramer J, Cummings SR 2000 Cognitive decline in women in relation to non-protein-bound oestradiol concentrations. Lancet 356:708–712

Endocrine Facets of Ageing.
Novartis 242

Print ISBN 0-471-48636-1 Online ISBN 0-470-84654-2

Alterations in the ageing corticotropic stress-response axis

Per Björntorp

Department of Heart and Lung Diseases, Sahlgren's Hospital, University of Göteborg, SE 413 45, Sweden

Abstract: The problem with determining whether ageing is followed by perturbations of the regulation of the hypothalamic–pituitary–adrenal (HPA) axis is that ageing *per se* is very difficult to separate from the effects of environmental insults. Data in ageing rodents indicate that with age the winding-down of cortisol after challenges is prolonged. This is probably due to an insufficient feedback regulation by glucocorticoid receptors in specific fields in the hippocampus. The functional and topographic characteristics of these changes are identical to those seen after prolonged stress, suggesting that such factors might be of significance. Data in humans also suggest that with age basal cortisol secretion, diurnal rhythm, and the early response to challenges are not affected but similar to animal data the return to baseline values after stimulation seems to be delayed, probably due to a diminished feedback control. Several studies suggest that common diseases of age, for example cardiovascular disease and type 2 diabetes mellitus, are associated with similar HPA axis perturbations as those seen in old subjects. Recent population studies indicate that adrenal hyperactivity, associated with a stressful environment, is generating risk factors for these diseases. This is likely to be dependent on genetic susceptibility, and associated polymorphisms have been found in several candidate genes of importance for neuroendocrine and autonomic regulations.

2002 Endocrine facets of ageing. Wiley, Chichester (Novartis Foundation Symposium 242) p 46–65

Stress and the function of the hypothalamic–pituitary–adrenal axis

The hypothalamic–pituitary–adrenal (HPA) axis regulates the secretion of cortisol. The net result of the output of cortisol from the adrenals is dependent on central stimulatory events and feedback inhibitory mechanisms. The central regulation takes the shape of a diurnal rhythm, which in humans consists of high activity in the early morning hours and low activity in the afternoon/evening. This basic pattern is changed by the experience of a variety of factors which are often grouped together under the common description of 'stress'.

'Stress' may be defined as factors which disturb the homeostasis of several neuroendocrine and autonomic systems. A more recent definition is the disturbance of allostasis. An example of allostasis is the maintenance of a normal adaptation of blood pressure to posture, or insulin secretion after a meal (McEwen 1998). Stress reactions have been defined by Henry & Stephens (1977) and may be divided into two distinct classes. One is the fight–flight reaction where a challenge is met by a typical neuroendocrine–autonomic reaction, primarily involving the sympathetic nervous system with elevation of heart rate and blood pressure. When the threat is dealt with successfully additional hormonal changes are occurring, which in males include an increase in testosterone production.

Another type of reaction is a depressive, defeat reaction, also described as a situation of helplessness. This reaction is seen when the threat is perceived as overwhelming and cannot be coped with. Mental depression also follows this reaction as well as typical neuroendocrine–endocrine reactions, including activation of the HPA axis with elevated cortisol secretion. When this is frequently repeated or chronic, other consequences follow. A primary reaction seems to be a delayed 'down-winding' of the response, with cortisol elevation remaining for longer than normal after a challenge. A second step is diminished responsiveness of the HPA axis. An early consequence of this seems to be decreased morning cortisol levels in humans. Finally a low, poorly responsive HPA axis results. Such neuroendocrine 'burn-out' has been described in war veterans and holocaust victims (McEwen 1998).

Along this pathway other neuroendocrine and autonomic adaptations are occurring. The growth hormone and hypothalamo–pituitary–gonadal axes are inhibited by the elevated HPA axis activity at several levels (Chrousos & Gold 1992). This results in decreased secretion and low concentrations of sex steroid and growth hormones. In addition, when the activity of the HPA axis is sufficiently down-regulated there seems to be a parallel activation of the sympathetic nervous system, which might be seen as a compensatory adjustment in an attempt to maintain homeostatic conditions (Plotsky et al 1989).

The activity of the HPA axis is controlled by a feedback inhibition mechanism where central glucocorticoid receptors are involved. When cortisol is bound to these receptors the activity of the HPA axis is diminished. When challenged with repeated activation of the HPA axis this control mechanism becomes less efficient, first apparently by a decrease of glucocorticoid receptor (GR) density and later by structural changes, including atrophy of the dendrites coupled to the inhibitory mechanism of the GR. Eventually brain substance loss occurs, which is visible as atrophy and the formation of lacunae. This is seen in Cushing's syndrome (with severe, constant overproduction of cortisol), in melancholic depression (another condition with chronically elevated cortisol), and in war veterans (presumably as a result of long-term, severe stress reactions in the battlefield). At which stage such

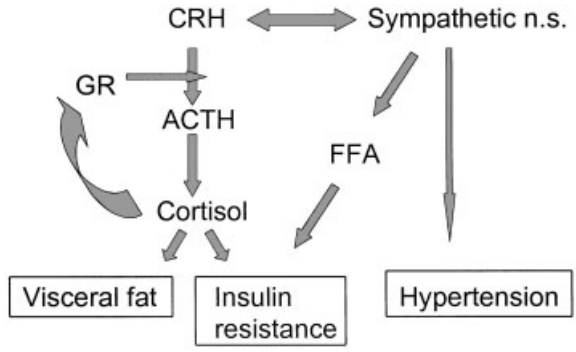


FIG. 1. The associations between stress reactions and pathways to disease generation. Activation of the HPA axis is followed by cortisol secretion, particularly when insufficiently controlled by the feedback loop involving central glucocorticod receptors (GRs). Androgens, probably partly of adrenal origin, are important in women. When prolonged, this activation causes insulin resistance and visceral accumulation of body fat, both cornerstones of the metabolic syndrome. A parallel activation of the central sympathetic nervous system is followed by hypertension and increased mobilisation of free fatty acids (FFA), which amplify insulin resistance. For details, see Björntorp (1996, 2002). CRH, corticotropin-releasing hormone; ACTH, adrenocorticotropic hormone; n.s., nervous system.

changes are reversible is not certain, but it seems unlikely that severe topographic losses can be normalized (McEwen 1998).

As described above, the reaction to stress involves a large number of reactions where the central sympathetic nervous system and the HPA axis are primary players. The inhibitions of other central neuroendocrine axes are probably secondary to the activation of the HPA axis but form an integrated part of the stress reaction participating in the damaging somatic consequences.

Both of these reaction types are often mixed, particularly in humans, and it may be difficult to separate one from the other. The peripheral consequences of stress reactions are therefore often a mixture of neuroendocrine/endocrine and autonomic reactions. As will be described later, such prolonged reactions are damaging to bodily systems and may end up in serious diseases (see schematic summary in Fig. 1).

Stress activators

What are the inducers of stress reactions? They consist of a large number of complex, variable external and internal events. External, psychological stressors may include socioeconomic handicaps such as demanding work environments or poor economic conditions. Psychosocial handicaps include divorce, living alone and bereavement. Other external stressors are a poor physical working situation

with noise, polluted air, toxicological hazards, etc. Internal stressors might be infections or other diseases, trauma and sleep deprivation. In addition to the large number of stress factors, different personalities perceive pressure differently: what one person counts as stressful does not necessarily apply to another person.

The complexities of this field have led researchers to define 'stress' as any factor that activates the stress axes: the HPA axis and/or the central sympathetic nervous system.

Ageing and the HPA axis

The background provided above is necessary for understanding the potential influence of ageing on the HPA axis and its associated tight network of other neuroendocrine reactions. One may ask whether this system is ageing by what might be called a 'normal' age-related process or if the wear and tear of today's complex, hectic living conditions are involved. As will be seen, it is difficult to separate these two factors.

In order to separate out the effects of a 'normal' ageing process from the effects of external factors, one would have to examine the HPA axis and its associated cascade of reactions in other neuroendocrine and autonomic systems in older subjects who have not been exposed to the wear and tear of life. This is of course not possible even if one were to find a population totally separated from modern, urbanized life, because there are always surrounding factors which activate our stress systems.

Animal studies

An approach to this problem might be to study the regulation of the HPA axis in the ageing laboratory rat. Such animals are probably less exposed to varying external stressors than humans are. Furthermore, the primitive mechanisms involved are probably common to most mammals because they are essential for survival, and the results from rats should therefore be reasonably applicable to humans. The results of such studies indicate that the ageing rat has an increased corticosterone secretion after a stress challenge (Sapolsky et al 1983). The down-winding of the corticosterone response seems to be delayed after a stress response. This varies among different rat strains (De Kloet 1992) and between genders (Brett et al 1986). The reason for the slow return to basal values seems to be a down-regulation of central GR density, particularly in certain fields of the hippocampus region (Sapolsky et al 1984a). This is apparently an effect of extended exposure to glucocorticoids (Sapolsky et al 1984b) and is amplified by toxins (Sapolsky 1985), including alcohol (Walker et al 1980). The mechanisms involved have been suggested to be effects of glucocorticoids on glucose uptake and utilization in the cells involved, in analogy with the well known sensitivity of brain cells to such

insults. This is seen after prolonged administration of glucocorticoids at concentrations corresponding to those after stress, resulting in about 50% lower GR density of hippocampal receptors (Sapolsky et al 1985a). Interestingly, the neonatal rat, which has a low number of GRs in critical areas of the brain that develop slowly to adult density, is sensitive to impacts during this developmental period. Interference with this development results in persistent hypersecretion of cortisol (Sapolsky et al 1985b).

The end result of such down-regulation of GRs will then be an inefficient feedback control of corticosterone production which will amplify further damage to the regulatory system. When exposure to noxious agents occurs for a shorter time, the changes are reversible, while longer periods of exposure are followed by slower or no restitution (Sapolsky 1985). This corresponds to a loss of glucocorticoid binding cells in the critical areas of the hippocampus which are replaced by glial cells. When the insults are sufficiently large and extended, loss of brain substance occurs.

Interestingly, these are the same changes that are seen in the ageing rat, and are the basis for the 'glucocorticoid cascade hypothesis' (Sapolsky et al 1986). This hypothesis suggests that repeated stress insults, particularly when accompanied with exposure to toxins, down-regulate GR density in specific areas of the hippocampus region in the brain. This is reversible up to a certain point, beyond which permanent, irreversible loss of hippocampal neurons occurs with persistent oversecretion of glucocorticoids as a result.

This hypothesis suggests that with ageing and the experience of repeated stress periods during life, such changes may occur. The best evidence for this possibility seems to be the identical functional and anatomical changes seen with ageing with less or no such exposure.

Studies in humans

The brain functions involved are those responsible for primitive survival reactions. Studies in non-human primates have shown essentially the same reaction pattern as in rodents (Uno et al 1989). Therefore a similar chain of reaction would also be expected in humans. The situation is, however, more difficult to evaluate in humans, who typically experience a wide variety of external challenges during a long life, with presumably varying vulnerability.

Several difficulties are apparent with the available human studies. These include variables such as the selection of study groups, whether the study group is healthy or not, sample size, the extent of the age differences in the groups, and perhaps the most important problem, the techniques utilized for assaying HPA axis function. This latter problem is particularly important in human studies because of the exquisite sensitivity of the reactions to be followed, as will be illustrated.

Furthermore, fully controlled experiments are difficult to perform in this area of research. These problems make many studies of limited value, which has been taken into account in the following evaluation.

Although the results are not totally consistent, they indicate that the basal, non-stimulated HPA axis and circadian rhythm are not disturbed in old subjects (Seeman & Robbins 1994). Stimulation by adrenocorticotropic hormone (ACTH) is apparently also followed by a normal initial response of cortisol while there might be a delayed return to normal values (Blichert-Toft 1975, West et al 1961). In general, studies with stimulation by corticotrophin-releasing hormone are difficult to evaluate, largely because of small sample sizes. However, one such study also suggests that the return to baseline cortisol values after such stimulations is prolonged in older individuals (Pavlov et al 1986).

Tests of the function of the feedback mechanism in humans usually involve the administration of dexamethasone and followed by the inhibition of cortisol secretion. These results are of particular importance in view of the evidence for a deficient feedback regulation in old animals. With the conventional dose of dexamethasone (1 mg) results have been largely normal, when calculated as inhibition below a certain given 'normal' level (Green & Friedman 1968), while old subjects seem to have higher mean absolute values after suppression (Tourigny-Rivard et al 1981). When inhibition with lower doses (0.5 mg) is tested to improve sensitivity, there is better evidence for a blunted inhibition in older subjects (Oxenkrug et al 1983, Branconnier et al 1984).

Most of the studies referred to have only measured the concentrations of circulating hormones, which are a result of secretion and clearance. One study indicates that there is no difference in clearance of cortisol (Barton et al 1993). The cortisol levels after various challenges are most likely a result of secretion without much influence of removal rates, although the latter cannot be stated with certainty.

Several studies have been performed in subjects with various diseases that might be suspected to be consequences of HPA axis activation over prolonged periods. Conditions with increased cortisol secretion due to tumours (such as Cushing's syndrome) or with elevated exposure to glucocorticoids due to therapeutic interventions are followed by insulin resistance, abdominal obesity, hypertension, dyslipidaemia, osteoporosis, cognitive impairments and immune deficiencies (Seeman & Robbins 1994). These are consequences of elevated glucocorticoid exposure where the mechanisms are essentially known.

Conditions with similar phylogenetic expression to these overexposures to glucocorticoids are hypertension, cardiovascular disease and type 2 diabetes mellitus. These conditions are suggested to have impairments of HPA axis regulation (Seeman & Robbins 1994). This might be a consequence of the diseased state, but it is possible that these diseases are in fact at least partly consequences of primary dysregulation of the HPA axis.

In an attempt to summarize the human data, there is no conclusive evidence that ageing *per se* is associated with a faulty regulation of the HPA axis, although this must be considered impossible to study in a meaningful way because it requires all external influences to be absent. There is, however, suggestive evidence that in the ageing person there is often a diminished rate of return of cortisol secretion to basal values after challenge, which may be due to an inefficient feedback regulation. This would then agree with the better-controlled animal data on the input of ageing. It is impossible, however, to judge whether this is a consequence of the ageing process or due to repeated exposure to stressful events during a long life. Normal ageing is unavoidably associated with exposure to the wear and tear of daily life. There are clearly individual differences in the sensitivity and perception of such challenges, and these differences most likely have a genetic basis. Increased frequency of periods of stress during a lifetime would be expected to increase the vulnerability to diseases via effects on metabolic and other processes, as will be discussed in a following section.

Another approach to this problem is the following. If it is supposed that the HPA axis and its associated cascade of events are affected by ageing, then it is interesting to know what characterizes subjects who have been able to maintain a normal HPA axis function into an advanced age. The function of the HPA axis has been followed longitudinally for a long period in a group of healthy subjects. Those without signs of deterioration of this function were characterized by good memory and selective attention (Lupien et al 1994) as well as preserved hippocampal structure (Lupien et al 1998). Whether these differences are genetically determined and localized to the HPA axis regulation itself, or to a resistance to environmental 'stressors', or both, is not known.

The impact of the HPA axis on human health

Since it is almost impossible to separate the effects of ageing and cumulative stress on the regulation of the HPA axis, it might be of interest to examine how the HPA axis activity is associated with diseases that are characteristic of ageing. This assumes that the insults on the HPA axis of wear and tear are an unavoidable consequence of ageing. Longevity runs in families and 'successful' ageing is likely to have genetic components. Perturbations of the regulation of the HPA axis and associated neuroendocrine reactions and disease risk factors are linked to molecular genetic characteristics. Although this molecular genetic information is so far preliminary and superficial, it might serve to generate ideas of new approaches to the problem of neurendocrine–endocrine and autonomic ageing.

This information has largely been obtained from two population studies, one in men and one in women. Since the primary aim of these studies was not to study the effects of ageing, they included subjects born in the same year, the men born in 1944

and the women in 1956. This approach was chosen to avoid the confounding influence of age. Nevertheless, the information obtained might be useful for the interpretation of the influence of daily life stressors on neuroendocrine health, or abnormalities and their associations to disease or disease precursors, characteristic of older subjects.

In these studies it was thought to be important to examine the status of the HPA axis during ordinary daily life. Measuring the easily disturbed HPA axis during abnormal, potentially stressful conditions, such as in a laboratory or hospital, does not provide the information needed in an attempt to examine the impact of daily life on, for example, cortisol secretion. If, in addition, venipuncture is involved, then the risk for disruption of a steady state increases due to the exquisite sensitivity of the systems being examined. For these reasons saliva cortisol was measured. The advantages of this procedure are that saliva can easily be collected under any conditions (except sleep) and saliva cortisol contains the free active fraction of circulating cortisol. Saliva was collected during several predetermined time points including morning, afternoon and evening, and perceived stress was registered by the proband. The reaction to a standardized lunch was determined. A dexamethasone suppression test was performed, also under 'free-living' conditions, with a lower (0.5 mg) than conventional (1.0 mg) dose in order to improve sensitivity. In this way, an impression of the basal and stimulated (food intake and reported perceived stress) activity of the HPA axis was obtained as well as the function of the feedback inhibitory loop. These measurements were then set in relation to a number of psychological, socioeconomic and lifestyle factors, other hormones, as well as conventional metabolic and haemodynamic risk factors for disease (Rosmond et al 1998, Baghaei et al 2001).

The sensitivity of the HPA axis as mirrored by saliva cortisol measurements is clearly demonstrated by another recent study where saliva cortisol was measured at random times during a working day, with perceived stress and mood being reported before each sampling. The results showed that the measurements were influenced not only by the stress perceived immediately before sampling, but also by the memory of previously experienced stress, anticipated stress and mood. Cortisol levels varied 20-fold over the day and were proportional to the reported perceived psychological impacts (Smyth et al 1998). This study demonstrates the necessity of measuring the activity of the HPA axis under ordinary, daily conditions, which are those presumably affecting disease-generating mechanisms over a prolonged period of time.

Men

The results in the men and women showed interesting differences. In the men it was possible to single out groups of HPA axis reactions. A normal group was

characterized by having high morning cortisol, rapidly decreasing before lunch, a response to lunch and then low cortisols in the afternoon/evening. This group consisted of about two-thirds of these essentially randomly selected men. About 25% of the men reported stress during the cortisol sampling and these men had lower morning values which did not wind down before lunch, and an accentuated lunch response. Afternoon and evening values did not differ from the normal group. This resulted in an elevation of total secreted cortisol over the day. These differences from the norm are characteristic of an HPA axis reaction which has been subjected to prolonged stress (Dallman et al 1992) and therefore suggest that these men had been repeatedly stressed by various factors before the day of measurements. The feedback inhibition also showed signs of a less than optimal function, presumably another sign of previous stress experiences.

There were statistical associations to socioeconomic and psychosocial handicaps as well as depressive and anxiety symptoms in these men, suggesting background factors to a perturbed function, or a hypersensitivity of the HPA axis.

In addition to these neuroendocrine perturbations, there were associations with conventional risk factors for disease, including abdominal accumulation of body fat, insulin resistance, dyslipidaemia and hypertension, a cluster of factors now called the 'metabolic syndrome'. It is predicted that these men have an increased risk of developing type 2 diabetes mellitus, cardiovascular disease and/or stroke.

The risk factor pattern can largely be explained by the elevated cortisol secretion, because cortisol directs an increased fraction of body fat to central depots through known mechanisms, where an increased density of GRs in visceral depots is a crucial characteristic (Björntorp 1996). This is seen dramatically in Cushing's syndrome. Furthermore, cortisol is well known to induce insulin resistance, and the associated dyslipidaemia may originate from the elevated insulin levels (Reaven 1988). The increased blood pressure is likely to be caused by a parallel activation of the sympathetic nervous system through mechanisms mentioned in the introduction. Taken together it is thus possible to visualize a chain of events starting out from environmental factors which activate the HPA axis, resulting in peripheral risk factors for diseases which increase dramatically in prevalence with age.

Among the men there were also a small group (less than 10%) where the HPA axis showed a low, constant, rigid activity, such as has been described for a neuroendocrine 'burn-out' (McEwen 1998). There were also signs of a poor suppression by dexamethasone. These men had low values of testosterone and growth hormone as well as robust associations to risk factors including elevated blood pressure. It is unlikely that elevated cortisol is responsible for these associations. Instead other hormonal and autonomic factors, taking part in the cascade of events following regulatory perturbations of the HPA axis, might be involved. We do not know the background to these changes, although there are

also associations to the presumed stress-generating factors mentioned above. Other factors, yet unknown, are probably also involved.

This description of detailed phylogenetic characteristics made it possible to approach genetic background factors by the candidate gene approach. Since the results pointed at a central origin of the findings, we focused on genes that control central neuroendocrine and autonomic regulations. This might be of interest from the aspect of ageing, because molecular genetic mechanisms might increase the susceptibility to develop various age-associated diseases.

A first target was the GR gene, because if the central GR is malfunctioning, the sensitivity of the HPA axis to various challenges would be increased due to a deficient feedback control, which would explain many of the phenomena described. The findings, in brief, have so far been the following. With restriction fragment length polymorphism technique it is possible to demonstrate polymorphisms that are strongly associated with basal and stimulated cortisol secretion as well as several of the crucial risk factors associated with a sensitive HPA axis. Furthermore, polymorphisms which are both activating or protecting from elevated activity of the sympathetic nervous system have been revealed (for review and detailed references, see Björntorp 2002). It would be interesting to examine whether these polymorphisms are associated with enhanced longevity. If they are of importance for the creation of disease risk factors, it seems likely that they would be associated with a shorter life due to development of morbidity and mortality.

Women

The study of women shows interesting differences in comparisons with the study in men. It turns out that androgens are the major associates to disease risk factors in women, showing stronger associations than various cortisol measurements (Baghaei et al 2001). This is also combined with low oestrogens. Such elevated androgen levels may have an adrenal origin because they are statistically connected with other hormones of adrenal origin. Furthermore, in women the endocrine perturbations also correlate with essentially the same psychosocial and socioeconomic handicaps as in men, including tendencies to anxiety and depression, expected to activate the HPA axis. It is likely that stressed men also secrete elevated amounts of adrenal androgens, but this is of no significance in the presence of a large input of gonadal testosterone. It seems likely from the results now available that certain common factors in men and women activate the HPA axis, with cortisol as the main trigger for adverse peripheral effects in men, while adrenal androgens are of more importance in women. These androgens in women may also have ovarian origin, but this has not yet been examined.

Androgens have several adverse effects on females. Muscular insulin resistance is created via known mechanisms and distribution of androgens to physically normal women is followed by accumulation of fat in the abdomen, both of which are corner stones of the metabolic syndrome (For review and references, see Björntorp 1996).

In women the candidate gene approach has followed different lines to the molecular genetic studies in men, guided by the results of the phylogenetic expression of symptoms. Special emphasis has been placed on the status of microsatellites in genes involved in steroid hormone regulations, because this has recently been found to be of particular interest in problems concerning steroid hormone metabolism and effects (Comings 1998). Studies have focused on the pathway of androgen metabolism and signalling to cells. Aromatase is the enzyme converting androgens to oestrogens, and might therefore be considered to protect women from a too pronounced an exposure to androgens. Elevated androgens in combination with low oestrogens, found in the women who were afflicted by disease risk factors in the population, may well be an indirect sign of less than optimal aromatase activity. The aromatase gene has a microsatellite in the fifth intron, which when short, previously has been found to be associated with increased risk of developing breast cancer and osteoporosis in women (Comings 1998). With hyperandrogenicity this microsatellite is also short (Baghaei et al 2001). This polymorphism seems particularly interesting because when women are examined who have signs of perfect somatic and psychological health, including low androgens and high oestrogens, their microsatellite in the aromatase gene is longer than average. In fact, both groups of women, one with a high androgen:oestrogen ratio and a number of disease risk factors as a consequence, and the other with general health and a low androgen:oestrogen ratio, have not only opposite phylogenetic characteristics, they form totally separate subgroups in their length of the microsatellite in the aromatase gene (L. Baghaei, R. Rosmond, L. Westberg, M. Hellstrund, M. Landen, E. Eriksson, G. Holm & P. Björntorp, unpublished results). This is such a striking, non-overlapping finding that it should motivate more detailed studies of the mechanisms involved.

The androgen receptor transfers the androgen signals to cells. Its gene has a microsatellite in the first exon, coding for the signalling part of the androgen receptor. Here a short repeat has also been found to be associated with serious disease both in men and women. Such a short stretch is associated with strong signals of androgens (Comings 1998). With hyperandrogenicity this microsatellite is also abnormally short, suggesting that the receptor transfers amplified signals to the cell, and would therefore worsen the effects of elevated androgens (Westberg et al 2001).

The stress reactions along the HPA axis and the central sympathetic nervous system are thus likely to be followed by predictors and subsequent precipitation

of diseases such as cardiovascular disease and type 2 diabetes mellitus. The presumed pathways for such a development are summarized schematically in Fig. 1. The neuroendocrine–endocrine profiles of such perturbations seem to be different in men and women. These perturbations have similarities with the characteristics of ageing. Detailed references and further discussion are found in reviews dealing with this problem (Björntorp 1996, 2002).

References

Baghaei F, Rosmond R, Westberg L et al 2001 Microsatellite stretches in the CYP450 aromatase gene are associated with androgens and risk factors in women, submitted

Barton RN, Horan MA, Weijers WM, Sakkee AN, Roberts NS, van Bezooijen CFA 1993 Cortisol production rate and the urinary excretion of 17-hydroxycorticosteroids, free cortisol and 6β-hydroxycortisol in healthy elderly men and women. J Gerontol 48:M213–M218 (erratum: 1994 J Gerontol 49:B35)

Björntorp P 1996 The regulation of adipose tissue distribution in humans. Int J Obes Relat Metab Disord 20:291–302

Björntorp P 2002 Hypothalamic origin of prevalent human disease. In: Pfaff D, Arnold A, Etgen A, Fahrbach S, Rubin R (eds) Hormones, brain and behavior. Academic Press, San Diego, in press

Blichert-Toft M 1975 Secretion of corticotrophin and somatotrophin by the senescent adenohypophysis in man. Acta Endocrinol Suppl 78:15–154

Branconnier RJ, Oxenkrug GF, McIntyre I, Pomara N, Harto NE, Gershon S 1984 Prediction of serum cortisol response to dexamethasone in normal volunteers: a multivariate approach. Psychopharmacology 84:274–275

Brett LP, Levine R, Levine S 1986 Bidirectional responsiveness of the pituitary-adrenal system in old and young male and female rats. Neurobiol Aging 7:153–159

Chrousos GP, Gold PW 1992 The concept of stress and stress system disorders. JAMA 267:1244–1252

Comings DE 1998 Polygenic inheritance and micro/minisatellites. Mol Psychiatry 3:21–31

Dallman MF, Akana SF, Scribner KA et al 1992 Stress, feedback and facilitation in the hypothalamo-pituitary-adrenal axis. J Neuroendocr 4:516–526

De Kloet ER 1992 Corticosteroids, stress, and aging. Ann NY Acad Sci 663:337–371

Green MF, Friedman M 1968 Hypothalamic-pituitary-adrenal function in the elderly. Gerontol Clin 10:334–339

Henry JP, Stephens PM 1977 Stress, health and the psychosocial environment: a sociobiological approach to medicine. Springer-Verlag, Berlin

Lupien S, Lecours AR, Lussier I, Schwartz G, Nair NPV, Meaney MJ 1994 Basal cortisol levels and cognitive deficits in human aging. J Neusrosci 14:2893–2903

Lupien SJ, DeLeon M, DeSanti S et al 1998 Cortisol levels during human aging predict hippocampal atrophy and memory deficits. Nat Neurosci 1:69–73 (erratum: 1998 Nat Neurosci 1:329)

McEwen BS 1998 Protective and damaging effects of stress mediators. New Engl J Med 338:171–179

Oxenkrug GF, Pomara N, McIntyre IM, Branconnier RJ, Stanley M, Gershon S 1983 Aging and cortisol resistance to suppression by dexamethasone: a positive correlation. Psychiatry Res 10:125–130

Pavlov EP, Harman SM, Chrousos GP, Loriaux DL, Blackman MR 1986 responses of plasma adrenocorticotropin, cortisol, and dehydroepiandrosterone to ovine corticotropin-releasing hormone in healthy aging men. J Clin Endocrinol Metab 62:767–772

Plotsky PM, Cunningham ET Jr, Widmaier EP 1989 Catecholaminergic modulation of cortocotropin-releasing factor and adrenocorticotropin secretion. Endocr Rev 10:437–458

Reaven GH 1988 Role of insulin resistance in human disease. Banting lecture 1988. Diabetes 37:1595–1607

Rosmond R, Dallman MF, Björntorp P 1998 Stress-related cortisol secretion in men: relationships with abdominal obesity and endocrine, metabolic and hemodynamic abnormalities. J Clin Endocrinol Metab 83:1853–1859

Sapolsky RM 1985 A mechanism for glucocorticoid toxicity in the hippocampus: increased vulnerability to metabolic insults. J Neurosci 5:1228–1232

Sapolsky RM, Krey LC, McEwen BS 1983 The adrenocortical stress-response in the aged male rat: impairment of recovery from stress. Exp Geront 18:55–62

Sapolsky RM, Krey LC, McEwen BS 1984a Glucocorticoid-sensitive hippocampal neurons are involved in terminating the adrenocortical stress response. Proc Natl Acad Sci USA 81:6174–6180

Sapolsky RM, Krey LC, McEwen BS 1984b Stress down-regulates corticosterone receptors in a site-specific manner in the brain. Endocrinology 114:287–292

Sapolsky RM, Krey LC, McEwen BS 1985a Prolonged glucocorticoid exposure reduces hippocampal neuron number: implications for aging. J Neurosci 5:1222–1227

Sapolsky RM, Meaney MJ, McEwen BS 1985b The development of the glucocorticoid receptor system in the rat limbic rain III. Negative feedback regulation. Brain Res 350:169–173

Sapolsky RM, Krey LC, McEwen BS 1986 The neuroendocrinology of stress and aging: the glucocorticoid cascade hypothesis. Endocr Rev 7:284–301

Seeman TE, Robbins RJ 1994 Aging and the hypothalamic-pituitary-adrenal response to challenge in humans. Endocr Rev 15:233–260

Smyth J, Ockenfels MC, Porter L, Kirschbaum C, Hellhammer DH, Stone AA 1998 Stressors and mood measured on a momentary basis are associated with salivary cortisol secretion. Psychoneuroendocrinology 23:353–370

Tourigny-Rivard MF, Raskind M, Rivard D 1981 The dexamethasone suppression test in an elderly population. Biol Psychiatry 16:1177–1184

Uno H, Tarara R, Else JG, Suleman MA, Sapolsky RM 1989 Hippocampal damage associated with prolonged and fatal stress in primates. J Neurosci 9:1705–1711

Walker DW, Barnes DE, Zornetzer SF, Hunter BE, Kubanis P 1980 Neuronal loss in hippocampus induced by prolonged ethanol consumption in rats. Science 209:711–721

West CD, Brown H, Simmins EL et al 1961 Adrenocortical function and cortisol metabolism in old age. J Clin Endocrinol Metab 21:1197–1207

Westberg L, Baghaei F, Rosmond R et al 2001 Polymorphisms in the androgen receptor gene and the estrogen receptor β gene are associated with androgen levels in women. J Clin Endocrinol Metab 86:2562–2568

DISCUSSION

Veldhuis: This area presents a lot of challenges to me. One of the difficulties I'm having as an endocrinologist with the testosterone theory, is that I don't see this syndrome X (metabolic syndrome) appearing across puberty in boys. At this stage their testosterone goes up 10–30-fold, but they get just mild insulin resistance. This makes me wonder whether insulin resistance is a marker for some other activity in

the system. Polycystic ovary syndrome (PCOS) patients (bearing in mind that this is a diverse category) appear to have primary insulin resistance. If PCOS patients who are not particularly obese but who are hyperandrogenaemic are given drugs or other interventions to lower their insulin levels, this also lowers androgen levels. If androgens were the only thing one measured and one knew nothing about insulin action through its tyrosine kinase pathways, one would say that it is the androgen that is the prime cause here.

Björntorp: I didn't have time to show that in the stressed men. Particularly in the small subset of 'burned out' men I referred to, testosterone levels are lowered. The hyperandrogenicity problem is probably a separate female issue. There is a sort of chicken and egg discussion about which comes first, the changes in the androgens or the changes in the oestrogens. But when we give androgens in moderate amounts to female rats, they become highly insulin resistant. When investigators have given androgens to women — there is one US study (Lovejoy et al 1996) and one Dutch study on transsexuals (Elbers et al 1997) — they also become insulin resistant. I believe that when you give testosterone, these things happen.

Veldhuis: There are a few exceptions. One data set from John Nestler showed that, when he reduced insulin with diazoxide, androgen levels also fell (Nestler et al 1989, 1990). Most experiments show the converse, but it is hard to give long-term androgens. When we monitor androgens, we may be looking at a marker of another underlying event that is driving syndrome X. We certainly see syndrome X all the time without any hint of hyperandrogenism. Could you clarify your thinking on the relationship between this group of women who are stressed and hyperandrogenaemic and the PCOS patients? Is PCOS simply an extended subgroup of this type of woman, or is PCOS a separate disease? It is such a tough area.

Björntorp: Yes. PCOS is a mess. First of all, I think we have to separate out PCOS and polycystic ovaries. There are reports now of polycystic ovaries in up to 20% of women. We have found around 25% of women to be hyperandrogenic. We are going to look at their ovaries. On the other hand, I have recently seen hints in the literature that stress might be involved in polycystic ovaries.

Veldhuis: There are all kinds of mild hints of HPA axis dysregulation in PCOS. Almost any mild degree of dysregulation feature that you wish to propose will have been reported, but not consistently. This is one of the problems that I have.

Prior: I'd like to expand the concepts a little more. Some time ago Kaplan and Adams showed that stressed, non-dominant premenopausal cyclic female monkeys continued to have regular cycles but had disturbed ovulation (Kaplan et al 1986, Adams et al 1985). Disturbed ovulation was associated with obliteration of the normal female protection against atherosclerosis. In those early studies, androgens weren't reported.

Björntorp: I know Carol Shively very well; we have a close contact. I asked her if she had looked at androgens. She tells me that they are elevated in females.

Prior: There are two types of non-ovulation that differ remarkably in many of their outcomes, especially in terms of what happens to bone. A simplistic way of looking at non-ovulation is as 'turned on' (meaning high luteinizing hormone [LH], ovaries enlarged and increased androgen manifestations) or 'turned off' (low gonadotropins, normal or low-ish oestrogen levels). But these two types of non-ovulation have a common morphology with polycystic ovaries. I think we should hone our terminology and stop defining a state that means androgen excess by the same non-specific morphological feature that also characterizes the very different hypothalamic non-ovulation (Prior 1997).

Burger: PCOS is a very difficult model or analogy to use in the present discussion. I have been quite taken by the sort of analysis that has come from Bart Fauser's group in Rotterdam, who has adopted a much more pragmatic approach to the whole concept of what we call PCOS (Imani et al 2000). He looks at the characteristics of women presenting because of infertility or coming to the gynaecological endocrine clinic. He takes a number of the classic features that we associate and then looks at their prevalence in individual women. He sees overlapping Venn diagrams of a group who have insulin resistance, a group who have hyperandrogenaemia, a group who have clinical hirsutism and a group with obesity. They overlap, but it is only a small proportion who have the full complement of all these features that were originally associated with the Stein–Leventhal description of PCOS. It is this sort of analysis that will help us to clarify what sort of terminology we should use. He uses it as a much more pragmatic approach to say, 'What is the prognosis of this individual and how should they be managed? Let's forget about trying to make a diagnosis of a category to which only very few will actually belong.' This is an illuminating perspective.

Veldhuis: To me, this is a more honest treatment of the heterogeneity that exists. This may help reduce some of the confusion caused by the clumping of patients in reports. I'm personally intrigued that insulin is driving androgen secretion. When we do *in vitro* studies with porcine theca cells, and transfect these with cDNA encoding the 17α-hydroxylase promoter, if we add minute amounts of insulin gene transcription goes up within 30–60 minutes. It is gorgeous! If you add a tiny bit of LH with the insulin, there is synergy. Most clinicians find that there are patients who are overly responsive to either insulin, or LH, or both. I want to be careful not to attribute something downstream, such as androgens, to events that are proximal or upstream. The theory of the serine phosphorylation defect has not actually been extended, but this is the kind of thinking that may eventually help us aggregate where appropriate and separate where appropriate. A particular patient might have a polymorphism for the androgen receptor,

because a slight decrease in androgen feedback due to a slightly defective receptor based on a polymorphism for the receptor causes her to oversecrete androgen. This may not be the same as another patient who is also anovulatory and hyperandrogenaemic with say a glutamine/alanine substitution in the promoter of the 17α-hydroxylase gene. She in turn is different from an obese syndrome X patient who has a primary defect in the insulin signalling pathway or in MAP kinase. I am struggling with the looseness of the biological endpoints that all of us depend on, and I am trying to drive some further precision in our focus of where the defects are, which would help us find out why the Venn diagrams that Henry Burger referred to are potentially separable.

Burger: Per Björntorp, how solidly established and how reproducible is the evidence of dehydroepiandrosterone sulfate (DHEAS) hypersecretion in chronic stress? My impression from having looked at the literature a while back is that it is not very consistent. Is this how we can explain some patients with hirsutism who otherwise have regular cycles and don't seem to fit in the PCOS grouping?

Björntorp: I can't answer that question.

Veldhuis: What might the notion of 'burn-out' better be defined to mean? Are you describing the chronically stressed animal without a novel stressor being introduced? Even a chronically stressed animal with a novel stressor will show a new stress response.

Björntorp: This is a confusing finding. This kind of burned-out HPA axis is seen in Vietnam veterans and Holocaust victims, for example, along with depression. People who are experts in the field believe that this is because the HPA axis is just worn out. In this situation there is apparently an increased activity of the sympathetic nervous system as a compensatory event. In the burned out men I showed here, which is about 9% of the population, we find background factors which we interpret as causing stress, but not dramatically more than in the less damaged men. My guess is that there is something else involved here.

Veldhuis: There are three clinical situations I can think of where the axis looks fairly flat. In the post-op Cushing's disease patients, the axis has been suppressed and is fairly flat, unless you infuse CRH and then the whole axis wakes up. Post-partum, there is a flat, monotonic, minimally pulsatile output. And there is a flat HPA axis in some cases of chronic fatigue syndrome (which is a bit like PCOS in that it is a poorly defined syndrome). Those three conditions involve inferential CRH deficiency states. If you model it on this basis, then you have to introduce something that I think is important in discussing these axes, and that is to view them as joint feedback and feed forward ensembles. For example, it would be inappropriate to talk only about feedforward, because this is irrelevant unless you can tell me the feedback state concurrently. If you give me any feedback state, I can make it irrelevant by driving feedforward appropriately. Neither feedback nor feedforward can be viewed in isolation. One of the things we may

need is a way to describe burn out profiles more formally. On the basis of all the known data on feedback, are there some conditions that would never produce that output in any sensible, easy manner? And are there other conditions that might? If you give me this information and I have the laboratory funds, I might then do the research to clarify some of the plausible options, and not immediately spend precious sponsorship funds on the least likely theories. One of the challenges we have clinically is that we have never assembled a clear, well defined, mapped network based on reasonable, sensible, minimal feedback connections.

Carroll: It is a very difficult area to nail down in humans for the reason that I mentioned before: the pituitary is sitting right in there interposed between your sampling zone and your zone of physiological interest — the brain. As long as that is the case, it is difficult to interpret. Most of the literature says that if you use ACTH and cortisol as your dependent variables for response to a stress, then in the context of chronic stress, acute stress challenges lead to impairment of the expected response. The same is true when CRH is administered. It is clear from the depression literature that if you provoke insulin hypoglycaemia, the ACTH and cortisol responses are blunted, and if you give CRH their response is blunted both in cortisol and ACTH. But you can't interpret that with reference to the brain because the pituitary is sitting right there. If what I was saying earlier today is correct, the pituitary is a prime site of GR action, and this may be the answer. We have to come up with alternative experimental strategies. Johannes Veldhuis and I have been looking at a low feedback strategy, but we don't have data analysed yet for that. This would be the approach: knockout the feedback signal with metyrapone, use ACTH as the dependent variable and then do things to the brain and see what happens. Plasma CRH is not a worthwhile measure in this context, for short-term studies, because it gets diluted in the general circulation.

Handelsman: Isn't it better for that experiment to use adrenalectomy rather than using drugs?

Carroll: That's not so easy to do in humans!

Velduis: This opens the question of stressing the axis, trying to pin-point responses and residual elements without watching the whole system change simultaneously. Even this is difficult, because of the triple nodes.

Carroll: Paul Plotsky and Paul Sovchenko refer to this as pharmacological adrenalectomy. You have to be very careful about timing and dosage to make it work.

Laron: The aetiology of so-called PCOS is more complicated. We have data that shows insulin-like growth factor (IGF)1 over-dosage causes a PCOS-like syndrome in females (Klinger et al 1998), and in males it increases LH (Laron & Klinger 1998). I don't think there are enough data showing whether insulin competes on the IGF1 receptor in the patients with so-called PCOS. There may

be interplay, but it seems that the synergism of IGF1 with androgens is stronger than that between insulin and androgens.

Veldhuis: When you say it produces a PCOS-like syndrome, given what we have heard about the Venn diagram multiplicity, what kinds of features are you referring to?

Laron: It causes hyperandrogenaemia, acne and interrupted menstruation, which are all reversible when the dose is reduced.

Veldhuis: Have the ovaries in these patients been examined by ultrasound?

Laron: Some have, but they did not show the typical changes of PCOS, namely the fibrosis.

Veldhuis: This highlights the complexity of watching an endproduct when you don't know which receptor pathways are triggered.

Prior: I was going to make the clinical observation that when women become overweight, they tend towards increased androgenicity; when men become overweight they tend towards decreased androgenicity. There are some fascinating epidemiological data taking the reported weight and height at age 18, and showing anovulatory infertility in those who have a body mass index higher than 24.5 (Rich-Edwards et al 1994). The relationship between body weight, LH and androgenicity is a key here, somehow.

Veldhuis: I would add insulin to this.

Björntorp: I know what you are saying. The problem with these studies as I see them is that people lump obesity into one pot. You have to separate central obesity and peripheral obesity.

Prior: That is the way I thought of it. Also, we must consider muscularity causing higher weight but not obesity.

Morley: Besides the paper on resistance, there is also a paper by Brüning et al (2000), in which the insulin receptor was specifically knocked out in the brain. These mice are very clearly hypogonadal. Females basically become fat, whereas males don't. This was used as an argument for insulin affecting feeding. In fact, if you knock out oestrogen you actually get fat females.

Brabant: It is unclear whether this is an effect mediated by insulin on body fat or simply via insulin acting on the gonadotroph.

Morley: The other thing in the recent literature that is going to change all of this is a paper showing that fat cells produce a PPARγ-related compound called resistin (Steppan et al 2001). If that is hormonal-dependent or changes hormone it will totally change the way we think of this. Most probably this is what is going to happen.

Haus: We studied over 750 24 h profiles (6–8 samples/24 h) of hormonal and biochemical variables in clinically healthy diurnally active subjects between 9 and >90 years of age. In comparison to young adult subjects (21 1.5 years of age), the elderly (77 7 years of age) showed a drop in their circadian mean of serum

ACTH levels (44.6 6.4 pg/ml vs. 25.0 1.6 pg/ml) while serum β-endorphin showed in the same subjects a statistically significant increase (3.9 0.2 pmol/l vs. 5.3 0.3 pmol/l) (Haus et al 1989, Nicolau et al 1991). This raises the question of a possible difference with ageing in the splitting of the parent molecule of proopiomelanocortin from which both ACTH and β-endorphin are derived.

Plasma cortisol 24 h mean values varied very little between the same age groups (10.5 0.3 μg/dl vs. 9.5 0.2 μg/dl) (Haus et al 1989, Lakatua et al 1992). However, in the plasma ACTH:plasma cortisol ratio there was a very marked age difference, which was strictly time dependent. In the morning, the ratio was comparable between the young and old subjects. However, during the afternoon and evening there was a substantial difference between the age groups. The younger subjects showed a high amplitude circadian rhythm with rising values of the ACTH:cortisol ratio in the afternoon reaching a peak at 00:00. With increasing age the amplitude of this rhythm became less and the rhythm almost disappeared in subjects above 80 (Lakatua et al 1992). This observation suggests in the elderly a higher sensitivity of the adrenal to ACTH during the evening and early night hours when the adrenal in the young subjects becomes less responsive to ACTH.

Veldhuis: This is the feedforward concept that I want to visualize.

References

Adams MR, Kaplan JR, Clarkson TB, Koritnik DR 1985 Ovariectomy, social status and atherosclerosis in cynomolgus monkeys. Arteriosclerosis 5:192–200

Brüning JC, Gautam D, Burks DJ et al 2000 Role of brain insulin receptor in control of body weight and reproduction. Science 289:2122–2125

Elbers JM, Asscheman H, Seidell JC, Megens JA, Gooren LJ 1997 Long-term testosterone administration increases visceral fat in female to male transsexuals. J Clin Endocrinol Metab 82:2044–2047

Haus E, Nicolau G, Lakatua DJ, Sackett-Lundeen L, Petrescu E 1989 Circadian rhythm parameters of endocrine functions in elderly subjects during the seventh to the ninth decade of life. Chronobiologia 16:331–352

Imani B, Eijkemans MJ, de Jong FH et al 2000 Free androgen index and leptin are the most prominent endocrine predictors of ovarian response during clomiphene citrate induction of ovulation in normogonadotropic oligomernorrheic infertility. J Clin Endocrinol Metab 85:676–682

Kaplan JR, Adams MR, Koritnik DR, Rose JC, Manuck SB 1986 Adrenal responsiveness and social status in intact and ovariectomized *Macaca fascicularis*. Am J Primatology 11:181–193

Klinger B, Anin S, Silbergeld A, Eshet R, Laron Z 1998 Development of hyperandrogenism during treatment with insulin-like growth factor I (IGF-I) in female patients with Laron syndrome. Clin Endocrinol 48:81–87

Lakatua DJ, Nicolau GY, Sackett-Lundeen L, Petrescu E, Ortmeier T, Haus E 1992 Circadian rhythm in adrenal response to endogenous ACTH in clinically healthy subjects of different age groups. 5th International Meeting on Chronopharmacology and Chronotherapeutics, Amelia Island, Florida, July 12–16, 1992, p 9–11

Laron Z, Klinger B 1998 Effect of insulin-like growth factor I treatment on serum androgens and testicular and penile size in males with Laron syndrome (primary growth hormone resistance). Eur J Endocrinol 138:176–180

Lovejoy JC, Bray GA, Bourgeois MO et al 1996 Exogenous androgens influence body composition and regional body fat distribution in obese postmenopausal women — a clinical research center study. J Clin Endocrinol Metab 81:2198–2203

Nestler JE, Barlascini CO, Matt DW et al 1989 Suppression of serum insulin by diazoxide reduces serum testosterone levels in obese women with polycystic ovary syndrome. J Clin Endocrinol Metab 68:1027–1032

Nestler JE, Singh R, Matt DW, Clore JN, Blackard WG 1990 Suppression of serum insulin level by diazoxide does not alter serum testosterone or sex hormone binding globulin levels in healthy, nonobese women. Am J Obstet Gynecol 163:1243–1246

Nicolau GY, Haus E, Lakatua DJ et al 1991 Circadian periodicity of pituitary-adrenal parameters in different age groups. 20th International Conference on Chronobiology, Tel Aviv, Israel, June 16–21, 1991

Prior JC 1997 Ovulatory disturbances: do they matter? Can J Diagnosis (February) p 64–80

Rich-Edwards JW, Goldman MB, Willett WC et al 1994 Adolescent body mass index and infertility caused by ovulatory disorder. Am J Obstet Gynecol 171:171–177

Steppan CM, Bailey ST, Bhat S et al 2001 The hormone resistin links obesity to diabetes. Nature 409:307–312

Endocrine Facets of Ageing.
Novartis 242

Print ISBN 0-471-48636-1 Online ISBN 0-470-84654-2

Male reproductive ageing: human fertility, androgens and hormone dependent disease

David J. Handelsman

ANZAC Research Institute, Department of Andrology, Concord Hospital, University of Sydney, Sydney, NSW 2139, Australia

Abstract. The waning of male virility with age, in all its ambiguities, has always intrigued humans, prompting innumerable approaches to staving it off. In modern terms, advancing age impacts on all aspects of male reproductive health — sexuality, fertility and androgenization — with differing extents and tempo. With ageing, male sexual function declines predominantly due to vasculogenic defects in cavernosal haemodynamics, whereas libido and ejaculation are less affected. This raises the potential for prevention and treatment of erectile dysfunction as an early clinical manifestation of atherosclerosis. After maturity, male fertility persists throughout life but decreases modestly with age presumably due to concomitant decline in sexual activity rather than in sperm output or function, although systematic population studies of the latter are difficult. Male ageing is associated with a progressive, partial and variable degree of androgen deficiency, but the clinical and public health significance remain to be established. Available evidence suggests that androgen supplementation is unlikely to prolong life expectancy but might improve quality of life through prevention of apparently age-related declines in androgen-sensitive tissues. The appropriate target population, treatment modality and objectives remain to be established by further controlled clinical studies.

2002 Endocrine facets of ageing. Wiley, Chichester (Novartis Foundation Symposium 242) p 66–81

Over 50 years ago WHO defined health as 'A state of complete physical, mental and social well-being and not merely the absence of disease or infirmity'. Analogously, male reproductive health can be considered as having three dimensions — sexuality, fertility and androgenization — each affected by ageing.

This review highlights two historical themes. The first is the importance of the great contributions of 20th century statistics to medicine — randomization and placebo controls — as the fundamentals of prospective or experimental studies. These features control not only known, but crucially unknown covariables, that influence outcomes. By contrast, retrospective or observational studies even if based on the counsel of perfection — valid population-based sampling frames

and objective validation of study measures — only control for known variables. This unique power gives salience to experimental over observational designs thereby creating a distinction between strong and weak inference in biomedical science. These ideas lie at the root of evidence-based medicine, a welcome modern trend in medical science even if somewhat misnamed. Its focus is not so much on evidence per se (as subjective or anecdotal experience constitute evidence), as on the quality of evidence. Ironically, the rigour of evidence-based medicine rests upon subjective judgement of the qualities of evidence. Nonetheless, the robustness of the criteria of randomization and placebo controls are themselves ultimately susceptible to objective judgement.

The second historical theme is the ramifications of Santayana's aphorism that those not familiar with their history are condemned to repeat it. This has particular resonance for male reproductive ageing research as medical history over the last two centuries has experienced repeated colourful episodes of rejuvenation quackery, including doctors, masquerading as science. Preying on the profound and endlessly resurgent but unattainable human desire for rejuvenation will always be fertile territory for the plausible quack to harvest rich pickings — and the crop is flowering once again.

Sexuality

Since antiquity, history and literature are replete with references to the waning of male virility, in all its ambiguities, with age. The seemingly inexhaustible repertoire of remedies to stave it off testifies to the ubiquity of this human fixation. In particular, the decline in male sexuality in old age, being both emblematic and problematic, it is not surprising that aphrodisiacs are among the most ancient and ubiquitous of folk remedies. The modern educated layman, however, having lost faith in rhino horn and tiger penis, appears now bedazzled by the misty allure of hormones, the contemporary folkloric embodiment of the fabled aphrodisiac. And for hormones, our social organization dictates that doctors remain its monopolistic dispensers.

Systematic sociological studies of human sexuality were pioneered by Kinsey in mid 20th century followed by more recent comprehensive national data from the UK (Wellings et al 1994), USA (Laumann et al 1994) and France (Messiah & Pelletier 1996). Their focus has been on men under 60 years leaving an incomplete picture. As an observational methodology, such research aspires to high standards in representative sampling; its limitations are in its lacking independent, objective validation of findings. It is hardly surprising that uncorroborated self-report (to strangers) of the most emotive, and hence least likely to be reliably reported, aspect of human behaviour would produce glaring discrepancies. For example, $\sim$50% of men but only $\sim$5% of women over the age

of 80 report ongoing sexual activity with a partner (Laumann et al 1994). If these facts are dubious, more subtle motivations and manifestations must be well beyond reach. Bedevilled by its subjectivity and imperilled by proximity to social mores, the prospects for more objective research into male sexual activity in the general population seem remote. Yet large lacunae in understanding of male sexuality remain, among them the role of prostitution which, given its ubiquity and pervasiveness, is remarkably little studied. If prostitution is the oldest profession, then use of its services must be the oldest male pastime. While the sociology of prostitutes is widely studied, their male clients remain obscure and ignored. It is hard to imagine that studies of such a ubiquitous and pervasive facet of male sexuality would not enlighten the understanding of male sexuality.

By contrast, medical complaints of male sexual dysfunction undoubtedly increase with age. While all functional aspects of male sexuality — libido, potency and ejaculation — are reduced in older men, the major impact of ageing is the exponential increase of erectile dysfunction with age (Ayta et al 1999). The last decade has seen a major paradigm shift in understanding of male sexual dysfunction. This includes a public relations makeover in nomenclature (impotence becoming erectile dysfunction), a shift in beliefs about causation (from prevailing Freudian psychoanalytic beliefs on psychosomatic causation, to erectile dysfunction being due to organic neurovascular disorders of cavernosal hydraulics) and development of the first effective pharmacotherapy.

The development of vasoactive cavernosal pharmacotherapy was inaugurated by the accidental discovery of papaverine effects during vascular surgery and soon surpassed by the equally fortuitous discovery of oral sildenafil during clinical drug evaluation for other purposes. Both are striking instances of Pasteur's dictum of chance favouring the prepared mind rather than outcomes of goal-oriented research. Stemming from recognizing the vasculogenic basis of most age-related erectile dysfunction, important issues arising include the role of erectile dysfunction as a sentinel presentation of cardiovascular disease, the potential for primary and secondary prevention strategies based on cardiovascular disease pathogenesis (Ayta et al 1999) and elucidating the pathogenic mechanisms of the most frequent iatrogenic cause of erectile dysfunction, anti-hypertensive drug therapy (Feldman et al 2000). While the lucrative new pharmaceutical market for vasoactive drugs will ensure expanding options for treatment of individuals with erectile dysfunction, the high and increasing prevalence means the prospective impact on public health policy and financing is daunting (Ayta et al 1999).

Fertility

Paternity is well established at the oldest age so the natural history of male fertility is concluded only by death. By contrast, female fertility, terminating naturally at

menopause, occupies only half of average adult life expectancy. Despite the enduring fertility potential, fathers older than 50 years are responsible for only ~1% of births in developed countries. Communal procreative patterns are determined by the similarity of couple's age and the overwhelming age-restriction of female fertility. Nevertheless, fertility concerns of men cannot be disregarded at any set age particularly with increasing remarriage to younger women.

Unfortunately the biomedical literature usually regards male fertility evaluation as synonymous with semen analysis. Such reductive logic risks overlooking important details of the whole picture and, coupled with the fact that men only provide semen samples when concerned about their fertility (Handelsman 1997), virtually all research on human sperm involves convenience samples inherently unrepresentative of the general male population. Tunnel vision in appraising the limitation of such research leads predictably to grievous misunderstanding (Handelsman 2000).

In evaluating age effects on male fertility, it is necessary to note that male fertility is measured by counting conceptions, not sperm. Consequently, estimation of male fertility has been largely the domain of demographers. They, in turn, focus almost exclusively on female fertility, for reasons only partly explained by the simplistic axiom that only maternity is ascertainable. The little evidence available suggests that male fertility declines modestly with age (Anderson 1975, Ford et al 2000). In these studies, it remains unclear if attempts to adjust for the highly correlated effects of wife's age are sufficient to exclude that the modest decline in male fertility is only illusory. If real, such modest declines in male fertility may be due to age-related reduction in coital rate, sperm production or function, none being easy to study in the general population.

Apart from indirect evidence from paternity, the few studies of sperm output available are restricted to small convenience samples of infertile, older men. These suggest sperm output is undiminished up to the age of 50 years but systematic studies of any quality are limited beyond that age to small convenience samples. Similarly, as both testicular histology and sperm function require biopsy and semen samples, respectively, there are equally no valid population-based data to evaluate age effects on spermatogenic histology or sperm function.

Given the difficulties of evaluating spermatogenesis by studies requiring semen analysis, valid surrogate variables would be useful. Indeed, semen analysis itself is an imperfect surrogate marker for male fertility for which questionnaire instruments have been developed (Levine 1988, Joffe 1997). Testicular volume provides an excellent surrogate marker of spermatogenesis because seminiferous tubules comprise the bulk of testis volume as noted in the clinical observation that testicular atrophy reliably connotes impaired spermatogenesis in infertile men. Lacking any longitudinal studies of testis size, the largest cross-sectional study indicates that testis volume is little affected by age until the eighth decade, if the

effects of terminal illness or vascular disease (Regadera et al 1985) are distinguished (Handelsman & Staraj 1985). Smaller post-mortem (Johnson 1986) and ultrasound (Lenz et al 1993) studies are consistent with these observations. Cross-sectional studies showing consistently that blood follicle-stimulating hormone (FSH) increases and inhibin B decreases progressively with advancing age while their inverse relationship is maintained suggest that these hormone markers, together with testis ultrasound, could constitute useful surrogate markers of spermatogenesis for future population-based studies of human ageing.

Androgen effects

The 20th century saw dramatic prolongation of life expectancy in developed countries. More people living longer in retirement creates a premium on strategies to promote healthy ageing (meaning, in this context, maintenance of enjoyable and independent living for the longest time with compression of morbidity into the shortest timeframe at the end of life). A focus on gainful coexistence with chronic ailments supplants the eradication of disease. Ameliorating disabilities that accrue during age could enhance quality of life regardless of its prolongation. Among strategies to improve health and well-being of the elderly, the judicious evidence-based use of hormonal therapy clearly warrants exploration. An important caveat is that since the best available evidence suggests that androgen replacement therapy does not influence life-expectancy (Handelsman 1998), beneficial effects of physiological androgen dosages are likely to be restricted to quality of life, for which instrumental measures remain inadequate.

'*You can't make a pig grow by weighing it*': *observational studies*

As testicular endocrine function can be evaluated from blood samples, large community-based epidemiological studies were feasible since the advent of radioimmunoassay in the 1960s. The now accepted consensus that blood testosterone concentrations decline gradually after mid-life (Gray et al 1991a) was secured by decisive evidence from the population-based Massachusetts Male Ageing Study (Gray et al 1991b). This decline of $\sim$1% per annum, accompanied by rising blood sex-hormone-binding globulin (SHBG), luteinizing hormone (LH) and FSH concentrations, is accelerated by concomitant medical disorders which accumulate with age. It is sobering that three generations of observational studies using various convenience samples had not achieved definitive conclusions, generating futile controversy for two decades, before the pivotal importance of representative sampling was fully appreciated. Key aspects of the

descriptive epidemiology of androgenic status of older men still requiring clarification include (a) whether androgenic thresholds, sensitivity and effects differ between ethnic groups, individuals and tissues, and (b) which older men and what biological effects are the best targets for androgen therapy.

'The Emperor's New Clothes': measuring testosterone

Like most steroids and drugs, testosterone binds with varying affinity to circulating proteins notably SHBG and albumin. It is often stated or assumed that various measures of 'free' testosterone are superior markers of androgen supply to, and/or net effects upon, androgen sensitive tissues than measurement of total testosterone, the gold standard for biochemical confirmation of androgen deficiency. The so-called 'free hormone' hypothesis has a long and tortuous history (Edwards & Ekins 1988, Pardridge 1988, Mendel 1989). Regarding testosterone, claims of superiority for derived testosterone assays are based exclusively on theoretical arguments without clinical validation; yet even in theory, freer movement of unbound testosterone into tissues would be just as likely a priori to result in faster metabolic inactivation as enhanced biological effects on target tissues.

Derived testosterone assays include direct measurement of free (non-protein-bound) testosterone by equilibrium dialysis, centrifugal ultrafiltration or analogue immunoassay, or indirect measures of 'bioavailable' (non-SHBG bound) testosterone. 'Free' testosterone can also be calculated from total testosterone and SHBG concentrations either as a simple ratio (Kapoor et al 1993) or a more complex calculation (Södergård et al 1982). Claims of superior clinical utility have not been supported by direct empirical proof; some derived testosterone measures are invalid on theoretical (Kapoor et al 1993) and/or empirical (Winters et al 1998) grounds. Given their weak rationale, absent empirical validation of alleged superiority and less accurate measurements, there seems little to recommend such measures in routine clinical use. The curious gulf between their validation and popularity may reflect their common distinctive feature, that of demonstrating a faster fall with age than total testosterone. Clearer separation between younger and older men serves to inflate the proportion of older men defined in this manner as having biochemical 'androgen deficiency'. This circular logic however cannot evade the need to prove efficacy and safety of androgen therapy in older men. Such confusion of ends and means may be alleviated by distinguishing androgen replacement therapy, which assumes a deficiency state to be rectified by physiological testosterone doses, and pharmacological androgen therapy which aims for proof of efficacy and safety regardless of drug, dose or deficiency state.

'If I had a hammer, I'd hammer in the morning': interventional therapeutic studies

Following the adage that when one's only tool is a hammer, remarkably soon all problems turn into nails, it is an understandable that androgen administration is proposed for older men. Such proposals long pre-date the first availability of testosterone (Hamilton 1937) with many bouts of rejuvenation quackery associated with the names of Brown-Séquard, Steinach and Voronoff into the early 20th century. Standard clinical endocrine practice includes replacement therapy for unequivocal hormonal deficiencies of the pancreas (insulin), thyroid (thyroxine), adrenal (glucocorticoid, mineralocorticoid) and gonads (oestrogen, androgen). Conversely, hormone replacement is not provided for other classical hormones such as prolactin, glucagon, somatostatin, calcitonin, calcitonin-gene related peptide and adrenalin, and other hormones (thyroid-stimulating hormone, LH, FSH, parathyroid hormone) are not replaced but substituted by simpler non-peptide end products. Generally, the criteria for a treatable hormonal deficiency include (a) a well defined clinical deficiency state, (b) availability of sufficient clinical grade hormones, and (c) convincing evidence for therapeutic efficacy and safety. The flexibility of this categorization is illustrated by the changing status of adult growth hormone replacement therapy based on emerging evidence (Carroll et al 1998).

Certain features of ageing men resemble those observed in androgen-deficient younger men, notably decreased lean body mass (muscle) and bone; reduced body hair growth, skin thickness and dermal sebum secretion; impaired cognitive function and mood; increased adiposity; and reduced strength, endurance, initiative, virility and sense of well-being. Since androgen replacement in younger men can reverse muscle, bone and mental changes of androgen deficiency, it is a reasonable postulate that the partial androgen deficiency may contribute to the physical frailty and mental torpor of older men. Nevertheless, it remains unclear whether (a) blood testosterone concentrations fall far enough to warrant replenishment, (b) tissue androgenic thresholds change with age, and (c) older tissues remain sufficiently androgen responsive. In short, the biological significance of the gradual, partial and variable decline in blood testosterone concentrations in older men cannot be established from observational studies alone but requires critical evaluation by interventional therapeutic studies.

Ad hoc trials of androgen therapy in ageing men dating back to the first availability of testosterone (Heller & Myers 1944) were unconvincing as they lacked the decisive features of placebo controls and randomization. Several recent small controlled studies of at least three months' duration demonstrate inconsistent benefits of testosterone supplementation (Marin et al 1992, Tenover 1992, Morley et al 1993, Sih et al 1997) but most had significant limitations in design (inadequate masking, poorly defined clinical end-points, low power).

A major study of generally healthy men over 65 years with a low plasma testosterone (<16.5 nmol/l) treated with daily transdermal testosterone for three years showed an improved sense of well-being, increased lean mass and decreased fat mass compared with placebo (Snyder et al 1999a,b). Crucially, however, testosterone did not consistently improve muscular function or bone density compared with placebo. Secondary analyses showed any benefits of testosterone in older men were limited to those with overt androgen deficiency. Consequently, at present androgen supplementation in ageing men is appropriate for overt androgen deficiency, but more liberal use is still best considered promising but unproven.

Future studies should aim for more powerful design, better focus on appropriate subgroups of men and end-points likely to benefit, and/or alternative hormonal regimens. Pharmacological androgen therapy, using supraphysiological doses or novel synthetic androgens, might improve muscle, bone or other androgen-dependent functions in older men regardless of androgen deficiency status, nature or dose of androgen. Viewed like any other anti-ageing treatment, this would require evidence of efficacy, safety and cost-effectiveness from controlled trials rather then relying on supposed replacement status to lighten the burden of proof for efficacy and safety. This approach would diversify androgen therapies to allow enhanced targeting of androgen therapy via exploiting variations in tissue selectivity and metabolic activation (5α reduction, aromatization) profiles (Sundaram et al 1994) that could be developed in novel potent designer androgens (Dalton et al 1998, Edwards et al 1998). Regardless of the specific androgen, the safety issues remain whether androgen therapy influences progression of prostate or cardiovascular disease or precipitates idiosyncratic effects (polycythaemia, sleep apnoea, fluid retention, behavioural disturbance). The key long-term effects on cardiovascular and prostate disorders would require large, long-term vigilance studies as were undertaken for oestrogen therapy in menopause (Handelsman 1998).

Androgen-dependent disorders (prostate and cardiovascular disease)

A salient fact of ageing men's health is that cardiovascular disease is the major cause of death, and prostate disease the leading cause of both major surgery and new internal cancers. Both prostate and cardiovascular disease demonstrate distinctive hormone-dependent features, with long incubation periods during early life culminating in exponentially increasing age-related incidence during late life. These features create opportunities for novel preventive strategies (Handelsman 1998). Among promising options for future developments is the potential to develop designer androgens (Dalton et al 1998, Edwards et al 1998) that exploit tissue-selective metabolic activation of testosterone (Sundaram et al 1994) via

the 5α reductase pathway, a local androgen amplification mechanism, or diversification of androgen action via aromatase which leads to effects mediated via the oestrogen receptor.

In the near future the most important developments with predictable potential for improved management of prostate disease in ageing men include (a) development of better tests to make wide-scale prostate cancer screening feasible and (b) Finasteride Prostate Cancer Chemoprevention study (Brawley & Parnes 2000) which will determine whether selective inhibition of 5α reductase can reduce prostate cancer diagnosis and mortality with great implications for development of prostate-sparing androgens for supplementation.

Arguably the most fundamental fact in cardiovascular epidemiology is that men have earlier and more severe morbidity and mortality than women of similar age. Despite prevailing orthodoxy based on retrospective case–control studies, it has long been evident that natural menopause does not accelerate cardiovascular disease (Heller & Jacobs 1978, Tunstall-Pedoe 1998), unlike breast cancer or bone density, where oestrogen dependency is clearer. This uncertainty is accentuated by the first prospective studies of oestrogen replacement therapy in menopausal women demonstrating no benefit, and even detrimental effects, on secondary prevention of cardiovascular disease (Hulley et al 1998). As life-long castration appears not to alter life-expectancy (Handelsman 1998), endogenous testosterone has at best only modest effects in promoting cardiovascular disease, perhaps only in subpopulations. While recent studies have rediscovered the vasodilatory properties of androgens (Jaffe 1977) and that low endogenous testosterone is a risk factor for cardiovascular disease (Alexandersen et al 1996), these findings need to be reconciled with the likelihood that androgens may also foster early stages of atherogenesis (McCrohon et al 1997, 1999, 2000, McCredie et al 1998).

Diagnosis and management of androgen deficiency in older men

The diagnosis of androgen deficiency, primarily a clinical diagnosis confirmed by appropriate hormone assays, is generally unambiguous in younger men with hypothalamic–pituitary or testicular disorders. Among older men the non-specific symptoms of androgen deficiency may have other causes and biochemical diagnosis is uncertain unless blood testosterone concentrations are unambiguously low. Appropriate reference ranges for blood testosterone concentrations are controversial — adopting the reference range of healthy young men assumes age-dependent changes in blood testosterone levels are inherently pathological whereas an age-adjusted reference range might overlook rectifiable androgen deficiency. A reliable, independent marker of net tissue androgen effect would help resolve this dilemma but none exists. The androgenic threshold for sexual function is low

whereas those for muscle, bone and cognitive function are unknown but likely to be higher, and may vary within and between individuals. Consequently, driven by the need for practical guidance, free of marketing hype in an environment of commercially driven disease-mongering, the Endocrine Society of Australia has recently published consensus best practice guidelines for androgen prescribing (Conway et al 2000) which were adopted by the national Pharmaceutical Benefits Scheme for subsidy of effective medicines. Revision based on further evidence and other national guidelines will undoubtedly emerge.

References

Alexandersen P, Haarbo J, Christiansen C 1996 The relationship of natural androgens to coronary heart disease in male: a review. Atherosclerosis 125:1–13

Anderson BA 1975 Male age and fertility: results from Ireland prior to 1911. Population Index 41:561–567

Ayta IA, McKinlay JB, Krane RJ 1999 The likely worldwide increase in erectile dysfunction between 1995 and 2025 and some possible policy consequences. BJU Int 84:50–56

Brawley OW, Parnes H 2000 Prostate cancer prevention trials in the USA. Eur J Cancer 36:1312–1315

Carroll PV, Christ ER, Bengtsson BA et al 1998 Growth hormone deficiency in adulthood and the effects of growth hormone replacement: a review. Growth Hormone Research Society Scientific Committee. J Clin Endocrinol Metab 83:382–395

Conway AJ, Handelsman DJ, Lording DW, Stuckey B, Zajac JD 2000 Use, misuse and abuse of androgens: The Endocrine Society of Australia consensus guidelines for androgen prescribing. Med J Aust 172:220–224

Dalton JT, Mukherjee A, Zhu Z, Kirkovsky L, Miller DD 1998 Discovery of nonsteroidal androgens. Biochem Biophys Res Commun 244:1–4

Edwards JP, West SJ, Pooley CL, Marschke KB, Farmer LJ, Jones TK 1998 New nonsteroidal androgen receptor modulators based on 4-(trifluoromethyl)-2(1H)-pyrrolidino[3,2-g] quinolinone. Bioorg Med Chem Lett 8:745–750

Edwards P, Ekins R 1988 The "Pardridge" hypotheses relating to the role of hormone binding proteins in hormone delivery: a critique. Steroids 52:367–368

Feldman HA, Johannes CB, Derby CA et al 2000 Erectile dysfunction and coronary risk factors: prospective results from the Massachusetts male aging study. Prev Med 30:328–338

Ford WC, North K, Taylor H, Farrow A, Hull MG, Golding J 2000 Increasing paternal age is associated with delayed conception in a large population of fertile couples: evidence for declining fecundity in older men. The ALSPAC Study Team (Avon Longitudinal Study of Pregnancy and Childhood). Hum Reprod 15:1703–1708

Gray A, Berlin JA, McKinlay JB, Longcope C 1991a An examination of research design effects on the association of testosterone and male aging: results of a meta-analysis. J Clin Epidemiol 44:671–684

Gray A, Feldman HA, McKinlay JB, Longcope C 1991b Age, disease, and changing sex hormone levels in middle-aged men: results of the Massachussetts Male Aging Study. J Clin Endocrinol Metab 73:1016–1025

Hamilton JB 1937 Treatment of sexual underdevelopment with synthetic male hormone substance. Endocrinology 21:649–654

Handelsman DJ 1997 Sperm output of healthy men in Australia: magnitude of bias due to self-selected volunteers. Hum Reprod 12:2701–2705

Handelsman DJ 1998 The safety of androgens: prostate and cardiovascular disease. In: Wang C (ed) Male reproductive function. Kluwer Academic Publishers, Norwell, MA, p 173–190
Handelsman DJ 2000 Myth and methodology in the evaluation of human sperm output. Int J Androl 23:50–53
Handelsman DJ, Staraj S 1985 Testicular size: the effects of aging, malnutrition and illness. J Androl 6:144–151
Heller CG, Myers GB 1944 The male climacteric, its symptomatology, diagnosis and treatment. J Am Med Assoc 126:472–427
Heller RF, Jacobs HS 1978 Coronary heart disease in relation to age, sex, and the menopause. Br Med J 1:472–474
Hulley S, Grady D, Bush T et al 1998 Randomized trial of estrogen plus progestin for secondary prevention of coronary heart disease in postmenopausal women. Heart and Estrogen/progestin Replacement Study (HERS) Research Group. JAMA 280:605–613
Jaffe MD 1977 Effect of testosterone cypionate on postexercise ST segment depression. Br Heart J 39:1217–1222
Joffe M 1997 Time to pregnancy: a measure of reproductive function in either sex. Asclepios Project. Occup Environ Med 54:289–295
Johnson L 1986 Spermatogenesis and aging in the human. J Androl 7:331–354
Kapoor P, Luttrell BM, Williams D 1993 The free androgen index is not valid for adult males. J Steroid Biochem Mol Biol 45:325–326
Laumann EO, Gagnon JH, Michael RT, Michaels S 1994 The social organization of sexuality. University of Chicago Press, Chicago, IL
Lenz S, Giwercman A, Elsborg A et al 1993 Ultrasonic testicular texture and size in 444 men from the general population: correlation to semen quality. Eur Urol 24:231–238
Levine RJ 1988 Monitoring fertility to detect toxicity to the male reproductive system. Reprod Toxicol 2:223–227
Marin P, Krotkiewski M, Björntorp P 1992 Androgen treatment of middle-aged, obese men: effects on metabolism, muscle and adipose tissues. Eur J Med 1:329–336
McCredie RJ, McCrohon JA, Turner L, Griffiths KA, Handelsman DJ, Celermajer DS 1998 Vascular reactivity is impaired in genetic females taking high-dose androgens. J Am Coll Cardiol 32:1331–1335
McCrohon JA, Death AK, Nakhla S et al 2000 Androgen receptor expression is greater in macrophages from male than from female donors. A sex difference with implications for atherogenesis. Circulation 101:224–226
McCrohon JA, Jessup W, Handelsman DJ, Celermajer DS 1999 Androgen exposure increases human monocyte adhesion to vascular endothelium and endothelial cell expression of vascular cell adhesion molecule-1. Circulation 99:2317–2322
McCrohon JA, Walters WA, Robinson JT et al 1997 Arterial reactivity is enhanced in genetic males taking high dose estrogens. J Am Coll Cardiol 29:1432–1436
Mendel CM 1989 The free hormone hypothesis: a physiologically based mathematical model. Endocr Rev 10:232–274
Messiah A, Pelletier A 1996 Partner-specific sexual practices among heterosexual men and women with multiple partners: results from the French national survey, ACSF. Analyse des Comportements Sexuel en France. Arch Sex Behav 25:233–247
Morley JE, Perry HM 3rd, Kaiser FE et al 1993 Effects of testosterone replacement therapy in old hypogonadal males: a preliminary study. J Am Geriatr Soc 41:149–152
Pardridge WM 1988 Selective delivery of sex steroid hormones to tissues by albumin and by sex hormone-binding globulin. Oxf Rev Reprod Biol 10:237–292
Regadera J, Nistal M, Paniagua R 1985 Testis, epididymis, and spermatic cord in elderly men. Correlation of angiographic and histologic studies with systemic arteriosclerosis. Arch Pathol Lab Med 109:663–667

Sih R, Morley JE, Kaiser FE, Perry HM 3rd, Patrick P, Ross C 1997 Testosterone replacement in older hypogonadal men: a 12-month randomized controlled trial. J Clin Endocrinol Metab 82:1661–1667

Snyder PJ, Peachey H, Hannoush P et al 1999a Effect of testosterone treatment on bone mineral density in men over 65 years of age. J Clin Endocrinol Metab 84:1966–1772

Snyder PJ, Peachey H, Hannoush P et al 1999b Effect of testosterone treatment on body composition and muscle strength in men over 65 years of age. J Clin Endocrinol Metab 84: 2647–2653

Södergård R, Bäckström T, Shanbhag V, Carstensen H 1982 Calculation of free and bound fractions of testosterone and estradiol-17 beta to human plasma proteins at body temperature. J Steroid Biochem 16:801–810

Sundaram K, Kumar N, Bardin CW 1994 7-α-methyl-19-nortestosterone: an ideal androgen for replacement therapy. Recent Prog Horm Res 49:373–376

Tenover JS 1992 Effects of testosterone supplementation in the aging male. J Clin Endocrinol Metab 75:1092–1098

Tunstall-Pedoe H 1998 Myth and paradox of coronary risk and the menopause. Lancet 351: 1425–1427

Wellings K, Field J, Johnson AM, Wadsworth J 1994 Sexual behaviour in Britain: the national survey of sexual attitudes and lifestyles. Penguin Books, London

Winters SJ, Kelley DE, Goodpaster B 1998 The analog free testosterone assay: are the results in men clinically useful? Clin Chem 44:2178–2182 (erratum: 1999 Clin Chem 45:444)

DISCUSSION

Veldhuis: This brings us well into the androgen discussion in ageing at the level of the target tissue. John Morley, I thought it would be good to give you the chance to comment.

Morley: What always amazes me is that David Handelsman and I agree on the conclusions, but for totally different reasons. Of the available data, I don't believe Snyder's study (Snyder et al 1999) was very rigorous for a variety of reasons. One was the addition of Ca^{2+} and vitamin D willy-nilly in a group who were chosen to be osteoporotic. This can affect muscle strength among other things. That alone questions that study. The other problem is that many of the men were not hypogonadal: as David Handelsman says (and I agree), the data suggest that you should treat hypogonadal males, not non-hypogonadal males who have drifted down into the normal range. I think that the strong data that we are about to see come from the unpublished study by Tenover. This study shows an improvement in strength as well as improvement in bone mineral density. Similar unpublished data are coming from the Baltimore group for upper limb strength. I think that the improvement of upper limb strength with testosterone may well be real in hypogonadal males. Bebb's data look very similar, but he is also going to take a while to publish. The Kenny paper is in press, and shows clear bone effects without Ca^{2+} and vitamin D addition in patients who were more hypogonadal than Snyder's. In a very small group, she found lower and upper limb strength increased, but not quite to a significant level. If you put these unpublished

studies together with the other published data, you can conclude that testosterone most probably has beneficial effects, but it is in people who are hypogonadal. This does not mean that every single man should get testosterone supplementation. The other unanswered question — which I think is the big one — is whether high, almost pharmacological doses are needed to produce effects, or whether lower doses, such as those delivered by patch techniques, are sufficient. We have a long way to go; we clearly need long, well-controlled trials. We also need to know who we are entering into these trials. I know some of these are in development, but so far I'm not aware of any funding agency that has been prepared to study the 3000–4000 men needed, equivalent to the women's health studies, to really answer these questions. Until we have these, we should treat men who are clearly hypogonadal, no matter what their age, and there will be a good outcome. There are three controlled trials that David didn't mention. Two of these are from Portland (Janowsky et al 1994, 2000) and there is another one from Matsumoto and Bremner's group (Cherrier et al 2001), all showing very positive effects on cognition. In fact, if you were to choose one thing that testosterone really seems to help for the majority of middle-aged to elderly men, it is cognitive function.

Handelsman: Both of our studies have a variety of cognitive measures. We also had gait, mobility and cognitive tests and quality of life measures: none were significantly improved. In particular, in the human chorionic gonadotropin (hCG) study we also had actigraphy measures for spontaneous physical activity as well as insulin clamps in 40 men before and after treatment. There was no significant change.

Veldhuis: And do you agree that the data are sparse on the route and dose-dependency of androgen repletion?

Handelsman: The data are sparse altogether. There is just one other comment I'd like to add putting the Snyder study in perspective, he got less than 100 patients out of screening 1000. You have to keep this high degree of selectivity in mind. This is with a testosterone level of <16 nM. If you insist on subjects being really hypogonadal (i.e. <10 nM), you will have 1 in 1000. This would be a very small subpopulation.

Morley: That is not necessarily true. He also acquired people who were osteopaenic to some extent. They had to have a lower bone mineral density. He had a number of other exclusion criteria. If you take the Massachusetts male ageing study, this does not fit at all with what the population numbers are. It is not what we found in California, and it is not what has been found by other people in other areas.

Handelsman: In our study we also recruited about 10%. The main reason for rejection was baseline testosterone concentrations.

Veldhuis: This permeates the area until we get a broad prospective open study. It makes the final results very subgroup dependent.

Burger: How does one define hypogonadism in the male who is said to be in the andropause? What are the criteria, and what range of testosterone concentrations are we to use? Is the young normal range the appropriate one or should we be working from a different one? These are crucial issues in this debate.

Veldhuis: Let me try to simplify this, does anyone have the nerve to give a number?

Handelsman: The Endocrine Society of Australia was bold enough to make a stab at this, and came up with a figure of 8 nM, independent of age (Conway et al 2000). Above this testosterone concentration, the diagnosis in absence of underlying pituitary or testis pathology was regarded as unequivocal.

Morley: The bioavailable testosterone or free testosterone index may be better. We use the bioavailable level of 70 ng/dl, which is about 3 nM. This is one of the choices.

Veldhuis: Is that independent of age? We would consider that level profoundly low in a young man.

Morley: In our lab, that is two standard deviations below the mean. We see no young men below 3 nM.

Wang: We have lots of debate in the USA about the cut-off level of testosterone to define the andropause. If serum testosterone levels are below 250 ng/dl, the men are hypogonadal and should be treated. The problem is when the levels are between 250 and 300. Unless we have large studies that can show that the benefit is outweighing the risk, we are hesitant to proceed.

Shalet: Is it possible to build a gonadotropin component into these definitions? What about raised LH?

Veldhuis: In the allostatic state that we learned about this morning, if the LH is elevated, you could argue that this is a physiological marker as an elevated thyroid stimulating hormone (TSH) is in early thyroid failure.

Laron: I think that is a wrong terminology. We measure male hypogonadism by millilitre volume of the testes. This shouldn't be confused with the quantitative secretion of androgens. They involve different cells.

Veldhuis: We heard that these volumes are pretty stable.

Morley: You could use andropause to get around this.

Veldhuis: I would argue that this is an age-dependent population-sensitive measure. It is assay dependent; you can't come up with a single number. I am with Henry Burger: we have to develop norms that are pertinent for the patient in question who is being compared. If the patient is a 78 year old individual, that clearly represents a potential population base that is readily distinguishable under usual health definitions for all body systems. Their glomerular filtration rate, lung diffusion capacity and bone mineral density are all reduced. If you are going to say whether a person has a disease, beyond the fact that they have survived to age 78, you have to show me other 78 year olds who are not complaining. He is entitled to

complain, but I may not attribute the complaint to that level solely if I find it matches those of all other men of similar age and disposition who are not complaining.

Riggs: Just to take the position of devil's advocate, we have struggled in the bone field about whether t scores or z scores are more important. In other words, should the normal range be that of young adults, or should it be age adjusted? We have come down strongly on the side of the former, because there is a critical value for bone density below which there is a marked increase in fracture risk, regardless of age.

Veldhuis: We don't have the targeted end point to tell us. I haven't heard this acknowledged. We need the dose-dependent target for different end points. They are not necessarily the same for osteopaenia as for muscle weakness and for decreased cognition.

Handelsman: If you are thinking about growth hormone studies in ageing, the advantage is that dose can be titrated with insulin-like growth factor (IGF)1 as an independent end-point. We don't have any equivalent form of titration with testosterone, which is a serious limitation. It is quite likely that the thresholds and/or sensitivity are different for different tissues. The sensitivity may not be the same for different age groups and populations.

Veldhuis: Absolutely. We have heard innuendos that the cognitive end-points might be more responsive, but this remains to be proven. It's an exciting issue. We picked muscle gene expression because Randy Urban in Texas showed increased IGF1 mRNA by two- to threefold in three weeks by titrated androgen replacement in six men.

Ruiz-Torres: We are interested in the question of why prostrate volume is so closely related to age, despite the fact that testosterone secretion declines. We thought that this could be due to an increased number of androgen receptors of the epithelium cells. However, we have found that the cytosolic receptors are increased, but the nuclear receptors do not show any change. One possible explanation is that the transfer from the nucleus to the cytoplasm is altered by ageing. In any case, it seems that androgens are not the cause of hypertrophic prostrate. Nevertheless, the benign prostatic hyperplasia (BPH) is mainly due to an increase in smooth muscle cells where testosterone could play a role as an anabolic effector. But normally in ageing the testosterone levels are low, so that this possibility concerns the treatment with androgens only.

Handelsman: It takes quite a bit of looking into what BPH and prostate growth with age means. BPH is actually nodular growth: I have always thought of this disease process as very much like fibroids. It doesn't necessarily occur smoothly, even though there is that famous study which suggests that there is a small rise (Berry et al 1984). In fact, it probably occurs in punctated bursts of growth, just like fibroids. The changes in the central zone are much more marked than those that

occur in the total prostate volume: we can see changes earlier and more prominently in the central zones. The fact is that with dihydrotestosterone (DHT) treatment, we didn't see any prostrate growth. My explanation for this is that using DHT you don't get any intraprostrate amplification of androgen action. Thus androgens which are not 5α reducible or activated would be expected to have less effect on the prostate. In balance, however, I have to add this may not actually be an advantage. There are reasons why having a prostate that doesn't have 5α reduction or full growth is a disadvantage. The disadvantage is that ejaculate volume is dependent on prostate fluids. If you don't have this, it could be that ejaculation may be experienced like in retrograde ejaculation, which may be symptomatically unacceptable.

Veldhuis: Are there any data on androgen receptor localization or activity in ageing? I recall diverse reports, one showing a change in the brain that was opposite to that in the prostate, and one showing no change. Per Björntorp, you alluded to an androgen receptor polymorphism. Do you know of any data on the effect of ageing on androgen receptor expression?

Björntorp: Yes, there are some microsatellites in the first exon which codes for the transduction domain. When this is too short, there is an increased risk for prostate cancer.

Veldhuis: That's a polymorphism without a known change in receptor function.

Björntorp: I think these recent microsatellite developments are very interesting. In the androgen receptor gene, for example, these microsatellites have been altered in transgenic mice. The shorter the microsatellite, the stronger the effect of the produced protein.

Ruiz-Torres: The number of androgen receptors in the prostatic epithelium depends on the location. We have found that the content of nuclear receptors of cells from the posterior zone — where cancer usually appears — is higher than in the case of the central zone, which is mostly affected by BPH.

References

Berry SJ, Coffey DS, Walsh PC, Ewing LL 1984 The development of human benign prostatic hyperplasia with age. J Urol 132:474–479

Cherrier MM, Asthana S, Plymate S et al 2001 Testosterone supplementation improves spatial and verbal memory in healthy older men. Neurology 57:80–88

Conway AJ, Handelsman DJ, Lording DW, Stuckey B, Zajac JD 2000 Use, misuse and abuse of androgens. The Endocrine Society of Australia consensus guidelines for androgen prescribing. Med J Aust 172:220–224

Janowsky JS, Oviatt SK, Orwoll ES 1994 Testosterone influences spatial cognition in older men. Behav Neurosci 108:325–332

Janowsky JS, Chavez B, Orwoll E 2000 Sex steroids modify working memory. J Cogn Neurosci 12:407–414

Snyder PJ, Peachey H, Hannoush P et al 1999 Effect of testosterone treatment on bone mineral density in men over 65 years of age. J Clin Endocrinol Metab 84:1966–1972

Endocrine Facets of Ageing.
Novartis 242

Print ISBN 0-471-48636-1 Online ISBN 0-470-84654-2

Male reproductive ageing: using the Brown Norway rat as a model for man

Christina Wang*, Amiya Sinha Hikim*, Monica Ferrini†, Juan J. Bonavera*, Dolores Vernet†, Andrew Leung*, Yan-He Lue*, Nestor F. Gonzalez-Cadavid† and Ronald S. Swerdloff*

*Divisions of *Endocrinology and †Urology, Departments of Medicine and Surgery, Harbor-UCLA Medical Center and Research and Education Institute, 1000 West Carson Street, Carson, CA 90509, USA*

Abstract: The Brown Norway (BN) rat is an excellent model for male reproductive ageing. We and others have shown that with ageing, the BN rat exhibits low serum testosterone, low Leydig cell steroidogenic capacity, decreased Sertoli cell function and number, marked reduction in seminiferous tubule volume and sperm content, and accelerated germ cell apoptosis. These testicular changes are the result of a combination of a primary testicular defect and a secondary hypothalamic dysfunction. Leydig cell dysfunction results from decreased activities of the steroidogenic enzymes and Leydig cell secretory capacity and is not corrected by daily administration of replacement luteinizing hormone (LH), suggesting a primary testicular defect. However ageing in male BN rats is associated with decreased LH pulse amplitude, reduced gonadotropin releasing hormone (GnRH) and gonadotropin responsiveness to excitatory amino acids, and decreased GnRH mRNA and peptide in the hypothalamus. We have further shown in the hypothalamus of ageing BN rats that while the excitatory amino acid receptor content is reduced, nitric oxide synthase (NOS) activity is increased which is due to increased inducible (iNOS) but not neuronal NOS (nNOS). The increased iNOS protein in the hypothalamus is associated with increased peroxynitrite formation and neuronal cell apoptosis. We conclude that increased hypothalamic levels of iNOS may result in neurotoxicity in the hypothalamus leading to loss of hypothalamic GnRH secretory cells and impaired GnRH pulsatile secretion that contributes to the abnormal Leydig cell function characteristic of male reproductive ageing.

2002 Endocrine facets of ageing. Wiley, Chichester (Novartis Foundation Symposium 242) p 82–97

Cross-sectional and longitudinal studies in men showed that serum testosterone declined with ageing (Vermeulen 1991). Even though serum total testosterone may be in the normal range, the free or bioavailable testosterone levels are frequently lower in elderly men (Gray et al 1991, Korenman et al 1990, Baker et al 1976, Harman & Tsitouras 1980). Low testosterone levels in older men are associated with sexual dysfunction, loss of bone, decreased muscle mass and

strength, increased body fat, frailty, and poorer quality of life (Swerdloff & Wang 1993). The low circulating serum testosterone levels are usually associated with elevated serum luteinizing hormone (LH) and follicle-stimulating hormone (FSH) levels in aged men. However, such increases in LH may be inappropriately low compared to those of young men with similar low serum testosterone levels. In search of an animal model to examine the process and mechanisms of reproductive ageing, we and others have defined the BN rat as the most suitable model for male reproductive ageing (Zirkin et al 1993, Wang et al 1993, Gruenewald & Matsumoto 1991). The BN rat is a better model to study male reproductive ageing than other strains because these rats have a longer lifespan, do not develop pituitary or testicular tumours, are not excessively obese, and manifest both testicular and hypothalamic-pituitary dysfunction with ageing. In this chapter, we will describe the work in our laboratory using the BN rat as a model for studying human male reproductive ageing.

Testicular dysfunction

Reproductive ageing in the BN rat is characterized by low serum testosterone levels (Zirkin et al 1993, Wang et al 1993, 1999). Low serum testosterone levels are also a hallmark of the ageing male demonstrated in both cross-sectional and longitudinal studies (Vermeulen 1991, Gray et al 1991). We studied serum testosterone levels and sperm concentration in 6, 9, 12, 15, 18 and 31 month-old BN rats. Beginning at 15 months, plasma testosterone and inhibin levels both showed a progressive decline with age (Fig. 1). Intratesticular testosterone and inhibin concentrations were not lower, and in fact appeared to be higher in testes that showed marked regression, because of the loss of seminiferous tubule content in the testis (Wang et al 1993, 1999). The decrease in serum testosterone was due to decreased Leydig cell secretory capacity as demonstrated by Zirkin et al (1993), Zirkin & Chen (2000) and Chen et al (1994, 1996). The Leydig cell dysfunction resulted from reductions in the levels and activities of steroidogenic enzymes (Luo et al 1996). Leydig cells isolated from old rats produced significantly less basal and LH-stimulated testosterone *in vitro*. If the Leydig cells were depleted by treatment with ethane dimethanesulfonate and then allowed to regenerate, the capacity of the Leydig cells in the old rats to produce testosterone was similar to young animals for up to 10 weeks. Whether the capacity of these repopulated Leydig cells in old rats would continue to produce testosterone in the older BN rats was not studied (Chen et al 1996). The same investigators administered contraceptive doses of testosterone (implants) to young and old rats for 8 months. Two months after removal of testosterone implants, the Leydig cells of both the young and old rats secreted high levels of testosterone. The investigators suggest that by placing the Leydig cells in 'hibernation' the decreased Leydig cell steroidogenesis associated

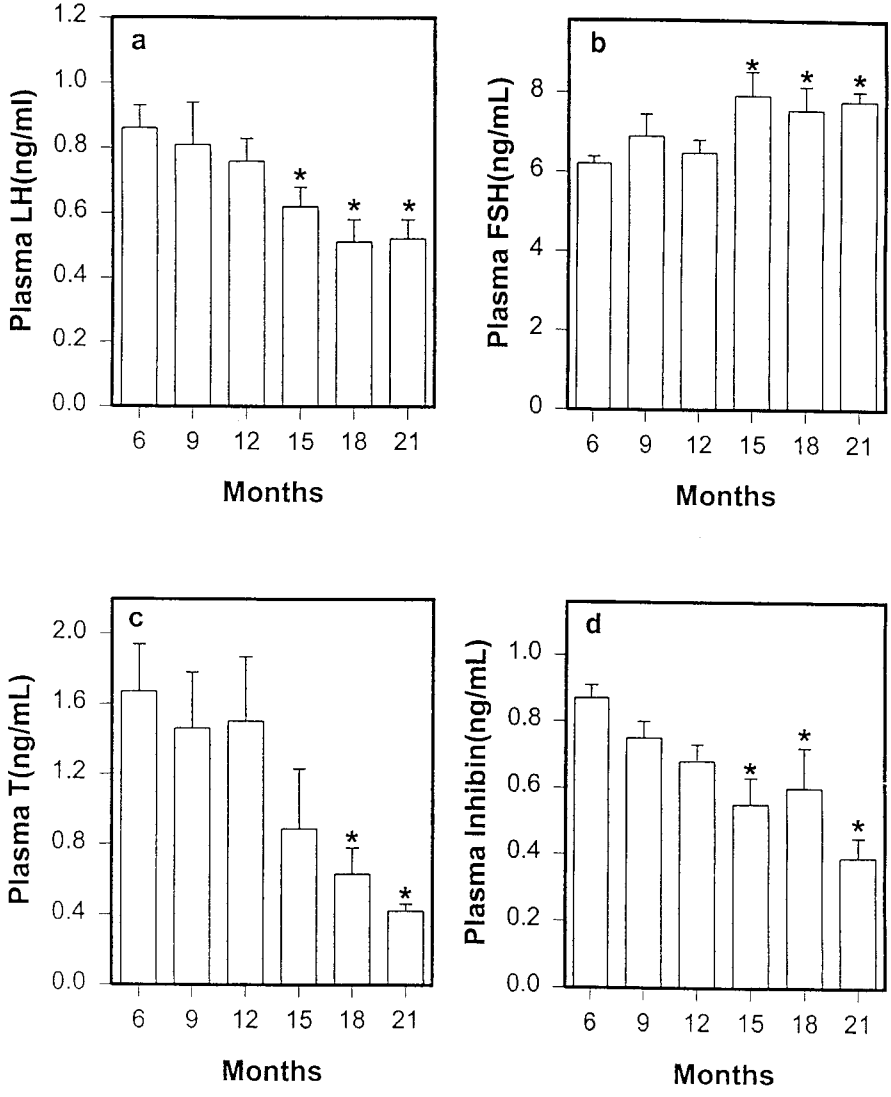


FIG. 1. Plasma LH (A), FSH (B), testosterone (C), and inhibin (D) concentrations in ageing rats. Data represent mean SE of 5–10 animals per group. *Indicates $P < 0.05$ when compared with young (6 month old) rats. Reproduced with permission from Wang et al (1999).

with ageing did not occur (Chen & Zirkin 1999). These gradual changes in serum testosterone levels in the BN rat mimicked closely the 'andropause' associated with ageing in men. Low serum testosterone levels are the cause of the low muscle mass, decreased bone density, frailty and might be partially responsible for the erectile dysfunction commonly encountered in older men as described in the chapter by Handelsman (2002, this volume).

We, as well as others (Wang et al 1993, 1999, Wright et al 1993), have shown that testicular weights were reduced in 21–22 month old BN rats to about 60–70% of those of young rats. Frequently a differential decrease in testis size was observed in the same animal. One testis was often much smaller ('regressed') and the other was relatively normal in weight. By 30 months the mean testicular weights were very small: only about 25–30% of those at 6 months. Mean testicular sperm concentration and total sperm content decreased progressively from 15 months (Fig. 2). Testicular histology in the relatively normal looking testis of 21–22 month old BN rats showed active spermatogenesis with large lumina and marked variation in the appearance of the germinal epithelium from relatively normal to a flattened epithelium lined by a single layer of Sertoli cells and a few spermatogonia. Many tubules had areas consisting of normal-looking tubules intermingled with groups of damaged tubules. In the regressed testis, the seminiferous tubule showed complete cessation of spermatogenesis. By 30 months, the testes were very small and showed features similar to those of the regressed testes of the 21–22 month old rats. Testicular stereologic analysis showed a marked reduction in the volumes of seminiferous tubules and tubular diameter. In old animals, Leydig cell volume was markedly lower but their number remained unchanged. There was a marked decrease in the number of Sertoli cell per testis in the regressed testes of 21–22 and 30 month old rats (Wang et al 1993, Wright et al 1993). We also showed that the decrease in germ cells was due to accelerated germ cell apoptosis (Fig. 3) involving spermatogonia, spermatocytes and spermatids (Wang et al 1999). In addition, there was marked variability in the apoptosis rate in different tubules. Germ cell apoptosis was most evidenced in stages XII–XIV when compared with young animals. It should be noted that in the regressed testis, the seminiferous tubules were lined with Sertoli cells and few apoptotic germ cells as well as Leydig and Sertoli cells (Wang et al 1999). The variable and irregular depletion of germ cells by apoptosis in the seminiferous tubules suggests that in addition to low testosterone levels, other factors such as decreased blood supply and increased cytotoxic agents (e.g. reactive oxygen species) might also play a role in germ cell degeneration. The changes in the seminiferous tubules in the BN rats are more pronounced than those reported in men. Semen analyses of healthy active grandfathers have been reported to be relatively normal (Nieschlag et al 1982), but others reported that the daily sperm production was decreased in ageing men (Neaves et al 1984).

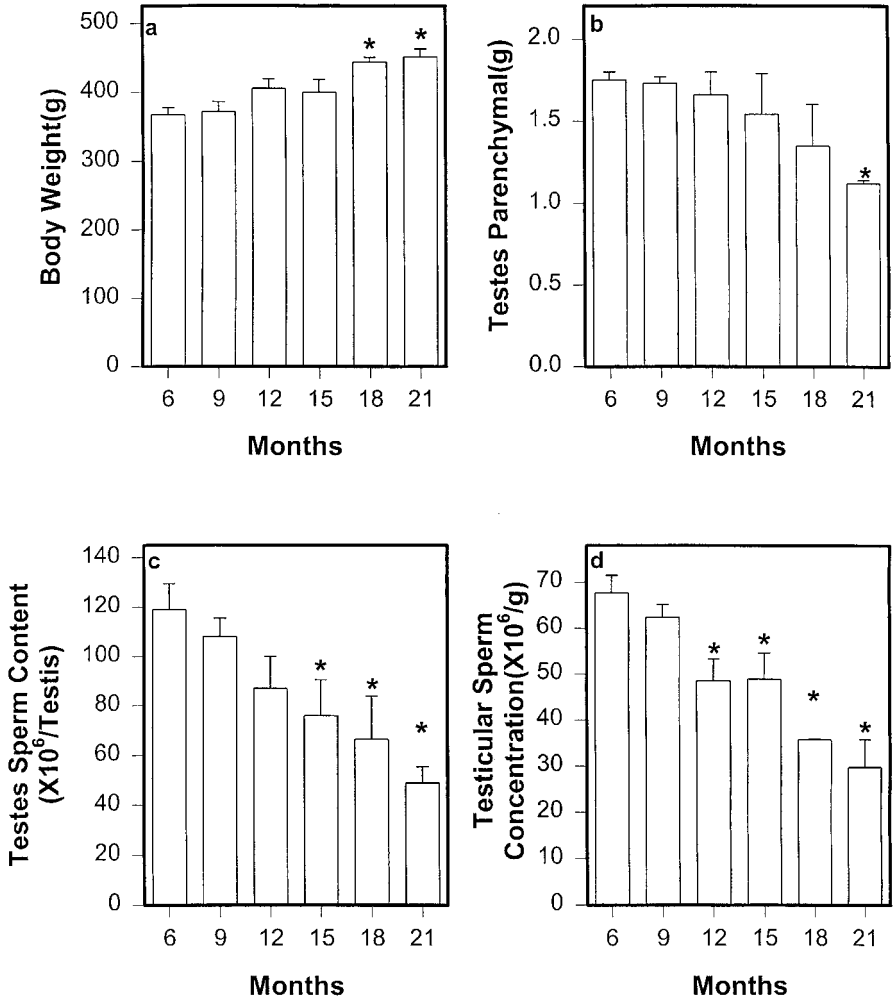


FIG. 2. Body weight (A), testicular parenchymal weight (B), sperm content (C), and sperm concentration (D) in ageing BN rats. Data represent mean SE of 5–10 animals per group. *Indicates significant difference, $P < 0.05$, when compared with young (6 month old) rats. Reproduced with permission from Wang et al (1999).

When rat LH was administered as a twice daily injection to 15 month old BN rats for 6 months, plasma hormone levels, testis weight, sperm concentration and content, and germ cell apoptosis rate remained the same as those of the untreated, control group. In the control group, three out of 10 testes were regressed whereas in the LH-treated group only one out of 12 testes was regressed. These results

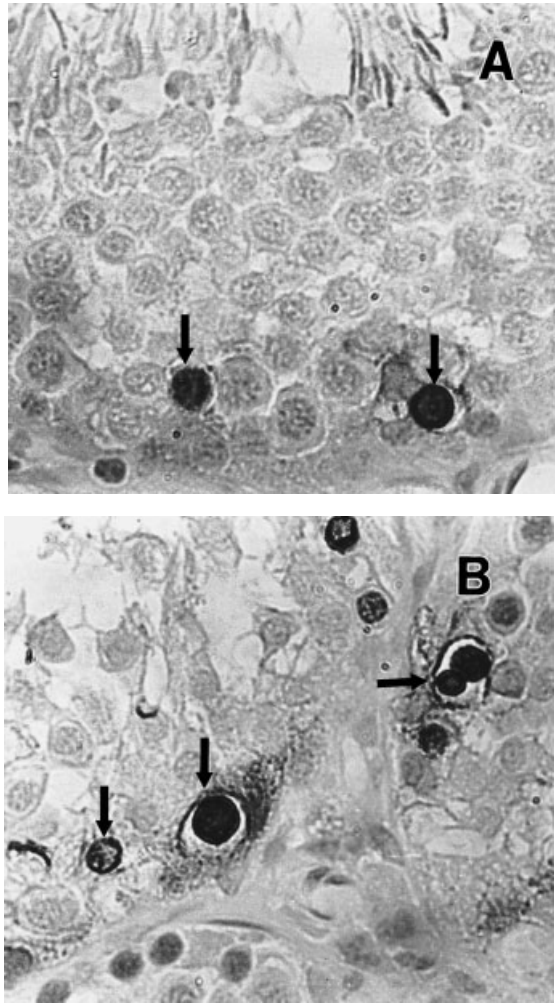


FIG. 3. Light micrographs of testicular sections from a 21 month old rat showing apoptotic germ cells (arrow) in the relatively normal looking (A) and regressed (B) testes. Visualization of apoptotic germ cells was by terminal deoxynucleotidyl transferase-mediated dUTP nick end labelling (TUNEL). Methyl green was used as a counterstain. Magnification

suggest that impaired hypothalamic–pituitary function may not be the only cause of testicular germ cell loss associated with ageing (Wang et al 1999). However the experimental conditions cannot exclude the possibility that the LH treatment might be inadequate because it was given as twice daily doses and not in a pulsatile fashion, and the testosterone response to the dose of LH administered might be insufficient to support spermatogenesis.

Hypothalamic–pituitary dysfunction

In addition to a primary testicular failure, the aged BN rat also showed features suggestive of a hypothalamic–pituitary dysfunction. Serum FSH levels were elevated in association with low serum inhibin and decreased spermatogenesis in older rats (Wang et al 1993, Gruenewald et al 1994). In contrast, despite the low serum testosterone levels, serum LH showed no change (Chen et al 1994, Gruenewald et al 1994) or a decrease in aged rats (Wang et al 1993, 1999) (Fig. 1). Moreover, the blunted rise in serum LH and FSH after castration in the old, when compared with the young animals provided further evidence for a hypofunctional hypothalamic–pituitary axis (Gruenewald et al 1994). We also showed that the pulsatile secretion of LH was characterized by a shortened pulse interval and reduced areas of the pulses in the old rats. LH pulse amplitude and total area of the LH pulses were also significantly lower in old than in young rats. In contrast, mean serum FSH levels in old rats were significantly higher than those observed in young rats. Mean areas but not amplitude of FSH pulses decreased significantly in the old rats. FSH pulse frequency increased and pulse interval decreased in the old rats (Fig. 4). Our results were corroborated by similar studies by Gruenewald et al (2000) who also demonstrated blunted circadian rhymicity of LH and testosterone secretion due to decreased gonadotropin releasing hormone (GnRH) production rather than decreased pituitary responsiveness to GnRH. These changes in the pulsatile secretion of the gonadotropins in the BN rat are similar to the human and are consistent with a hypothalamic GnRH pulse generator dysfunction. In man, high amplitude LH peaks tend to fall and frequency of low amplitude LH peaks tend to rise in older men (Veldhuis et al 1992, Mulligan et al 1995). The decreased LH secretory burst amplitude correlated with serum free testosterone in elderly men. In contrast, serum FSH showed an increase in basal secretion rate and increased FSH secretory burst mass and amplitude in old compared to young men (Veldhuis et al 1999). The neuroendocrine mechanisms underlying the discrepant LH and FSH pulsatile secretions occurring with ageing are not known.

It has been previously shown that excitatory amino acids acting through their receptors have a primary role in the regulation of the pulsatile secretion of GnRH and LH. GnRH peptide content, synthetic capacity and gene expression are reduced in aged male rats (Gruenewald & Matsumoto 1991, Gruenewald et al 2000). We hypothesize that decreased GnRH responsiveness to excitatory amino acids may occur with ageing. To test this hypothesis, we first demonstrated that *in vivo* serum FSH or LH responsiveness to GnRH was not altered in the old rats. We then showed that administration of an excitatory amino acid (glutamate) receptor agonist *N*-methyl-D-aspartate (NMDA) induced higher LH and prolactin releases in young versus old animals. Moreover, using hypothalamus fragments, we showed that the *in vitro* GnRH efflux in response to NMDA was lower in old rats

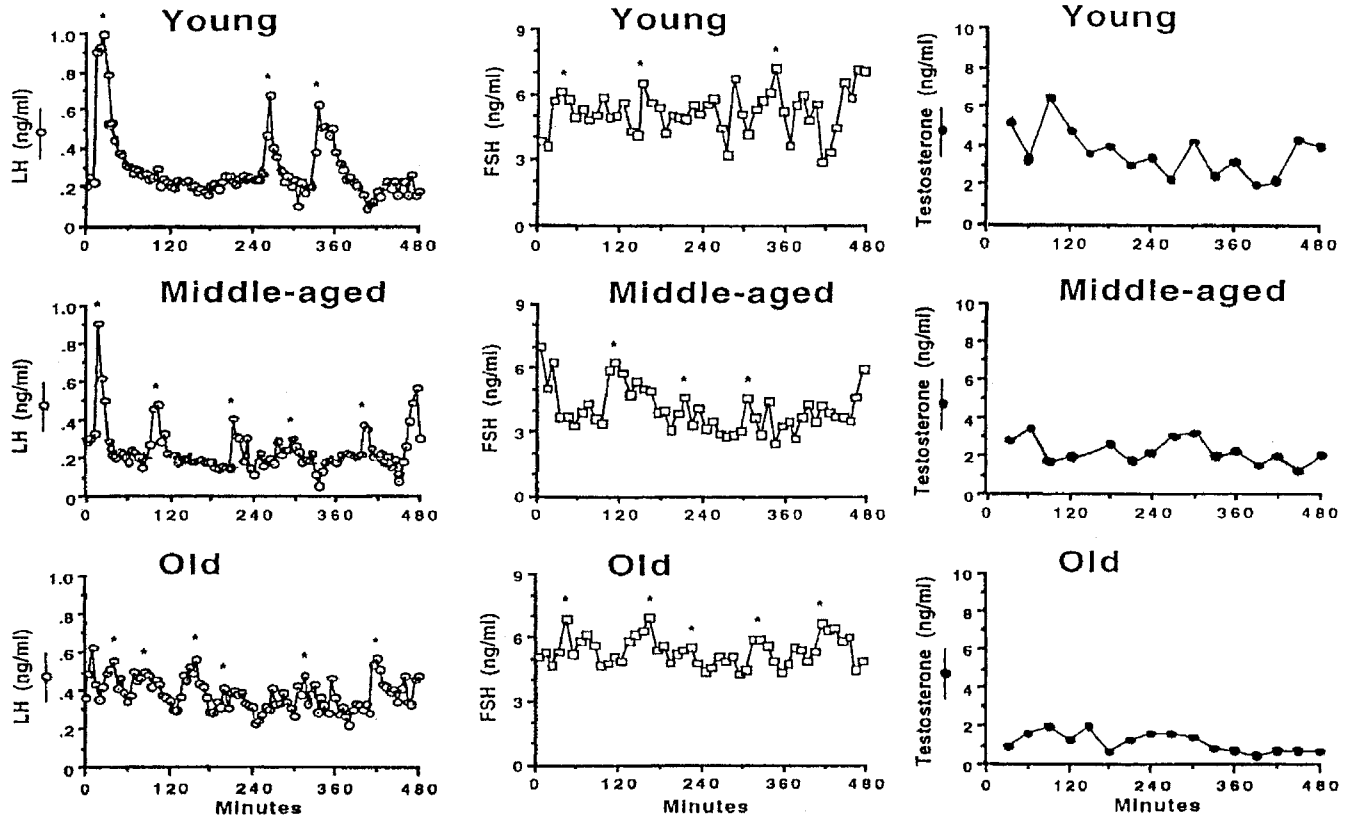


FIG. 4. Plasma luteinizing hormone (LH), follicle-stimulating hormone (FSH), and testosterone (T) levels over the 8 h sampling period of one representative male rat of each age group. *Denotes pulses identified by cluster analysis.

compared to young rats. In the hypothalamus from old rats there was significant reduction in the content of glutamine and γ-aminobutyric acid (Bonavera et al 1998). Taken together, these results showed that the NMDA–GnRH–LH axis was altered in old rats, and the decreased hypothalamic content of some of the excitatory amino acids and reduced responsiveness of GnRH neurons to NMDA, both *in vivo* and *in vitro*, may play a role in the altered LH pulsatile secretion observed in older rats.

Nitric oxide synthase and reproductive ageing

The excitatory amino acid receptors (NMDA receptors) are considered to be the main neurotransmitter receptors mediating fast synaptic excitation in the central nervous system (CNS) through the opening of Ca^{2+} channels triggering a series of neuronal cascades. One of the mechanistic pathways is the stimulation of neuronal nitric oxide synthase (nNOS) resulting in the synthesis of nitric oxide which has been presumed to be the mediator of some of the neuroendocrine effects of the excitatory amino acid glutamine specifically on GnRH and LH pulsatile secretions (Braun et al 1997). In the hypothalamus, nNOS is the predominant NOS isoform and has been demonstrated to colocalize with or adjacent to the NMDA receptors and GnRH neurons in the medial preoptic area (Grossman et al 1994). Thus in ageing, it is conceivable that the hypothalamic NMDA receptor/nNOS pathway may play a role in hypothalamic dysfunction. NMDA receptors might be decreased accompanied by a decrease in nNOS activity in the brain and other organs outside the CNS.

An alternative hypothesis is that ageing is associated with an excessive synthesis of NO resulting in the accumulation of its cytotoxic metabolites such as peroxynitrite, leading to neuronal apoptosis. The process might affect the hypothalamic neurons including those that secrete GnRH. Such cytotoxic effects of excessive levels of NO could result in accelerated apoptosis of other components of the reproductive axis such as the testes. The high NO levels in tissues of ageing animals may occur as a result of excessive stimulation of nNOS by activation of NMDA receptors or the spontaneous expression of inducible NOS (iNOS), the NOS isoform that is induced during autoimmunity, inflammation and degeneration. In normal physiological conditions iNOS is undetectable in the organs of adult laboratory animals and is expressed in high levels only after exogenous cytokine stimulation and in inflammatory or infectious processes. To study the role of NOS in reproductive ageing, our laboratories investigated the NMDA receptor content and binding, nNOS and iNOS levels, and activity in the brain and in the testes of the ageing BN rat.

The NMDA receptor binding activity in the hypothalamus of old rats was 66% lower than that of adult animals. The results of the binding activity were confirmed

by the demonstration that the NMDA receptor content was also decreased by 34% compared to young animals. To our surprise, the decrease in NMDA receptors was associated with a 67% increase in NOS activity in the hypothalamus of old rats when compared to the adult animals, while nNOS content was not different between the two groups. In contrast, iNOS content in the hypothalamus of old rats were increased by 3.8-fold compared with adult animals (Fig. 5). The increase in iNOS content was demonstrated not only in the hypothalamus but also in the frontal and parietal cortex, and in the cerebellum (Vernet et al 1998). Our results showed that ageing in the BN rat was associated with high NO synthesis in the hypothalamus and other regions of the brain. This occurred independently of the NMDA receptors (which were decreased) and nNOS activity (which was unchanged). We thus concluded that increased iNOS might result in neurotoxicity which could be involved in the impaired GnRH pulsatile secretion and also a possible inducer of age-associated cell loss in the brain and other organs such as the testes.

We then proceeded to investigate the role of iNOS in male reproductive ageing in the hypothalamus and the end organ, the testis. Using immunohistochemistry we found significant increases in iNOS immunostaining in the supraoptic and paraventricular nucleus and the preoptic area of the hypothalamus in the old rats. Nitrotyrosine, a marker for peroxynitrite formation (a cytotoxic product of excess NO and reactive oxygen species interaction) was also elevated in the same areas of the hypothalamus of old rats. The accumulation of peroxynitrite was accompanied by an increase in the apoptotic index of neurons in the supraoptic, paraventricular and arcuate nucleii as well as in the preoptic area of the hypothalamus of old rats. Apoptosis of neurons is extremely rare in the hypothalamus and other areas of the brain of young animals. We thus hypothesized that neuronal apoptosis could be the cause of the reduction of GnRH neurons detected by *in situ* hybridization by Gruenewald et al (2000). In contrast, ageing did not affect nNOS expression. We further examined the anatomical relationship of iNOS and GnRH-positive neurons in the hypothalamus using double immunofluorescence technique in combination with confocal laser scanning microscopy. The iNOS staining co-localized with GnRH staining in the same regions of the hypothalamus of the rat brain. These preliminary studies showed iNOS expression in the hypothalamus of the affected regions of the brain known to control the synthesis and release of GnRH, confirming our hypothesis that iNOS may indeed play a role in the reduction of GnRH pulsatile secretion resulting in reproductive dysfunctions such as lowered testosterone in the ageing males. Ongoing studies aim to demonstrate iNOS co-localization with apoptotic cells in the hypothalamus of old rats.

We also demonstrated that similar changes in NOS activity occurred in the testes of old BN rats. In the regressed testes of old animals NOS activity was increased

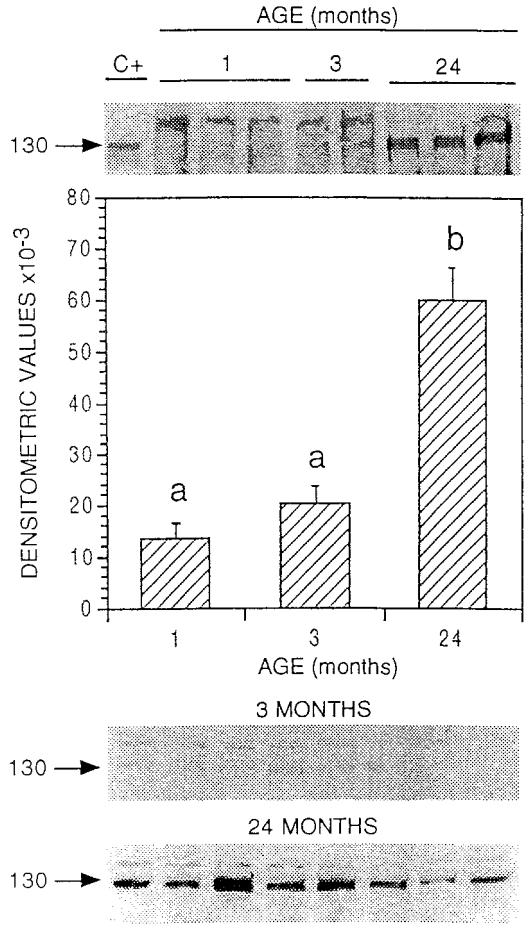


FIG. 5. Effect of ageing on iNOS levels in the rat hypothalamus. Top panel, autoradiography of the 130 kDa bands on a typical Western blot of the postmitochondrial supernatants (80 mg protein/lane) with an antibody against mouse iNOS and visualization with a luminol reaction. Middle panel, Mean intensity of the respective bands determined by densitometry

compared with younger adults. The expression of iNOS assessed by Western blot assay was present in 3 month old animals but increased by 2.5-fold in the relatively normal testes and fourfold in the regressed testes of the old rats. No significant changes were noted in nNOS levels between the testes obtained from young or

old animals. Immunohistochemistry showed that iNOS expression was most striking in Leydig cells with little or no immunoreactivity in the Sertoli and germ cells of young rats. In contrast, there was a marked increase in iNOS immunoreactivity in the Sertoli cells and some staining in the germ cells of old rats. Neuronal NOS was detectable in Leydig and Sertoli cells in both young and old animals but unlike iNOS, no age-related differences were apparent. Our results showed that ageing in the BN rats, in a similar fashion to changes in the hypothalamus and other brain regions, was associated with iNOS induction and high iNOS synthesis in the testis. We also speculate that the increased iNOS through the formation of cytotoxic products may be an important mediator of age-related activation of germ cell apoptosis.

Summary

In this chapter we have described the changes in the male reproductive axis with ageing in the BN rat as a model for human reproductive dysfunction. We have shown that the reproductive axis in the rat sustained dual hits at the testis and the hypothalamus. We showed that these hits caused an accelerated neuronal and germ cell apoptosis presumably as a result of oxidative damage by excessive accumulation of the inducible NOS. The dual dysfunction at both the testicular and hypothalamic regions possibly resulted in impaired Leydig cell function and decreased spermatogenesis characteristic of male reproductive ageing in men.

References

Baker HWG, Burger HG, de Kretser DM et al 1976 Changes in the pituitary-testicular system with age. Clin Endocrinol 5:349–372

Bonavera J J, Swerdloff RS, Sinha-Hikim AP, Lue YH, Wang C 1998 Aging results in attenuated gonadotropin releasing hormone–luteinizing hormone axis responsiveness to glutamate receptor agonist N-Methyl-D-aspartate. J Neuroendocrinol 10:93–99

Braun DW, Bhat GK, Lamar CA, Mahesh VB 1997 Gaserous transmitters and neuroendocrine regulation. Neuroendocrinology 65:385–395

Chen H, Zirkin BR 1999 Long-term suppression of Leydig cell steroidogenesis prevents Leydig cell aging. Proc Natl Acad Sci USA 96:14877–14881

Chen H, Hardy MP, Huhtaniemi I, Zirkin BR 1994 Age-related decreased Leydig cell testosterone production in the Brown-Norway rats. J Androl 15:551–557

Chen H, Huhtaniemi I, Zirkin BR 1996 Depletion and repopulation of Leydig cells in the testes of aging Brown Norway rats. Endocrinology 137:3447–3452

Gray A, Berlin JA, McKinlay JB, Longcope C 1991 An examination of research design effects on the association of testosterone and male aging: results of a meta-analysis. J Clin Epidemiol 44:671–684

Grossman AB, Rosmanith WG, Kabigtin EB, Cadd G, Clifton D, Steiner RA 1994 The distribution of hypothalamic nitric oxide synthase mRNA in relation to gonadotropin-releasing hormone neurons. J Endocrinol 140:R5–R8

Gruenewald DA, Matsumoto AM 1991 Age-related decreases in serum gonadotropin levels and gonadotropin-releasing hormone gene expression in the medial preoptic area of the male rat are dependent upon testicular feedback. Endocrinology 129:2442–2450

Gruenewald DA, Naai MA, Hess DL, Matsumoto AM 1994 The Brown Norway rat as a model of male reproductive aging: evidence for both primary and secondary testicular failure. J Gerontol 49: B42–B50

Gruenewald DA, Naai A, Marck BT, Matsumoto AM 2000 Age-related decrease in hypothalamic gonadotropin-releasing hormone (GnRH) gene expression but not pituitary responsiveness to GnRH, in the male Brown Norway rat. J Androl 21:72–84

Handelsman DJ 2002 Male reproductive ageing: human fertility, androgens and hormone dependent disease. In: Endocrine facets of ageing. Wiley, Chichester (Novartis Found Symp 242) p 66–81

Harman SM, Tsitouras PD 1980 Reproductive hormones in aging men. I. Measurement of sex steroids, basal luteinizing hormone and Leydig cell response to human chorionic gonadotropin. J Clin Endocrinol Metab 51:35–40

Korenman SG, Morley JE, Mooradian AD et al 1990 Secondary hypogonadism in old men: its relation to impotence. J Clin Endocrinol Metab 71:963–969

Luo L, Chen H, Zirkin BR 1996 Are Leydig cell steroidogenic enzymes differentially regulated with aging? J Androl:509–515

Mulligan T, Iranmanesh A, Gheorghiu S, Godschalk M, Veldhuis JD 1995 Amplified nocturnal lutienizing hormone (LH) secretory burst frequency with selective attenuation of pulsatile (but not basal) testosterone secretion in healthy aged men: possible Leydig cell desensitization to endogenous LH signalling — a clinical research center study. J Clin Endocrinol Metab 80:3025–3031

Neaves WB, Johnson L, Porter JC, Parker CR, Petty CS 1984 Leydig cell numbers, daily sperm production and serum gonadotropin levels in aging men. J Clin Endocrinol Metab 59:756–763

Nieschlag E, Lammers U, Freischem CW, Langer K, Wickings EJ 1982 Reproductive functions in young fathers and grandfathers. J Clin Endocrinol Metab 55:675–681

Swerdloff RS, Wang C 1993 Androgens and aging in men. Exp Gerontol 28:435–446

Veldhuis JD, Urban RJ, Lizarralde G, Johnson ML, Iranmanesh A 1992 Attenuation of luteinizing hormone secretory burst amplitude is a proximate basis for the hypoandrogenism of healthy aging in men. J Clin Endocrinol Metab 75:707–713

Veldhuis JD, Iranmanesh A, Demers LM, Mulligan T 1999 Joint basal and pulsatile hypersecretory mechanisms drive the monotropic follicle-stimulating hormone (FSH) elevation in healthy older men: concurrent preservation of the orderliness of the FSH release process: a general clinical research center study. J Clin Endocrinol Metab 84:3506–3514

Vernet D, Bonavera JJ, Swerdloff RS, Gonzalez-Cadavid NF, Wang C 1998 Spontaneous expression of inducible nitric oxide synthase in the hypothalamus and other brain regions of aging rats. Endocrinology 139:3254–3261

Vermeulen A 1991 Androgens in the aging male. J Clin Endocrinol Metab 73:221–224

Wang C, Leung A, Sinha-Hikim AP 1993 Reproductive aging in the male Brown-Norway rat: a model for the human. Endocrinology 133:2773–2781

Wang C, Sinha-Hikim AP, Lue YH, Leung A, Baravarian S, Swerdloff RS 1999 Reproductive aging in the Brown Norway rat is characterized by accelerated germ cell apoptosis and is not altered by luteinizing hormone replacement. J Androl 20:509–518

Wright WW, Fiore C, Zirkin BR 1993 The effect of aging on the seminiferous epithelium of the Brown-Norway rat. J Androl 14:110–117

Zirkin BR, Santulli R, Strandberg JD, Wright WW, Ewing LI 1993 Testicular steroidogenesis in the aging Brown-Norway rat. J Androl 14:118–123

Zirkin BR, Chen H 2000 Regulation of Leydig cell steroidogenic function during aging. Biol Reprod 63:977–981

DISCUSSION

Handelsman: Can I draw you out on one aspect you didn't mention, which is the opioid control of GnRH neurons. As far as I understand it, what you are working on is within the GnRH neuron itself. Is there any relationship between opioid effects and iNOS?

Wang: We haven't studied this. Opiates exert negative effects on GnRH hormones. We don't know whether the iNOS is important as the mechanism of action, but this is what we are pursuing. We are looking at both the brain and the testis.

Müller: You have shown an increase of iNOS in both the testis and the brain. In the brain iNOS increases in many areas beside the hypothalamus. Can you speculate on this finding a little bit? Could it be that NOS increase is not only a marker of decreased fertility but also a general phenomenon? We also have some indirect data in this respect as far as growth hormone (Rigamonti et al 1999), and sexual function (Melis et al 2001) are concerned.

Wang: There are data even in the human brain showing that iNOS activity may be increased in older subjects, but these data are not as solid as the rat data. The NMDA receptor has also been implicated in a stress-related increase in cortisol, causing damage to the brain, especially in rat models.

Björntorp: I am going to ask a terribly ignorant question. How do you imagine that the NOS is acting here?

Wang: We think that ageing causes decreasing blood flow and increased ROS generation, including iNOS. I believe there is also an increase in cytokines in the brain. The peroxynitrite products of iNOS are cytotoxic and kill the neurons that secrete GnRH and oxytocin, which causes some of the changes we observed. We also have evidence that the iNOS is colocalized in cells undergoing apoptosis.

Laron: You said that this is the best model for ageing studies in humans. We learned earlier about the characteristics of ageing in humans. What are the characteristics of ageing in the rat?

Wang: I think osteoporosis is present. I have no idea about muscle. The only reason we think this is a good model is that the testosterone is low, caused by both testicular and hypothalamic–pituitary dysfunction.

Morley: You are looking at 50 year olds. These could very well be human data, but you would have to go to 27–28 month old rats to be looking at the equivalent of 'old' subjects. The use of 'old' is a misnomer in this situation.

Wang: Initially we did experiments on 30 month old rats, but in these rats the testis is so small we cannot do many experiments. The supply of very old rats can be

limited and 50% of the rats die by the age of 30 months. With the iNOS knockout mice we wish to study them when they are very young and then sacrifice them at different ages until old age.

Morley: You also didn't look at 12 month old rats. In our paper we found that some of those increases were present at 12 months (Morley et al 1996). It really is a maturational change with the iNOS. We have found a reduction of NOS mRNA in older animals.

Wang: We have looked at 3, 6, 9, 12 and 18 month old animals, because we want to characterize the changes so that we can do interventions. This is why we need to know when they start to change with ageing.

Handelsman: A point of clarification. You mentioned transgenics in your paper. Are you planning to do knockouts in the mouse? If so, is the mouse the same as the Brown Norway rat?

Wang: We plan to use iNOS knockout mice. We have studied both the testis and the brain of the mouse, we can see an increase in iNOS with ageing. We have not done the pulsatile LH secretion; this is very difficult in the mouse.

Brabant: With the maturational change of iNOS decreasing in the old animals, it would be interesting to know what happens if you castrate young animals.

Wang: We haven't done castration experiments, although we really should. Al Matsumotos's group did castration experiments in the young and old rats, and compared the GnRH content in hypothalamic neurons (Gruenewald & Matsumoto 1991, Gruenewald et al 1994, 2000). They showed that GnRH mRNA and content are decreased in castrated ageing BN rats. They did not measure iNOS. We also haven't done testosterone replacement experiments in these rats.

Veldhuis: There may be a slightly different question here: in the female rat, dozens of reports have shown that high dose oestradiol is neurotoxic to certain of these centres, while at the same time being protective against brain ischaemia and hypoxia. Georg Brabant is raising an interesting point: could you prevent ageing of the GnRH neurons by castration?

Wang: Castration has not been shown to be protective in the hypothalamus. There was a recent paper in which researchers gave contraceptive doses of testosterone into the old BN rat, which caused the suppression of testosterone and spermatogenesis (Chen & Zirkin 1999). The treatment caused the Leydig cells to go into what they called 'hibernation'. If this was done for 6 months and then the treatment was stopped, the testosterone production in the young and old rats became similar.

Veldhuis: What makes you say that the upstream activators of this iNOS are probably cytokines? What evidence do you have that cytokines are increased in the ageing male rat in these areas?

Wang: There is scattered information about increased cytokine levels, both in the rat brain and the human brain. iNOS is traditionally induced by inflammatory responses or cytokines.

Veldhuis: So the neuronal death could be a glial event?

Wang: Yes.

Laron: What do you know about the influence of insulin-like growth factor (IGF)1 on the ageing brain? A few months ago I went through the data on IGF1 in the brain, and it seems to be anti-apoptotic. You showed increased apoptosis.

Wang: I don't know.

Veldhuis: Is this the same model in which a single group has reported that fetal neuronal transplantation has restored potency in the old male rat? This raises the possibility that there is structural loss of GnRH neurons, and it is not just functional. I didn't believe that this was the case. I had always preferred the hypothesis that there was functional loss of input signals to the GnRH ensemble, or loss of coordinate secretion. Your data are suggesting that there is increased cell death.

Prior: Tomorrow we will be talking about the perimenopause in women. To use similar language and call it the 'andropause' is to make light of the huge number of changes that occur over 5–10 years in women, versus the very gradual, slow changes that occur in men. I wish we could take away that word and not use it. Perhaps the 'andropause' applies to the Norway rat, but I don't think it applies to human males.

References

Chen H, Zirkin BR 1999 Long-term suppression of Leydig cell steroidogenesis prevents Leydig cell aging. Proc Natl Acad Sci USA 96:14877–14881

Gruenewald DA, Matsumoto AM 1991 Age-related decreases in serum gonadotropin levels and gonadotropin-releasing hormone gene expression in the medial preoptic area of the male rat are dependent upon testicular feedback. Endocrinology 129:2442–2450

Gruenewald DA, Naai MA, Hess DL, Matsumoto AM 1994 The Brown Norway rat as a model of male reproductive aging: evidence for both primary and secondary testicular failure. J Gerontol 49: B42–B50

Gruenewald DA, Naai A, Marck BT, Matsumoto AM 2000 Age-related decrease in hypothalamic gonadotropin-releasing hormone (GnRH) gene expression but not pituitary responsiveness to GnRH, in the male Brown Norway rat. J Androl 21:72–84

Melis MR, Succu S, Spano MS et al 2001 Penile erection induced by EP 80661 and other hexarelin peptide analogues: involvement of paraventricular nitric oxide. Eur J Pharmacol 411:305–310

Morley JE, Kumar VB, Mattammal MB, Farr S, Morley PM, Flood JF 1996 Inhibition of feeding by a nitric oxide synthase inhibitor: effects of aging. Eur J Pharmacol 311:15–19

Rigamonti AE, Cella SG, Marazzi N, Müller EE 1999 Nitric oxide modulation of the growth hormone-releasing activity of hexarelin in young and old dogs. Metabolism 48:176–182

Endocrine Facets of Ageing.
Novartis 242

Print ISBN 0-471-48636-1 Online ISBN 0-470-84654-2

Mechanisms of conjoint failure of the somatotropic and gonadal axes in ageing men

J. D. Veldhuis*, A. Iranmanesh†, T. Mulligan‡ and C. Y. Bowers§

**Division of Endocrinology, University of Virginia School of Medicine, Charlottesville, Virginia 22908-0202, †Endocrine Section, Salem Veterans Affairs Medical Center, Salem, Virginia 24153, ‡Geriatrics and Extended Care Service, McGuire Veterans Affairs Medical Center, Richmond, Virginia 23249, and §Department of Medicine SL53, Tulane University Medical Center, New Orleans, Louisiana 70112-2699, USA*

Abstract. Endogenous growth hormone (GH) production falls by 50% every 7 years and bioavailable testosterone concentrations decline concomitantly by 12–15% every decade in ageing men. Despite this temporal parallelism, the neuroendocrine bases of the somatopause and gonadopause are not known. This knowledge deficit contrasts with the recent unfolding of new insights into the nature of oestrogen-dependent control of the GH–insulin-like growth factor (IGF)1 axis in pre- and postmenopausal women. The present overview examines the postulate that the pathophysiology of somatopause and gonadopause in ageing men is bidirectionally linked. According to this broader thesis, hyposomatotropism accentuates Leydig cell steroidogenic failure and, conversely, progressive androgen deficiency exacerbates the decline in GH–IGF1 output in ageing.

2002 Endocrine facets of ageing. Wiley, Chichester (Novartis Foundation Symposium 242) p 98–124

Clinical features of ageing include variably impaired psychological well being, cognitive function and quality of life; decreased libido and/or sexual function; reduced physical productivity; diminished muscle and bone mass; and increased visceral obesity, dyslipidemia and risk of cardiovascular events. These attributes collectively heighten the potential for frailty, disability, suffering and loss of independent living status. From an endocrine perspective, ageing in the male is accompanied by a progressive and dual decline in the bioavailability of growth hormone/insulin-like growth factor (IGF)1 and testosterone, which otherwise jointly support tissue anabolism (Iranmanesh et al 1994, Veldhuis et al 1995; Fig. 1). The terms somatopause and gonadopause highlight the foregoing age-related impoverishment of GH/IGF1 and androgen output, respectively.

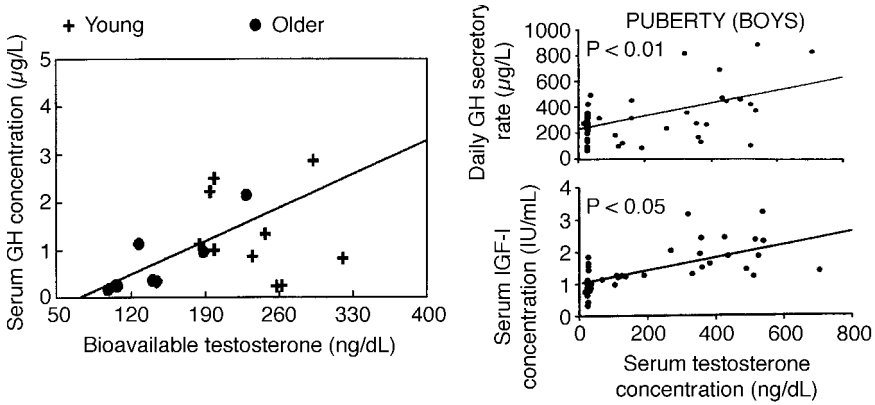


FIG. 1. (*Left*) Relationship between overnight serum bioavailable testosterone and GH concentrations in a group of 10 young and older healthy men (Mulligan et al 1999a, Veldhuis et al 1999b, 2000b). (*Right*) Correlations between serum total testosterone and 24 h GH and IGF1 output in a cohort of 46 healthy pubertal boys (Martha et al 1992).

However, virtually nothing is known about the basic clinical pathophysiology underlying the interlinked attrition of these pivotal trophic–hormone axes in ageing.

The present overview develops the bipartite thesis that ageing-associated hyposomatotropism and hypogonadism arise from conjoint pathophysiologies. We hypothesize that failing GH/IGF1 output worsens the age-related decline in luteinizing hormone (LH)-stimulated testicular steroidogenesis, and, conversely, that waning androgen availability blunts hypothalamo–pituitary drive of the GH/IGF1 axis. This notion reflects extensive basic science and clinical data (examined below), which collectively indicate that GH and androgen show anabolic synergy and mechanistic coupling in normal physiology.

Physiological synergy between the actions of GH and testosterone

GH and testosterone stimulate skeletal and muscular growth synergistically in late puberty (Giustina & Veldhuis 1998, Keenan et al 1993, Klindt et al 1990, Zachmann 1992). Recent studies also document target-tissue synergy between GH and androgen in healthy older men. Thus, restoring both GH and testosterone output in the older male by nearly physiological means would be important. However, the precise mechanistic basis for joint failure of GH and testosterone secretion in ageing is not known. To this end, a better comprehension of their interactive neuroendocrine control will be required.

Basic linkages between the somatotropic and gonadal axes

Extensive clinical data affirm that testosterone bioavailability and GH secretion rise in parallel in puberty and fall concurrently in ageing (Fig. 1; Copinschi & Van Cauter 1994, Dudl et al 1973, Giustina & Veldhuis 1998, Corpas et al 1992, Fryburg et al 1997, Hartman et al 1991, Iranmanesh et al 1991, 1994, Martha et al 1992, Mulligan et al 1999a, Veldhuis et al 1991, 1995). Causality is inferable since, at least in androgen-deficient individuals, administration of testosterone effectually drives the secretion of both GH and IGF1, and augments local tissue-specific IGF1 activity (Fryburg et al 1997, Gentili et al 2000, Snyder et al 1999, Urban et al 1995, Wennink et al 1990). Conversely, GH and IGF1 amplify the biosynthesis and tissue actions of androgens (Balducci et al 1993, Carani et al 1999, Giustina & Veldhuis 1998, Kulin et al 1981, Lin et al 1986). Thus, testosterone positively modulates the secretion and activity of the GH/IGF1 system (Fryburg et al 1997, Gentili et al 2000, Veldhuis et al 1997), whereas GH and IGF1 impact the production and effects of testosterone. Deficits in one or more of the foregoing interactions may contribute to the pathophysiology of combined GH and androgen deficiency in ageing, systemic illness and debilitated states.

Our recent clinical studies document that testosterone retains full efficacy in driving threefold amplification of pulsatile, 24 h rhythmic and entropic GH secretion in older men, as otherwise observed in boys and young men (Fryburg et al 1997, Gentili et al 2000, Shah et al 1999a, Veldhuis et al 1997, 2000a). However, the exact neuroendocrine mechanisms that mediate these stimulatory actions of testosterone are not established at any age. The following observations underscore possible clues to and the implications of elucidating such basic mechanistic pathways.

GH and IGF1 versus co-gonadotropins

GH and IGF1 can be considered as 'co-gonadotropins', in view of their ability to facilitate LH and/or follicle-stimulating hormone (FSH)-stimulated gonadal steroidogenesis in the rat and human (Lin et al 1986, Veldhuis 1996). Clinical studies corroborate a co-gonadotropic action of GH in ovulation induction in hypopituitary, but not eusomatotropic women. Analogously, hypopituitary, but not GH-sufficient, boys and men respond to GH repletion with enhanced human chorionic gonadotropin (hCG)-stimulated testosterone secretion (Balducci et al 1993, Carani et al 1999, Kulin et al 1981). Thus, maximal hCG/LH-driven gonadal steroidogenesis appears to require a GH-sufficient state. This emergent notion has never been tested in hyposomatotropic ageing men, who are in fact hypopituitary by young-adult standards (Giustina & Veldhuis 1998, Martha et al 1992, Veldhuis et al 1995, 1997, 1999a). To address this issue, we have developed a

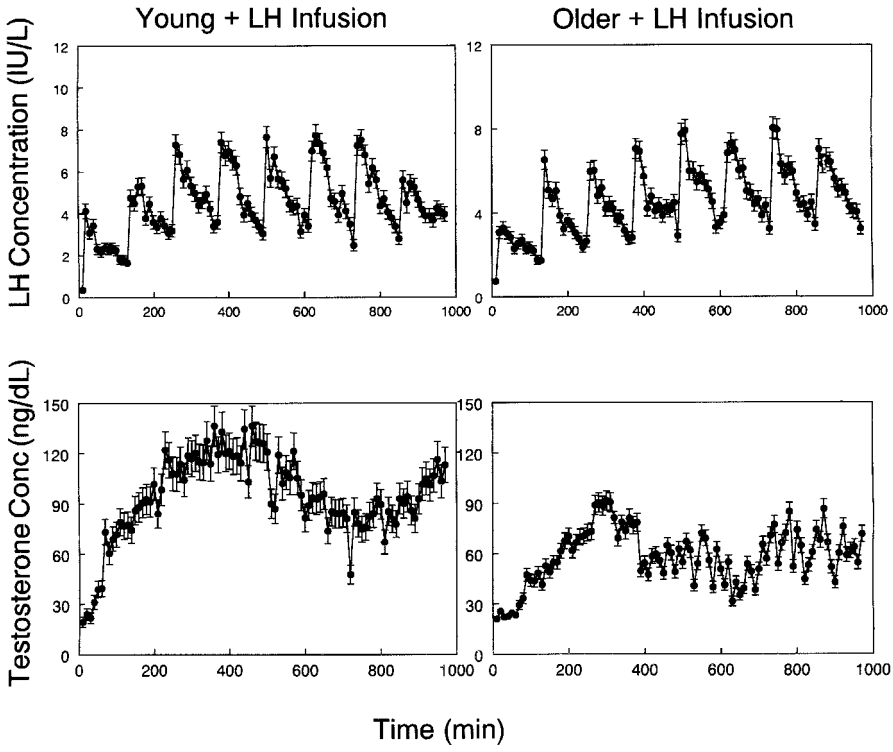


FIG. 2. Illustrative serum LH and testosterone concentration profiles obtained in one young and one older man pretreated with leuprolide 3–4 weeks earlier to down-regulate endogenous gonadotropin secretion. Data reflect 10 min blood sampling and eight consecutive intravenous pulses of recombinant human LH (50 IU, Serono) (Mulligan et al 2000).

novel experimental regimen of physiologically i.v. pulsatile GH 'addback' for 2 weeks to test the GH dependence of hCG-stimulated Leydig-cell steroidogenesis in older men. This mechanistic consideration will be important to address definitively (Blackman 1987, degli Uberti et al 1997, Giustina & Veldhuis 1998, Haji et al 1980, Mulligan et al 1999a, Shah et al 1999b, Veldhuis 1996).

Our pilot studies show that a pulsatile i.v. infusion of recombinant human (rh) GH every 90 min for 14 days (0.33 μg/kg/pulse) will restore 24 h serum GH concentration profiles, elevate plasma IGF1 concentrations and heighten 4 h mean Leydig-cell testosterone secretion stimulated by a single i.m. injection of 2000 IU hCG. In preliminary experiments designed to eliminate the use of the less physiological hCG stimulus, we have tested Leydig-cell responsiveness to pulsatile i.v. infusions of rh LH in 15 leuprolide-down-regulated young and older men (Mulligan et al 2000; Fig. 2). To improve study design further, we

have shown that a single s.c. dose (2 mg) of a gonadotropin-releasing hormone (GnRH) antagonist, ganirelix, lowers the serum testosterone concentration within 6 h by $>85\%$ for 16–24 h in young men (T. Mulligan, A. Iranmanesh & J. D. Veldhuis, unpublished data). We earlier reported a comparably rapid time course of D-Nal-Glu GnRH's inhibitory actions in young men. Studies by Tenover et al (1990), also showed that young and older men manifest equivalent (age-independent) gonadal-axis suppression by a GnRH antagonist.

Documenting GH-dependent enhancement of gonadotropin-supported Leydig-cell steroidogenesis in older men during controlled pulsatile LH drive would directly link hyposomatotropism with impaired testicular steroidogenesis in older men. To our knowledge, such a causal association would represent a novel finding in any ageing mammalian species. Conversely, we posit that androgen feedback on the GH axis enhances somatotropic activity. This thesis is discussed next from a general and then mechanistic perspective.

Overview of the ensemble GH/IGF1 axis

The GH/IGF1 axis comprises a feedback ensemble controlled jointly by: (i) GH-releasing hormone (GHRH) feedforward; (ii) somatostatin (SS) inhibition; (iii) a GH-releasing oligopeptide (GHRP) signalling pathway; and (iv) GH and IGF1 autonegative feedback (Mueller et al 1999, Argente et al 1991, Baumann & Maheshwari 1997, Baumbach et al 1998, Bowers et al 1990, Bowers 1993, Carlsson et al 1990, Frohman & Jansson 1986, Giustina & Veldhuis 1998, Jaffe et al 1993, Kojima et al 1999, Smith et al 1997, Hofland & Lamberts 1996). This simplified core construct is illustrated in Fig. 3.

GHRH is an established primary agonist driving the biosynthesis and pulsatile secretion of GH in all mammalian species (Giustina & Veldhuis 1998). Conversely, somatostatin (SS) is a dominant inhibitory signal, which antagonizes the exocytotic secretion of GH, but not its biosynthesis or storage (Hofland & Lamberts 1996). GHRPs are potent and selective oligopeptidyl GH secretagogues (Bowers et al 1984), mimicked by certain non-peptidyl agonists and exemplified endogenously by a ^{3}Ser-octanoylated 28-amino acid GHRP-like ligand (Smith et al 1997, Howard et al 1996, Kojima et al 1999, Mueller et al 1999). The foregoing trilogy of neuropeptidyl regulators controls GH secretion via topographically and biochemically distinct receptors and secondary signalling molecules (Barinaga et al 1985, Giustina & Veldhuis 1998, Mayo 1992, Mueller et al 1999). These diverse, but uniquely interactive, features create: (a) cooperative mechanisms of biological control, and (b) multiple vulnerable loci for disrupted neuroregulation in ageing and/or hypogonadism. We believe that such interactive properties further mandate the particularly careful design of

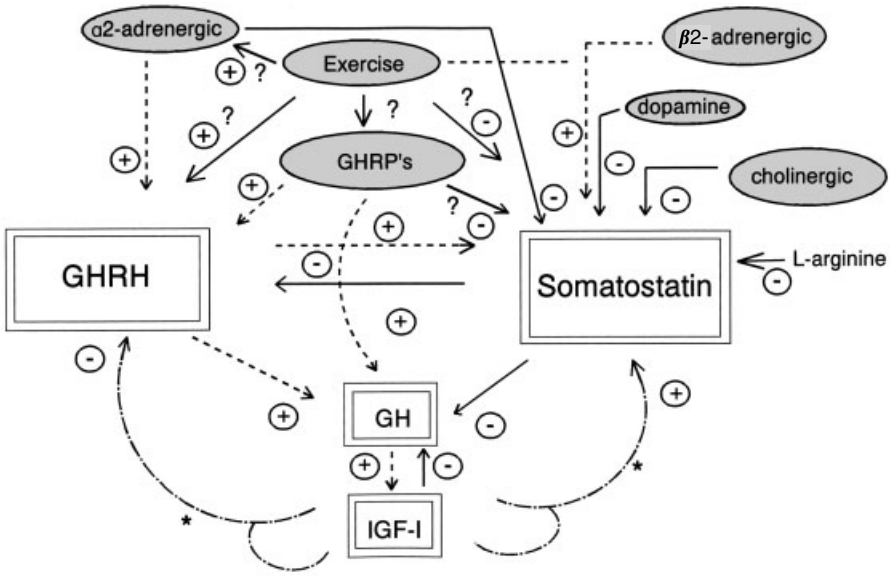


FIG. 3. Working experimental schema of primary tri-peptidyl control of the human GH axis (Giustina & Veldhuis 1998).

experimental interventions, e.g. wherein one 'clamps' two (of the three) input signals in order to assess (sex-steroid induced) changes in the third.

Altered GH neuroregulation in ageing and/or hypogonadism

Precisely how ageing and/or androgen deficiency impacts the GH/IGF1 neuroregulatory unit is not known (Giustina & Veldhuis 1998). However, both animal and human studies make an ensemble perspective essential. For example, clinically, only L-arginine (an agent believed to withdraw SS) combined with (a) GHRH, (b) GHRP or (c) both GHRH and GHRP can normalize GH secretion acutely, when judged against similarly stimulated young adults (Ghigo et al 1990, Khorram et al 1997, Veldhuis & Giustina 2000). Specifically, no single GHRH or GHRP agonist, or SS antagonist, can restore GH/IGF1 output completely in older volunteers (Veldhuis & Giustina 2000, degli Uberti et al 1997, Giustina & Veldhuis 1998). Notably, sustained exogenous GHRH stimulation or GHRP2 drive only partially reconstitutes the GH/IGF1 axis in elderly humans, thus pointing to a relative deficiency of each agonist (Corpas et al 1992, Evans et al 2000, Iranmanesh et al 1998, Khorram et al 1997). However, the GH-stimulating effect of GHRH infused i.v. over 3 days is blunted by increasing age and relative hypoandrogenaemia (Iranmanesh et al 1998; Fig. 4).

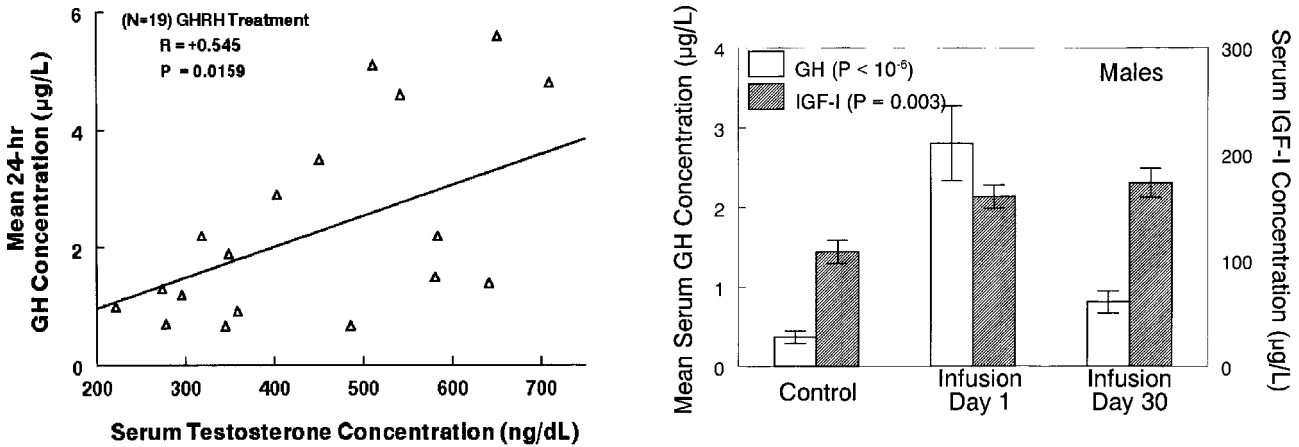


FIG. 4. (*Left*) Testosterone-dependent decline in 24 h GH secretion driven by pulsatile i.v. infusion of GHRH (0.33 μg/kg/pulse) every 90 min for 3 days in 19 men (Iranmanesh et al 1998). (*Right*) Partial reconstitution of daily GH and IGF1 production by continuous s.c. GHRP2 infusion for 30 days in 12 healthy older men.

Thus, we propose that multifold neuroregulatory failure underlies impoverished GH secretion in ageing, namely, combined GHRH and/or GHRP deficiency and SS excess. Several plausible mechanistic considerations render this thesis more compelling.

GHRP and GHRH

GHRPs act at both hypothalamic and pituitary loci (Smith et al 1997). At CNS sites, these agonists stimulate electrical firing and c-*fos* gene expression in rodent NPY and GHRH neurons, elicit GHRH secretion acutely into sheep portal blood, and induce somnolence or alter appetite (Arvat et al 1998, Dickson et al 1995, Giustina & Veldhuis 1998, Guillaume et al 1994, Iranmanesh et al 2000, Locke et al 1995). At the pituitary level, GHRPs stimulate GH secretion directly (albeit less powerfully) *in vitro*, and putatively act at joint hypothalamo–pituitary loci to enhance somatotrope GH gene expression in the infant rat *in vivo* (Bowers et al 1984). Central (hypothalamic) actions of GHRPs are critical clinically, since hypothalamo–pituitary interruption virtually abolishes GHRP-stimulated GH secretion, even when somatotrope responsiveness to GHRH is preserved (Giustina & Veldhuis 1998, Mueller et al 1999). Maximal effects of GHRPs also require a functional GHRH receptor, as inferred from genetic studies in mice (lit/lit mutation) and humans (dwarfs of Sindh) (Baumann & Maheshwari 1997, Frohman 1996), and based on the ability of a selective GHRH-receptor antagonist to suppress GHRP-stimulated GH secretion in young men.

Most studies document prominent *in vivo* synergy between the secretagogue effects of GHRP and GHRH in the human, pig and rodent (Bowers 1998, Giustina & Veldhuis 1998, Mueller et al 1999, Bowers et al 1990; Fig. 5). How testosterone modulates the foregoing GHRH–GHRP synergy at any age remains unknown.

Somatostatin and GHRPs

GHRPs act as so-called 'functional SS antagonists' by increasing the ID_{50} of SSs inhibition of spontaneous and GHRH-stimulated GH secretion by two–threefold *in vitro* and by 8–10-fold *in vivo* (Bowers 1998, Smith et al 1997). GHRPs partially oppose central SSergic activity, but do not directly block SS binding to pituitary cells or SS secretion into portal blood (Bowers 1998, Guillaume et al 1994).

Somatostatin, GHRH and GHRP triology

Tri-peptidyl interactions operate in the rat and human, since experimentally reducing SSergic input will markedly amplify *in vivo* dual-agonist (GHRH/GHRP)

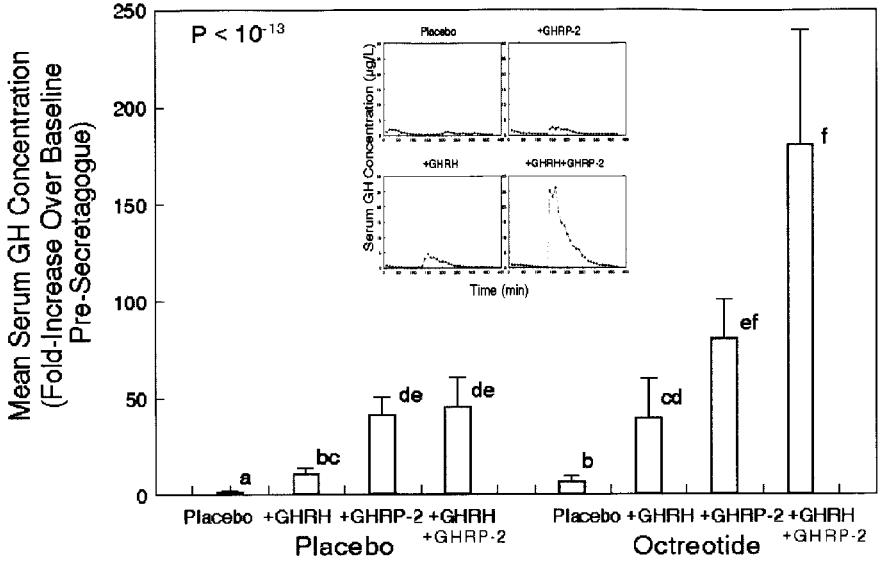


FIG. 5. Synergistic stimulation of GH secretion by bolus injection of GHRH (1 μg/kg) and GHRP2 (1 μg/kg) in middle-aged men, which can be enhanced further by post-SS (octreotide) rebound. See text for details.

synergy (Arvat et al 1998, Bowers 1998, Smith et al 1997). Indeed, co-infusion of L-arginine (to withdraw SS) and GHRH or GHRP, or all three stimuli combined, will evoke supra-additive GH secretion in older humans, which is equivalent to that achieved in young adults (Arvat et al 1998, Bowers 1998). These data are consistent with excessive SSergic restraint and combined GHRH/GHRP deficiency in ageing (Bowers 1998, Giustina & Veldhuis 1998). In contrast, the magnitude of bihormonal GHRH/GHRP synergy (without L-arginine) wanes substantially in older humans. Accordingly, we propose to examine the endogenous control of all three (SS, GHRH, and GRHP) signalling pathways to better explicate the mechanisms of hyposomatotropism in the older male.

GH autonegative feedback

An abrupt increase in the GH concentration feeds back physiologically to limit further secretion (Berman et al 1994, Chapman et al 1997, Clark et al 1988, Giustina & Veldhuis 1998, Harel & Tannenbaum 1992, Rosenthal et al 1986). This time-lagged and reversible autoregulatory action probably sustains normal GH pulsatility (Frohman 1996). Autonegative feedback is mediated via GH's

stimulation of hypothalamic SS release and reciprocal inhibition of GHRH secretion, without any direct action on somatotropes (Frohman 1996, Smith et al 1997). The ability of testosterone to stimulate GH and IGF1 production simultaneously would suggest that testosterone may act to override such autoinhibition by GH (Gentili et al 2000, Giustina et al 1997, Giustina & Veldhuis 1998, Veldhuis et al 1997).

Integrative issues

The foregoing interactive features of GH neuroregulation highlight the crucial need to explore interactive mechanisms subserving impoverishment of GH/IGF1 output in the ageing and relatively hypogonadal male. Below we evaluate several such presumptive neuroregulatory mechanisms, which could plausibly mediate testosterone's failing drive of the GH/IGF1 axis in older men.

Hypothesis 1: testosterone deprivation accentuates GH autonegative feedback in the ageing male

Autonegative feedback denotes the recognized ability of a GH pulse to inhibit subsequent GH secretion, whether driven spontaneously or by an exogenous GHRH or GHRP stimulus (Carlsson et al 1990, Chihara et al 1981, Clark et al 1988, Rosenthal et al 1986). GH autoinhibition occurs by way of central nervous system (CNS) (rather than pituitary) pathways, which rapidly stimulate SS and repress GHRH secretion (Chihara et al 1981, Pellegrini et al 1996). Autofeedback is non-trivial, since molecular defects of the GH receptor (or IGF1) gene evoke marked secondary GH hypersecretion (Frohman 1996). Conversely, GH secretagogues that putatively oppose SS release, such as L-arginine, overcome GH autofeedback. Thus, GH autonegative feedback influences the activity of each primary neuroregulatory peptide (SS, GHRH and GHRP; Fig. 3).

We have shown that a single i.v. pulse of rhGH reproducibly inhibits subsequent spontaneous (endogenous) GH secretion by 75–80%, as well as that stimulated acutely by bolus GHRH (by 60–75%) and GHRP2 (by 35–50%). GH feeds back less effectively to suppress the GHRP than GHRH stimulus, presumptively because GHRPs partially antagonize SS's actions (Guillaume et al 1994, Bowers 1998). These data suggest the experimental question: does testosterone depletion in older men limit GH and IGF1 output in part by accentuating (hypothalamic SS-mediated) GH autonegative feedback? This issue is additionally noteworthy in view of the recent finding that brain GH receptor density falls in older humans. Since CNS GH receptors mediate GH autofeedback (Giustina & Veldhuis 1998), their attrition in ageing could forecast a countervailing interpretation of waning GH autofeedback. However, oestrogen

elevates hypothalamic (and represses liver) GH receptor expression, at least in the rat. Thus, relative oestrogenization in ageing men might actually accentuate GH receptor-dependent autonegative feedback. Given these provocative and divergent regulatory issues, we believe that GH autofeedback studies will be important to pursue in androgen-deficient and androgen-replete older men.

Hypothesis 2: testosterone depletion reduces the potency and/or efficacy of GHRH's actions

GHRH stimulates three major responses in somatotropes: (i) exocytotic release of stored GH (immediate effect); (ii) *de novo* GH gene transcription and GH synthesis (acute and short-term actions); and (iii) somatotrope cellular hypertrophy and proliferation (longer-term response) (Giustina & Veldhuis 1998, Mueller et al 1999). Whereas few if any studies show consistently positive regulation of the GHRHergic pathway by oestrogen (Argente et al 1991), in the male rat (non-aromatizable) androgens up-regulate hypothalamic GHRH gene and peptide expression and enhance pituitary responsiveness to GHRH (Jansson et al 1993, Mueller et al 1999, Argente et al 1991). Likewise, in leuprolide-down-regulated young men, testosterone addback restores the acute stimulatory effect of GHRH (Devesa et al 1991). Notably, several independent clinical studies suggest that endogenous GHRHergic activity is reduced in the ageing male: (1) intermittent i.v. infusion of GHRH (0.33 μg/kg every 90 min for 72 h) amplifies pulsatile GH secretion by several fold in older men, albeit not to young-adult levels (Iranmanesh et al 1998); (2) a GHRH-receptor antagonist inhibits GH secretion more effectively in older than young individuals, consistent with reduced hypothalamic GHRH secretion and/or SS excess (Russell-Aulet et al 1999); and (3) post-SS rebound GH secretion is impaired in the elderly, suggesting limited endogenous GHRH drive (degli Uberti et al 1997). However, how testosterone modulates the GHRHergic pathway in ageing humans remains unknown.

Based on the foregoing physiology, and testosterone's ability to rescue hypo-somatotropism in older men (Gentili et al 2000), we postulate that testosterone can (a) enhance endogenous GHRH secretion and/or (b) facilitate somatotrope responsiveness to GHRH. To this end, we are carrying out clinical experiments to drive the GHRHergic signalling pathway in older men while simultaneously fixing inputs by SS and GHRP before and after testosterone supplementation.

Hypothesis 3: Testosterone deficiency increases the hypothalamic release and/or actions of SS in ageing men

Beyond its primary repressive role (above), SS paradoxically maintains somatotrope responsiveness to recurrent stimulation by secretagogues (Baumbach et al 1998,

Kraicer et al 1986, Sugihara et al 1989). Specifically, intermittent SS exposure obviates the biochemical down-regulation of GH secretion induced by repeated GHRH and GHRP stimuli (Smith et al 1997). Thus, a critical mechanistic question is whether testosterone depletion limits GH secretion in part by augmenting sustained rather than intermittent SSergic activity (Fryburg et al 1997, Giustina et al 1997, Giustina & Veldhuis 1998, Veldhuis et al 1997).

Clinical studies are particularly pertinent here, in view of possible species differences in SS and androgen physiology (Argente et al 1990, Painson et al 2000, Pincus et al 1997). For example, in the rat, non-aromatizable androgens (but not oestrogen) stimulate GH secretion and up-regulate hypothalamic SSergic activity. In contrast, in the human, non-aromatizable androgens (stanozolol, fluoxymesterone, oxandrolone and 5α-DHT) do not stimulate GH secretion consistently (Devesa et al 1991, Fryburg et al 1997, Giustina & Veldhuis 1998, Keenan et al 1993, Veldhuis et al 1997). Thus, aromatizable and nonaromatizable androgen actions are readily distinguishable in the two species, whereas testosterone's impact on SSergic signalling may not be distinctive.

Clinical studies of this issue are further relevant in older humans, in whom accentuated SS inhibition is widely inferred, but its susceptibility to relief by sex-steroid hormone repletion is unknown (Chihara et al 1981). Our pilot data document that short-term testosterone administration in older men exerts qualitatively identical actions to those reported in hypogonadal boys and young men (Fryburg et al 1997, Gentili et al 2000, Giustina et al 1997, Veldhuis et al 1997); specifically: (i) augmentation of GH secretory burst mass and basal GH release; (ii) amplification of the 24 h rhythmicity of GH secretion; (iii) heightening of the irregularity (approximate entropy) of GH release; and (iv) elevation of plasma IGF1 concentrations (Fig. 6). All four responses are strongly controlled by SS under various pathophysiological conditions (Giustina & Veldhuis 1998, Mueller et al 1999, Mulligan et al 1999b). For example, we have shown that SS or octreotide infusions selectively suppress GH secretory burst mass and basal GH release in young and older men (Mulligan et al 1999b). Likewise, SSergic inputs likely influence the 24 h rhythmicity of GH secretion, since the latter persists during an unvarying exogenous GHRH or GHRP2 infusion (Evans et al 2000, Iranmanesh et al 1998, Shah et al 1999a, 2000). In corollary, SS signalling governs the quantifiable irregularity (entropy) of GH secretory patterns (Gevers et al 1998, Pincus et al 1996, Straume et al 1995). Lastly, longer-term inhibition of GH output by SS clearly lowers IGF1 production. Testosterone strongly impacts each of these four categories of GH/IGF1 responsiveness in older men (Gentili et al 2000), thus pointing to (but not proving) its ability to control SSergic signalling the elderly male.

Available clinical data do not exclude an opposing hypothesis that an aromatizable androgen actually elevates SSergic input, as reported for both

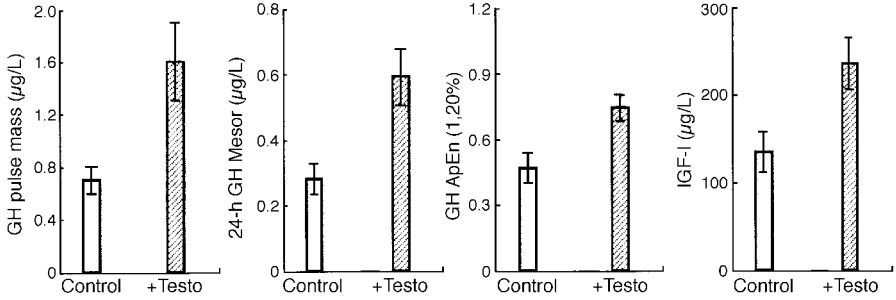


FIG. 6. Fourfold stimulation by short-term (3 week) parenteral testosterone supplementation of: (i) GH secretory burst mass; (ii) 24 h rhythmic GH release; (iii) GH ApEn (irregularity measure); and (iv) serum IGF1 concentrations in older men

aromatizable and non-aromatizable androgens in the rodent (Giustina & Veldhuis 1998, Mueller et al 1999, Chihara et al 1981, Frohman 1996). Indeed, diethylstilbestrol administration to young men and the normal preovulatory milieu in young women enhance GH release stimulated by secretagogues that putatively withdraw SSergic restraint (Frantz & Rabkin 1965). Given such divergent clinical data, it will be crucial to clarify how testosterone deficiency modulates SSergic signalling in the human.

Hypothesis 4: testosterone deprivations attenuate activity of the GHRP pathway in older men

Several pivotal factors motivate the important, but unproven, consideration that deficient endogenous GHRPergic activity contributes to the hyposomatotropism of ageing. Foremost among these are partial reconstitution of GH/IGF1 axis output by continuous s.c. GHRP2 infusion or oral MK0677 (a non-peptidyl agonist) administration in older individuals, and diminished hypothalamic GHRP receptor density in ageing humans (Chapman et al 1996, Mueller et al 1999). The timeliness of this query is highlighted by the recent cloning of a natural ^{3}Ser-octanoylated 28 amino-acid ligand of the GHRP receptor, ghrelin, in the rat and human. Ghrelin gene transcripts are expressed by RT-PCR in the brain, and ghrelin peptide is detected by immunocytochemistry in the arcuate nucleus of the hypothalamus and by radioimmunoassay in peripheral human blood (Kojima et al 1999). Since neither the native peptide nor derivative antagonists are available for clinical use, we and others have probed responsiveness of the human GHRP-receptor/effector pathway using a potent and highly specific synthetic (hexapeptide) agonist, such as GHRP2 (our FDA IND #38 149) (Bowers 1993, 1998, Bowers et al 1990, Pihoker et al 1998, Shah

et al 1998a,b, 1999a,c, 2000). GHRP2 is the most effective GHRP-receptor agonist available for investigational use in the human. GHRP2 synergizes with GHRH in stimulating GH secretion in healthy men (Fig. 4), and enhances oestradiol's drive of GH secretion in postmenopausal women (Evans et al 2000, Shah et al 1998a,b, 1999c, 2000). Other clinical studies show that GHRPs exert maximal acute stimulatory effects in mid-to-late puberty when sex-steroid hormone concentrations peak. Moreover, a single i.m. injection of testosterone in boys and brief oestrogen exposure in girls double GH stimulation by a near-maximally effective dose of the GHRP, hexarelin (Loche et al 1997). Thus, sex steroids may modulate GHRP-receptor/effector activity in the human, as inferred recently in the postnatal rat and GH-transgenic mouse. Indeed, the promoter of the human GHRP-receptor gene contains a hemioestrogen-responsive element. Thus, we postulate that testosterone could act either pre- or post-aromatization to up-regulate responsiveness of this key secretagogue pathway.

Not all studies point to sex-steroid dependent control of the GHRP pathway. For example, GH responses to a single (near-maximal) i.v. bolus dose of a GHRP did not: (i) vary within the menstrual cycle; (ii) differ in women and men; or (iii) change following low-dose (0.05 mg daily) transdermal oestradiol administration in postmenopausal individuals . However, the foregoing analyses were carried out in the face of variable (and, hence, potentially confounding) endogenous SSergic and GHRH inputs. In fact, based on a simplified tri-peptidyl model of GH neuroregulation (Fig. 3), we reason that testosterone's reported facilitation of maximal GHRP stimulation in prepubertal boys (Loche et al 1997) could reflect testosterone's (non-exclusive) ability to: (a) facilitate GHRP-receptor/effector signalling (Mueller et al 1999); (b) reduce concomitant SSergic activity; and/or (c) augment the release and/or actions of endogenous GHRH. Only the first interpretation would be consistent with GHRP-receptor/effector up-regulation. The second consideration arises because SS partially opposes GHRP's actions (Bowers 1998, Iranmanesh et al 1999, Wideman et al 2000a,b). The third postulate is significant, since GHRPs can release and act synergistically with GHRH (above). Accordingly, further experimental studies will be needed to resolve these key mechanistic distinctions.

In addition to receptor-level regulation, the secretion of endogenous GHRP-like ligands could be controlled by androgen. Since ghrelin is also produced by gastric (oxyntic) cells (Kojima et al 1999), simply assaying its concentration in peripheral blood would not be fully informative. Accordingly, to explore testosterone's regulation of endogenous GHRP release and actions, one would need to block SS secretion and fix GHRH inputs simultaneously (Bray et al 1999, degli Uberti et al 1997, Dickerman et al 1993, Giustina & Veldhuis 1998). This stratagem should appraise how testosterone modulates non-SS- and non-GHRH-dependent (i.e. putative GHRP-like) endogenous drive of the human GH axis

(Bray et al 1999, Giustina & Veldhuis 1998, Iranmanesh et al 2000, Shah et al 1998a,b, 1999c, Wideman et al 2000a,b).

Distinctions in the actions of androgen and oestrogen on the GH/IGF1 axis

The neuroendocrine mechanisms by which testosterone governs pulsatile GH secretion in the human have remained elusive, in part because of species differences in the nature of androgenic control of the GH/IGF1 axis and in part due to the tripeptidyl control of this specialized axis (Giustina & Veldhuis 1998). Moreover, known actions of oestrogen are not necessarily equivalent to those postulated for testosterone. Notably, in clinical studies testosterone, but not oestradiol, consistently stimulates both GH and IGF1 production and elevates (non-pulsatile) GH secretion (Bellantoni et al 1991, Devesa et al 1991, Giustina & Veldhuis 1998, Karlsson et al 1990, Shah et al 1999b, van Kesteren et al 1996, Blumenfeld et al 1992, De Leo et al 1993). Thus, for example, the putative neuroendocrine mechanism of oestrogen's unleashing of GH secretion in postmenopausal women by way of reduced systemic IGF1 feedback cannot be facilely invoked to explicate testosterone's combined stimulation of GH and IGF1 secretion in ageing and/or hypogonadal men.

Summary

Advances in GHRH, GHRP and SS peptide chemistry, receptorology and neuroregulatory physiology now create a unique platform for more informative and insightful clinical studies of the mechanisms of testosterone's control of the ageing human GH/IGF1 axis. We suggest that the relatively hyposomatotropic and hypoandrogaemic older male should afford an excellent clinical context in which to explore such issues. In addition, based on the cardinal role of sex steroid hormones in sustaining GH secretion throughout the adult human lifetime (Giustina & Veldhuis 1998), such studies should also provide significant corollary insights into the regulatory pathophysiology of the GH/IGF1 axis during early puberty and other hypogonadal states.

References

Argente J, Chowen BJ, Steiner RA, Clifton DK 1990 Somatostatin messenger RNA in hypothalamic neurons is increased by testosterone through activation of androgen receptors and not by aromatization to estradiol. Neuroendocrinology 52:342–349

Argente J, Chowen JA, Zeitler P, Clifton DK, Steiner RA 1991 Sexual dimorphism of growth hormone-releasing hormone and somatostatin gene expression in the hypothalamus of the rat during development. Endocrinology 128:2369–2375

Arvat E, Ceda GP, Di Vito L et al 1998 Age-related variations in the neuroendocrine control, more than impaired receptor sensitivity, cause the reduction in the GH-releasing activity of GHRP's in human aging. Pituitary 1:51–58

Balducci R, Toscano V, Mangiantini A, Bianchi P, Guglielmi R, Boscherini B 1993 The effect of growth hormone administration on testicular response during gonadotropin therapy in subjects with combined gonadotropin and growth hormone deficiencies. Acta Endocrinol 128:19–23

Barinaga M, Bilezikjian LM, Vale WW, Rosenfeld MG, Evans RM 1985 Independent effects of growth hormone releasing factor on growth hormone release and gene transcription. Nature 314:279–281

Baumann G, Maheshwari H 1997 The Dwarfs of Sindh: severe growth hormone (GH) deficiency caused by a mutation in the GH-releasing hormone receptor gene. Acta Pediatr Suppl 432:33–38

Baumbach WR, Carrick TA, Pausch MH et al 1998 A linear hexapeptide somatostatin antagonist blocks somatostatin activity in vitro and influences growth hormone release in rats. Mol Pharmacol 54:864–873

Bellantoni MF, Harman SM, Cho DE, Blackman MR 1991 Effects of progestin-opposed transdermal estrogen administration on growth hormone and insulin-like growth factor-I in postmenopausal women of different ages. J Clin Endocrinol Metab 72:172–178

Berman M, Jaffe CA, Tsai W, DeMott-Friberg R, Barkan AL 1994 Negative feedback regulation of pulsatile growth hormone secretion by insulin-like growth factor I: involvement of hypothalamic somatostatin. J Clin Invest 94:138–145

Blackman MR 1987 Pituitary hormones and aging. Endocrinol Metab Clin North Am 16:981–994

Blumenfeld Z, Barkey RJ, Youdim MB, Brandes JM, Amit T 1992 Growth hormone (GH)-binding protein regulation by estrogen, progesterone, and gonadotropins in human: the effect of ovulation induction with menopausal gonadotropins, GH, and gestation. J Clin Endocrinol Metab 75:1242–1249

Bowers CY 1993 GH releasing peptides — structure and kinetics. J Pediatr Endocrinol 6:21–31

Bowers CY 1998 Synergistic release of growth hormone by GHRP and GHRH: scope and implication. In: Bercu BB, Walker RF (eds) Growth hormone secretagogues. Springer-Verlag, New York, p 1–25

Bowers CY, Momany FA, Reynolds A, Hong A 1984 On the in vitro and in vivo activity of a new synthetic hexapeptide that acts on the pituitary to specifically release growth hormone. Endocrinology 114:1537–1545

Bowers CY, Reynolds GA, Durham D, Barrera CM, Pezzoli SS, Thorner MO 1990 Growth hormone (GH)-releasing peptide stimulates GH release in normal men and acts synergistically with GH-releasing hormone. J Clin Endocrinol Metab 70:975–982

Bray M, Shah N, Veldhuis JD 1999 Impact of estrogen on the dose-dependent inhibition by somatostatin of GH secretion in postmenopausal women. Presented at the 81st Annual Endocrine Society Meeting, San Diego, California, June 12–15

Carani C, Granata AR, De Rosa M et al 1999 The effect of chronic treatment with GH on gonadal function in men with isolated GH deficiency. Eur J Endocrinol 140:224–230

Carlsson LMS, Clark RG, Robinson IC 1990 Sex difference in growth hormone feedback in the rat. J Endocrinol 126:27–35

Chapman IM, Bach MA, Cauter EV et al 1996 Stimulation of the growth hormone (GH)-insulin-like growth factor I axis by daily oral administration of a GH secretagogue (MK-677) in healthy elderly subjects. J Clin Endocrinol Metab 81:4249–4257

Chapman IM, Hartman ML, Pezzoli SS et al 1997 Effect of aging on the sensitivity of growth hormone secretion to insulin-like growth factor-I negative feedback. J Clin Endocrinol Metab 82:2996–3004

Chihara K, Minamitani N, Kaji H, Arimura A, Fujita T 1981 Intraventrically injected growth hormone stimulates somatostatin release into rat hypophyseal portal blood. Endocrinology 109:2279–2281

Clark RG, Carlsson LMS, Robinson ICAF 1988 Growth hormone (GH) secretion in the conscious rat: negative feedback of GH on its own release. J Endocrinol 119:201–209

Copinschi G, Van Cauter E 1994 Pituitary hormone secretion in aging: role of circadian rhythmicity and sleep. Eur J Endocrinol 131:441–442

Corpas E, Harman SM, Pineyro MA, Roberson R, Blackman MR 1992 Growth hormone (GH)-releasing hormone-(1-29) twice daily reverses the decreased GH and insulin-like growth factor-I levels in old men. J Clin Endocrinol Metab 75:530–535

De Leo V, Lanzetta D, D'Antona D, Danero S 1993 Growth hormone secretion in premenopausal women before and after ovariectomy: effect of hormone replacement therapy. Fertil Steril 60:268–271

degli Uberti EC, Ambrosio MR, Cella SG et al 1997 Defective hypothalamic growth hormone (GH)-releasing hormone activity may contribute to declining GH secretion with age in man. J Clin Endocrinol Metab 82:2885–2888

Devesa J, Lois N, Arce V, Diaz MJ, Lima L, Tresguerres JA 1991 The role of sexual steroids in the modulation of growth hormone (GH) secretion in humans. J Steroid Biochem Mol Biol 40:165–173

Dickerman Z, Guyda H, Tannenbaum GS 1993 Pretreatment with somatostatin analog SMS 201-995 potentiates growth hormone (GH) responsiveness to GH-releasing factor in short children. J Clin Endocrinol Metab 77:652–657

Dickson SL, Leng G, Dyball RE, Smith RG 1995 Central actions of peptide and non-peptide growth hormone secretagogues in the rat. Neuroendocrinology 61:36–43

Dudl RJ, Ensinck JW, Palmer HE, Williams RH 1973 Effect of age on growth hormone secretion in man. J Clin Endocrinol Metab 37:11–16

Evans WS, Bowers CY, Hull LT, Anderson SM, Veldhuis JD 2000 Effects of estradiol and of continuous 24-hour GHRH- and GHRP-2 stimulation in women on pulsatile and entropic GH secretion. Presented at the Endocrine Society 82nd Annual Meeting, Toronto, Canada, June 21–24 Abstract # 2016

Frantz AG, Rabkin MT 1965 Effects of estrogen and sex difference on secretion of human growth hormone. J Clin Endocrinol Metab 25:1470–1480

Frohman LA 1996 New insights into the regulation of somatotrope function using genetic and transgenic models. Metabolism 45:1–3

Frohman LA, Jansson JO 1986 Growth hormone-releasing hormone. Endocr Rev 7:223–253

Fryburg DA, Weltman A, Jahn LA et al 1997 Short-term modulation of the androgen milieu alters pulsatile, but not exercise or growth hormone releasing hormone-stimulated GH secretion in healthy men. J Clin Endocrinol Metab 82:3710–3719

Gentili A, Mulligan T, Godschalk M et al 2000 Testosterone supplementation increases growth hormone secretion in older but not young men. Presented at the Endocrine Society 82nd Annual Meeting, Toronto, Canada, June 21–24 Abstract # 1944

Gevers E, Pincus SM, Robinson IC, Veldhuis JD 1998 Differential orderliness of the GH release process in castrate male and female rats. Am J Physiol 274:R437–R444

Ghigo E, Goffi S, Nicolosi M et al 1990 Growth hormone (GH) responsiveness to combined administration of arginine and GH-releasing hormone does not vary with age in man. J Clin Endocrinol Metab 71:1481–1485

Giustina A, Veldhuis JD 1998 Pathophysiology of the neuroregulation of growth hormone secretion in experimental animals and the human. Endocr Rev 19:717–797

Giustina A, Scalvini T, Tassi C et al 1997 Maturation of the regulation of growth hormone secretion in young males with hypogonadotropic hypogonadism pharmacologically

exposed to progressive increments in serum testosterone. J Clin Endocrinol Metab 82:1210–1219

Guillaume V, Magnan E, Cataldi M et al 1994 Growth hormone (GH)-releasing hormone secretion is stimulated by a new GH-releasing hexapeptide in sheep. Endocrinology 135:1073–1076

Haji M, Kato KI, Nawata H, Ibayashi H 1980 Age-related changes in the concentrations of cytosol receptors for sex steroid hormones in the hypothalamus and pituitary gland of the rat. Brain Res 204:373–386

Harel Z, Tannenbaum GS 1992 Synergistic interaction between insulin-like growth factors-I and -II in central regulation of pulsatile growth hormone secretion. Endocrinology 131:758–764

Hartman ML, Faria AC, Vance ML, Johnson ML, Thorner MO, Veldhuis JD 1991 Temporal structure of in vivo growth hormone secretory events in man. Am J Physiol 260:E101–E110

Hofland LJ, Lamberts SWJ 1996 Somatostatin receptors and disease: role of receptor subtypes. Baillieres Clin Endocrinol Metab 10:163–176

Howard AD, Feighner SD, Cully DF et al 1996 A receptor in pituitary and hypothalamus that functions in growth hormone release. Science 273:974–977

Iranmanesh A, Lizarralde G, Veldhuis JD 1991 Age and relative adiposity are specific negative determinants of the frequency and amplitude of growth hormone (GH) secretory bursts and the half-life of endogenous GH in healthy men. J Clin Endocrinol Metab 73:1081–1088

Iranmanesh A, Grisso B, Veldhuis JD 1994 Low basal and persistent pulsatile growth hormone secretion are revealed in normal and hyposomatotropic men studied with a new ultrasensitive chemiluminescence assay. J Clin Endocrinol Metab 78:526–535

Iranmanesh A, South S, Liem AY et al 1998 Unequal impact of age, percentage body fat, and serum testosterone concentrations on the somatotrophic, IGF-I, and IGF-binding protein responses to a three-day intravenous growth-hormone-releasing-hormone pulsatile infusion in men. Eur J Endocrinol 139:59–71

Iranmanesh A, Gupta PM, Bowers CY, Veldhuis JD 1999 GHRH and GHRP-2 interact to stimulate GH secretion in men in the presence of low-dose synthetic somatostatin (octreotide): evidence for a novel collaboration between GHRH and GHRP. Presented at the 81st Annual Endocrine Society Meeting, San Diego, California, June 12–15

Iranmanesh A, Bowes CY, Hussein RH, Veldhuis JD 2000 Singular and interactive impact of acute infusions of GHRH, GHRP-2, and octreotide pretreatment on GH neuroregulation in middle-aged men. Presented at the Endocrine Society 82nd Annual Meeting, Toronto, Canada, June 21–24 Abstract # 2019

Jaffe CA, Friberg RD, Barkan AL 1993 Suppression of growth hormone (GH) secretion by a selective GH-releasing hormone (GHRH) antagonist. Direct evidence for involvement of endogenous GHRH in the generation of GH pulses. J Clin Invest 92:695–701

Jansson JO, Staffan E, Isaksson OGP 1993 Sexual dimorphism in the control of growth hormone secretion. Endocr Rev 128:150–168

Karlsson R, Eden S, von Schoultz B 1990 Altered growth hormone secretion during oral contraception. Gynecol Obstet Invest 30:234–238

Keenan BS, Richards GE, Ponder SW, Dallas JS, Nagamani M, Smith ER 1993 Androgen-stimulated pubertal growth: the effects of testosterone and dihydrotestosterone on growth hormone and insulin-like growth factor-I in the treatment of short stature and delayed puberty. J Clin Endocrinol Metab 76:996–1001

Khorram O, Laughlin GA, Yen SS 1997 Endocrine and metabolic effects of long-term administration of [Nle27]growth hormone-releasing hormone-(1-29)-NH2 in age-advanced men and women. J Clin Endocrinol Metab 82:1472–1479

Klindt J, Ford JJ, Macdonald GJ 1990 Synergism of testosterone propionate with growth hormone in promoting growth of hypophysectomized rats: effect of sexual differentiation. J Endocrinol 127:249–256

Kojima M, Hosoda H, Date Y, Nakazato M, Matsuo H, Kangawa K 1999 Ghrelin is a growth-hormone-releasing acylated peptide from stomach. Nature 402:656–660

Kraicer J, Cowan JS, Sheppard MS, Lussier B, Moor BC 1986 Effect of somatostatin withdrawal and growth hormone (GH)-releasing factor on GH release in vitro: amount available for release after disinhibition. Endocrinology 119:2047–2051

Kulin HE, Samojlik E, Santen RJ, Santner S 1981 The effect of growth hormone on the Leydig cell response to chorionic gonadotropin in boys with hypopituitarism. Clin Endocrinol 15:463–472

Lin T, Haskell J, Vinson N, Terracio L 1986 Direct stimulatory effects of insulin-like growth factor 1 on Leydig cell steroidogenesis in primary culture. Biochem Biophys Res Commun 137:950–956

Loche S, Colao A, Cappa M et al 1997 The growth hormone response to hexarelin in children: reproducibility and effect of sex steroids. J Clin Endocrinol Metab 82:861–864

Locke W, Kirgis HD, Bowers CY, Abdoh AA 1995 Intracerebroventicular growth-hormone-releasing peptide-6 stimulates eating without affecting plasma growth hormone responses in rats. Life Sci 56:1347–1352

Martha PM Jr, Gorman KM, Blizzard RM, Rogol AD, Veldhuis JD 1992 Endogenous growth hormone secretion and clearance rates in normal boys as determined by deconvolution analysis: relationship to age, pubertal status and body mass. J Clin Endocrinol Metab 74:336–344

Mayo KE 1992 Molecular cloning and expression of a pituitary-specific receptor for growth hormone-releasing hormone. Mol Endocrinol 6:1734–1744

Müller EE, Locatelli V, Cocchi D 1999 Neuroendocrine control of growth hormone secretion. Physiol Rev 79:511–607

Mulligan T, Iranmanesh A, Kerzner R, Demers LW, Veldhuis JD 1999a Two-week pulsatile gonadotropin releasing hormone infusion unmasks dual (hypothalamic and Leydig cell) defects in the healthy aging male gonadotropic axis. Eur J Endocrinol 141:257–266

Mulligan T, Jaen-Vinuales A, Godschalk M, Iranmanesh A, Veldhuis JD 1999b Synthetic somatostatin analog (octreotide) suppresses daytime growth hormone secretion equivalently in young and older men: preserved pituitary responsiveness to somatostatin's inhibition in aging. J Am Geriatr Soc 47:1422–1424

Mulligan T, Kuno H, Clore J, Iranmanesh A, Veldhuis JD 2000 Pulsatile infusions of recombinant human LH in leuprolide-downregulated older vs young men unmask an impoverished Leydig-cell secretory response in aging to mid-physiological LH stimuli. Presented at the Endocrine Society 82nd Annual Meeting, Toronto, Canada, June 21–24 Abstract #249

Painson JC, Veldhuis JD, Tannenbaum GS 2000 Single exposure to testosterone in adulthood rapidly induces regularity in the growth hormone release process. Am J Physiol 278:E933–E940

Pellegrini E, Bluet-Pajot MT, Mounier F, Bennett P, Kordon C, Epelbaum J 1996 Central administration of a growth hormone (GH) receptor mRNA antisense increases GH pulsatility and decreases hypothalamic somatostatin expression in rats. J Neurosci 16:8140–8148

Pihoker C, Kearns GL, French D, Bowers CY 1998 Pharmacokinetics and pharmacodynamics of growth hormone-releasing peptide-2: a phase I study in children. J Clin Endocrinol Metab 83:1168–1172

Pincus SM, Gevers E, Robinson IC et al 1996 Females secrete growth hormone with more process irregularity than males in both humans and rats. Am J Physiol 270:E107–E115

Pincus SM, Veldhuis JD, Mulligan T, Iranmanesh A, Evans WS 1997 Effects of age on the irregularity of LH and FSH serum concentrations in women and men. Am J Physiol 273:E989–E995

Rosenthal SM, Hulse JA, Kaplan SL, Grumbach MM 1986 Exogenous growth hormone inhibits growth hormone-releasing factor-induced growth hormone secretion in normal men. J Clin Invest 77:176–180

Russell-Aulet M, Jaffe CA, Friberg RD, Barkan AL 1999 In vivo semiquantification of hypothalamic growth hormone-releasing hormone (GHRH) output in humans: evidence for relative GHRH deficiency in aging. J Clin Endocrinol Metab 84:3490–3497

Shah N, Anderson SM, Azimi PP, Evans WS, Bowers CY, Veldhuis JD 1998a Estradiol augments basal GH pulse mass and amplifies GH-secretory responsiveness to low doses of GHRP-2. Presented at the Fifth International Pituitary Congress, Naples, Florida, June 28–30

Shah N, Anderson SM, Azimi PP, Evans WS, Bowers CY, Veldhuis JD 1998b Growth hormone releasing peptide-2 (GHRP-2) induced growth hormone (GH) release is influenced by oral estrogen replacement in postmenopausal women. Presented at the Growth Hormone Research Society Conference, San Francisco, California, September 3–6

Shah N, Evans WS, Bowers CY, Veldhuis JD 1999a Tripartite neuroendocrine activation of the human growth-hormone (GH) axis in women by continuous 24-hour GH-releasing peptide infusion: pulsatile, entropic, and nyctohemeral mechanisms. J Clin Endocrinol Metab 84:2140–2150

Shah N, Evans WS, Veldhuis JD 1999b Actions of estrogen on the pulsatile, nyctohemeral, and entropic modes of growth hormone secretion. Am J Physiol 276:R1351–R1358

Shah N, Bowers CY, Veldhuis JD 1999c Pulsatile nyctohemeral and entropic modes of GH secretion after estradiol (E2), GHRP-2 or the combinatin in post-menopausal women. Presented at the Southern Regional Meeting of the American Federation of Medical Resarch, New Orleans, Louisiana, February 18–20

Shah N, Evans WS, Bowers CY, Veldhuis JD 2000 Oral estradiol administration modulates continuous intravenous growth hormone (GH)-releasing peptide-2 driven GH secretion in postmenopausal women. J Clin Endocrinol Metab 85:2649–2659

Smith RG, van der Ploeg LH, Howard AD et al 1997 Peptidomimetic regulation of growth hormone secretion. Endocr Rev 18:621–645

Snyder PJ, Peachey H, Hannoush P et al 1999 Effect of testosterone treatment on body composition and muscle strength in men over 65 years of age. J Clin Endocrinol Metab 84:2647–2653

Straume M, Chen L, Johnson ML, Veldhuis JD 1995 Systems-level analysis of physiological regulation interactions controlling complex secretory dynamics of growth hormone axis: a connectionist network model. Methods Neurosci 28:270–310

Sugihara H, Minami S, Wakabayashi I 1989 Post-somatostatin rebound secretion of growth hormone is dependent on growth hormone-releasing factor in unrestrained female rats. J Endocrinol 122:583–591

Tenover JS, Dahl KD, Vale WW, Rivler JE, Bremner W J 1990 Hormonal responses to a potent gonadotrophin hormone-releasing hormone antagonist in normal elderly men. J Clin Endocrinol Metab 71:881–888

Urban RJ, Bodenburg YH, Gilkison C et al 1995 Testosterone administration to elderly men increases skeletal muscle strength and protein synthesis. Am J Physiol 269:E280–E286

van Kesteren P, Lips P, Deville W et al 1996 The effect of one-year cross-sex hormonal treatment on bone metabolism and serum insulin-like growth factor-1 in transsexuals. J Clin Endocrinol Metab 81:2227–2232

Veldhuis JD 1996 Male hypothalamic-pituitary-gonadal axis. In: Lipshultz LI, Howards SS (eds) Infertility in the male. Mosby, Philadelphia, PA, p 23–58

Veldhuis JD, Giustina A 2000 Neuroendocrinology strategies for appraising suspected growth hormone deficiency in the adult. In: Loriaux LD (ed) The endocrinologist. Lippincott Williams & Wilkins, Baltimore, MD, vol 10:34–50

Veldhuis JD, Iranmanesh A, Ho KK, Waters MJ, Johnson ML, Lizarralde G 1991 Dual defects in pulsatile growth hormone secretion and clearance subserve the hyposomatotropism of obesity in man. J Clin Endocrinol Metab 72:51–59

Veldhuis JD, Liem AY, South S et al 1995 Differential impact of age, sex-steroid hormones, and obesity on basal versus pulsatile growth hormone secretion in men as assessed in an ultrasensitive chemiluminescence assay. J Clin Endocrinol Metab 80:3209–3222

Veldhuis JD, Metzger DL, Martha PM Jr et al 1997 Estrogen and testosterone, but not a non-aromatizable androgen, direct network integration of the hypothalamo-somatotrope (growth hormone)-insulin-like growth factor I axis in the human: evidence from pubertal pathophysiology and sex-steroid hormone replacement. J Clin Endocrinol Metab 82:3414–3420

Veldhuis JD, Evans WS, Shah N, Story S, Bray MJ, Anderson SM 1999a Proposed mechanisms of sex-steroid hormone neuromodulation of the human GH–IGF-I axis. In: Veldhuis JD, Giustina A (eds) Sex-Steroid interactions with growth hormone. Springer-Verlag, New York, p 93–121

Veldhuis JD, Iranmanesh A, Mulligan T, Pincus SM 1999b Disruption of the young-adult synchrony between luteinizing hormone release and oscillations in follicle-stimulating hormone, prolactin, and nocturnal penile tumescence (NPT) in healthy older men. J Clin Endocrinol Metab 84:3498–3505

Veldhuis JD, Roemmich JN, Rogol AD 2000a Gender and sexual maturation-dependent contrasts in the neuroregulation of growth hormone secretion in prepubertal and late adolescent males and females — a general clinical research center-based study. J Clin Endocrinol Metab 85:2385–2394

Veldhuis JD, Iranmanesh A, Godschalk M, Mulligan T 2000b Older men manifest multifold synchrony disruption of reproductive neurohormone outflow. J Clin Endocrinol Metab 85:1477–1486

Weltman A, Weltman JY, Hartman ML et al 1994 Relationship between age, percentage body fat, fitness and 24-hour growth hormone release in healthy young adults: effects of gender. J Clin Endocrinol Metab 78:543–548

Wennink JMB, Delemarre-van de Waal HA, Schoemaker R, Blaauw G, van den Braken C, Schoemaker J 1990 Growth hormone secretion patterns in relation to LH and testosterone secretion throughout normal male puberty. Acta Endocrinol 123:263–270

Wideman L, Weltman JY, Patrie JT et al 2000a Synergy of L-arginine and GHRP-2 stimulation of growth hormone in men and women: modulation by exercise. Am J Physiol 279:R1467–R1477

Wideman L, Weltman JY, Patrie JT et al 2000b Synergy of L-arginine and growth hormone (GH)-releasing peptide-2 on GH release: influence of gender. Am J Physiol 279:R1455–R1466

Zachmann M 1992 Interrelations between growth hormones and sex hormones: physiology and therapeutic consequences. Horm Res 38:1–8

DISCUSSION

Björntorp: You measured these things every second minute, and then you have a pulse of say LH. This then has to reach the testosterone-producing site, causing testosterone secretion. This then has to feedback. Is this actually happening? I can

understand the nervous circuit, because this is quick, but the circulatory circuit is slower.

Veldhuis: Yes, there is feedback. One of the things that is strangely lacking is the exact kinetics of the feedback time delays. I have spent a year reading 600 papers and cannot find exact time delays. We are going to use the drug ketoconazole, which blocks cytochrome P450 activity at high doses and thus steroidogenesis. One dose of this drug lowers testosterone concentrations overnight from 25 nM to about 3 nM. Now you have a testosterone-withdrawn state in which you can clamp feedback. At midnight, we start a constant testosterone infusion that is about one-third of the expected amount produced endogenously over the same time interval. At 8 a.m. the next morning we pulse in a 6 min waveform of testosterone and monitor feedback timing in young and older men. Currently, we are guessing feedback timing on the basis of cross-correlation data, which means that we take 15 older men and 15 younger men, and sample for LH and T simultaneously for 24 h. We then have paired series. We ask the question, 'whenever T goes down, how long does it take for LH to go up?' In the human, this negative feedback has about a 60–90 min delay in the young and 0–60 min delay in the older male. Feedback is occurring, but this is the only way that we have estimated how long it takes for the system to react.

Björntorp: You are not saying that the decrease of the LH peak is dependent on an immediate feedback.

Veldhuis: No, that is a good point — it is unlike the cortisol–adrenocorticotropic hormone (ACTH) axis where there is some evidence for that. This is not so rapid.

Björntorp: So the LH peaks are sort of automatic?

Veldhuis: That is what we believe, but we think the pulse generator frequency is under T control in the human.

Handelsman: It is a wonderfully subtle illustration of entropy. The power of the approach is enormous. I just wondered whether you have thought about this another way.

Veldhuis: This is the advantage of the current formulation by Steve Pincus, which we used in Pincus et al (1996). It is a lag-independent cross-approximate entropy (ApEn) metric. One of the disadvantages of simple linear cross-correlation is that it is assumed that each subject has roughly the same relationship between the two hormones in time, and also that within any one person there is a similar relationship across the day and night. This may not be true. When we run windows of cross-correlation, we find that the strength of feed-forward coupling varies across 24 h. It sounds intuitively obvious, but it is very clear for ACTH–cortisol and LH–T. The beauty of cross-ApEn is that it is lag-independent. It basically asks the question, given standardized z-score transforms of the original time series to make them scale independent, if there are some wiggles in the first series of hormones, do they ever happen in the second? If

they never do, they are not very synchronous. If they do happen a lot together, they are fairly synchronous. If they are happening almost all the time, they are highly synchronous. It matches templates up- and downstream independently of location.

Handelsman: One of the beauties of this technique is that it is model-independent. However, the difficulty is that it makes entropy seem such an abstract notion. To be a bit more concrete, in the studies where you deliberately gave LH pulses, how does that look under your model? Can you override the apparent age-related increase in entropy by administering clear and coherent LH pulses?

Veldhuis: We intend to look at this. When we use the GnRH pump in older men, we see a result that at first is counterintuitive. There is a more random output of LH in younger and older men under perfectly regular 90 min experimentally enforced pulsatile GnRH drive (Mulligan et al 1999). Why is this? We are actually monitoring minute-to-minute feedback activity between T and/or LH under the GnRH stimulus and not the 90 min pulse. This is a microanalysis that is checking feedback adjustment (Veldhuis 1999a). When the system is forced with fixed input, this abolishes feedback. Thus, ApEn of LH actually goes up on the GnRH pump in young men. The axis essentially becomes a clamped system with a non-dynamic quality of feedback. The feedback elegance is abolished by the clamp.

Handelsman: The physiological pharmacodynamic models of Jusko and colleagues are very similar; they are constructed of components like that. Are your thoughts going in this direction?

Veldhuis: This is the idea. We have a couple of papers out using testosterone, which took us five years to write, because there are surprisingly large subtleties in how to build a dose–response curve and prove that the set of equations is realizable mathematically (Keenan & Veldhuis 1998, 2001, Keenan et al 2000). There are some things that are produced only in the square root of minus one, which is an answer that has no utility to us as clinicians, being an undefined term. We have now done this for ACTH and we are just getting there for GH (Farhy et al 2001). The idea is only to take the core components. The first time we did this for GH, we tried to do it comprehensively (Straume et al 1995). We ended up with 87 parameters, and it may take a few dozen years for computers to be developed with the power to optimize this collection of parameters. Now we are down to 12 parameters for GH and about 10–12 for LH. Without infusing LH, I'd like to know that the older Leydig cell of the mouse, human or rat is unresponsive to LH. How do I do that? I have to watch LH and T move together in young and older men and calculate the endogenous dose–response curve, without ever seeing it. I can't do that without a correct statement of how the dose–response curve primarily operates. If I can do that, I can tell you the dose–response curve without injecting anything.

Robertson: I'm not as familiar with chaos theory applied to the endocrine system as I am for example with its application to heart rate. But looking at your data I

would never have guessed you would be able to account for nearly everything that you see by the interactions of these two variables. What percentage of influences do you think are outside this paradigm?

Veldhuis: That is a gorgeous question: I just wish you had been a reviewer. A reviewer once said that we didn't need any stochastic, random element that is unexplained, but that we should just draw the correct feedback loop. My response was that nothing is absolutely constant. If I stand up, my LH distribution volume is slightly different. If I walk, my testis bloodflow is changed. Everything is changing slightly outside the idealized dose–response. The aggregate uncertainty can be instilled in a stochastic differential equation. Our stochastic term is only 2–3% of the data, but it is critical. Where is the stochastic element? Firstly, the pulse generator doesn't fire exactly as a clock ticks. We allow it some variability (Veldhuis 1999a, Urban et al 1988). Second, we say that the feedback equations we are talking about are idealized equations. They are never observed. The feedback equations are dancing slightly, but at all times. We put in a little stochastic term to allow the parameters of the feedback equations to jiggle by 2–3%. This gives our realistic data profiles. Otherwise one observes gorgeous curves that you and I only see one in 20 profiles.

Handelsman: This is such a highly deterministic system. One feature is that you can explain virtually all T secretion by LH pulsatility as shown by the fact that you can switch it completely off with antagonists or steroids. This makes it reasonable to say that you can predict nearly all the components, because we can easily identify proximate determinants that can switch it completely off. This isn't true for most other physiological systems, where there is a very lower proportion of variance explained.

Veldhuis: It is strongly deterministic with just 2–3% stochastic. This fits with the fact that we are not a couple of molecules reacting in free solution.

Giustina: One point we know from the ageing GH axis is that ageing is interacting with obesity in creating a loss of GH secretion. What about your model of LH and testosterone in obesity?

Veldhuis: All we know is that in general the literature agrees that LH pulse amplitude is damped in some manner by visceral obesity in particular. Most studies show this effect to be quite strong, and at least as strong as the age effect in middle age. What isn't clear to me is what is mediating this effect. It could be the insulin levels.

Shalet: Where does oestradiol fit in to this?

Veldhuis: That is a frightening question because the situation is getting more and more complicated. The oestrogen receptor knockout or the aromatase gene-defective animal have about a doubling of LH output. We find the same with the drug anastrozole, an aromatase inhibitor. *In situ* aromatase activity appears to be important in negative feedback. It isn't clear whether this is controlling only

amplitude or frequency. We have had to study 31 men to try to get a clear answer. We have used three different pulse methods to see whether we can get a consistent opinion on it. The amplitude clearly changes, so there is amplitude drive. I had been puzzled why men show an increased LH pulse frequency on clomifene or tamoxifen, both antioestrogens, but when you infuse peripheral oestrogen in the human, you can almost never demonstrate a suppression of LH pulse frequency (Veldhuis et al 1984, Veldhuis & Dufau 1987, Urban et al 1988). The exception is a study in which we put an oestrogen-containing silastic ring intravaginally in postmenopausal women, delivering oestradiol (Veldhuis et al 1987). There, on day 5, LH pulse frequency fell with amplitude and then recovered. In the monkey, one can infuse peripheral oestrogen, which decreases hypothalamic multiunit firing within minutes. But, excluding those exceptions, people cannot readily demonstrate oestradiol negative feedback on frequency in the male. This is puzzling. Is it the *in situ* hypothalamic oestradial that suppresses the pulse generator frequency? As far as I can tell, this would explain the tamoxifen/clomifene and anastrozole data. These drugs would block oestradiol produced in the hypothalamus. This would also explain the fact that peripheral oestradiol infusion, in almost everybody's hands, mainly blocks GnRH-driven LH amplitude at the pituitary level. The aromatase inhibitor study will be key to try to dissect whether that is true.

Laron: What down-regulates prolactin?

Veldhuis: I would love to know. Older men are hypoprolactinaemic (Iranmanesh et al 1999). So are type I diabetic patients (Iranmanesh et al 1990). There are a collection of curious situations where there is reversible hypoprolactinaemia. But I don't know other data that give us clear evidence for dopamine excess.

Müller: There could be an up-regulation of the dopamine receptor.

Veldhuis: That would be beautiful.

Müller: We have data from experiments in older rats showing increased pituitary sensitivity to the prolactin-lowering effect of bromocriptine (Cocchi et al 1984). Moreover in aged female rats presenting with marked alterations in the tuberoinfundibular dopaminergic neuronal function, pituitary binding sites for [^{3}H] spiroperidol, a neuroleptic, are increased (Govoni et al 1980).

Laron: This relates to my first question: how much of the dose–response is actually linked to the number of receptors?

Veldhuis: The data in the rat do not consistently show loss of GnRH receptor or GnRH activity with ageing.

Wang: The GnRH responsiveness is normal in old rats.

Veldhuis: In our hands it is also normal to enhanced in the human (Mulligan et al 1999, Zwart et al 1996).

Morley: Johannes, you have done a great job of trying to take the complex and make it intelligible. Unfortunately, I prefer complexity. As I look at the literature,

when we start to look closely, young and old are not young and old: there are at least four separate phases. There is the 20–32 year old who we could call normal young. Somewhere after 32 we start to lose some testosterone in almost all studies. When you get to early middle age (40–55), there are some hints that there is excess opioid secretion at this stage and these people are very responsive to opiate antagonism in their LH levels. Then we get the group you have been talking about, the 60–75 year olds. In longitudinal studies by ourselves and others, when you get to 80+ the LH suddenly takes off and gets to 25–30 IU/L. These are clearly a different group. I guess the question is, how do you put this together with the entropy and what causes that sudden release in very old age to going from a quasi secondary hypogonadism to a primary hypogonadism?

Veldhuis: That is why I was so excited by Christine Wang's data suggesting that there may be some fixed GnRH defect. I had assumed that the late LH rise was due to end-stage Leydig cell failure (Veldhuis et al 1999b). Of course, no one has done the kind of near-physiological drive of the Leydig cells that modern tools allow us to do. The idea of formalizing several levels in the axis that are points of lesioning in ageing is that you can then test them and their implications. Certain nodes clearly don't lead to exactly what you predict. The reason is that if there is a non-linear interactive, time-delayed system, intuition is stymied (Keenan & Veldhuis 1998, 2001, Keenan et al 2000). However, what we thought we would do is create a model in which we let the computer run overnight, producing a seven year lifespan. We let it gradually trickle down one of the feedback constants, and then introduce a couple of lesions on top of that. This gives an idea of whether it is possible to unmask the phases. The other real challenge is the between-individual variability. There you have individuals who appear to be absolutely normal in their entropy scores, and yet their pulse generator looks awfully good.

Morley: Could you go back and look at the people you studied a long time ago? This is probably the key to understanding all this.

Veldhuis: Believe it or not, since you like complexity, I will soon have data of 10 min LH ApEn analyses collected for four consecutive days, to watch the pulse generator unfold and fluctuate over the day and night. We intend to compare this in older and young men. We postulate that as one gets into these metastable conditions (by which I mean that they are not absolutely normal, but they are not pathological), the stability of the pulse generator over four days will be degraded (Pincus et al 1996). We may be wrong, and it may prove to be even more stable in the elderly, which would be a more exciting paper.

References

Cocchi D, Novelli A, Ganzetti I, Müller EE 1984 In vivo supersensitivity of the anterior pituitary of old female rats to dopaminergic inhibition of prolactin secretion. Gerontology 30:345–349

Farhy LS, Straume M, Johnson ML, Kovatchev BP, Veldhuis JD 2001 A construct of interactive feedback control of the GH axis in the male. Am J Physiol 281:R38–R51

Govoni S, Memo M, Saiani L, Spano PF, Trabucchi M 1980 Impairment of brain neurotransmitter receptor in aged rats. In: Mechanisms of aging and development, chapter 1–2. Elsevier Sequoia, Lausanne, p 39–46

Iranmanesh A, Veldhuis JD, Carlson EC et al 1990 Attenuated pulsatile release of prolactin in men with insulin-dependent diabetes mellitus. J Clin Endocrinol Metab 71:73–78

Iranmanesh A, Mulligan T, Veldhuis JD 1999 Mechanisms subserving the physiological nocturnal relative hyperprolactinaemia of healthy older men: dual decline in prolactin secretory burst mass and basal release with preservation of pulse duration, pulse frequency, and interpulse interval: a general clinical research center study. J Clin Endocrinol Metab 84:1083–1090

Keenan DM, Veldhuis JD 1998 A biomathematical model of time-delayed feedback in the human male hypothalamic–pituitary–Leydig cell axis. Am J Physiol 275:E157–E176

Keenan DM, Veldhuis JD 2001 Hypothesis testing of the aging male gonadal axis via a biomathematical construct. Am J Physiol Regul Integr Comp Physiol 280:R1755–R1771

Keenan DM, Sun W, Veldhuis JD 2000 A stochastic biomathematical model of the male reproductive hormone system. SIAM J Appl Math 61:934–965

Mulligan T, Iranmanesh A, Kerzner R, Demers LW, Veldhuis JD 1999 Two-week pulsatile gonadotropin releasing hormone infusion unmasks dual (hypothalamic and Leydig cell) defects in the healthy aging male gonadotropic axis. Eur J Endocrinol 141:257–266

Pincus SM, Mulligan T, Iranmanesh A, Georghiu S, Godschalk M, Veldhuis JD 1996 Older males secrete luteinizing hormone and testosterone more irregularly, and jointly more asynchronously, than younger males. Proc Natl Acad Sci USA 93:14100–14105

Straume M, Chen L, Johnson ML, Veldhuis JD 1995 Systems-level analysis of physiological regulation interactions controlling complex secretory dynamics of growth hormone axis: a connectionist network model. Methods Neurosci 28:270–310

Urban RJ, Evans WS, Rogol AD, Kaiser DL, Johnson ML, Veldhuis JD 1988 Contemporary aspects of discrete peak detection algorithms. I. The paradigm of the luteinizing hormone pulse signal in men. Endocr Rev 9:3–37

Veldhuis JD 1999a Male hypothalamic-pituitary-gonadal axis. In: Yen SSC, Jaffe RB, Barbieri RL (eds) Reproductive Endocrinology. W.B. Saunders Co., Philadelphia p 622–631

Veldhuis JD 1999b Recent insights into neuroendocrine mechanisms of aging of the human male hypothalamo-pituitary-gonadal axis. J Androl 20:1–17

Veldhuis JD, Dufau ML 1987 Estradiol modulates the pulsatile secretion of biologically active luteinizing hormone in man. J Clin Invest 80:631–638

Veldhuis JD, Rogol AD, Samojlik E, Ertel NH 1984 Role of endogenous opiates in the expression of negative feedback actions of androgen and estrogen on pulsatile properties of luteinizing hormone secretion in man. J Clin Invest 74:47–55

Zwart AD, Urban RJ, Odell WD, Veldhuis JD 1996 Contrasts in the gonadotropin-releasing dose–response relationships for luteinizing hormone, follicle-stimulating hormone, and α subunit release in young versus older men: appraisal with high specificity immunoradiatric assay and deconvolution analysis. Eur J Endocrinol 135:399–406

Endocrine Facets of Ageing.
Novartis 242

Print ISBN 0-471-48636-1 Online ISBN 0-470-84654-2

Effects of growth hormone and insulin-like growth factor 1 deficiency on ageing and longevity

Zvi Laron

Endocrinology & Diabetes Research Unit, Schneider Children's Medical Center of Israel and Sackler Faculty of Medicine, Tel Aviv University, Israel

Abstract: Present knowledge on the effects of growth hormone (GH)/insulin-like growth hormone (IGF)1 deficiency on ageing and lifespan are reviewed. Evidence is presented that isolated GH deficiency (IGHD), multiple pituitary hormone deficiencies (MPHD) including GH, as well as primary IGF1 deficiency (GH resistance, Laron syndrome) present signs of early ageing such as thin and wrinkled skin, obesity, hyperglycemia and osteoporosis. These changes do not seem to affect the lifespan, as patients reach old age. Animal models of genetic MPHD (Ames and Snell mice) and GH receptor knockout mice (primary IGF1 deficiency) also have a statistically significant higher longevity compared to normal controls. On the contrary, mice transgenic for GH and acromegalic patients secreting large amounts of GH have premature death. In conclusion longstanding GH/IGF1 deficiency affects several parameters of the ageing process without impairing lifespan, and as shown in animal models prolongs longevity. In contrast high GH/IGF1 levels accelerate death.

2002 Endocrine facets of ageing. Wiley, Chichester (Novartis Foundation Symposium 242) p 125–142

In contrast to growth and development, ageing is a progressive process orchestrated by decreasing synthesis and secretion of numerous factors and hormones; among them growth hormone (GH) and its anabolic effector hormone, insulin-like growth factor 1 (IGF1). Therefore, ageing is often compared with growth hormone deficiency (GHD) (Toogood & Shalet 1998). This assumption is based on the evidence that pituitary GH secretion and serum IGF1 concentrations decline with increasing age (Gil-Ad et al 1984, Arvat et al 2000), reaching low levels in late adulthood, and have similarities to changes of body appearance, composition and function (Carroll et al 1998, Toogood & Shalet 1998) (Table 1). These findings led to trials of GH treatment in elderly people (Rudman et al 1990). The finding that GH increased lean body mass, decreased adiposity and improved apparent skin changes gave birth to an

TABLE 1 Similarities between GH deficiency and ageing

Thinning of skin (wrinkling)
Excess adipose tissue (obesity)
Rise in insulin resistance (tendency for diabetes)
Decline in β cell function
Reduced lean body (muscle) mass
Reduced physical performance
Reduced mineral density (osteoporosis)
Lowered venous access
— — — — — — — — — — — —
Rise in serum cholesterol

approved (Butterfield et al 1997) and non-approved administration of GH to ageing people, at 'so-called' rejuvenation clinics. These medical acts were reinforced by reports that GH deficiency increases the risk for cardiovascular disease (Rosén et al 1993) and leads to premature mortality (Rosén & Bengtsson 1990).

In order to analyse the present knowledge on the possible role of GH and IGF1 in ageing and lifespan, this paper reviews states of congenital (i.e. primary) GH and/or IGF1 deficiency in humans and animals. Attention is paid as to whether patients or animals with GH/IGF1 deficiency present early signs of ageing and effects on the duration of their lifespan.

Congenital isolated GH deficiency (IGHD)

Previously called idiopathic GHD, modern laboratory technology has shown that this state can be caused by molecular defects of the GH releasing hormone (GHRH) gene or receptor, or the hGH gene (Laron 2001). These patients are presently diagnosed at an early age and treated with the unlimited amounts of available biosynthetic hGH. Therefore, there is little information on studies of adult patients with isolated GH deficiency (IGHD). In 1969 Merimee and colleagues reported 31 patients with hereditary IGHD, whose age ranged from 13–78 years (Merimee et al 1969). Among the clinical descriptions Merimee & Laron (1996) wrote that wrinkling of the skin often began early in life and these patients consequently looked prematurely old (Table 2). Ten out of 13 males and 9 of 18 females had wrinkled skin. Rimoin et al (1966) performed skin biopsies and found decreased soluble collagen in the dermis of two thirds of these patients. The histological changes found were probably the underlying cause of thinning and

TABLE 2 Clinical characteristics of adult patients with untreated IGHD

n = 31	*13 males*	*18 females*
Age (years)		13–78
Height (cm)		110–140
Wrinkled skin	10	9
High pitched voice	12	5

Based on data from Merimee & Laron (1996).

wrinkling of the skin, characteristic of GH/IGF1 deficiency, and these in turn are caused by the lack of anabolic effect on collagen and hydroxyproline of these hormones. The only other histological data on the skin have been obtained by Abramovici et al (1983) who studied the skin biopsies of 35 children and adolescents including 18 with IGHD. These latter investigators found that patients with IGHD lack elastic fibres in the skin papillary layer and an uneven distribution of elastic fibres in the reticular layer.

Congenital multiple pituitary hormone deficiencies (MPHD) including growth hormone

In 1988, we had the opportunity to examine six out of 10 living dwarfed patients, part of 24 related patients recorded on the island of Krk in the Adriatic Sea. They belong to two villages near to each other, and were known to have existed since the end of the 19th century (Hanhart 1925). DNA from these patients revealed a mutation in the *PROP1* gene (a transcription factor) causing MPHD (thyroid-stimulating hormone, prolactin, luteinizing hormone, follicle-stimulating hormone and GH deficiencies) (Krzisnik et al 1999). They were treated by thyroxine, which some took irregularly. Only one 14 year old girl received GH. The five adult patients ranged from 47 to 68 years (3 males, 2 females). In addition to short stature (120–139 cm), they were obese, sexually immature and had a very wrinkled skin (Fig. 1). Notably the patients did not have any grey hair despite their advanced adult age. This was also seen on a picture of a 70 year old patient found on his tombstone (Fig 2). The information obtained for four deceased patients revealed that they had died at ages 68, 77, 83 and 91.

Laron syndrome (primary GH resistance)

The following describes adult patients with primary IGF1 deficiency due to primary GH resistance or insensitivity (i.e. Laron syndrome). In 1966 and in 1968 our group described a new hereditary syndrome resembling IGHD, but

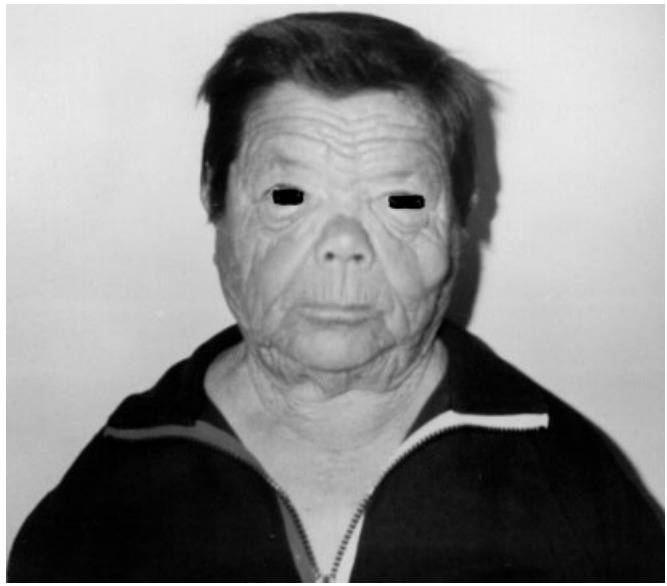


FIG. 1. Appearance of a 58 year old patient with GH deficiency due to a *PROP1* gene mutation. Note wrinkled and loose skin.

FIG. 2. Appearance of a 70 year old patient with GH deficiency due to a *PROP1* mutation. Note absence of grey hair and loose skin. For details see text. Reproduced with permission from Krzisnik et al (1999).

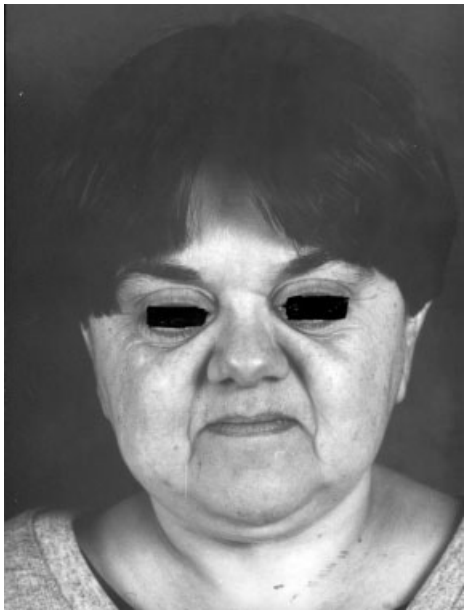


FIG. 3. Early ageing appearance of a 39 year old female with Laron syndrome.

with very high serum hGH levels (Laron et al 1968). Since then we have been following in Israel a cohort of 51 patients from infancy to adulthood (Laron 1999a, Laron & Parks 1993). Since the first description several hundred patients, or their descendants, have been described with Laron syndrome, mainly in Mediterranean and Mid-Eastern populations (Rosenfeld et al 1994, Laron 1999a). This syndrome is caused by deletions or mutations in the GH receptor or postreceptor pathways (Godowski et al 1989, Amselem et al 1996, Laron 1999a, 1999b), leading to an inability by the liver and possible other tissues to generate IGF1 (Laron et al 1971), the anabolic effector hormone of GH (Laron 1999c). Studying adult patients with Laron syndrome (Laron & Klinger 1993, 1994, Laron 1999b) we observed that these patients remain very short (females, 108–136 cm; males, 119–142 cm; adult height), have an early ageing appearance (such as a wrinkled face at an early adult age; Fig. 3), and relatively thin skin on their hands. Abramovici et al (1983) performed skin biopsies in six children and late adolescents and found that patients with Laron syndrome had bundles of thickened elastic fibres in the upper dermis.

Even young adult patients presenting with Laron syndrome develop marked general and visceral obesity (Fig. 4), high cholesterol levels (Laron & Klinger 1993), reduced muscular strength (Brat et al 1997), insulin resistance (Laron et al

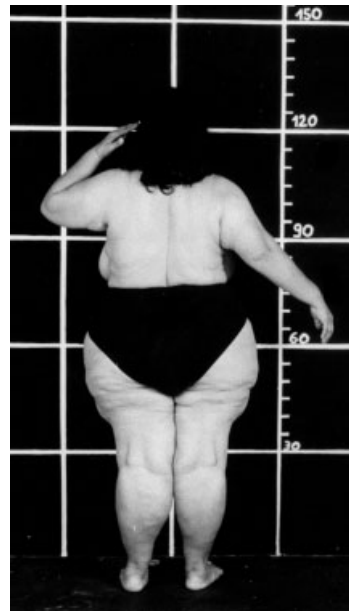


FIG. 4. Marked obesity in a 41 year old female with Laron syndrome.

1997), osteoporosis (Laron & Klinger 1994), and/or suffer from psychological deficiencies (Galatzer et al 1993), all features characteristic for normal ageing and usually apparent at a later age. The oldest patient followed by us is a 73 year old male; one lady examined by us only once and suspected (but not proven) to have Laron syndrome died at age 53. She had suffered from asthma and coronary heart disease (Laron 1999b). Also, adult patients in the large Ecuadorian cohort of Laron syndrome patients have been reported to have reached ages of 70 years or more (Rosenbloom et al 1999). It is of note that with one exception none of our adult patients has grey hair. However, they have a tendency for baldness (in males) and thin hair (in females) (Laron et al 2001).

In conclusion, the relatively small number of adult patients with IGHD or MPHD never previously treated with GH, as well as patients with primary IGF1 deficiency (Laron syndrome) not treated by IGF1, show a series of early developing characteristics compatible with ageing such as thinning and wrinkling of skin, obesity, muscle weakness, osteoporosis and hyperlipidermia.

In contradistinction to the postulation of Rosén & Bengtsson (1990) that hypopituitary patients have premature mortality due to cardiovascular disease (Rosén et al 1993), the patients with GH and IGF1 deficiency live a long life, despite the signs of early ageing. One big difference between our patients and

those reported by Rosén et al (Rosén & Bengtsson 1990, Rosén et al 1993) is that almost all of those reported by Rosén and colleagues had tumours, mostly pituitary adenomas, and were treated either by surgery or irradiation; all were MPHD and received a combination of hormone replacement treatments with the exception of GH. Therefore, those patients cannot be compared to the patients with IGHD and/or IGF1 deficiency, and the statement that GH or IGF1 deficiency shortens the lifespan seems incorrect.

A review of animal studies using models of GH or IGF1 deficiency also revealed that the lifespan in these animals is prolonged compared to intact animals.

Ageing and lifespan in GH/IGF1-deficient mice

Several mouse models with GH/IGF1 deficiency are available to study the influence of these hormones on ageing and longevity (lifespan). Due to the IGF1 deficiency all homozygous affected mice are dwarfed and they divide as the human models into MPHD including GH (e.g. the Ames dwarf mice and the Snell dwarf mice) and primary IGF1 deficiency (the Laron mouse).

The Ames dwarf mice (df/df)

These mice first described by Schaible & Gowen (1961) have a mutation in a transcription factor for all anterior pituitary hormones (GH, prolactin, thyroid-stimulating hormone and sex hormones) called Prophet of Pit.1 (Prop-1) (Sornson et al 1996), which is located on chromosome 11. Homozygous mice for the df/df mutation are dwarfed, and have a longer life span than control animals, which is not related to caloric intake or the reduced body temperature (Bartke 1998).

Snell dwarf mice (dw/dw)

Described in 1929 this type of dwarfed mouse has been subsequently shown to be caused by a mutation of the transcription factor Pit-1 (Li et al 1990) which is involved in the differentiation of somatotrophs, lactotrophs and thyrotrophs (Sornson et al 1996). Phenotypically, the Ames and Snell dwarfed mice are very similar with the exception that in Snell mice the gonadal development is more advanced.

Snell mice have extremely low serum levels of IGF1 (van Buul Offers et al 1986). They have also been described to have delayed ageing and a longer lifespan than normal animals from the same strain (Bartke 2000).

The ageing symptoms of these two types of MPHD mice are their retarded sexual development, reduced activity (not in all), progressive obesity and hair

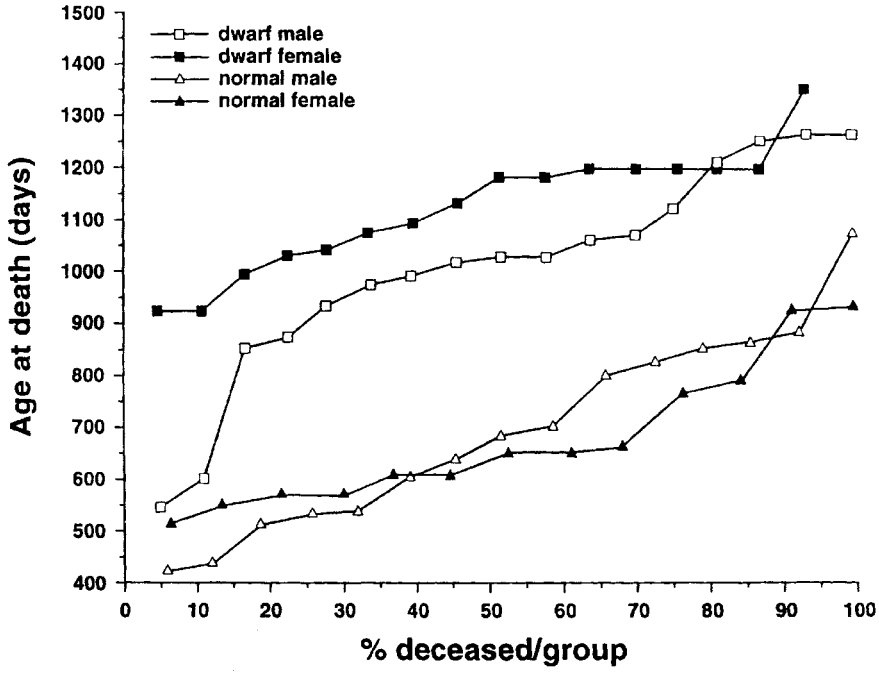


FIG. 5. Increased longevity in Ames dwarfed mice compared to normal controls of the same breed. Reproduced with permission from Bartke (2000).

loss (in part of the animals). Nevertheless, these animals appear to remain in excellent general condition for longer periods than their normal siblings (Bartke 2000). A group of Ames dwarfed mice outlived a control group of normal mice by more than one year (Bartke 2000) (Fig. 5). This extension of lifespan was longer in female mice.

The GH receptor/BP gene-disrupted mice (the Laron mouse)

A model of isolated IGF1 deficiency was created by disrupting the GH receptor (GHR) gene in mice (Zhou et al 1997). This model bears many similarities to the human primary GH resistance (GH insensitivity, i.e. Laron syndrome) (Kopchick & Laron 1999), such as high GH and low IGF1 and IGF binding protein (IGFBP)3 levels, dwarfism and organomicria, typical characteristics of Laron syndrome (Laron 1999b). Calculating average lifespans for each genotype (+/+, +/−, −/−) and gender, we observed that the homozygous mice for the GHR mutation had a significantly longer lifespan than the unaffected and heterozygote mice (Coschigano et al 2000) (Table 3).

TABLE 3 Lifespan of GHR/BP gene-disrupted mice

Gender	*Genotype*	*n*	*Lifespan (days)**
Males	+/+	7	629 ± 72
	+/−	8	668 ± 51
	−/−	7	975 ± 106[a]
Females	+/+	13	749 ± 41
	+/−	19	701 ± 36
	−/−	11	1031 ± 41[b]

*Mean SE.
[a]$P < 0.02$ compared to +/+.
[b]$P < 0.005$ compared to +/+.
Reproduced with permission from Coschigano et al (2000).

GH transgenic mice

In contrast to the previously described observations, prolonged elevation of serum GH, as occurs in GH transgenic mice, is associated with a reduced lifespan (Bartke 1998), which may reach half of that in normal mice of the same species. This is similar to findings in patients with acromegaly. Thus, the question arises whether high levels of GH increase mortality. In effect, treatment of rats with high doses of GH accelerates the death of the animals (Groesbeck et al 1987). Although the conditions may be different, one should remember that GH treatment of chronically ill patients in intensive care units was also found to increase mortality (Takala et al 1999).

At present it is not clear how GH/IGF1 deficiency prolongs the lifespan in mice. It is possible that certain genes are involved. Mutations of a recently described insulin receptor like-gene, *Daf2*, result in increased longevity (Kimura et al 1997). This receptor, possibly homologous to the mammalian IGF1 receptor, mimics primary IGF1 deficiency. Nor do we fully understand how GH excess shortens the lifespan. This may be partly due to the water and electrolyte retention induced by GH/IGF1 and/or by the well-documented cardiotrophic effects of these hormones.

Although it may sound anecdotal it should be mentioned that there is evidence that within species, lifespan is negatively correlated with body size. Thus dogs from small breeds live longer than dogs from large breeds and small mice live longer than large mice (Bartke 2000). Last but not least, food-restricted animals (which are smaller), live longer than those fed *ad libitum* (Masoro 1992), with the exception of Ames and Snell mice.

The premature ageing syndromes

It was of interest to find out whether the rare genetic disorders known as premature ageing syndromes (Pesce & Rothe 1996) are related to a disorder in the secretion of GH or IGF1. All are characterized by marked growth retardation associated with early and fast ageing, various dermal changes (wrinkling, loose skin), hypotrichosis and early greying of the hair, and early death mostly by heart attacks due to atherosclerosis or congestive heart failure.

Progeria (Hutchinson–Gilford disease)

First described in 1886 by Hutchinson, the incidence is estimated at 1 per million live births. The syndrome is characterized by increased hyaluronic acid excretion. We found only one report by Villee et al (1969) who studied two boys with classical progeria and found lack of GH response to insulin-induced hypoglycemia.

Werner's syndrome (adult progeria)

First described in 1904 this disease is clinically characterized by short stature, skin changes (soft tissue wasting ulcerated hyperkeratosis, pigmentation) fine hair and alopecia; early greying of hair and a tendency to malignancies and insulin-resistant diabetes. There is only one report on GH deficiency in Werner syndrome (Rubin & Reed 1996).

Cockayne's syndrome

Reported in 1936 and 1946 by Cockayne, it is characterized by dwarfism associated with retinal atrophy and deafness as well as skin atrophy. Endocrine function in a group of patients revealed normal GH response to stimulation tests in eight patients, decreased response in four, and an exaggerated response in three children (Nance & Berry 1992).

Bloom syndrome

Bloom syndrome is a rare autosomal recessive disorder characterized by growth deficiency, skin changes, photosensitivity, variable degrees of immunodeficiency, predisposition to malignancies, type 2 diabetes and early death. It is caused by mutations in the gene BLM. Growth retardation is a major characteristic of Bloom syndrome but GH deficiency has so far not been documented.

Rothmund–Thomson syndrome

This disease first described in 1986 is a rare autosomal recessive disorder characterized by short stature, skin changes (consisting of atrophy and telangiectasis) and hair loss (Kaufmann et al 1986). The skin changes resemble Bloom syndrome. Kaufmann et al (1986) reported GH deficiency in an 11 year old girl when tested by arginine, L-Dopa and GH-releasing hormone (GRF 1–44). No similar reports have come to our attention.

In summary, the finding of only very few patients with GH deficiency among the patients with 'premature ageing syndromes' of genetic origin, the majority of whom have normal pituitary functions, indicates that their accelerated ageing and various complications are not related to GH or IGF1. The rare instances of GH deficiency must be considered coincidental.

Conclusion

From the clinical and experimental studies reviewed it ensues that longstanding GH/IGF1 deficiency of genetic origin does not shorten lifespan. On the contrary, it may prolong it, as is clearly evident from animal models. This occurs simultaneously with the development of some characteristic changes of early ageing (thinning of skin, wrinkling, obesity, reduction in lean body mass) but arrest of other signs, such as greying of hair. It has also been shown that high levels of GH accelerate death. How exactly GH and IGF1 affect the ageing process and duration of life remains to be established.

References

Abramovici A, Josefsberg Z, Mimouni M, Liban E, Laron Z 1983 Histopathological features of the skin in hypopituitarism and Laron-type dwarfism. Isr J Med Sci 19:515–519

Amselem S, Sobrier ML, Dastot F, Duquesnoy P, Duriez B, Goossens M 1996 Molecular basis of inherited growth hormone resistance in childhood. Baillière's Clin Endocrinol Metab 10:353–369

Arvat E, Broglio F, Ghigo E 2000 Insulin-like growth factor I: implications in aging. Drugs Aging 16:29–40

Bartke A 1998 Growth hormone and aging. Endocrine 8:103–108

Bartke A 2000 Delayed aging in Ames dwarf mice. Relationships to endocrine function and body size. In: Hekimi (ed) The molecular genetics of aging. Results and problems in cell differentiation. Springer-Verlag, Berlin, vol 26:181–202

Brat O, Ziv I, Klinger B, Avraham M, Laron Z 1997 Muscle force and endurance in untreated and human growth hormone or insulin-like growth factor-I-treated patients with growth hormone deficiency or Laron syndrome. Horm Res 47:45–48

Butterfield GE, Thompson J, Rennie MJ, Marcus R, Hintz RL, Hoffman AR 1997 Effect of rhGH and rhIGF-I treatment on protein utilization in elderly women. Am J Physiol 272:E94–E99

Carroll PV, Christ ER, Bengtsson BA et al 1998 Growth hormone deficiency in adulthood and the effects of growth hormone replacement: a review. Growth Hormone Research Society Scientific Committee. J Clin Endocrinol Metab 83:382–395

Coschigano KT, Clemmons D, Bellush LL, Kopchick J J 2000 Assessment of growth parameters and life span of GHR/BP gene-disrupted mice. Endocrinology 141:2608–2613

Galatzer A, Aran O, Nagelberg N, Rubitzek J, Laron Z 1993 Cognitive and psychosocial functioning of young adults with Laron syndrome. In: Laron Z, Parks JS (eds) Pediatric and adolescent endocrinology, vol 24: Lessons from Laron syndrome (LS) 1966–1992. Karger, Basel, p 53–60

Gil-Ad I, Gurewitz R, Marcovici O, Rosenfeld J, Laron Z 1984 Effect of aging on human plasma growth hormone response to clonidine. Mech Ageing Dev 27:97–100

Godowski PJ, Leung DW, Meacham LR et al 1989 Characterization of the human growth hormone receptor gene and demonstration of a partial gene deletion in two patients with Laron-type dwarfism. Proc Natl Acad Sci USA 86:8083–8087

Groesbeck MD, Parlow AF, Daughaday WH 1987 Stimulation of supranormal growth in prepubertal adult plateaued and hypophysectomized female rats by large doses of rat growth hormone: physiological effects and adverse consequences. Endocrinology 120:1963–1975

Hanhart E 1925 Uber heredodegenerativen Zwergwuchs mit Dystrophia adiposo-genitalis. Arch Julius Klaus Stift Vererbungsforsch Sozialanthropol Rassenhyg 1:235–253

Kaufmann S, Jones M, Culler FL, Jones KL 1986 Growth hormone deficiency in the Rothmund-Thomson syndrome. Am J Med Genet 23:861–868

Kimura KD, Tissenbaum HA, Liu Y, Ruvkun G 1997 *daf-2,* an insulin receptor-like gene that regulates longevity and diapause in *Caenorhabditis elegans*. Science 277:942–946

Kopchick JJ, Laron Z 1999 Is the Laron mouse an accurate model of Laron syndrome? Mol Genet Metab 68:232–236

Krzisnik C, Kolacio Z, Battelino T, Brown M, Parks JS, Laron Z 1999 The 'Little People' of the Island of Krk — revisited. Etiology of hypopituitarism revealed. J Endocrine Genet 1:9–19

Laron Z 1999a Laron syndrome: primary growth hormone resistance. In: Jameson JL (ed) Hormone resistance syndromes. Contemporary endocrinology. Humana Press, Clifton, NJ, vol 2:17–37

Laron Z 1999b Natural history of the classical form of primary growth hormone (GH) resistance (Laron syndrome). J Pediatr Endocrinol Metab 12:231–249

Laron Z 1999c Somatomedin-1 (recombinant insulin-like growth factor-1): clinical pharmacology and potential treatment of endocrine and metabolic disorders. BioDrugs 11:55–70 (erratum: 1999 BioDrugs 11:114)

Laron Z 2001 Molecular mutations in the human growth hormone axis. In: Eugster EA, Pescovitz OH (eds) Developmental endocrinology from research to clinical practice. Humana Press, Clifton, NJ

Laron Z, Klinger B 1993 Body fat in Laron syndrome patients: effect of insulin-like growth factor I treatment. Horm Res 40:16–22

Laron Z, Klinger B 1994 IGF-I treatment of adult patients with Laron syndrome: preliminary results. Clin Endocrinol 41:631–638

Laron Z, Parks JS 1993 Pediatric and adolescent endocrinology, vol 24: Lessons from Laron syndrome (LS) 1966–1992. Karger, Basel

Laron Z, Pertzelan A, Karp M 1968 Pituitary dwarfism with high serum levels of growth hormone. Isr J Med Sci 4:883–894

Laron Z, Pertzelan A, Karp M, Kowadlo-Silbergeld A, Daughaday WH 1971 Administration of growth hormone to patients with familial dwarfism with high plasma immunoreactive growth hormone: measurement of sulfation factor, metabolic and linear growth responses. J Clin Endocrinol Metab 33:332–342

Laron Z, Avitzur Y, Klinger B 1997 Insulin resistance in Laron syndrome (primary insulin-like growth factor-I (IGF-I) deficiency) and effect of IGF-I replacement therapy. J Pediatr Endocrinol Metab 10:105–115

Laron Z, Ben-Amitai D, Lurie R 2001 Impaired hair growth and structural defects in patients with Laron syndrome (Primary IGF-I deficiency). Pediat Res 49:76A

Li S, Crenshaw BE 3rd, Rawson EJ, Simmons DM, Swanson LW, Rosenfeld MG 1990 Dwarf locus mutants lacking three pituitary cell types result from mutations in the POU-domain gene *pit-1*. Nature 347:528–533

Masoro EJ 1992 Retardation of aging processes by nutritional means. Ann NY Acad Sci USA 673:29–35

Merimee T, Laron Z 1996 Modern endocrinology and diabetes, vol 4: Growth hormone, IGF-I and growth: new views of old concepts. Freund Publishing House Ltd, Tel Aviv

Merimee T, Rimoin DL, Hall JD, McKusick VA 1969 A metabolic and hormonal basis for classifying ateliotic dwarfs. Lancet 1:963–965

Nance MA, Berry SA 1992 Cockayne syndrome: review of 140 cases. Am J Med Genet 42:68–84

Pesce K, Rothe MJ 1996 The premature aging syndromes. Clin Dermatol 14:161–170

Rimoin DL, Merimee TJ, McKusick VA 1966 Sexual ateliotic dwarfism: a recessively inherited isolated deficiency of human growth hormone. Trans Assoc Am Physicians 79:297–304

Rosén T, Bengtsson BA 1990 Premature mortality due to cardiovascular disease in hypopituitarism. Lancet 336:285–288

Rosén T, Edén S, Larson G, Wilhelmsen L, Bengtsson BA 1993 Cardiovascular risk factors in adult patients with growth hormone deficiency. Acta Endocrinol (Copenh) 129:195–200

Rosenbloom AL, Guevara-Aguirre J, Rosenfeld RG, Francke U 1999 Growth hormone receptor deficiency in Ecuador. J Clin Endocrinol Metab 84:4436–4443

Rosenfeld RG, Rosenbloom AL, Guevara-Aguirre J 1994 Growth hormone (GH) insensitivity due to primary GH receptor deficiency. Endocr Rev 15:369–390

Rubin CD, Reed B 1996 Twenty-four hour growth hormone secretion in a patient with Werner's syndrome. Exp Gerontol 31:557–561

Rudman D, Feller AG, Nagraj HS et al 1990 Effects of human growth hormone in men over 60 years old. N Engl J Med 323:1–6

Schaible R, Gowen JW 1961 A new dwarf mouse. Genetics 46:896

Sornson MW, Wu W, Dasen JS et al 1996 Pituitary lineage determination by the prophet of Pit-1 homeodomain factor defective in Ames dwarfism. Nature 384:327–333

Takala J, Ruokonen E, Webster NR et al 1999 Increased mortality associated with growth hormone treatment in critically ill adults. N Engl J Med 341:785–792

Toogood AA, Shalet SM 1998 Ageing and growth hormone status. Baillieres Clin Endocrinol Metab 12:281–296

van Buul-Offers S, Veda I, van den Brande JL 1986 Biosynthetic somatomedin C (SM-C/IGF-I) increases the length and weight of Snell dwarf mice. Pediatr Res 20:825–827

Villee DB, Nichols G Jr, Talbot NB 1969 Metabolic studies in two boys with classical progeria. Pediatrics 43:207–216

Zhou Y, Xu BC, Maheshwari HG et al 1997 A mammalian model for Laron syndrome produced by targeted disruption of the mouse growth hormone receptor/binding protein gene (the Laron mouse). Proc Natl Acad Sci USA 94:13215–13220

DISCUSSION

Shalet: Just a point of fact. Elderly patients with pituitary disease are very different in their GH secretion when compared with age-matched controls. Just

to equate a so-called somatopause with pituitary tumour patients that have organic GH deficiency is incorrect. The difference in the 24 h profile is some 90%. The patients with pituitary tumours and organic GH deficiency have a GH reduction of 90%, mainly consisting of a decrease in the amplitude of the GH pulse. This needs to be stated up-front.

I was surprised at the way you were pushing us at the end into thinking it is better to be IGF1 and GH deficient. I find that puzzling for a man who has spent so many years of his life fighting to get IGF1 replacement for patients with GH insensitivity. It is a curious contradiction in terms of your policy. Does this mean that you will no longer replace GH and IGF1 in children who are GH-deficient or insensitive, respectively, for fear of reducing their potential life expectancy?

Laron: You don't have to exaggerate. If you have muscle weakness, short stature and osteoporosis, this should be treated. What I wanted to point out is the following. (a) The general statement that hyperpituitarism reduces the lifespan is not true, unless you analyse the precise nature of the hyperpituitarism. (b) Too much GH is dangerous. We should learn how to administer GH replacement in order to prevent its negative effects. I am not saying we shouldn't treat true GH/IGF1 deficiency. However, the dose one should use in ageing adults is still controversial.

Giustina: I have a couple of points. The parallelism between greying hair and longevity is not proven. I am not sure there are data showing that people with no grey hair live longer. Moreover, acromegalic patients do not have very early greying of hair. This fact raises some doubts in the relationship with GH. When you quote the data from Rosén and Bengtsson on lifespan, if you look at the real data, there is no big difference between control and population studies. This means that when you study a very small population such as yours, and say that lifespan is not reduced, statistically this is not correct. This is because GH-deficient subjects do not die at a very young age. However, they have been proven to have a reduced lifespan with respect to a comparable population in the same country in the same registry. If you want to demonstrate what you are saying you need to look at the comparable population in your territory and see whether on a statistical basis there is a reduction or not in lifespan. Otherwise, what you are saying is that these people may sometimes live long and sometimes not. This is a descriptive concept that has to be proven on a statistical basis.

Laron: One thing is clear. The animal models with isolated IGF1 deficiency or multiple hormone deficiency, including GH, live longer. In humans, having 'congenital GH or IGF1 deficiency' does not shorten the lifespan, as stated by Rosén & Bengtsson (1990). The population they studied were patients after operation or irradiation for pituitary tumours.

Giustina: But you didn't prove it.

Laron: Their statements have been cited in many papers and even textbooks.

Giustina: You need to prove that in your control population in that territory, the lifespan is the same. Otherwise you are not proving the concept. You are just describing the fact that some of the patients may live a long time. There is no statistical evidence supporting your concept.

Laron: With regard to cancer and IGF1, there was a meeting in Halle in September 2000 which had a clear message. People genetically susceptible to cancer are very susceptible to IGF1, as they are to sex hormones. In those who are not genetically susceptible, IGF1 does not induce cancer.

Monson: Concerning the comparison of different populations, the Lund series (Bülow et al 1997) and the Gothenburg series (Rosén & Bengtsson 1990) examined patients from the mid-1950s, when the concept of lipid lowering and healthy lifestyle was less defined. So these were observational, epidemiological studies in patients who by current standards may have had suboptimal care. We are superimposing on that background a group of patients who by virtue of small size may not have sufficient power to show a difference in mortality, and who also by virtue of one's interest in their clinical problem, are likely to have had more interventions. It is therefore very difficult to be certain that lifespan is reduced.

We talk about the Lund study and the Gothenberg study as demonstrating increased mortality related to GH deficiency, but these patients were predominantly pan hypopituitary. This is a demonstration of increased mortality in hypopituitary populations, who may have had variable quality of cortisol replacement. We know that GH deficiency itself alters the relationship between cortisol and cortisone conversion. This is likely to be accentuated in hepatocytes and adipocytes. Arterial intima–media thickness is increased in hypopituitary patients and this is partially reversed by GH replacement. Nonetheless, we should be wary about concluding that GH of itself has any true impact on atherogenesis and that there is reversibility in terms of GH replacement. Having said this, I agree with you that the animal data are extremely compelling in terms of the effect of GH and IGF1 on longevity.

Handelsman: I would add that in several of those studies they did not have adequate reproductive hormone replacement, either.

Laron: In view of the alleged neuroprotective and neurotrophic actions of IGF, in people with Laron syndrome there is a decline in the central nervous system (CNS) function and when they are treated with IGF there is amelioration, as observed in the treated children. We have not treated adults long enough, but we have recently found, by MRI, changes in the brains of IGF1-deficient adult patients, and no defect whatsoever in patients treated from childhood onwards. The longest treatment in paediatric patients is 10 years. It is difficult to compare adults with children, but judging by the size of the head (which indicates brain growth; Laron et al 1992) and by psychological tests (Galatzer et al 1993), the

intrauterine and perinatal IGF1 is of great importance for its neurotrophic effect. This seems to be preventable by treatment.

Riggs: Are individuals with congenital IGF1 or GH deficiency truly susceptible to osteoporosis? Outside of the bone field it is not always appreciated that what is measured by dual-energy X-ray absorptiometry (DEXA) is areal bone density and not volumetric density. There will be a built in error if you have small bones. It is possible to correct this through formulae. It would be interesting to see whether you could do this. I guess the compelling question is, is there enough follow-up on these patients with regard to whether or not they develop vertebral fractures?

Laron: There aren't vertebral fractures, but there is cervical stenosis which develops in adulthood. In children who have been treated for a long time we don't see these changes. We know that IGF1 is an anabolic hormone that influences the connective tissue. I wish to mention one more important issue that relates to GH or IGF replacement therapy, namely quality of life. This is a difficult subject to study.

Shalet: Adults with childhood-onset GH deficiency are as a group significantly osteopaenic. Middle-aged subjects with adult-onset GH-deficiency are also osteopaenic, but less so. The elderly-onset GH-deficient patients are not significantly different in terms of bone mineral density measurements from age-matched controls.

Monson: Should we really be using the term osteoporosis in relation to childhood-onset GH deficiency? Isn't this a peak bone mass issue?

Riggs: Osteoporosis can arise from lack of developing optimal peak bone mass. But the point is that even though DEXA is universally used, it is not widely appreciated that if the size of the bone is different from normal, the normative values are not useful.

Monson: I was thinking about structure, also. A similar bone mineral density deficit in a child that has failed to reach peak bone mass, compared with a 60 year old with hypopituitarism, will be associated with quite different bone morphology in terms of what will have happened to the struts.

Riggs: That is correct.

Ruiz-Torres: From a gerontological point of view, there are two contradictory facts that are apparent. On the one hand, GH deficiency is not related to short life. Furthermore, there are experiments, such as those of A. V. Everitt in Australia, which show that hypophysectomy has lifespan-prolonging effects similar to those of dietary restriction. On the other hand, the opposite point is that well being is related to GH concentration, as we have seen in healthy people in agreement with your GH deficiency results.

Laron: Patients with Laron syndrome are short and have reduced muscle mass, which contributes to the development of osteoporosis. They are hindered in normal life to varying degrees. These patients also have varying deficits in mental

abilities. They should be diagnosed prenatally, and ideally start treatment prenatally — and certainly no later than at birth.

Veldhuis: Do they tend to have a slightly low core body temperature? We heard yesterday how fasting tends to reduce mean core temperature. Does anyone know whether the mice deficient in mitochondrial uncoupling protein are also thermogenic? This would test the theory of thermogenesis, which is painfully broad.

Handelsman: To avoid therapeutic nihilism about the use of GH, what about using IGF1 as a way of dose titration? How important is this?

Laron: It is used as a marker for acromegaly. We use IGF1 as a marker for testing whether the dose of IGF1 is sufficient.

Shalet: Remember that 50% of middle-aged hypopit patients start with a normal IGF1 level. In adult patients we keep the IGF1 between −2 and +2 SDs. We don't give them supraphysiological doses of GH because we would then exceed the IGF1 SD score of +2. Clearly, at the other end, for a patent starting with a low IGF1 level, monitoring the IGF1 level helps assess compliance. Otherwise, in terms of optimizing GH replacement therapy, IGF1 SD score doesn't really help.

Laron: There is debate concerning whether one has to look at both IGF1 and also IGF binding protein 3. The consensus now is that IGF1 suffices, and only in early infancy when IGF1 is low should IGFBP3 be measured.

Elahi: I have reviewed the literature and been unable to find a consistent number for what is considered a low IGF1 at any age. Professor Shalet, what number do you use? We use $\leqslant 135$ pg/mol as our low value.

Shalet: There is no number. We have a service provided by a pharmaceutical company that makes the GH. Our patients are in an international surveillance program. We take blood, the sample is sent off to that particular lab and they have normative data that are decade based and gender based, and they then issue a standard deviation score that takes into account the normal age-matched value.

Haus: The circadian peak in plasma GH decreases in the elderly and some suggestions have been made that the functional state of elderly subjects could be improved by GH substitution. This clearly is not supported by Professor Laron's observation on the life expectancy of subjects with genetic and/or acquired conditions of habitually high or low GH concentrations. In this context, an observation on the correlation of the circadian means and amplitudes in plasma GH concentrations with the functional state of the subjects may be of interest. Comparing the circadian mean and amplitude of plasma GH (obtained in 279 profiles in 149 subjects, 77 8 years of age with 6 measurements over a 24 h span) with the functional state of the subjects as evaluated by the Index of Independence in Activities of Daily Living (ADL, Katz) and the Mental Status Index (MSI) we found a statistically significant positive correlation of the growth hormone levels

and amplitudes with functional impairment rather than with functional capacity (Haus et al 1989).

References

Bülow B, Hagmar L, Mikoczy Z, Nordstrom CH, Erfurth EM 1997 Increased cerebrovascular mortality in patients with hypopituitarism. Clin Endocrinol 46:75–81

Galatzer A, Aran O, Nagelberg N, Rbitzek J, Laron Z 1993 Cognitive and psychosocial functioning of young adults with Laron syndrome. In: Laron Z, Parks JS (eds) Lessons from Laron syndrome (LS) 1966–1992. Pediatr Adolesc Endocrinol 24:53–60

Haus E, Nicolau GY, Robu E, Petrescu E, Sackett-Lundeen L 1989 The functional state of elderly subjects related to the circadian and circannual mean and amplitude in plasma growth hormone. Chronobiologia 16:142–143

Laron Z, Anin S, Klipper-Aubach Y, Klinger B 1992 Effects of insulin-like growth factor on linear growth, head circumference and body fat in patients with Laron-type dwarfism. Lancet 339:1258–1261

Rosén T, Bengtsson BA 1990 Premature mortality due to cardiovascular disease in hypopituitarism. Lancet 336:285–288

Endocrine Facets of Ageing.
Novartis 242

Print ISBN 0-471-48636-1 Online ISBN 0-470-84654-2

The role of insulin-like growth factor 1 and insulin in ageing and atherosclerosis

Antonio Ruiz-Torres

Instituto Universitario de Investigación, Gerontológica y Metabolica (University Research Institute of Ageing), Hopital de la Princesa, Diego de León 62, 28006 Madrid

Abstract. With advancing age insulin-like growth factor (IGF)1 blood levels decrease continuously, but with great interindividual differences. There is a relationship between the IGF1 serum concentration and biomarker behaviour, indicating that growth hormone (GH) secretion is a determinant of organismic well being and surviving in advanced age. In contrast, the secretion of insulin rises with age, which is related to both increasing body fat mass and ageing itself. *In vitro* insulin stimulates the proliferation, migration and collagen secretion of human vascular smooth muscle cells (SMCs). The mechanism underlying these processes mainly involves occupancy of IGF1 receptors by insulin, with the exception of migration. With advancing age of the donor, the *in vitro* proliferation rate and migration capacity of SMCs decreases. When insulin or IGF1 is added, there is no reversibility, so that there is no recovery to the values of SMCs from young donors. The blockade of Ca^{2+} channels by diltiazem inhibits the *in vitro* stimulation by IGF1 and insulin on SMC proliferation and migration. We conclude that the acceleration of ageing is related to the decline of IGF1 in such a manner that ageing rates progress as GH secretion diminishes. Biomarkers are affected correspondingly. The role of insulin in atherogenesis is related to hyperinsulinaemia, but the increase in insulin secretion belongs to the process of ageing regulation. Nevertheless, the effect of insulin in changing the phenotype of SMCs is atherogenic. Diltiazem may therefore act as an antiatherogenic agent. In advanced age the risk of atherogenesis decreases because of lowered propensity of SMCs to proliferate and migrate, which is probably due to a greater proportion of senescent cells.

2002 Endocrine facets of ageing. Wiley, Chichester (Novartis Foundation Symposium 242) p 143–160

After reaching a maximum level at the end of the puberty, insulin-like growth factor 1 (IGF1) blood levels decline, indicating that the genetic programme of growth and differentiation has finished and adulthood has begun. The involution of the organism appears simultaneously with a decrease of IGF1 levels and growth hormone (GH) secretion (Iranmanesh & Veldhuis 1992, Vahl et al 1996). From a gerontological point of view, it is not possible to distinguish the signs of the

involutive process and those of ageing. For this reason, the continuous decline of IGF1 secretion may be considered a marker of biological ageing.

IGF1 and insulin secretion during ageing

The secretion of GH decreases with advancing age, mainly through a decrease in the amplitude of GH pulses (Veldhuis et al 1995). Mean 24 h GH concentrations decline from late puberty into old age (Rudman et al 1981) due to decreased GH production and an increased clearance rate (Iranmanesh et al 1991). The changes in IGF1 levels throughout life appear to mimic those of GH (Ho et al 1987).

Accordingly, in a cross-sectional study of healthy adults with similar lean body mass (LBM), the IGF1 blood levels decreased with population age (Ruiz-Torres & Corpas 1993), independently of adiposity (Copeland et al 1990). We observed that the slope of the corresponding curve shows differences dependent on the age range considered. In the range between 20 and 50 years the slope is clearly steeper than when all age groups up to a very advanced age are included. This indicates that people with low levels of IGF1 die earlier so that the average value in the surviving group is increased. Consequently, the curve obtained up to around 50 years age (and then extrapolated) represents a more realistic behaviour of IGF1 and should be considered as a standard reference curve.

These results could help explain the role of IGF1 levels on age-related differences in, for example, testosterone, thyroid hormone and procollagen III peptide blood concentrations that disappear when comparing young and old persons with similar IGF1 values (Table 1). Furthermore, individuals of very advanced age show IGF1 blood levels clearly above the standard regression curve, the behaviour of which is not affected by the selection of individuals by mortality (Soares de Melo 1997).

In a similar defined population of healthy individuals (as mentioned earlier) insulin levels are usually increased with advancing age (Ruiz-Torres et al 1996).

TABLE 1 Differences between young[a] and old[b] depending on IGF1 blood level in males

	IGF1	*Testosterone*	*PIIIP*	*LBM*
Young versus old	Decreased $P < 0.0001$	Decreased $P < 0.0001$	Increased $P < 0.0001$	Decreased $P < 0.0001$
Lowest range in young versus highest in old of IGF1	NS	NS	Increased $P < 0.01$	NS

[a]20–39 years age, $n = 22$.
[b]70–92 years age, $n = 33$.
LBM, lean body mass; NS, difference not significant; PIIIP, procollagen III peptide.

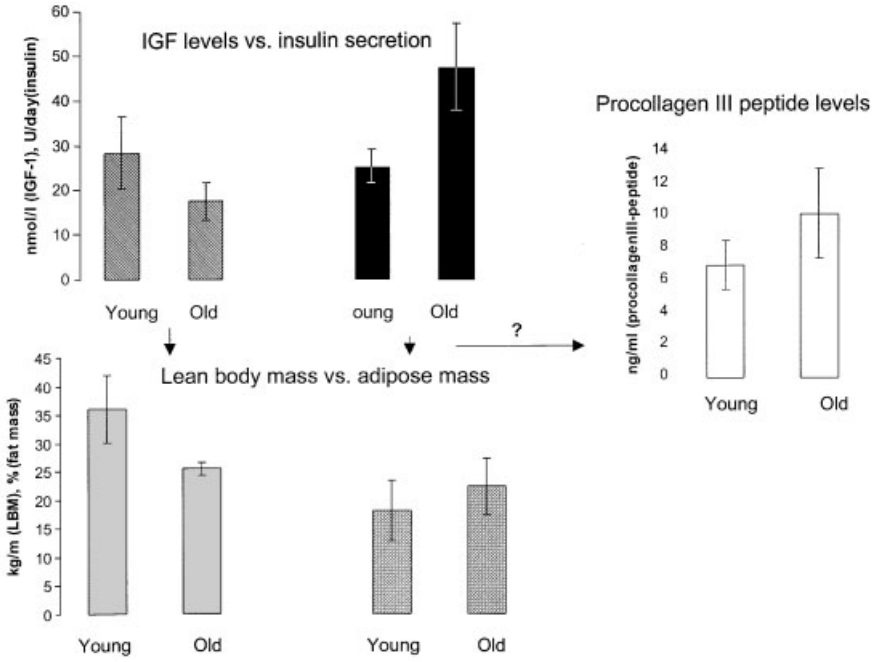


FIG. 1. IGF1 serum levels and insulin secretion of young (20–39 years old, $n = 22$) and old (70–92, $n = 33$) healthy men with corresponding anthropometrical manifestations. On the right are blood concentrations of the N-terminal peptide of procollagen type III (PIIIP). The figure shows the opposite age-dependent behaviour of the hormones mentioned. IGF1 concentrations were determined after alcohol extraction by radioimmunoassay and PIIIP. Daily insulin secretion was by means of 24 h C-peptide excretion, corrections and normalization of results as described (Ruiz-Torres et al 1996); LBM calculated according to Forbes & Bruining (1976); and adipose mass worked out on the basis of skin fold thickness and body density according to Durnin & Womersley (1974).

This elevation of insulin is related to the increased body fat mass and reduced muscle mass, the latter primarily due to the progressive decrease of GH/IGF1 secretion (Fig. 1). Moreover, the reverse correlation between insulin secretion and IGF1 blood levels could be understood as a wear and tear effect. Nevertheless, it needs to be stressed that all regulatory processes have some side effects, in this case those concerning excessive amounts of insulin with or without hyperinsulinaemia.

Atherogenity of insulin

Clinical studies have demonstrated that those processes linked to hyperinsulinaemia, such as type 2 diabetes or obesity, show a higher mortality due to coronary or cerebral atherosclerosis (Pyörälä et al 1985). Furthermore,

experimental results show that insulin acts on the vascular wall, either producing hypertension and endothelial changes, or influencing the smooth muscle cells (SMCs) to proliferate. It is well known that endothelial lesions and SMC proliferation are basic steps of atherogenesis (Ross 1993).

For a better understanding of the role of SMCs in atherosclerosis, it is worth mentioning that these cells and collagen represent the main content of the atheroma plaque. SMCs migrate from the media crossing the intima to accumulate and release collagen. Two distinctive phenotypes of SMC are known: contractile and synthetic. Contractile SMCs respond to agents inducing vasomotor changes, whereas the synthetic SMCs are capable of expressing genes for growth regulators and collagen synthesis. Normally, in adult life the vascular wall has only postmitotic SMCs which are contractile. Therefore, muscle cells of atheromas should have changed their differentiated phenotype to a synthetic one, with the capability to proliferate, migrate and finally secrete collagen. At present, it is believed that oxidized low density lipoprotein (LDL) alters the endothelium, producing a cascade of events including SMC migration (for review see, Massy & Keane 1996). The question is whether insulin is able to change the SMC phenotype and, if so, by what mechanism.

Effects of insulin and IGF1 on SMCs

More than a decade ago different publications showed that insulin stimulates SMC proliferation *in vitro* (Stout 1990). We have observed that in non-cultured cells, SMCs directly taken from the human artery, insulin stimulates collagen secretion. This effect was probably produced by activation of the IGF1 receptors, because addition of insulin receptor-blocking antibodies did not show any inhibition. On the contrary, antibodies blocking IGF1 receptors inhibited the insulin-induced collagen secretion. We concluded that insulin is able to change the SMC phenotype by acting as a growth factor (Ruiz-Torres et al 1998).

Moreover, insulin stimulates the chemotaxis of SMCs directly dispersed from the human artery (Muñoz et al 1998). In these experiments this migration could be inhibited by insulin receptor-blocking antibodies, so we assumed that insulin was acting here through its specific receptors. Nevertheless, these results point out a very close relationship between the stimulating effect of insulin on both collagen secretion and migration, in spite of apparently acting through different types of receptor. Further experiments were required to clarify how insulin stimulates SMC migration.

The effect of insulin and IGF1 on the cytoskeleton in relation to SMC migration

The cytoskeleton is a complex network of protein filaments extending throughout the cytoplasm that is involved in a range of cell processes. Of the three main

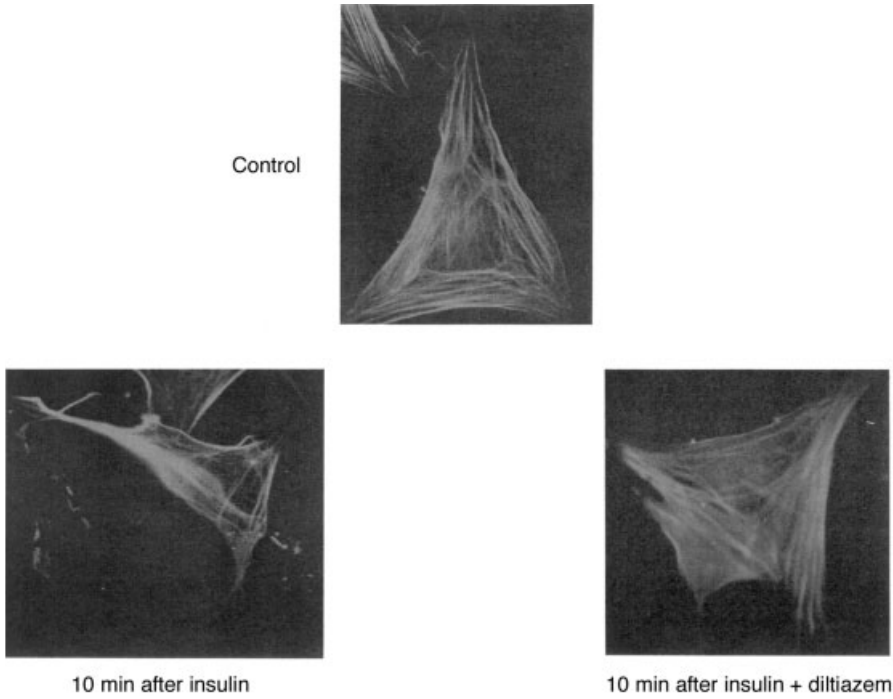


FIG. 2. Insulin-induced F-actin reorganization near the membrane of human vascular smooth muscle cells showing a ruffling which does not appear when Ca^{2+} channels of these cells are blocked by diltiazem. The results are similar in the case of IGF1 (unpublished).

cytoskeletal types, the actin filaments are primarily responsible for many cell movements, for example for SMC migration (Alberts et al 1994, p 787–803). According to Bretscher's model of fibroblast locomotion, actin filaments depolymerize ahead of the nucleus, generating actin subunits which diffuse to the cell's front where actin filaments polymerize at the leading edge (Bretscher 1996). Chemoattractant receptors contribute to the promotion of actin assembly at the leading cell edge (Stossel 1993). Ca^{2+} ions play an important role in the process of assembly and deassembly of actin filaments. Insulin induces these specific rearrangements in mesangial cells *in vitro*. Fluorescent staining for F-actin with phalloidin normally shows actin filaments spanning the entire cell in all directions. After insulin treatment, the cells show peripheral ruffling and lifting off from the substrate as attachments are released (Berfield et al 1996). We have shown that insulin, like IGF1, induces membrane ruffling of F-actin in human vascular SMCs *in vitro*. This effect appears very quickly, just 5 minutes after hormone application. As expected, the blockade of Ca^{2+} channels by diltiazem does not allow insulin to induce ruffling (Fig. 2). The result is similar for IGF1.

These data suggest that insulin influences SMC movements with a mechanism probably similar to that of IGF1.

IGF1-like mechanism of insulin to induce proliferation but not migration of SMCs

A large number of well known factors modulate SMC migration, such as platelet-derived growth factor (PDGF) and IGF1. Most have similar proliferation effects. It is therefore possible that both the migratory and proliferative responses have a common intracellular mechanism, but the results obtained postulate some differences (Schachter 1997). The concentration of IGF1 required to induce migration is substantially lower than that needed for proliferation (Bornfeldt et al 1994). Regarding the effect of Ca^{2+} channels blockade on migration, diltiazem inhibits IGF1 and insulin stimulation in a similar manner (Fig. 3).

Insulin stimulates SMCs to proliferate similarly to IGF1. Our recent unpublished results obtained from *in vitro* experiments with cultured human arterial SMCs show that the mitotic induction of insulin occurs by occupation of the IGF1 receptors. This has been demonstrated using receptor-blocking antibodies. The blockade of insulin receptors does not inhibit the insulin-induced induction of SMC proliferation, but the addition of IGF1 receptor blocking antibodies does. The opposite results have been obtained in chemotaxis experiments (Fig. 4). The addition of IGF1 receptor-blocking antibody has no effect on migration, whereas insulin receptor-blocking antibody does.

As expected, both the proliferation and migration capability of SMCs may be inhibited by blockade of Ca^{2+} channels as with diltiazem. These results support the possibility of preventing atherosclerosis by treating with Ca^{2+} antagonists (Marche et al 1997).

The results above are in agreement with those — also mentioned in this chapter — on the insulin mechanism stimulating collagen secretion. On this basis and considering the induction of F-actin ruffling, we therefore postulate that there are no differences between IGF1 and insulin with respect to their effect in changing the phenotype of SMCs. Extrapolating these results to *in vivo*, insulin may be behaving as a growth factor rather than a metabolic effector because of its presence in higher than normal concentrations. From this point of view, whether insulin is atherogenic or not would depend primarily on its concentration.

Atherogenesis and ageing

The culture of SMCs is a useful model system because under these conditions the cells show many of the functional changes characteristic of atherogenesis, in particular the ability to proliferate and to secrete collagen (Campbell & Campbell

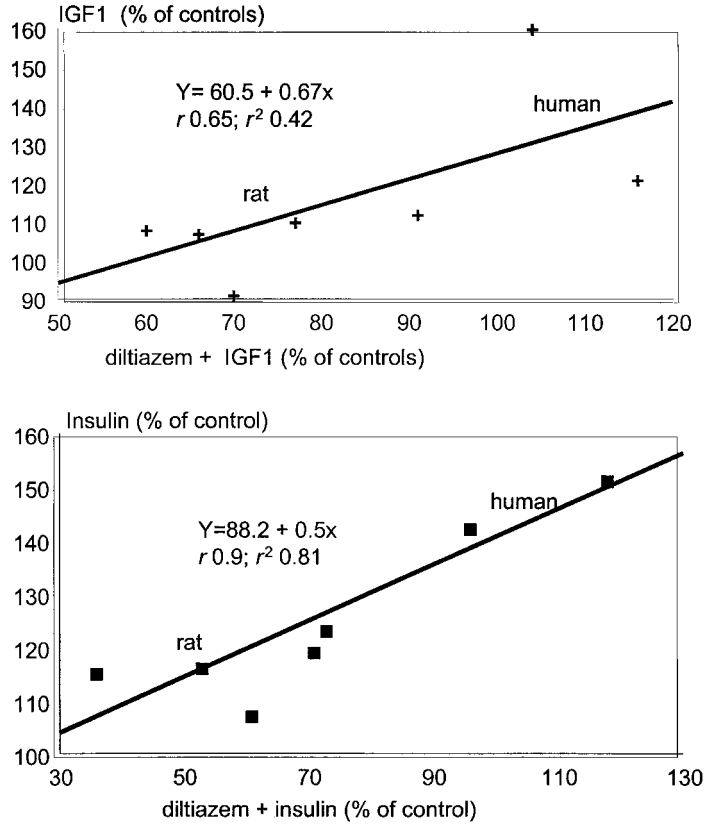


FIG. 3. Relationship between hormone-induced migration and corresponding inhibition due to Ca^{2+} channel blockade by diltiazem in vascular smooth muscle cells from rat aorta and human femoral artery. The inhibition decreases directly proportionally to stimulation level either by IGF1 or insulin (bottom). Unpublished experiments performed in the Boyden chamber.

1987, Massy & Keane 1996). The *in vitro* proliferating SMCs develop cytoskeletal features similar to those observed in fetal and pathological states (Skalli et al 1986). Essentially, SMCs exhibit two phenotypes in culture: one able to proliferate and the other to differentiate, recalling the *in vivo* fetal and contractile phenotypes, respectively. The terminal differentiation in culture resembles the behaviour observed in other cells such as melanocytes (Medrano et al 1994). Proliferating SMCs *in vivo* assume fetal phenotypic features which are also observed in cultured cells (Desmoulière & Gabbiani 1992).

As the proliferation of human vascular SMCs characterizes the atherogenesis, it seems of interest to investigate the mitotic activity in cultured SMCs dependent on

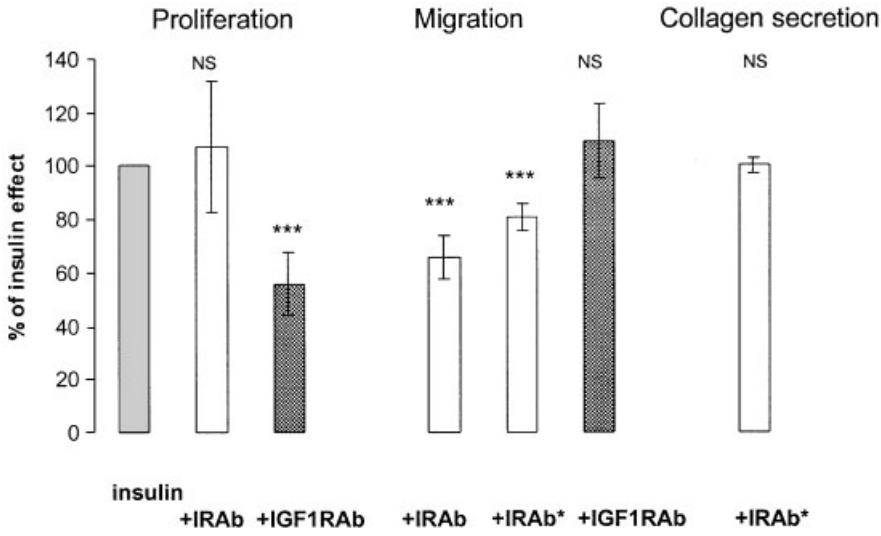


FIG. 4. Inhibition of insulin stimulation of proliferation, migration and collagen secretion in human vascular smooth muscle cells (hvSMCs) due to receptor blocking antibodies, indicating the mechanism of action. The addition of IGF1 receptor blocking antibody inhibits the proliferative effect of insulin, whereas the opposite occurs in the case of migration. Similarly to proliferation, the insulin receptor blocking antibody does not show any effect on collagen secretion in spite of its inhibition of glucose uptake. White columns: incubations of hvSMCs with insulin and specific receptor blocking antibody (IRAb). Hatched columns: those with IGF1-receptor blocking antibody (IGF1RAb). *Dispersed cells from artery; the others from the culture (2–4 passage). ***$P < 0.0001$; NS, not statistically significant (results partially published in Ruiz-Torres et al 1998, Muñoz et al 1998).

the age of the donor. The proliferation rate of SMCs decreases in the culture as donor age advances (Ruiz-Torres et al 1999). The decline reaches the zero point that is the total loss of proliferative activity, at age over 100 years, near the limit of maximum life potential for human beings. Therefore age is a factor which decreases the ability of vascular SMCs to proliferate. The meaning is the same for *in vivo* as *in vitro*. Nevertheless, cultured cells age according the passage numbers as described by Hayflick (Smith & Hayflick 1974).

The cellular expression of ageing *in vivo* or *in vitro* is the progressive appearance of senescent cells, so that cells have a finite life potential (Hayflick 1987). Senescent cells lose their capacity to proliferate despite potentially remaining in the tissue or culture for a relatively long time (Matsumura et al 1979). A similar phenomenon occurs regarding migration capacity. We recently found that the basal migration of human vascular SMCs decreases as donor's age advances. The slope of the curve is similar to that of proliferation, showing the total loss near the age of maximum life potential (Ruiz-Torres et al 1999). Consequently, the interpretation is the same in

TABLE 2 Decrease of migration by age: chemotaxis (basal and induced) of cells from young and old male donors

Age (years)	*43*		*74*	
Number of experiments	3		3	
Migrated cells:				
Basal	930	36	540	43*
1 nM insulin	1110	165	740	106*/**
1 nM IGF1	1153	117	710	10*/***

Results using the Boyden chamber. *young:old $P < 0.05$; **with basal young $P < 0.07$; ***with basal young $P < 0.05$.

both cases: during adulthood, increasing age decreases the sensitivity of SMCs to phenotypic change (Table 2). The high content of senescent cells in advanced age would remarkably diminish the risk for atherogenesis. The opposite would happen when relatively young SMCs coexist with risk factors such as hypercholesterolaemia and hyperinsulinaemia. This state corresponds to the age range within the 3rd and 5th decades according to our studies and in agreement with epidemiological results on cardiovascular degenerative diseases.

Acknowledgements

This work was made possible by a grant of the Fondo de Investigación Sanitaria (FIS), Spanish Ministry of Health, Nr 98/0177. The unpublished results described here were obtained with the collaboration of Raffaele Carraro, Rosario Lozano, Manuel Macía and Marcia Soares de Melo.

References

Alberts B, Bray D, Lewis J, Raff M, Roberts R, Watson JD 1994 Molecular biology of the cell. Garland, London

Berfield AK, Raugi GJ, Abrass CK 1996 Insulin induces rapid and specific rearrangement of the cytoskeleton of rat mesangial cells *in vitro*. J Histochem Cytochem 44:91–101

Bornfeldt KE, Raines EW, Nakano T, Graves LM, Krebs EG, Ross R 1994 Insulin-like growth factor I and platelet-derived growth factor-BB induce directed migration of human arterial smooth muscle cells via signaling pathways that are distinct from those of proliferation. J Clin Invest 93:1266–1274

Bretscher MS 1996 Getting membrane flow and the cytoskeleton to cooperate in moving cells. Cell 87:601–606

Campbell GR, Campbell JH 1987 Smooth muscle cells. In: Olson AG (ed) Atherosclerosis and clinical science. Churchill Livingstone, New York, p 105–115

Copeland KC, Colletti RB, Devlin JT, McAulife TL 1990 The relationship between insulin-like growth factor-1, adiposity and aging. Metabolism 39:584–587

Desmoulière A, Gabbiani G 1992 The cytoskeleton of arterial smooth muscle cells during human and experimental atheromatosis. Kidney Int Suppl 37:S87–S89

Durnin JV, Womersley J 1974 Body fat assessed from total body density and its estimation from skinfold thickness: measurements on 481 men and women aged from 16 to 72 years. Br J Nutr 32:77–97

Forbes GB, Bruining GJ 1976 Urinary creatinine excretion and lean body mass. Am J Clin Nutr 29:1359–1366

Hayflick L 1987 Origins of longevity. In: Warner HR, Butler RN, Sprott RL, Schneider EL (eds) Modern biological theories of aging. Raven Press, New York, p 21–34

Ho KY, Evans WS, Blizzard RM et al 1987 Effects of sex and age on the 24-hour profile of growth hormone secretion in man: importance of endogenous estradiol concentrations. J Clin Endocrinol Metab 64:51–58

Iranmanesh A, Lizarralde G, Veldhuis JD 1991 Age and relative adiposity are specific negative determinants of the frequency and amplitude of growth hormone (GH) secretory bursts and the half-life of endogenous GH in healthy men. J Clin Endocrinol Metab 73:1081–1088

Iranmanesh A, Veldhuis JD 1992 Clinical pathophysiology of the somatotropic (GH) axis in adults. Endocrinol Metab Clin North Am 21:783–816

Marche P, Hérembert TH, Zhu DL 1997 Pharmacologic treatment of atherosclerosis: beyond lipid-lowering therapy. Int J Cardiol 62:S17–S22

Massy ZA, Keane WF 1996 Pathogenesis of atherosclerosis. Semin Nephrol 16:12–20

Matsumura T, Zerrudo L, Hayflick L 1979 Senescent human diploid cells in culture: survival, DNA synthesis and morphology. J Gerontol 34:328–334

Medrano EE, Yang F, Boissy R et al 1994 Terminal differentiation and senescence in human melanocyte: repression of tyrosine-phosphorylation of the extracellular signal-regulated kinase 2 selectively defines in two phenotypes. Mol Biol Cell 5:497–509

Muñoz FJ, Jiménez M, Melón J, Ruiz-Torres A 1998 Phenotypic changes in vascular smooth muscle cells during aging: insulin effect on migration. Gerontology 44:149–152

Pyörälä KE, Savolainen E, Kaukolas S, Haapakoski J 1985 Plasma insulin as coronary heart disease risk factor: relationship to other factors and predictive value during 9 and a half-years follow-up of the Helsinki Policeman Study population. Acta Med Scand Suppl 701:38–52

Ross R 1993 The pathogenesis of atherosclerosis: a perspective for the 1990s. Nature 362: 801–809

Rudman D, Kutner MH, Rogers CM, Lubin MF, Flemming GA, Bain RF 1981 Impaired growth hormone secretion in the adult population: relation to age and adiposity. J Clin Invest 67:1361–1369

Ruiz-Torres A, Corpas E 1993 Anthropometrical changes in aging: Their relationship with GH secretion and its possible metabolic consequences. In: Laron Z, Butenandt O (eds) Growth hormone replacement therapy in adults: pros and cons. Freund Publishing House, London, p 123–136

Ruiz-Torres A, Melón J, Munöz FJ 1998 Insulin stimulates collagen synthesis in vascular smooth muscle cells from elderly patients. Gerontology 44:144–148

Ruiz-Torres A, Vicent D, Sanchez de Paco G, Munoz FJ, Gimeno A, Carraro R 1996 Increase in insulin secretion with age: its clinical importance in evaluating abnormal secretions focused on diabetes type II and obesity. Arch Gerontol Geriatr 22:39–47

Ruiz-Torres A, Gimeno A, Melón J, Mendez L, Munöz FJ, Macía M 1999 Age-related loss of proliferative activity of human vascular smooth muscle cells in culture. Mech Ageing Dev 110:49–55

Schachter M 1997 Vascular smooth muscle cell migration, atherosclerosis, and calcium channel blockers. Int J Cardiol 62:S85–S90

Skalli O, Bloom WS, Ropraz p, Azzarone B, Gabbiani G 1986 Cytoskeletal remodeling of rat aortic smooth muscle cells *in vitro*: relationships to culture conditions and analogies to *in vivo* situations. J Submicrosc Cytol 18:481–493

Smith JR, Hayflick L 1974 Variation in the life-span of clones derived from human diploid cell strains. J Cell Biol 62:48–53

Soares de Melo M 1997 Concentración serica de IGF-1 durante la vida adulta. Su relación con el estado de conservación biologica y capacidad de supervivencia. PhD thesis. Universidad Autonoma de Madrid, Spain

Stossel TP 1993 On the crawling of animal cells. Science 260:1086–1094

Stout RW 1990 Insulin and atheroma, 20-yr perspective. Diabetes Care 13:631–654

Vahl N, Jorgensen JOL, Jurik AG, Christiansen JS 1996 Abdominal adiposity and physical fitness are major determinants of the age associated decline in stimulated GH secretion in healthy adults. J Clin Endocr Metab 81:2209–2215

Veldhuis JD, Liem AY, South S et al 1995 Differential impact of age, sex steroid hormones, and obesity on basal versus pulsatile growth hormones secretion in men as assessed in an ultrasensitive chemiluminiscence assay. J Clin Endocrinol Metab 80:3209–3222

DISCUSSION

Veldhuis: One of the issues I am perceiving is that the distinction is sometimes blurred between what we implicitly view as the reversible facets of ageing and those that aren't. Your extrapolation of the IGF1 levels down to about age 110 was exciting. I guess for testosterone we'd live to be about 250, so we will run out of other things before testosterone.

Handlesman: Tangential to this, there is a great deal of re-thinking going on in atherogenesis, particularly as people are starting to recognize that a lot of the accepted explanations about oestrogens preventing cardiovascular disease are based almost solely on retrospective case control data. In collaboration with some of our cardiology colleagues, we have done a series of studies looking at elements of the process of atherogenesis. In particular, we have studied adhesion and migration of macrophages to human endothelial cells. It really looks as if testosterone itself has significant effects, increasing adhesion by a VCAM mechanism, including NF-κB and Ca^{2+}. This is very similar to what you are describing. It is somewhat surprising that the insulin effects seem to be inhibiting this when the epidemiology suggests that insulin levels rise when atherogenesis increases. Perhaps part of the picture is that sex steroids, and particularly androgens, have a role that is so far not fully recognized.

Veldhuis: In part, this illustrates the complexity of these system interactions. One has conditions in the prostate where testosterone will induce binding proteins for IGF1 and conversely GH alters testosterone's conversion to dihydrotestosterone in the liver. The gemisch of these changes across axes is going to be tough to sort out, with the incremental changes within each axis being so small. I am struck by the enormous range of similarity of mild glucocorticoid excess to ageing and syndrome X. Working in the GH field I've always attributed this to GH

deficiency. Here we have two systems where arguing for a slight increase in corticol net activity (associated with the stress in daily living), and a decline in GH. Each directional change produces a similar outcome. Altogether, I'm surprised we're still alive here!

Prior: I would like to understand the interrelationships between insulin/IGF1 and body weight, and in particular changes with endurance or aerobic exercise. There is some key related to exercise that will enlighten us about the rise in insulin and decrease in IGF with ageing.

Elahi: I don't know much about IGF1 with respect to body composition, but it is well established that hyperinsulinaemia not only causes overall increased adiposity, but also deposition of subcutaneous fat in the abdomen which may consist of two metabolically active types.

Veldhuis: We found that fit young adults tend to undersecrete insulin, presumably because of good insulin sensitivity in the periphery (Engdahl et al 1995). They have well-organized low amplitude insulin pulses that are sufficient to maintain euglycaemia, whereas older people show some clear disruption of several features of insulin release (Meneilly et al 1997, 1999).

Monson: Cortisol metabolism and GH are not necessarily distinct from each other. Adrenocorticotropic hormone (ACTH) feedback controls normal circulating free cortisol. However, at a tissue level, increased 11β-HSD1 activity in the adipocyte consequent upon GH deficiency will shift the set point in favour of increased local exposure to cortisol in both the liver and the adipocyte. It isn't surprising, therefore, that there may be phenotypic similarities. I am not saying that all the effects of GH on fat accumulation are mediated through cortisol, but there is a potential link there. The other important hormonal modulator of 11β-HSD1 is insulin.

Carroll: To add to that, there is yet another way that the two interact. Under stress conditions, and certainly in human depression, GH secretion at night is practically eliminated.

Veldhuis: There is a cortisol–GH connection at a couple of interesting neuroendocrine levels, as well (Giustina & Veldhuis 1998, Giustina et al 1994).

Giustina: A slight cortisol excess may decrease GH secretion via an increase in hypothalamic somatostatin. We have good evidence both in normal volunteers and in patients with autoimmune diseases that you can get a decrease in GH secretion after short- and long-term glucocorticoid administration. In addition, GH inhibition is observed in patients with Cushing's disease.

Veldhuis: This is where I betray my own intuition that with ageing being such a minimally incremental, subtle process, these interactions between systems, particularly when they have common adverse effects on certain target cells, are probably especially important to understand. We may be wrong in thinking of

ageing as a monohormonal problem. We do this only because we work more easily in one hormone system at a time.

Handelsman: Is there evidence that mild hypercortisolism actually accelerates atherogenesis?

Veldhuis: Can the Rotterdam group help us with this? Their data are more correlated with decreased bone mass and decreased quality of life with high cortisol, than death from atherosclerosis.

van den Beld: I investigated the relationship between cortisol and intima medial thickness in the distensibility of the carotid artery, and couldn't find any correlation between this and low insulin/cortisol.

Carroll: In relation to that, there are many more ways to die than just by atherosclerosis. Atherosclerosis is a major cause of premature mortality. We are constantly riding the boundary between gerontology and geriatrics here.

Handelsman: In a population, the gender disparity in life expectancy is about 5–7 years. This is precisely the difference in the peak and the rates of cardiovascular deaths in the community. It isn't affecting just premature deaths, it is the overall pattern of death. This gender disparity remains less well understood than it was once thought.

Laron: One factor in atherosclerosis that hasn't been mentioned is lipoprotein (a), which is regulated by IGF1 and insulin. IGF1 suppresses it; GH elevates it.

How do you explain the effect on procollagen III? We know that IGF raises it. Is it a competitive effect with the insulin receptors?

Ruiz-Torres: You are right. The blood level curve of procollagen III peptide shows a biphasic behaviour. During young adulthood, there is a decrease from a maximum reached in the first phase of life. Then after age 30 there is another rise, in spite of continuous diminution of IGF1. This dissociation is probably due to insulin, whose secretion increases with advancing adult age. As shown, insulin acts in a similar way to IGF1. Furthermore, the more insulin released, the higher the level of procollagen III, as we have seen in hyperinsulinaemic type II diabetes patients.

Laron: Procollagen III is also related to the collagen in the elastic fibres. We know that there is a reduction in elastic fibres in IGF1 deficiency, but this does not fit the levels that you showed.

Ruiz-Torres: The procollagen III levels we see in the second phase of adulthood are mainly related to the accumulation of collagen in the arterial wall. The best example is myocardial infarction patients with high procollagen III peptide blood levels.

Giustina: I would like to make a general point about IGF1 and ageing. One thing we haven't discussed so far, but which is important, is the concept of the frail elderly. These people are different from the normal elderly subjects.

Endocrinologists could help understand this. It has been suggested that IGF1 could be a marker of the frail elderly.

Ruiz-Torres: My intention was to explain the role of insulin in atherogenesis. I have presented results which would lead to the conclusion that the way by which insulin induces smooth muscle cells to proliferate is through IGF1 receptors. On the other hand, I have also presented results that show that IGF1 serum levels are related to well being in advanced age. These data indicate that not only muscle mass but also other ageing parameters are less influenced when IGF1 levels are relatively high.

Giustina: Is IGF1 acting on the endothelium or on the smooth muscle cells?

Ruiz-Torres: We haven't studied this point specifically, but I know that both endothelium and smooth muscle cells are sensitive to IGF1 as well as insulin. From a gerontological point of view, the latter seems to be the more important atherogenic effector because it is increased in ageing, as opposed to IGF1 which decreases. Regarding the question of whether atherogenesis is a normal manifestation of ageing or a disease, I would like to underline how difficult it is to find a correct answer. My personal opinion is that atherosclerosis is a 'side-effect' of hormonal changes linked to the regulatory process of ageing.

Giustina: The frail elderly are the men or women who are likely to be prone to immobility and diseases.

Ruiz-Torres: Compared with young individuals you would say that they are ill, but considering a normal follow-up of ageing the state of these individuals would only express the final phase of life.

Giustina: Clinically speaking there are scales that can be used to quantify the frailty of the population.

Morley: The problem with frailty is that everybody knows what it is, but there is no good definition. The Baltimore group have a definition that looks pre-dominantly at muscle strength. We have to be careful about these definitions, because each group has gone out and defined something. If I see a patient I know if they are frail, but when stick to the definitions they don't necessarily categorize the people we would say are frail into those definitions. The problem with IGF1 as a marker of frailty is that it is almost certainly a marker because it is a marker of malnutrition. Frail people are almost always somewhat malnourished, so this is where we run into trouble with it as a marker. There is a nice study coming out on centenarians showing that the subjects with really low IGF1s are those with low albumins and tend to be malnourished (Arai et al 2001). Again, you are looking at the problem of interactions, and nutrition becomes a major hormonal interactor for IGF1. This is where we are running into trouble. None of this is easy: we desperately need a good definition for frailty and also for sarcopenia.

Riggs: We have defined sarcopenia on the basis of established values in young people, rather than on a functional basis.

Handelsman: As an outsider reading the gerontology literature I see the issue of malnutrition raised all the time. I wonder how much of it is pushed by the for-profit US nursing homes. Is this the real issue? Is this true for non-institutionalized people out in the community?

Morley: It is a huge problem. In my practice I see a lot of malnourished old people. Old people run into trouble when they get sick, and many of them become malnourished. 10–20% of the really frail old people are malnourished. As they get more frail they get more malnourished. Depression is a big factor in inducing malnutrition.

Laron: I would like to clarify the relationship between malnutrition and IGF1. Malnutrition results in secondary GH resistance and low IGF1. I showed that IGF1 deficiency does not cause malnutrition; on the contrary, it causes obesity, but with reduced lean body mass. The frailty and lack of appetite in old people must have another aetiology than IGF1 deficiency, as must their lack of thirst.

Morley: There is a large literature on the factors involved in causing anorexia in ageing, which has nothing to do with IGF1. It is just one of the markers of malnutrition, and it is one that may be less affected by cytokines, but I still haven't seen enough data to convince me that it is not cytokine dependent.

Veldhuis: Malnutrition in ageing is a fascinating issue. It is a complex cascade spanning factors from depression to socioeconomic status. I agree that it looks like a mild GH resistance state. In a fasting animal, GH receptor signalling is down-regulated (Giustina & Veldhuis 1998).

Björntorp: With regard to cortisol as a risk factor for atherosclerosis, as far as I can see there are no prospective studies on this. This is probably due to the fact that this whole field has been so focused on details of lipid fractions, hypertension, smoking and insulin. On the other hand, an increased mass of visceral fat, which might be considered as a surrogate measurement of chronic elevation of cortisol secretion, is a powerful risk factor for atherosclerotic manifestations.

Veldhuis: This is what I mean when I talk about these devilish interrelationships.

Carroll: If you are going to do studies on the cortisol relationship, you want to be looking at the night levels of cortisol, not the daytime measures. This is the approach the MacArthur Foundation is taking with their epidemiological measures of allostatic load. They are measuring overnight urinary free cortisol excretion among other things.

Björntorp: Are they doing prospective studies?

Carroll: Yes.

Brabant: I wanted to come back to the question of the endothelium. What is NO doing in your concept? NO deficiency is a marker of atherosclerosis in animal models, where there is a decrease of NO along with atherosclerosis. On the other

hand, NO is closely controlled by the GH–IGF1 system. How does this fit into your concept?

Ruiz-Torres: Our studies have been focused on finding a relationship between the hormonal state in ageing and the phenotypic change of vascular smooth muscle cells representing the first step in atherogenesis. This change means that a contractile, non-mitotic cell becomes able to proliferate, migrate and produce collagen. It is known that NO is anti-atherogenic, but especially because of its interaction with angiotensin II. Additional to this, NO is likely to help stabilize the postmitotic state of smooth muscle cells, but its role here is not clear. IGF1 induces NO production in many cells, but it also inhibits NO production in smooth muscle cells after interleukin 1β. In any case, we have to bear in mind that in ageing both GH and IGF1 secretion are remarkably reduced.

Prior: I don't know about the IGF1–insulin relationship to NO, but we recently showed in an intra-arterial cross-over randomized study that oestrogen and progesterone have similar NO-mediated effects to increase forearm blood flow (Mather et al 2000).

Veldhuis: I think that there is prompt vasoarterial dilation in the human with IGF infusion. It is thought to operate through NO. GH itself increases NO products in the urine (Giustina & Veldhuis 1998). There are some exciting linkages.

Handelsman: Using the tracheal ultrasound technique to measure flow-mediated dilatation, it is possible to show that castration in men increases vascular activity as do oestrogens. In transexuals and normal physiological young men, androgens decrease vascular reactivity but also increase vessel diameter. There are quite a number of direct sex steroid relationships with NO. There were placebo-controlled studies in the 1950s and 1960s of cardiac ischaemia clearly showing that testosterone is a vasodilator under some circumstances.

Morley: There is a study from Newcastle showing that angina can be treated with testosterone (English et al 2000). The problem with NO is that it may actually be bad to vasodilate. If there is an unstable plaque, vasodilation will increase shear force which could result in popping the clot. It is one of those questions that hasn't been answered: most deaths from atherosclerosis involve the rupture of a clot, not blocked arteries. This may be one of the reasons that the HERS study showed early death (Hulley et al 1998).

Giustina: As to whether the dilatation effect is good or bad, this probably depends on the cardiovascular situation at the outset. If a patient has the mild degree of heart failure that is often observed in older people, the vasodilating effect will probably be beneficial. Studies show that if GH is infused acutely or if it is given on a subchronic basis, cardiovascular function is improved.

Morley: We are dependent on waiting for the MRI techniques to get good enough to pick up the unstable plaques. When we can do this, we will have much more insight as to who to give vasodilators to and who not to.

Müller: The involvement of the NO system in neuroendocrine control is a well known phenomenon. For instance, the ability of growth hormone (GH)-releasing peptides (GHRPs) to increase GH secretion in both old and young dogs is strikingly increased by NO donors, and counteracted by NOS inhibitors (Rigamonti et al 1999). GH secretagogues, in addition to their endocrine actions, exert other important extra-endocrine actions, and they too appear to require NO involvement. Some GHRPs stimulate sexual function in rats following intracerebral administration, and NOS inhibitors prevent this action (Melis et al 2001); in addition, the NO system would be involved in the prevention of injury and dysfunction of the vascular endothelium effected by GHRPs in a rat heart preparation undergoing low-flow ischaemia and reperfusion (De Gennaro Colonna et al 1997). Interestingly, in hearts from aged rats GHRP fail to interfere positively with the endothelium-dependent relaxant mechanism, suggesting the existence of an impaired NO function (Rossoni et al 1998).

Morley: We were discussing anorexia in ageing. To complete the cycle, our data suggest fairly strongly that anorexia in ageing is related to NO malfunction both within the hypothalamus and also in the fundus of the stomach. Many older people cannot open up their fundus (the top part of their stomach), so they get early satiation because the food just goes straight into the antrum where dilation produces rapid feedback, resulting in anorexia. Our animal studies suggest NO is involved, and the human studies agree.

Veldhuis: Is any more known about NO induction by sex steroids?

Morley: The simple answer is that testosterone is permissive for the development of NO synthase. If you have no testosterone at all, you don't get NO synthase. If you have some, you tend to get NO synthase. We have a paper in press looking at Viagra (sildenafil) failures in people who are hypogonadal. If you also treat them with testosterone, Viagra will work. I think oestrogen is similar.

Wang: Nestu Cadavid's group has shown that NOS in the penis is responsive to androgens (Garban et al 1995).

References

Arai Y, Hirose N, Yamamura K et al 2001 Serum insulin-like growth factor 1 in centenarians: implications of IGF1 as a rapid turnover protein. J Gerontol 56:M79–M82

De Gennaro Colonna V, Rossoni G, Bernareggi M, Müller EE, Berti F 1997 Cardiac ischemia and impairment of vascular endothelium function in hearts from growth hormone-deficient rats: protection by hexarelin. Eur J Pharmacol 334:201–207

Engdahl J, Veldhuis JD, Farrell PA 1995 Altered pulsatile insulin secretion associated with endurance training. J Appl Physiol 79:1977–1985

English KM, Steeds RP, Jones TH, Diver MJ, Channer KS 2000 Low-dose transdermal testosterone therapy improves angina threshold in men with chronic stable angina: a randomized, double-blind, placebo-controlled study. Circulation 102:1906–1911

Garban H, Marquez D, Cai L, Rajfer J, Gonzalez-Cadavid NF 1995 Restoration of the normal adult penile erectile response in aged rats by long-term treatment with androgens. Biol Reprod 53:1365–1372

Giustina A, Veldhuis JD 1998 Pathophysiology of the neuroregulation of GH secretion in experimental animals and the human. Endocr Rev 19:717–797

Giustina A, Bresciani E, Bossoni S et al 1994 Reciprocal relationship between the level of circulating cortisol and growth hormone secretion in response to growth hormone-releasing hormone in man: studies in patients with adrenal insufficiency. J Clin Endocrinol Metab 79:1266–1272

Hulley S, Grady D, Bush T et al 1998 Randomized trial of estrogen plus progestin for secondary prevention of coronary heart disease in postmenopausal women. Heart and estrogen/progestin replacement study (HERS) research group. JAMA 280:605–613

Mather KJ, Norman EG, Prior JC, Elliott TG 2000 Preserved forearm endothelial responses with acute exposure to progesterone: a randomized cross-over trial of 17-b estradiol, progesterone and 17-b estradiol with progesterone in healthy menopausal women. J Clin Endocrinol Metab 85:4644–4649

Melis MR, Succu S, Spano MS et al 2001 Penile erection induced by EP 80661 and other hexarelin peptide analogues: involvement of paraventricular nitric oxide. Eur J Pharmacol 411:305–310

Meneilly GS, Ryan AS, Veldhuis JD, Elahi D 1997 Increased disorderliness of basal insulin release, attenuated insulin secretory burst mass, and reduced ultradian rhythmicity of insulin secretion in older individuals. J Clin Endocrinol Metab 82:4088–4093

Meneilly GS, Veldhuis JD, Elahi D 1999 Disruption of the pulsatile and entropic modes of insulin release during an unvarying glucose stimulus in elderly individuals. J Clin Endocrinol Metab 84:1938–1943

Rigamonti AE, Cella SG, Marazzi N, Müller EE 1999 Nitric oxide modulation of the growth hormone releasing activity of hexarelin in young and old dogs. Metabolism 48:176–182

Rossoni G, De Gennaro Colonna V, Bernareggi M, Polvani GL, Müller EE, Berti F 1998 Protectant activity of hexarelin and growth hormone against post ischemic ventricular dysfunction in hearts from aged rats. J Cardiovasc Pharmacol 32:260–265

Endocrine Facets of Ageing.
Novartis 242

Print ISBN 0-471-48636-1 Online ISBN 0-470-84654-2

The ageing female reproductive axis I

Henry G. Burger*, Emma Dudley†, Pam Mamers‡, David Robertson*, Nigel Groome§ and Lorraine Dennerstein†

**Prince Henry's Institute of Medical Research, Monash Medical Centre, 246 Clayton Road, Clayton, Australia, †Office for Gender & Health, The University of Melbourne, 6th Floor, Charles Connibere Building, Royal Melbourne Hospital, Vic 3050, Australia, ‡Department of Obstetrics & Gynaecology, Monash Medical Centre, Clayton, Australia and §School of Biological & Molecular Sciences, Oxford Brookes University, Gipsy Lane Campus, Headington, Oxford, UK*

Abstract. The female reproductive axis includes the hypothalamo–pituitary unit, the ovaries and the uterus. While changes in the brain may contribute to reproductive ageing, the major focus of current research is on the ovary, where the progressive loss of follicles ultimately leads to absent follicular function and consequent permanent cessation of menstruation, the menopause. The pituitary gonadotropins, follicle-stimulating hormone (FSH) and luteinizing hormone, stimulate ovarian secretion of oestradiol and the inhibins from follicular granulosa cells, and androgens from interstitial cells, including the theca. A primary event in the ageing of the reproductive axis appears to be a decline in the secretion of inhibin B as follicle numbers fall. This leads to a slow rise in FSH in women who continue to cycle regularly, particularly in the last decade of reproductive life. As the menopause approaches, decreasing concentrations of both oestradiol and inhibin B lead to more marked increases in the gonadotropins, which reach their postmenopausal peak 2–3 years after final menses. In contrast, total testosterone concentrations are maintained across the menopausal transition, with a fall in sex hormone binding globulin (SHBG) and hence a rise in free testosterone.

2002 Endocrine facets of ageing. Wiley, Chichester (Novartis Foundation Symposium 242) p 161–171

In women as opposed to men, there is a striking and readily observable marker of reproductive ageing, the spontaneous cessation of menstrual bleeding or menopause. In this paper, the characteristics of the hypothalamo–pituitary–gonadal axis are summarized with respect to contemporary research on the inhibins in particular. The overall perspective put forward is that the endocrine changes which characterize female reproductive ageing are the consequence of the progressive decline in ovarian follicle number, with the ultimate loss of granulosa cell function. The precise mechanisms underlying follicular loss remain to be characterized fully. While much research has concentrated on changes in

oestradiol levels and their consequences, this review also summarizes data on the inhibins and on androgens, particularly testosterone.

The hypothalamo–pituitary–ovarian axis

The hypothalamo–pituitary–ovarian axis is a classical example of a closed loop endocrine negative feedback system. Pulsatile secretion of gonadotropin-releasing hormone (GnRH), from hypothalamic peptidergic neurons drives the pulsatile secretion of luteinizing hormone (LH) and follicle-stimulating hormone (FSH) from the anterior pituitary. Whilst LH secretion depends on GnRH input, there is a GnRH-independent component of FSH secretion, probably driven by intrapituitary secretion of activin (Corrigan et al 1991), and illustrated by the ability of dispersed pituitary cell cultures to continue to secrete FSH, but to cease secreting LH (Farnworth et al 1988). The gonadotropins in turn drive ovarian production of steroid and peptide hormones. Oestradiol is the major steroid secretory product of the granulosa cell, where it is formed as a result of the action of aromatase on testosterone derived from the surrounding theca under LH control. Progesterone is the product of granulosa–lutein cell secretion following formation of the corpus luteum. The inhibins are gonadal glycoprotein hormones, again predominantly the product of the ovarian granulosa cell, though there is also evidence of thecal inhibin secretion. Two major types of inhibin have been isolated, inhibin A and inhibin B. Evidence from the localization of inhibin subunits within the ovary (Roberts et al 1993) and from studies of circulating inhibin levels (Groome et al 1996), indicates that inhibin A is a product primarily of the dominant follicle. Inhibin B on the other hand is the product of the cohort of growing follicles from which the dominant follicle is selected. The secretory patterns of the two inhibins differ. Inhibin A levels are quantitatively low throughout much of the follicular phase of the cycle and show a late follicular phase rise in parallel with the preovulatory rise in serum oestradiol. Inhibin A levels peak at mid cycle, fall briefly and then rise to reach their highest levels during the luteal phase, when inhibin A is a product of the corpus luteum, its secretion being parallel to that of progesterone. In the late luteal phase, circulating concentrations of oestradiol, progesterone and inhibin fall. Inhibin B, on the other hand, shows an early follicular phase rise and fall, parallel to the rise and fall in circulating FSH. A mid cycle peak occurs in parallel with that of inhibin A, but following this peak, inhibin B falls to low concentrations and remains low throughout the luteal phase until the initiation of the luteal–follicular transition, when its levels rise, closely associated with the intercycle rise in FSH. The inhibins specifically inhibit the synthesis and secretion of pituitary FSH by mechanisms that have not yet been clarified. Recently, two types of molecules have been recognized which may function as components of an inhibin receptor system, betaglycan

(Lewis et al 2000) and p120 (Chong et al 2000). The precise signalling mechanisms are still being elucidated. Oestradiol also exerts negative feedback effects on pituitary FSH and LH secretion, and acts predominantly at the hypothalamic level. Under certain circumstances, oestradiol exerts a paradoxical positive feedback effect involved in the generation of the mid-cycle LH surge. When operating as a negative feedback system, the pituitary–gonadal axis can be conceptualized as a system in which FSH and LH drive the ovarian production of oestradiol and the inhibins, which in turn feed back to negatively regulate gonadotropin secretion. In this model, a primary defect in ovarian inhibin secretion, for example, would be expected to lead to a monotropic increase in circulating FSH levels.

Ovarian morphology as a function of age

The numbers of ovarian primordial follicles reach their peak during fetal life. About one million are present at birth and the number then decreases progressively with age. Semi-logarithmic plots of ovarian follicle numbers as a function of age suggests that there is an increase in the rate of follicle loss at approximately age 38, so that the curve appears to be biexponential (Richardson et al 1987). This interpretation of the data has been challenged (Leidy et al 1998). Few if any follicles remain at the time of the cessation of menses and follicular numbers in older women whose cycles have become irregular are about 1/10 of those in women of similar age in whom ovarian cyclicity continues. Whether or not the rate of loss of follicles changes at age 38, there is clearly an alteration in oocyte function as fecundity decreases markedly at around that time (Schwartz & Mayaux 1982).

Endocrine markers of follicle number

Several studies of the pituitary–ovarian axis as a function of increasing age in regularly cycling women have indicated that the most striking observable change is a progressive rise in early follicular and mid cycle FSH levels without significant change in the levels of LH, oestradiol or progesterone (Lee et al 1988). This monotropic rise in FSH is explicable on the basis of changes in the circulating concentrations of inhibin B (Klein et al 1996, Burger et al 2000a). Therefore the authors (Burger et al 2000b) measured the circulating concentrations of oestradiol, FSH and the inhibins in the early follicular phase of 66 regularly cycling women aged 20–50. Serum FSH, inhibin A and oestradiol were all positively correlated with age between years 20 and 50, the increase in FSH being particularly striking in women over the age of 40. Inhibin B levels were not significantly correlated with age, though they showed a tendency to fall slowly, but in women over the age of 40

there was a highly significant inverse correlation between inhibin B and FSH ($r = -0.61$, $P < 0.001$). When log FSH was modelled as a function of log inhibin B and log oestradiol, with age fitted as a co-variate, only inhibin B was a significant independent predictor of FSH. Other studies have also shown a decline in circulating serum inhibin B levels as a function of age with no change or even an increase in oestradiol and inhibin A (Danforth et al 1998, Welt et al 1999). Thus it can be postulated that inhibin B is the main form of inhibin regulating FSH during the follicular phase of the menstrual cycle. Inhibin B levels may therefore be a marker of ovarian follicular numbers and/or function.

The importance of inhibin B as a regulator of the pituitary–gonadal axis becomes more evident at the time of onset of menstrual irregularity, marking the beginning of the menopausal transition or perimenopause. Studies from the authors' laboratory have shown that the most clear-cut change in pituitary ovarian function in women who had developed irregular menstrual cycles was a profound fall in inhibin B without significant change in inhibin A and oestradiol, and with a small but statistically non-significant increase in FSH (Burger et al 1998). As progression through the menopause transition occurs, inhibin A and oestradiol levels also fall with further rises in serum FSH. From this prospective study of a community based sample of women experiencing the menopause, it was concluded that at the time of final menses, circulating FSH levels (48.4 Iu/l) were approximately 50% of those which would ultimately be found postmenopausally, whilst circulating oestradiol was also approximately 50% of its early follicular phase levels at about 113 pmol/l (Burger et al 1999). Nadir oestradiol levels are reached two to three years postmenopausally as are peak concentrations of FSH. At this time inhibin B is undetectable and inhibin A is also very low or undetectable.

Consequences of the fall in circulating oestradiol

The clinical markers signalling the end of reproductive function in the female are symptoms resulting from loss of ovarian oestradiol secretion. The most striking and characteristic symptom is the hot flush which shows a variable prevalence in different communities, but affects 65–75% of women in developed countries. Broad correlations between circulating oestradiol concentrations and hot flush frequency have been documented in various studies (Guthrie et al 1996). In the Melbourne Women's Midlife Health project, longitudinal observations have confirmed that the only symptoms clearly related to the transition from early to late perimenopause when the profound fall in oestradiol levels occurs are hot flushes, night sweats and vaginal dryness (Dennerstein et al 2000). The other symptom which changes at this time is that of breast tenderness which falls significantly as oestradiol levels fall. The profound drop in oestradiol

concentrations, more than 90% across the menopausal transition, may have later important health consequences. There is a clear-cut acceleration in the rate of loss of bone mineral at the time of menopause (Guthrie et al 1998), continuing for five to eight years and predisposing women, in particular, to later postmenopausal osteoporotic fracture. Whether the change in circulating oestradiol also predisposes to the occurrence of cardiovascular disease, independently of the ageing process, remains a controversial issue. Long-term consequences of oestrogen deprivation may result in an increased risk of the development of Alzheimer's dementia and possibly other disorders.

Changes in circulating androgens

The significance of changes in androgen secretion is a neglected area of female reproductive ageing. One important study has documented a 50% fall in circulating total and free testosterone concentrations in normal regularly cycling women between the ages of 20 and 40 (Zumoff et al 1995). This has been postulated to reflect declining levels of adrenal androgen precursor secretion. Across the menopausal transition itself, studies from the authors' laboratory indicate that there is no significant change in the circulating concentrations of total testosterone, whilst there is a fall in sex hormone binding globulin and an increase in free androgen index (Burger et al 2000b). Studies from other investigators suggest that there may be a further increase in circulating androgen levels in the late 50s and 60s (Laughlin 2000). The precise consequences of these changes in androgen for womens' health in general are unknown. On the other hand, loss of androgen as may occur following ovariectomy may lead to significant consequences of loss of drive and energy and loss of sexual interest which can be corrected by androgen substitution (Shifren et al 2000).

Conclusions

The most striking endocrine marker of female reproductive ageing is a progressive increase in the concentrations of circulating FSH, possibly beginning in the 20s and becoming more obvious from approximately age 40. This increase in FSH may be the result primarily of a decrease in the concentrations of circulating inhibin B, acting as a marker of primordial follicle number. When follicle numbers fall to critical levels, granulosa cell function becomes impaired and subsequently ceases with falls in circulating oestradiol and inhibin A and further increases in FSH. Changes in circulating androgens appear to occur particularly during reproductive life when levels fall between ages 20 and 50. The implications of ageing of the female reproductive axis for health and health policy remain to be fully determined.

References

Burger HG, Cahir N, Robertson DM et al 1998 Serum inhibins A and B fall differentially as FSH rises in perimenopausal women. Clin Endocrinol 48:809–813 (erratum: 1998 Clin Endocrinol 49:550)

Burger HG, Dudley EC, Hopper JL et al 1999 Prospectively measured levels of serum follicle-stimulating hormone, estradiol and the dimeric inhibins during the menopausal transition in a population-based cohort of women. J Clin Endocrinol Metab 84:4025–4030

Burger HG, Dudley E, Mamers P, Groome P, Robertson DM 2000a Early follicular phase serum FSH as a function of age: the roles of inhibin B, inhibin A and estradiol. Climacteric 3: 17–24

Burger HG Dudley EC, Cui J, Dennerstein L, Hopper JL 2000b A prospective longitudinal study of serum testosterone dehydroepiandrosterone sulfate and sex hormone-binding globulin levels through the menopause transition. J Clin Endocrinol Metab 85:2832–2938

Chong H, Pangas SA, Bernard D J et al 2000 Structure and expression of a membrane component of the inhibin receptor system. Endocrinology 141:2600–2607

Corrigan AZ, Bilezikjian LM, Carroll RS et al 1991 Evidence for an autocrine role of activin B within rat anterior pituitary cultures. Endocrinology 128:1682–1684

Danforth DR, Arbogast LK, Mroueh J et al 1998 Dimeric inhibin: a direct marker of ovarian aging. Fertil Steril 70:119–123

Dennerstein L Dudley EC, Hopper JL, Guthrie JR, Burger HG 2000 A prospective populated-based study of menopausal symptoms. Obstet Gynecol 96:351–358

Farnworth PG, Robertson DM, de Kretser DM, Burger HG 1988 Effects of 31 kilodalton bovine inhibin on follicle-stimulating hormone and luteinizing hormone in rat pituitary cells *in vitro*: actions under basal conditions. Endocrinology 122:207–213

Groome NP, Illingworth PJ, O'Brien M et al 1996 Measurement of dimeric inhibin B throughout the human menstrual cycle. J Clin Endocrinol Metab 81:1401–1405

Guthrie JR, Dennerstein L, Hopper JL, Burger HG 1996 Hot flushes, menstrual status and hormone levels in a population-based sample of midlife women. Obstet Gynecol 88:437–442

Guthrie JR, Ebeling PR, Hopper JL et al 1998 A prospective study of bone loss in menopausal Australian-born women. Osteoporos Int 8:282–290

Klein NA, Illingworth PJ, Groome NP, McNeilly AS, Battaglia DE, Soules MR 1996 Decreased inhibin B secretion is associated with the monotropic FSH rise in older, ovulatory women: a study of serum and follicular fluid levels of dimeric inhibin A and B in spontaneous menstrual cycles. J Clin Endocrinol Metab 81:2741–2745

Laughlin GA, Barrett-Connor E, Kritz-Silverstein D, von Mühlen D 2000 Hysterectomy, oophorectomy, and endogenous sex hormone levels in older women: the Rancho Bernado study. J Clin Endocrinol Metab 85:645–651

Lee SJ, Lenton EA, Sexton L, Cooke ID 1988 The effect of age on the cyclical patterns of plasma LH, FSH, oestradiol and progesterone in women with regular menstrual cycles. Hum Reprod 3:851–855

Leidy LE, Godfrey LR, Sutherland MR 1998 Is follicular atresia biphasic? Fertil Steril 70: 851–859

Lewis KA, Gray PC, Blount AL et al 2000 Betaglycan binds inhibin and can mediate functional antagonism of activin signalling. Nature 404:411–414

Richardson SJ, Senikas V, Nelson JF 1987 Follicular depletion during the menopausal transition: evidence for accelerated loss and ultimate exhaustion. J Clin Endocrinol Metab 65:1231–1237

Roberts VJ, Barth S, el-Roeiy A, Yen SS 1993 Expression of inhibin/activin subunits and follistatin messenger ribonucleic acids and proteins in ovarian follicles and the corpus luteum during the human menstrual cycle. J Clin Endocrinol Metab 77:1402–1410

Schwartz D, Mayaux MJ 1982 Female fecundity as a function of age: results of artificial insemination in 2193 nulliparous women with azoospermic husbands. Federation CECOS. Federation CECOS. N Engl J Med 306:404–406

Shifren JL, Braunstein GD, Simon JA et al 2000 Transdermal testosterone treatment in women with impaired sexual function after oophorectomy. N Engl J Med 343:682–688

Welt CK, McNicholl DJ, Taylor AE, Hall JE 1999 Female reproductive aging is marked by decreased secretion of dimeric inhibin. J Clin Endocrinol Metab 84:105–111

Zumoff B, Strain GW, Miller LK, Rosner W 1995 24 h mean plasma testosterone concentration declines with age in normal premenopausal women. J Clin Endocrinol Metab 80:1429–1430

DISCUSSION

Veldhuis: Is the quality of current ultrasonography such that one can argue whether the amount of inhibin produced per antral follicle is declining, or there are just fewer follicles entering the antral stage and then eventually being selected?

Burger: The evidence would be very strongly that the number of antral follicles developing decreases as the total primordial follicle pool falls. This has been modelled by Faddy & Gosden (1995) among others. Their model strongly indicates that the number of follicles recruited gets progressively smaller. To my knowledge there is only one study done ultrasonographically that specifically looked at this, and it does show a marked decline in the numbers of visible follicles with increasing age. This is a potentially fertile area for more study.

Veldhuis: What is the evidence that follicle function is decreasing? You are saying that it may not be. If one gave these women recombinant human FSH under GnRH antagonist blockade, would one expect inhibin B secretion to respond normally?

Burger: This is a difficult study, which we have been talking about doing. My prediction would be that the response would decrease, because there will be a smaller number of follicles. This is precisely the problem I have had in trying to think about how to design the study. How do you nail whether it is a per follicle or total numbers issue?

Veldhuis: There was a paradoxical oestradiol elevation in the face of a granulosa cell that under-secretes inhibin. What do you postulate is happening here?

Burger: I don't have a good explanation, except the differential regulation phenomenon that I postulated. If the follicle starts to drop off its inhibin secretion, this allows FSH to rise. Our postulate is that oestradiol and inhibin normally contribute about 50% each. This is based on physiological oestradiol replacement therapy in post-menopausal women, for example, and looking at the suppression of FSH that results from elevating oestradiol to follicular phase levels. The feedback is about 50:50, and if you drop out one of the feedback factors, if the oestradiol was thinking about switching off as well, the higher FSH will stimulate it into keeping going for a while.

Veldhuis: So you don't find elevated oestradiol without elevated FSH.

Burger: Our data are on a once-a-year sample basis. They are not sufficient to answer that. I am not far enough through the analysis to tell you too much about another study which I have done in collaboration with Britt-Marie Landgren in Stockholm. She has done a longitudinal study of about 15 women who have been studied annually from age 45–47 until they reach their menopause. They have had blood sampling three times a week through one cycle each year for up to eight years. I am hoping to be able to dissect out some of those questions in that group. Interestingly, it seems that the women who continue to have ovulatory cycles with normal luteal phases don't show much of a rise in FSH in the follicular phase. It is only the women who start to show luteal insufficiency, or who have anovulatory cycles of various kinds, who do. My preliminary take on that number is that about 50% of the women continue to have ovulatory cycles right to the end of the transition, even though they start to become irregular, whereas the others have all sorts of cycle patterns even though their degree of irregularity doesn't seem too different from those who don't.

Prior: From some of the data, it looks like there are more follicular type cysts in the ovaries of perimenopausal women. Are you firm on the idea that each follicle makes the same amount of inhibin?

Burger: I am not firm on the idea; I have no reason to believe they wouldn't. The cysts may reflect the higher FSH level.

Björntorp: Could you expand on the regulation of the androgen production? Also, we are planning to treat our slightly hypoandrogenic women, who we think are sick. They are all premenopausal. If we are going to treat them with androgen, what is the best way of doing this?

Burger: The regulation of post-menopausal ovarian androgen secretion has not been very fully worked on. There do appear to be a small number of subjects (presumably those with the highest androgen levels) who have stromal hyperplasia, even though by other criteria they haven't had polycystic ovary syndrome. There may be some degree of autonomy about the androgen secretion in those with stromal hyperplasia. My hypothesis is that the stromal and interstitial cells that remain in the ovary are the source of androgen. There must be some aromatase there because some post-menopausal ovaries do continue to secrete a small amount of oestrogen. If you measure the ovarian venous arterial difference, there is evidence that oestradiol is being made by the ovary. I suspect that the androgen secretion is primarily LH driven, as it is premenopausally, and that when oestrogen is given, it does two things: it suppresses gonadotropin secretion and therefore also the androgen secretion to a degree, and if the oestrogen is given orally it will also increase SHBG levels such that the free androgen levels will drop disproportionately. Our suspicion is that a number of women who are given standard hormone replacement therapy either continue to have or develop

symptoms of androgen deficiency, including loss of libido, because their free androgen levels have been dropped heavily. I don't know of many systematic studies that have really looked at the regulation of androgen secretion. It is not an easy area to study.

Handelsman: Can I draw you out on the fall in testosterone in younger women? Are there longitudinal data to support this, as claimed by some, and what do you think the mechanisms are?

Burger: I don't know of any longitudinal data in the younger age group. As you saw, our studies began with women who were already 45. There are few longitudinal studies in this area, and yet they are crucial. I have no idea about the mechanism.

Riggs: Have you attempted to do regression analyses to see whether there is a threshold level of oestradiol at which point bone loss begins? And does it continue linearly or does it accelerate as oestradiol levels decrease?

Burger: The people primarily involved in the bone studies with us are John Wark and Peter Ebeling. We didn't see a high degree of correlation between change in oestradiol and change in bone density between measurements. I don't think we have identified a threshold point, and from our limited data the rate of loss was maximal in the group right at the time they became post-menopausal. Those who remained post-menopausal already had a lesser rate of change than that very early group.

Carroll: Women in this age group have a fairly high incidence of mood disorder. Depending on whom you read, 15–30% may have depression at some time in the period that you are studying. I see you have Lorraine Dennerstein (a psychiatrist) as a collaborator. Can you make any comments on the timing of menopausal events *vis-à-vis* presence of mood disorder?

Burger: Lorraine Dennerstein is the principal investigator for our overall study, and I represent the endocrine arm of it. When we did a longitudinal analysis of symptoms, which included some of the symptoms you would associate with mildly depressed mood, we couldn't establish a correlation between these and the occurrence of menopause. There are three things that come out of that longitudinal analysis: hot flushes/night sweats and vaginal dryness are the two symptoms positively related to the occurrence of menopause, and the third one is disappearance of breast tenderness.

Carroll: My question was whether a history of depression predicts an earlier onset of the menopausal events.

Burger: I am not aware of it, and I think the relationship between mood disorder and menopause has become clouded. To my knowledge it is much more related to premenopausal experiences and psychosocial background than it is to the menopause itself. In contrast to that, there was a paper published recently from the group at NIH (Schmidt et al 2000). They did a prospective randomized

controlled trial in which they administered transdermal oestradiol to a group of women who were moderately depressed at the time of menopause. This showed a positive short-term effect.

Shalet: I think you said you thought it was pretty useless to measure FSH and oestradiol in terms of predicting the development of the menopause. Is this also true for an inhibin B measurement? As I recollect, Faddy & Gosden (1996) describe a change in the rate of atresia around about 37–38 years of age. Is that correct, and if so what causes it?

Burger: My grip on the data as far as inhibin B is concerned is that it probably is not a lot better. We need to examine this more critically. It may turn out to be better. It certainly is an area which people working in assisted reproductive technology (ART) programmes have addressed quite carefully. Is inhibin B predictive of reproductive outcome in response to ART? Although this is controversial, the best studies have shown that it is not predictive. In terms of the rate of atresia, the semi-logarithmically plotted data do suggest that there is a break in the line around age 38 with an increased rate of loss beyond this. This has been challenged in a paper in *Fertility and Sterility* by Leidy et al (1998). They argue that this was a mathematical artefact. In contrast, it is around age 38 that the decline in fertility really kicks in. Clearly, the phenomenon that explains follicle loss in atresia appears to be apoptosis. To me this only takes the question back one stage: why does apoptosis occur?

Laron: The IGF1 decline may play a role in apoptosis; it is known that IGF1 delays or prevents apoptosis.

Morley: I was interested in your comment that testosterone goes up post-menopausally. As you know, muscle also falls off fairly dramatically post-menopausally. It is not likely to be related to oestrogen. How convinced are you that testosterone goes up over this period, and in particular bioavailable testosterone? What is its role in replacement post-menopausally?

Burger: In our data, there was a fairly slow increase in free androgen index across the menopause. Trying to continue a longitudinal study like this is extremely difficult to get funding for, because the agencies say that we have already had enough money and want to start funding elsewhere. I can't tell you any more about our longitudinal study, which I think is the way to solve this. The data I am talking about are cross-sectional and from at least three groups. The most recent was a paper from Laughlin et al (2000) who looked at over 600 women. They showed that from the late 50s right up to 80, there was an increase in total testosterone and also an increase in SHBG. Thus free androgen index didn't change. I don't know what the function of testosterone is at that stage. Perhaps the most striking place where a function of testosterone is seen is in the young woman who has had her ovaries removed. If you give her adequate oestrogen and progesterone replacement therapy, often she will continue not to be in

optimal health: she will lack energy and drive, and have low libido. If you give her physiological level testosterone replacement it is strikingly beneficial. There was a recent paper from Jan Shifren in the *New England Journal of Medicine*, who gave testosterone transdermally strictly in the physiological dosage range to oophorectomized women (Shifren et al 2000). This showed benefit in the older age group. I believe from clinical practice that in a subset of women who present peri- or post-menopausally with poor energy and loss of libido, in whom testosterone levels are at the low end of the range and in whom you cannot discern a relationship problem, testosterone is highly clinically beneficial.

Handelsman: The free androgen index in men is particularly inappropriate because it cannot measure free testosterone (Kapoor et al 1993). The assumption is that the testosterone is negligible related to SHBG concentrations (100-fold difference), which is not the case in men. However, this is true in women, and free androgen index does not have the same theoretical problems in women.

References

Faddy MJ, Gosden RG 1995 A mathematical model of follicle dynamics in the human ovary. Hum Reprod 10:770–775

Faddy MJ, Gosden RG 1996 A model conforming the decline in follicle numbers to the age of menopause in women. Hum Reprod 11:1484–1486

Kapoor P, Luttrell BM, Williams D 1993 The free androgen index is not valid for adult males. J Steroid Biochem Mol Biol 45:325–326

Laughlin GA, Barrett-Connor E, Kritz-Silverstein D, vov Muhlen D 2000 Hysterectomy, oophorectomy, and endogenous sex hormone levels in older women: the Rancho Bernardo Study. J Clin Endocrinol Metab 85:645–651

Leidy LE, Godfrey LR, Sutherland MR 1998 Is follicular atresia biphasic? Fertil Steril 70: 851–859

Shifren JL, Braunstein GD, Simon JA et al 2000 Transdermal testosterone treatment in women with impaired sexual function after oophorectomy. N Engl J Med 343:682–688

Schmidt PJ, Nieman L, Danaceau MA et al 2000 Estrogen replacement in perimenopause-related depression: a preliminary report. Am J Obstet Gynecol 183:414–420

Endocrine Facets of Ageing.
Novartis 242

Print ISBN 0-471-48636-1 Online ISBN 0-470-84654-2

The ageing female reproductive axis II: ovulatory changes with perimenopause

Jerilynn C. Prior

Division of Endocrinology and Metabolism, University of British Columbia, Vancouver Hospital and Health Sciences Centre, Vancouver, BC, Canada V5Z 1C6

Abstract. Perimenopause, a complex physiological transition for midlife women, begins with changes in experiences many years before cycles become irregular, oestradiol levels decrease or follicle-stimulating hormone levels increase. Erratic and average higher oestradiol levels as well as shorter luteal phase lengths and lower progesterone levels occur during perimenopause. These ovarian changes may be causally related to lower inhibin production but the dynamic prospective inter-relationships within women are not well documented. This review will first define perimenopause and then explore the limited published data on ovulatory characteristics in perimenopause. In addition, it will report preliminary prospective observational data on menstrual cycles and ovulation in initially ovulatory women followed through the perimenopause. Prospective data suggest that ovulation disturbances begin early in perimenopause and increase with irregular cycles. Combined with higher oestradiol levels they may cause menorrhagia. It is not yet known whether disturbances of ovulation relate to bone loss in perimenopausal, as in premenopausal, women. It is also not known whether progesterone therapy can effectively counteract the end organ (breast, endometrial, brain) effects of higher/erratic oestradiol levels and effectively treat perimenopausal vasomotor and other symptoms.

2002 Endocrine facets of ageing. Wiley, Chichester (Novartis Foundation Symposium 242) p 172–192

Each woman is born with an average of over a million follicles in her two ovaries, and each follicle contains an egg that could potentially be released and fertilized. The life cycle of each woman's cohort of follicles is not well known but includes continuous maturation (that may manifest as ovarian cysts; Merrill 1963) and atresia of immature follicles that begin long before puberty. Therefore, independent of pituitary stimulation or ovulation, follicle numbers steadily decrease. Prior to puberty, the ovaries enlarge and cystic activity increases but the first 10–12 years following menarche is required before the majority of women consistently and normally ovulate (Vollman 1977). This review of ovulation will focus on ovulation during perimenopause, the final portion of the life cycle of ovarian follicles.

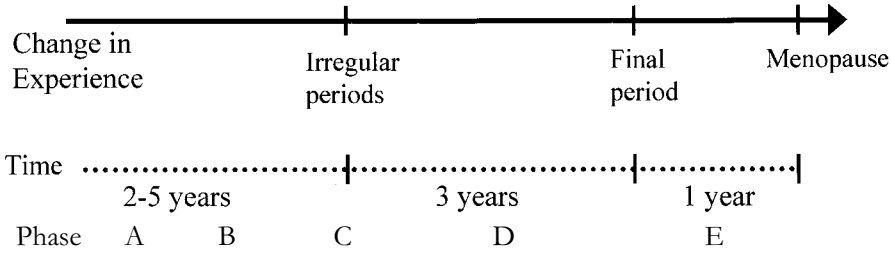


FIG. 1. This time line diagram shows the approximate time intervals for the characteristic changes of the perimenopause. Phases A and B occur before irregular periods begin. Phases C and D occur before the year of final menstrual flow that is phase E of the perimenopausal transition (Prior 1998).

Perimenopause is understood to begin when the number of remaining ovarian follicles reaches some (unknown) low point. Cross-sectional ovarian histological studies suggest that the rate of follicle 'loss' accelerates abruptly in the late 30s or early 40s (Richardson et al 1987). On the face of it, that information suggests that ovulation becomes more prevalent during perimenopause. Perimenopause, for this review, begins when a woman reports a change in experience (such as increased breast tenderness and premenstrual symptoms with or without night sweats and sleep disturbances, Fig. 1). That the ovaries become depleted of follicles fits with the prevalent notion that perimenopause is a time of ovarian senescence during which 'oestrogen deprivation' develops. In fact, cross-sectional data have been interpreted to support such an oestradiol decline (Burger et al 1995) although a meta-analysis of all controlled studies in which oestradiol levels have been systematically measured in perimenopausal and premenopausal control women shows that oestradiol levels are significantly higher in perimenopausal women (Prior 1998). These higher and erratic oestradiol levels are postulated to be due to decreasing ovarian inhibin production (Burger et al 2002, this volume) and others (Klein et al 1996, Welt et al 1999). These lower inhibin levels allow follicle-stimulating hormone (FSH) levels to rise slightly but not significantly, which stimulates multiple follicles each of which secretes some oestradiol. The resulting oestradiol levels are not only higher but because of disturbed pituitary–ovarian feedback, are also non-suppressible (Fig. 2) (Prior 1998).

Rising and uninhibited FSH levels stimulate maturation and oestradiol production by more follicles but it is not clear whether or not ovulation and progesterone production are also hyperstimulated. More ovarian cysts, that suggest anovulation, appear to be present (Djerassi et al 1995). These women, were they to become pregnant, would more likely produce non-identical twins (Gilfillan et al 1996). However, all of the few studies in which ovulation has been

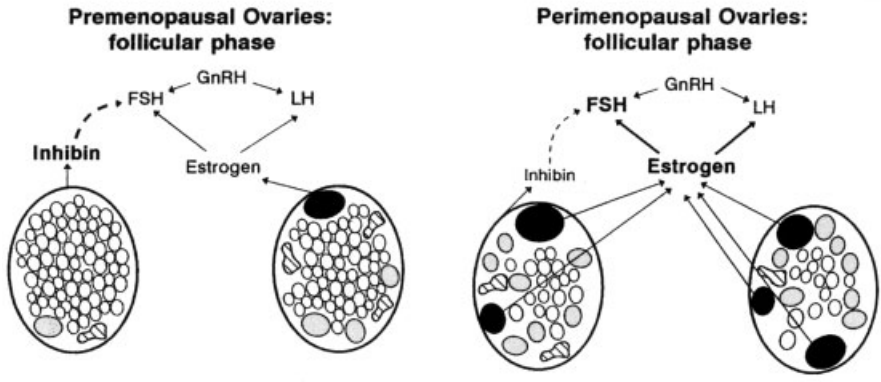


FIG. 2. Diagram of the ovaries in the follicular phase and the inter-relationships among ovarian oestradiol, inhibin and FSH productions during the premenopausal and perimenopausal years. Lacking adequate inhibin production from ovarian follicles (dark ovoids), perimenopausal FSH levels rise slightly and the increasing oestradiol levels are not sufficient, with lower inhibin levels, to suppress them. Dotted line, inhibition; solid line, stimulation; shaded ovoids, growing follicles, striped bodies, atretic corpus luteum. Reprinted from Prior (1998) with permission of the Endocrine Society.

documented prospectively and some cross-sectional studies indicate that disturbances of ovulation are an integral part of the ageing of the reproductive system for women.

The purpose of this review is to examine reproductive ageing in women as it relates to ovulation and progesterone production throughout the several postulated phases of the perimenopause (Table 1) (Prior 1998). This paper will focus not only on available data concerning ovulation in perimenopause, but also highlight the potential physiological and clinical consequences of such ovulation disturbances. Finally, it will review the potential data from studies in progress and propose therapy with progesterone for symptomatic perimenopausal women.

It is important first, to define ovulation disturbances and indicate how they are diagnosed. The term 'ovulation disturbance' includes a spectrum of changes in progesterone production by the corpus luteum including short luteal phase (SLP) and anovulatory cycles (Anov) (Prior et al 1990a). An SLP lasts less than 12 days from the luteinizing hormone (LH) peak or has fewer than 10 days of significant basal temperature elevation (Vollman 1977). Ovulation disturbances and luteal phase length can be documented by quantitative basal temperature measurement methods using least squares or mean temperature analysis to determine whether and where in the cycle a significant temperature increase occurs (Prior et al 1990b). Ovulation disturbances can also be diagnosed using daily or intermittent measurements of blood (Welt et al 1999), saliva or urinary progesterone breakdown products such as pregnanediol corrected for creatinine

(PdG) (Santoro et al 1996). However, no single gold standard for ovulation exists and criteria for diagnosing ovulation and luteal phase length with each method are not well described and validated. Importantly, no ovulation documentation methods are suitable for continuous monitoring over long periods of time and in random participants from populations.

Ovulation disturbances and perimenopause

The following will briefly describe the perimenopausal prevalence and incidence of ovulation disturbances while expanding on a previous review (Prior 1998). Cross-sectional data will be reviewed before the prospective and epidemiological data are examined.

The earliest cross-sectional studies of ovulation in 40–50 year old women used non-quantitative basal temperature methods. Collett et al (1954) documented 302 cycles from 146 women aged 17–50 using the basal temperature nadir (assessed visually from graphed data) as the day of ovulation. By these imperfect temperature analysis criteria, 15.1% of cycles in women ages 40–50 were anovulatory. This contrasted with an average of about 2% anovulatory cycles in women aged 25–34. Doring (1969), also using non-quantitative basal temperature methods, showed SLP and anovulatory cycles were more prevalent in cyclic women of increasing age.

Vollman, a Swiss physician, collected basal temperatures for 14 848 cycles in over 500 women and analysed them using an innovative quantitative mean basal temperature (QBT) analysis method (that has subsequently been validated against the midcycle LH peak; Prior et al 1990b). Women were stratified by gynaecological age (years since menarche); the prevalence of ovulation disturbances was documented to be almost 60% in the first year and about 21% in the cycles at highest gynaecological ages (Vollman 1977) (Fig. 3). Results from the three large basal temperature-based data sets differ from those of the majority of smaller cross-sectional studies using hormonal measurements. Four careful studies did not show lower progesterone or PdG levels in older women (Lee et al 1988, Reame et al 1996, Reyes et al 1977, Welt et al 1999). However, two hormone-based cross-sectional studies did show significantly lower progesterone levels or PdG excretions in older women (Ballinger et al 1987, Santoro et al 1996). One study tested PdG from daily overnight urine samples across one cycle in women with regular cycles, contrasting 11 women over age 47 with 11 under 36. The older women had significantly lower PdG levels as well as higher urinary total oestrogen excretions (Santoro et al 1996). Similar results were obtained using weekly serum sampling (Ballinger et al 1987).

Thus cross-sectional studies suggest but do not support the hypothesis that ovulation disturbances are more prevalent in perimenopause. However, in none

TABLE 1 Proposed phases of the perimenopause: clinical and hormonal characteristics

	Phase A	*Phase B*	*Phase C*	*Phase D*	*Phase E*
Duration	~2–24 months	~2–24 months	~1–2 years	~1–2 years	1 year
Menstrual cycles	Regular — cycle length shorter; short FP+. Usually ovulatory	Regular — cycle length shortest; short FP+. Onset of ovulatory disturbances — short luteal phase and anovulation	Irregular — alternating short and long cycles, normal ovulation less than 50%	Oligomenorrhoea. Rare normal ovulatory cycles	Amenorrhoea
Flow	Increased or the same	Often increased in duration and amount	↑↑ with flooding or ↓; often alternating	Spotting alternating with flooding is common	None
Menstrual cycle-related symptoms	↑ breast tenderness, mood swings and swelling before flow*. Dysmenorrhoea ±↑ Headaches ↑ and/or migraines start	↑↑ breast tenderness, mood swings and swelling before flow*. Often ↑↑dysmenorrhoea. Headaches in migraine sufferers often severe	Less cyclic breast, mood and fluid symptoms*, or present and unpredictable. Dysmenorrhoea less related to flow. May occur at midcycle or for many days in the cycle	Breast tenderness persistent in some women ± other premenstrual symptoms*. Dysmenorrhoea may be ↓ or persistent in a few. Sometimes is relieved with flow	Breast tenderness, mood and fluid symptoms less*. Sometimes occur without subsequent flow. Dysmenorrhoea usually gone

Vasomotor symptoms	Often begin. First onset, cyclic before and during flow usually in the night or very early morning hours. May occur at midcycle. Rare daytime VMS	Cyclic related to flow and usually still night sweats during or at the end of sleep. Fewer VMS in midcycle Rare daytime VMS	Night sweats still tend to be cyclic, but less predictable. For some women VMS symptoms ↓ but others begin to have marked ↑. First daytime VMS	Night and day VMS no longer predict flow. For some women they may be very severe both day and night, especially in long cycles	Night and day VMS may become daily and incessant. In others they are rare, have decreased or generally disappear
Hormonal characteristics	↑ E2 in early FP and premenstrually. FSH normal	↑↑ E2 especially with ovulatory disturbances. FSH intermittently	E2 normal alternating with ↑↑, especially around flow	E2 normal except ↑↑ levels intermittently in long cycles. ↑FSH	E2 normal or slightly low but ↑↑ intermittently. ↑↑ FSH
	LH normal ? Inhibin B ↓ Inhibin A normal	LH normal. Inhibin B ↓. Inhibin A ↓ with ovulatory disturbances	↑ FSH more common. ↑ LH occasionally. Inhibin B ↓↓. Inhibin A ↓.	↑LH now common. Inhibin B ↓↓. Inhibin A ↓↓.	↑↑ LH much of the time. Inhibins B and A both below assay sensitivity

↑, moderately increased; ↑↑, very high; ?, uncertain; E2, oestradiol; +FP, follicular phase; *, premenstrual symptoms; VMS, vasomotor symptoms.
Modified from Prior (1998).

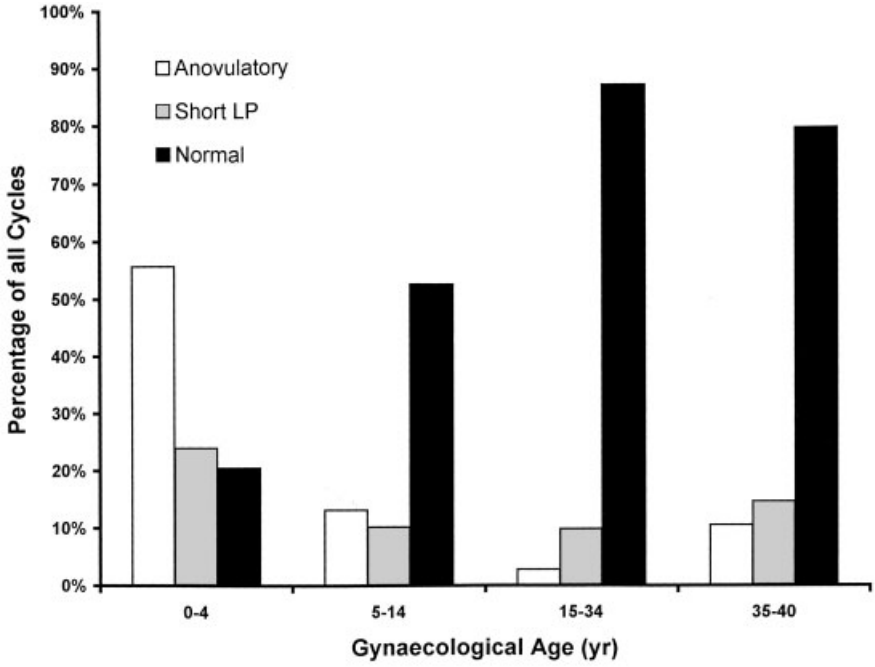


FIG. 3. This bar diagram of ovulation disturbances cross-sectionally shows the percentages of cycles in women of different gynaecological ages that are anovulatory (open bars), have SLPs (short LP, grey bars) and are normally ovulatory (normal, black bars). Note that anovulatory cycles increase in women of gynaecological ages 35–40 but that SLPs increase only slightly. Adapted to represent data by Vollman (1977).

of these cross-sectional studies are the necessary clinical or prospective menstrual cycle data reported that would allow a diagnosis of perimenopause or assessment of the phase of participants.

There are even fewer prospective studies of ovulation and its disturbances during perimenopause. The majority of those that do exist poorly describe recruitment criteria and the participants' physiological characteristics and clinical experiences. Most well designed longitudinal studies report menstrual cycle data without documentation of ovulation (Brambilla et al 1994, Kaufert et al 1987, Treloar 1981). A number of other important longitudinal studies collecting intermittent hormonal data have also not documented ovulation (Guthrie et al 1998, Longcope et al 1986). Even if ovulatory status is determined, luteal phase lengths are not assessed systematically by any longitudinal data sets except those of Vollman (1977). Others have only documented anovulatory but not SLP cycles, because weekly urine sampling does not allow luteal length quantification (Metcalf 1979, Brown 1985).

The available prospective data consistently show that ovulation disturbances increase in perimenopause. Vollman (1977) diagrams the final 100 cycles in one woman who experienced 16% of cycles as anovulatory. Vollman also noted that within one woman the basal temperatures decreased (in both the follicular and luteal phases) in cycles during the 3rd and 4th decades from menopause compared with the 2nd and 1st (Vollman 1977). Temperatures were lower by −0.1 °C in the follicular and by –0.15 °C in the luteal phase, suggesting an added effect of lower progesterone production in addition to the effect of an apparently lower metabolic rate (documented earlier, Collett 1949).

Although detailed cycle data are graphed for three or four cycles each from only four women, Shideler shows the variability of both flow and ovulation (Shideler et al 1989). Figure 3 shows that each woman in phase C of perimenopause experienced variations in both luteal phase and in cycle lengths and that each experienced ovulation disturbances. These women, in contrast to many reported in the above cross-sectional studies, were perimenopausal by cycle variability and/or vasomotor symptoms (VMS) (Shideler et al 1989).

A much longer set of weekly urinary hormonal data are available from each of two women over the final 6–7 years of the perimenopausal transition (Brown 1985), and from one woman over 108 consecutive weeks (Metcalf & Donald 1979). The two women studied by Brown recorded 15 and 23 episodes of flow and both had several months of no flow at the end of monitoring. Using 5 as the PdG cut-off for ovulatory cycles, 8 of 15 and 10 of 23 cycles — only 48.4% — were ovulatory (Brown 1985). The longitudinal data in one woman, however, appeared to be earlier in the perimenopausal transition and showed that 86% of cycles were ovulatory (18 of 21 flow episodes were ovulatory by PdG) (Metcalf & Donald 1979).

In a larger and more systematic study, and the only one that had as its primary focus as the prospective study of ovulation in perimenopause, Metcalf systematically documented weekly urinary PdG over three cycles in 139 women over 40 years of age (Metcalf 1979). 93% of the 86 participants in whom all of the documented cycles were of normal cycle length (21–35 days) were ovulatory (although some probably were SLP cycles) as were 95% of the cycles in the 81 women who reported no recent cycle changes. However, if cycles were irregular either by history (in 58 women) or by prospective documentation (in 53 women), the percentage of the three cycles that were consistently ovulatory decreased to 39.7% and 34%, respectively (Metcalf 1979). These latter women who were perimenopausal by epidemiological criteria based on irregular cycles had an average of 36.9% of consistently ovulatory cycles (Metcalf 1979). Despite this, PdG levels did not differ with age and 10.3% of women who had gone three months without flow subsequently ovulated (Metcalf 1979).

The final prospective study documented three women aged 37–47 for whom one cycle of daily hormonal data were available approximately 10 years previously and graphically displayed two cycles of hormonal data 11 years apart from one woman (Welt et al 1999). Mean progesterone levels were lower in women studied 10 years later (4.9 versus 11.7 ng/ml) in addition to lower luteal phase inhibin A levels and borderline lower follicular phase inhibin B levels. No significant prospective differences in LH, FSH or oestradiol were documented (Welt et al 1999). From graphed data, luteal phase lengths shortened in one woman followed over 11 years (10 and 11 versus 12 and 13 days from the LH peak) (Welt et al 1999).

A large prospective epidemiological study from Malmo, Sweden, providing the only population-based data of ovulation available, studied over 150 women for 12 years with serum hormone levels measured at 3–6 monthly intervals (Rannevik et al 1995). This study followed women through menopause and for several years following it. In women who were 72–61 months before their final flow, 62.2% of cycles were ovulatory with a mean progesterone level of 27.3 nmol/l. However, women in the 0–6 months before the final flow experienced ovulatory cycles only 4.8% of the time although the average progesterone level was unchanged at 22.4 nmol/l (Rannevik et al 1995). These data were presented as a summary in the text but no primary progesterone data were provided.

Thus prospective data consistently document ovulation disturbances as a part of perimenopause and reproductive ageing in women. In women who were symptomatic with new cycle irregularity or vasomotor symptoms the incidence of ovulation disturbances was increased. However, the literature contains information about prospective variations in ovulation in relatively few women and includes no primary data from epidemiological samples. These prospective results (Fig. 4) suggest that ovulation disturbances do not generally increase until phase C of perimenopause when cycle intervals start becoming variable.

Clinical effects of ovulation disturbances

The focus on ovulation disturbances in this paper has so far only discussed their prevalence and incidence. It is likely however, that any SLP or persistent ovulation disturbances present in phases A and B when cycles are still regular causes dysfunctional bleeding and menorrhagia in women experiencing high oestradiol levels. It is also likely that premenstrual symptoms are increased in cycles with ovulation disturbances in addition to high oestradiol levels (Wang et al 1996). Severe mood swings, increased stretchy mucus and breast tenderness prior to and during flow commonly coexist with disturbed perimenopausal ovulation. Ballinger described heavy flow in such cycles (Ballinger et al 1987). In addition, the cycles with ovulation disturbances may be at increased risk for VMS based on data suggesting increased VMS prior to

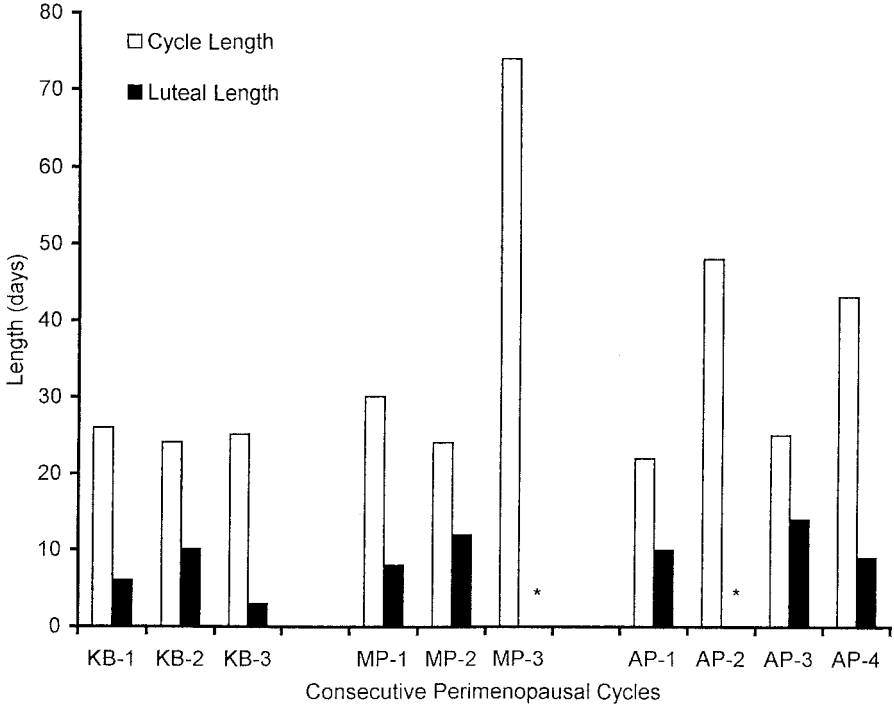


FIG. 4. The ovulatory characteristics of three or four consecutive cycles from three perimenopausal women. The cycle lengths are shown as open bars and luteal phase lengths are shown in black. Using 10 days as the lower limit of normal, each woman had at least one SLP and two of three women had anovulatory cycles (*). Redrawn from data by Shideler et al (1989).

flow and that progesterone/progestin therapy is an effective treatment. Ovulation disturbances are related to rapid cancellous bone loss in regularly cycling, initially ovulatory premenopausal women (Prior et al 1990a) and cyclic medroxyprogesterone therapy increases bone density in women with menstrual cycle and ovulation disturbances (Prior et al 1994). It is probable, therefore, that ovulation disturbances in any phase of perimenopause, but especially phases C and D contribute to the increased bone loss that occurs at that stage of the transition (Okano et al 1998, Prior 1998).

In summary, ovulation disturbances are probably of clinical as well as physiological consequence in the perimenopause and may relate to menorrhagia, VMS, mood disturbances and accelerated bone loss. Studies are needed to document this because therapy with cyclic progesterone or medroxyprogesterone may well be effective in symptomatic perimenopausal women.

Research in progress

Prospective data from the Vancouver Ovulation and Bone Change Cohort (Prior et al 1996, 1990a) are still being collected as these women become perimenopausal or menopausal. Some of those women continue to keep quantitative basal temperature and Daily Perimenopause Diary (Prior 1999) records, and will potentially provide important comparisons of ovulation and experiences in premenopausal and perimenopausal cycles in relation to subsequent menopause and bone density. As an illustration, one year of ovulatory characteristics (analysed by the QBT least squares method; Prior et al 1990b) are shown from the initial study and 10 years later in one woman (Fig. 5). This woman's initial cycles averaged 29.3 1.6 days in length and became two days shorter (27.0 1.5 days, $P = 0.0004$). The changes in luteal phase length were even more dramatic with reduction from a mean of 11.2 2.0 to 5.4 2.7 days over the 10 years ($P < 0.0001$). Note that she had no normally ovulatory cycles in 1994–1995 even though she continued to have regular cycles.

A large selected population to represent the US ethnic mix is being studied with US National Institutes of Health funding (Sowers et al 2000). Although over 3000 women will be followed prospectively, only limited, intermittent documentation of hormone levels is being performed.

The Canadian Multicentre Osteoporosis Study (CaMOS), a national population-based study that includes approximately 35 premenopausal and 60 perimenopausal women in each of nine centres (Kreiger et al 1999), is just completing a three-year follow-up in women who were aged 40–60 at baseline. The 3 year questionnaire includes information about cycle regularity and the experience of premenstrual symptoms and night sweats as well as molimina (Prior 1997). We are currently in the process of evaluating the molimina question in relation with the characteristics in premenopausal women as well as validating its assessment of ovulation. Eventually, prospective population-based data about experiences, weight, diet and exercise as well as bone changes will be available from CaMOS data. Unfortunately, no current methods for prospective continuous ovulation documentation are sufficiently convenient and reliable that they can be used in epidemiological samples.

Summary

This review of ovulation in midlife shows that ovulation disturbances are an important characteristic of endocrine ageing in women and are likely to be prevalent in all phases of perimenopause. At present only limited prospective data are available and these data generally lack clinical correlation and recording of women's experiences. The ovarian hormonal physiology, sociocultural context,

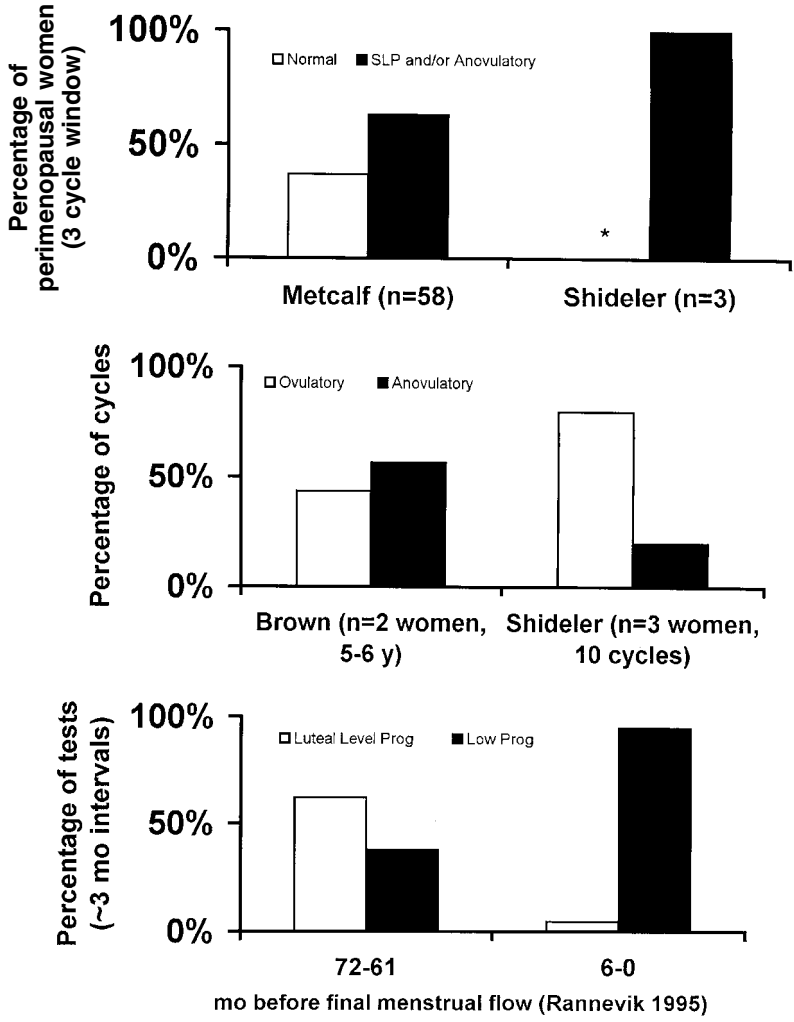


FIG. 5. This three-part figure summarizes prospective data on ovulation disturbances during the perimenopause. The top section shows the proportion of three women experiencing three consecutive cycles that are normal (open bar) or showed ovulation disturbances (short luteal phase [SLP] and/or anovulatory in black). Metcalf's data in women with irregular cycles are shown on the left ($n = 58$) and on the right prospective data for 3–4 consecutive cycles in three women (see Fig. 4). The middle portion of the diagram shows prospective data drawn as percentage of ovulatory (open bar, [includes SLP for Shideler data]) and anovulatory cycles (black bar). The bottom panel shows the percentage of sera with luteal levels of progesterone (Luteal Levels Prog, open bar) or low progesterone levels (Low Prog, black bar) during the 72–61 versus 6–0 months before the final menstrual flow from Rannevik data. mo, months. All data redrawn from published work (Metcalf 1979, Shideler et al 1989, Brown 1985, Rannevik et al 1995).

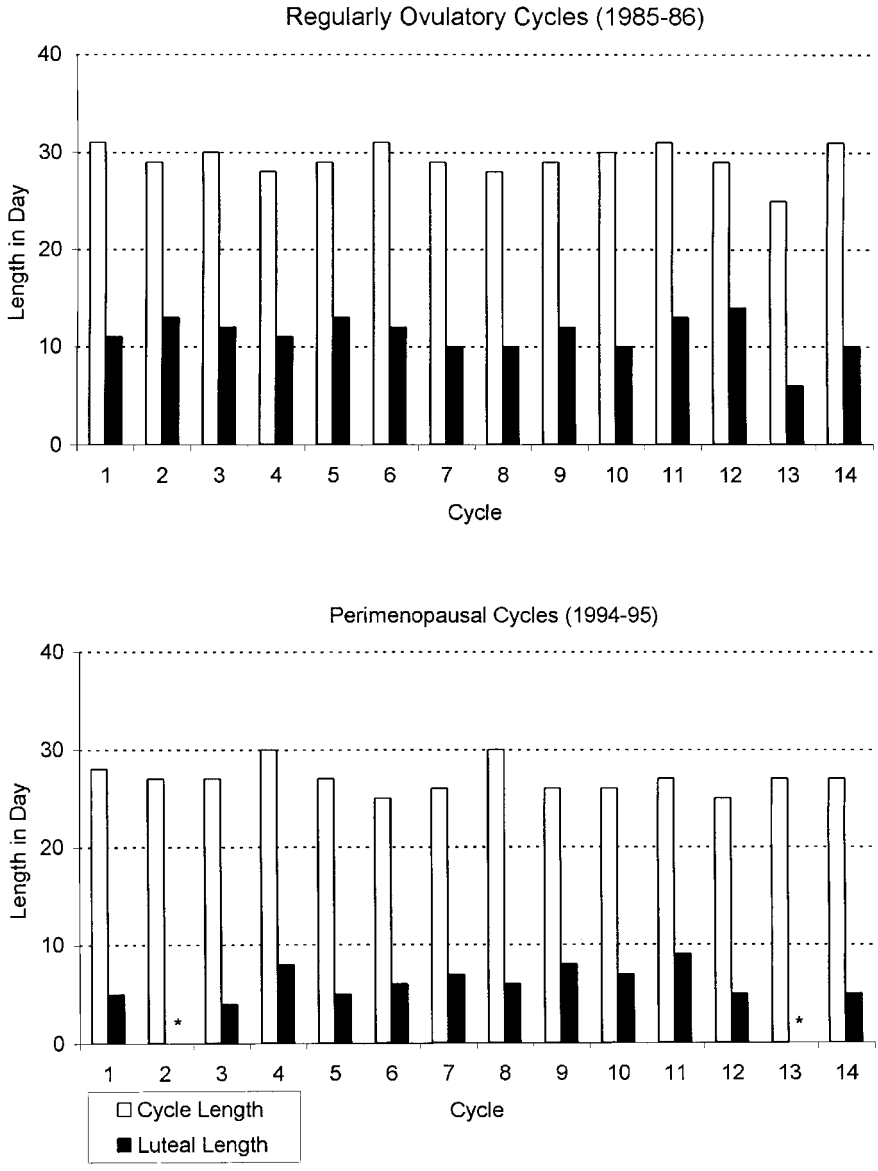


FIG. 6. Diagram contrasting 14 consecutive cycles in one ovulatory woman during 1985–1986 and 1994–1995 showing cycle length (open bars) and luteal phase lengths (black) using quantitative basal temperature analysis with least mean squares statistics (Prior et al 1990b). The normal luteal phase length of 10 days is virtually consistent in the earlier years but is never present despite 86% continued ovulatory cycles when she experienced vasomotor symptoms and sleep disturbances although remained in phase B of perimenopause.

experiential and clinical consequences as well as genetic background of perimenopausal ovulation disturbances need to be systematically documented in prospective studies.

References

Ballinger CB, Browning NC, Smith AH 1987 Hormone profiles and psychological symptoms in peri-menopausal women. Maturitas 9:235–251

Brambilla DJ, McKinlay SM, Johannes CB 1994 Defining the perimenopause for application in epidemiologic investigations. Am J Epidemiol 140:1091–1095

Brown JB 1985 Return of fertility after childbirth and during lactation, and changes in the climacteric. J Biosoc Sci Suppl 9:5–27

Burger HG, Dudley EC, Hopper JL et al 1995 The endocrinology of the menopausal transition: a cross-sectional study of a population-based sample. J Clin Endocrinol Metab 80:3537–3545

Burger HG, Dudley E, Mamers P, Robertson D, Groome N, Dennerstein L 2002 The ageing female reproductive axis I. In: Endocrine facets of ageing. Wiley, Chichester (Novartis Found Symp 242) p 161–171

Collett M 1949 Basal metabolism at the menopause. J Appl Physiol 1:629–636

Collett ME, Wertenberger GE, Fiske VM 1954 The effect of age upon the pattern of the menstrual cycle. Fertil Steril 5:437–448

Djerassi A, Coutifaris C, West VA et al 1995 Gonadotroph adenoma in a premenopausal woman secreting follicle-stimulating hormone and causing ovarian hyperstimulation. J Clin Endocrinol Metab 80: 591–594

Doring GK 1969 The incidence of anovular cycles in women. J Reprod Fertil Suppl 6:77–81

Gilfillan CP, Robertson DM, Burger HG, Leoni MA, Hurley VA, Martin NG 1996 The control of ovulation in mothers of dizygotic twins. J Clin Endocrinol Metab 81:1557–1562

Guthrie JR, Ebeling PR, Hopper JL et al 1998 A prospective study of bone loss in menopausal Australian-born women. Osteoporos Int 8:282–290

Kaufert PA, Gilbert P, Tate R 1987 Defining menopausal status: the impact of longitudinal data. Maturitas 9:217–226

Klein NA, Illingworth PJ, Groome NP, McNeilly AS, Battaglia AS, Soules MR 1996 Decreased inhibin B secretion is associated with the monotropic FSH rise in older, ovulatory women: a study of serum and follicular fluid levels of dimeric inhibin A and B in spontaneous menstrual cycles. J Clin Endocrinol Metab 81:2742–2745

Kreiger N, Tenenhouse A, Joseph L et al 1999 The Canadian Multicentre Osteoporosis Study (CaMos): Background, rationale, methods. Can J Aging 18:376–387

Lee SJ, Lenton EA, Sexton L, Cooke ID 1988 The effect of age on the cyclical patterns of plasma LH, FSH, estradiol and progesterone in women with regular menstrual cycles. Hum Reprod 3:851–855

Longcope C, Franz C, Morello C, Baker RS, Johnston CC Jr 1986 Steroid and gonadotropin levels in women during the peri-menopausal years. Maturitas 8:189–196

Merrill JA 1963 The morphology of prepubertal ovary: relationship to the polycystic ovary syndrome. Southern Med Journal 56:225–231

Metcalf MG 1979 Incidence of ovulatory cycles in women approaching the menopause. J Biosoc Sci 11:39–48

Metcalf MG, Donald RA 1979 Fluctuating ovarian function in a perimenopausal women. N Z Med J 89:45–47

Okano H, Mizunuma H, Soda M et al 1998 The long-term effect of menopause on postmenopausal bone loss in Japanese women: results from a prospective study. J Bone Miner Res 13:303–309

Prior JC 1997 Ovulatory disturbances: they do matter. Can J Diagnosis (Feb issue) 64–80
Prior JC 1998 Perimenopause: the complex endocrinology of the menopausal transition. Endocr Rev 19:397–428
Prior JC 1999 The puzzle of perimenopause — using the daily perimenopause diary. Video produced by University of British Columbia, distributed by BC Endocrine Research Foundation, British Columbia
Prior JC, Vigna YM, Schechter MT, Burgess AE 1990a Spinal bone loss and ovulatory disturbances. N Eng J Med 323:1221–1227 (erratum: 1990 N Eng J Med 328:1724)
Prior JC, Vigna YM, Schulzer M, Hall JE, Bonen A 1990b Determination of luteal phase length by quantitative basal temperature methods: validation against the midcycle LH peak. Clin Invest Med 13:123–131
Prior JC, Vigna YM, Barr SI, Rexworthy C, Lentle BC 1994 Cyclic medroxyprogesterone treatment increases bone density: a controlled trial in active women with menstrual cycle disturbances. Am J Med 96:521–530
Prior JC, Vigna YM, Barr SI, Kennedy S, Schulzer M, Li DK 1996 Ovulatory premenopausal women lose cancellous spinal bone: a five year prospective study. Bone 18:261–267
Rannevik G, Jeppsson S, Johnell O, Bjerre B, Laurell-Borulf Y, Svanberg L 1995 A longitudinal study of the perimenopausal transition: altered profiles of steroid and pituitary hormones, SHBG and bone mineral density. Maturitas 21:103–113
Reame NE, Kelche RP, Beitins IZ, Yu MY, Zawacki CM, Padmanabhan V 1996 Age effects of follicle-stimulating hormone and pulsatile luteinizing hormone secretion across the menstrual cycle of premenopausal women. J Clin Endocrinol Metab 81:1512–1518
Reyes FI, Winter JS, Faiman C 1977 Pituitary-ovarian relationships preceding the menopause. I. A cross-sectional study of serum follicle-stimulating hormone, luteinizing hormone, prolactin, estradiol and progesterone levels. Am J Obstet Gynecol 129:557–564
Richardson SJ, Senikas V, Nelson JF 1987 Follicular depletion during the menopausal transition: evidence for accelerated loss and ultimate exhaustion. J Clin Endocrinol Metab 65:1231–1236
Santoro N, Brown JR, Adel T, Skurnick JH 1996 Characterization of reproductive hormonal dynamics in the perimenopause. J Clin Endocrinol Metab 81:1495–1501
Shideler SE, DeVane GW, Kalra PS, Bernirschke K, Lasley BL 1989 Ovarian-pituitary hormone interactions during the perimenopause. Maturitas 11:331–339
Sowers M, Crawford SL, Sternfeld B et al 2000 SWAN: a multicenter, multiethnic, community-based cohort study of women and the menopausal transition. In: Lobo RA, Kelsey J, Marcus R (eds) Menopause: biology and pathobiology. Academic Press, San Diego, p 175–188
Treloar AE 1981 Menstrual cyclicity and the pre-menopause. Maturitas 3:249–264
Vollman RF 1977 The menstrual cycle. Major Probl Obstet Gynecol 7:1–193
Wang M, Seippel L, Purdy RH, Backstrom T 1996 Relationship between symptom severity and steroid variation in women with premenstrual syndrome: study on serum pregnenolone, pregnenolone sulfate, 5alpha-pregnane-3,20-dione, and 3alpha-hydroxy-5a-pregnan-20-one. J Clin Endocrinol Metab 81:1076–1082
Welt CK, McNicholl DJ, Taylor AE, Hall JE 1999 Female reproductive aging is marked by decreased secretion of dimeric inhibin. J Clin Endocrinol Metab 84:105–111

DISCUSSION

Veldhuis: One of the distinctions I see between your work and Henry Burger's is that you propose that oestradial elevations occur before FSH. Therefore, you have the task of accounting for an isolated oestradiol increase. Is that right?

Prior: Yes. My hypothesis would be that FSH isn't measurably higher (Burger 1994), but this doesn't mean that it isn't occasionally higher and at a level sufficient to recruit a larger cohort of follicles.

Veldhuis: We do come to a distinction that will probably need more careful study, as to whether the antral follicle cohort is increased or not. High quality ultrasonogrophy prospectively could be marvellous.

Is it your view that the luteal phase defects are a marker of the primary ovarian disease associated with follicle attrition, or is this a neuroendrocrine disorder reflected in due course in an abnormal cytodifferentiation?

Prior: I would guess that it is the organization of the stimuli to the follicle, but I don't know the answer.

Burger: I don't know the answer either, but I have normally interpreted luteal phase defects as related to a defect in follicular function. However, it is very hard to diagnose them in young reproductive-age women. It is a woolly area.

Veldhuis: I don't know whether iNOS is in the ovary, but the NOergic pathway is clearly there — it is partly driven by FSH. Can you think of a mechanism that would drive inappropriate oestradiol episodically leading to some defect in follicle growth, which is a mixture of oestradiol, FSH, insulin-like growth factor (IGF)1 and also negative regulators producing orderly follicle growth, yet enough oestradiol to drive a surge, and then complex organizaton of that follicle into a corpus luteum? Could the ovarian ageing seen in the human be the analogue of the CNS ageing seen in the rat? In the female rat there is strong evidence for CNS-dependent ageing, rather than primary ovarian ageing, based on the ovarian transplantation studies (Evans et al 1992). Is there a possible model for iNOS being involved in the early changes in the ovary? The species difference is not the basic issue to me, but rather the site where the defect first emerges.

Prior: I don't know, but this is a useful thing to start thinking about.

Handelsman: The point that Jerilynn Prior made was that all the flushing episodes seem to start through sleep. It has always struck me that there are clearly oestrogen-dependent symptoms and then there are the rest. I have always assumed that some of these are due to sleep disturbance from flushing during the night. If this was so, it should be oestrogen sensitive. It appears that this is not the case. Is sleep disturbance important?

Prior: I would say that it is very important. Something happens to sleep physiology, because there is sleep disturbance in women who have never reported hot flushes. There is some neuroendrocrine change that occurs at that stage of life.

Veldhuis: There aren't many data. We wrote one paper showing this LH–FSH synchrony loss in the monotrophic FSH elevation stage of the premenopause (Matt et al 1998a) and in ageing men (Pincus et al 1997). It is clearly abnormal, but I don't know that it can't be explained by a downstream problem in variable

oestrogen overproduction and then withdrawal, leading sometimes to high FSH. In the men it seems clear that there is some central nervous system (CNS) dysregulation of the outflow control of several factors, some neurogenic, some hormonal, and some across axes (Pincus et al 1996, Veldhuis et al 1999a,b, 2000). But whether these derangements are in any sense primary is unclear. The sleep disorder raises the question of whether CNS alterations are precursory.

Müller: Referring to this argument, in addition to their genomic actions, steroids can have non-genomic effects that occur with a shorter time lapse. They can act directly at the level of hormone-sensitive neurotransmitter receptors present on cellular membranes of neuronal cells, and can also be synthesized in the CNS, independently from their peripheral sources (Baulieu 1997). For instance, precursors (pregnenolone sulfate, dehydroepiandrosterone) or metabolites (allopregnanolone) of progesterone, act respectively as antagonists and agonists of the $GABA_A$ receptor, thus influencing neuronal activity within large parts of the CNS (Genazzani et al 1996). If they can modulate GABAergic or glutaminergic function, we should not be surprised that they can induce behavioural alterations.

For instance, insomnia may be an expression of latent depression. Many of the symptoms such as irritability and depressed mood can be interpreted as the action of these steroids directly on the brain.

It is interesting that in rats, also, before the complete arrest of the oestrus cyclicity there is a phase called constant oestrus, involving continuous activation of oestrogen production (Kalra et al 1993). This is reminiscent of the high variability of circulating oestradiol levels of women in the late perimenopause (Burger 1999).

Veldhuis: In the rat there is a blunting of LH surge in middle-aged females, well before their oestrus cyclicity changes, pointing to a CNS component (Matt et al 1998b). This may be a feature of the rodent (Evans et al 1992). Others would say it is also a feature of the human.

Burger: I have debated this issue at some length with Phyllis Wise. I challenged her about the relevance of the rodent model to an understanding of the human endocrinology. I must say that she has persuaded me that there are similarities that warrant the continued exploration of that model.

Laron: Coming back to the issue of sleep disturbance, we know that in puberty children love to sleep for a long time. Would this mean that they have much more of what the menopausal women lack?

Veldhuis: GH secretagogues are thought to be somnogenic (Giustina & Veldhuis 1998). There are data showing that the actions of GH secretagogues favour sleep (Thorner et al 1990).

Carroll: With regard to Dr Müller's point about the neurosteroids, they tend to have GABAergic and somewhat sedative properties. As I was preparing my review

I came across another reference to yet another alleged explanation for the night sweats and vasomotor instability of menopausal women. This is increased cell number and enlarged cell size of neurokinin B neurons in the infundibular nucleus of the hyothalamus. This is another theory as to why they develop vasomotor instability.

I agree that the menopausal period is one of stress. In general, in the epidemiological studies of depression that I talked about yesterday, an important point to make is that depressive symptoms seem to count, rather than a formal psychiatric diagnosis of major depression. The traditional clinical psychiatric distinction between minor depression and major depression appears to be unimportant in these longitudinal studies.

Müller: There are data showing that the latest antidepressants are effective in blocking some of these postmenopausal symptoms. This has been shown for instance with inhibition of hot flushes by venlafaxine, a specific blocker of noradrenergic re-uptake (Loprinzi et al 2000).

Veldhuis: Does it affect the sleep disturbance?

Müller: I don't remember.

Haus: We have been talking about sleep disturbances, but nobody has mentioned melatonin. In the USA, melatonin can be purchased over the counter and many people who develop sleep disturbances take it, some under medical direction, many others without. What role might melatonin play in the treatment of sleep disturbances in the elderly? Some publications on this topic have previously appeared (e.g. Dagan et al 1998).

Prior: I haven't seen any data on this.

Riggs: I want to ask about the important studies you did in 1990 on the relationship between decreased luteal phase duration and bone loss. The limitation of these studies is that it takes so long to get an accurate estimate of bone loss by bone densitometry. What has changed since then is that we now have the biochemical markers, so it is possible to see changes in bone turnover within a single cycle. Have you had a chance to look at this, to follow up on the earlier studies?

Prior: Progesterone works on bone formation and not on bone resorption. The markers that we have for bone formation are influenced by bone resorption. This means that we don't have a specific marker that we could use to document the progesterone effect on bone.

Riggs: I disagree with that. Formation and resorption are linked together, so the markers for each will be linked together. If you measured resorption and formation, and looked at the gap between them, you ought to be able to see a difference, if there is a difference, within the individual cycle. This might be worth doing.

Prior: There have been several attempts to look at changes in bone resorption across normal ovulatory menstrual cycles. The data are wild. There is obviously a

lag of a few days between a time of change in oestrogen and a change in those markers. It is complicated to do this study; I haven't yet got sufficient funding to do it. I have applied to do a study in which I will control resorption in perimenopause, give progesterone cyclically or daily, and see what happens to bone.

Robertson: The vasomotor symptoms of menopause are so dramatic and so discrete. I am amazed we don't have a better handle on them. What is the current understanding of the kindling features: the things that are occurring in the minute or two before the onset of these? What are the mediators of the vasomotor symptoms?

Prior: That's a good question. It is clear that there is an adrenergic activation prior to any temperature change. The women will describe that they woke up suddenly and they felt anxious or angry, and then they felt hot. The better studies of the temperature change suggest that there is a tiny increase in core temperature which the hypothalamus then decides is not OK. It begins the process of vasodilitation that leads to heat dissipation. The core temperature then drops. Some women will get quite chilled afterwards and will describe 'cold sweats'. Hot flushes are increased by social stress (Swartzman et al 1990). For example, people who are having a number of hot flushes will sometimes get a flush if they hear an argument in the next office. Early on in some women flushes are triggered by even a small sip of alcohol. We have used a diary system so that each day a person integrates their assessment of the intensity of the hot flush on a 0–4 scale, the number of discrete episodes during the waking day, and the number and intensity during sleep. We have those data tracked longitudinally in quite a large number of women. I think we are getting a better descriptive idea about it, but the endocrinology and neurobiochemistry are complex.

Burger: There are so many things about hot flushes that are not understood. One of the curiosities is that in population surveys of women there seems to be a baseline hot flush frequency reporting rate of about 10% in the normal community under the age of 40. If you ask women whether they have had hot flushes in the last two weeks, about 10% will say they have. I see a lot of patients referred for hot flushes for which I can find no cause whatsoever.

Prior: To put a different perspective on it, I would think that many of those women are in the earliest phases of perimenopause (Prior 1998). If you were to follow them over time, they will begin to show short follicular phases and ovulation disturbances.

Ruiz-Torres: Have you any data on leptin interactions?

Prior: No, but that's an interesting question.

Handelsman: One of the purposes of this meeting is to identify things that we don't understand. The most striking thing for me is watching those log linear declines in primordial follicles, which are disappearing at a phenomenal rate.

What controls this atresia? With the time decay in a log linear fashion, menopause could be delayed by years. Yet we have no idea what controls this atresia that dictates age at menopause.

Veldhuis: First, the ovarian physiologists are going to have to teach us what local factors are produced by the granulosa cell that talk to the theca cell and egg, and vice versa.

References

Baulieu EE 1997 Neurosteroids: of the nervous system, by the nervous system, for the nervous system. Recent Prog Horm Res 52:1–32

Burger HG 1994 Diagnostic role of follicle-stimulating hormone (FSH) measurements during the menopausal transition — an analysis of FSH, oestradiol and inhibin. Eur J Endocrinol 130:38–42

Burger HG 1999 The endocrinology of the menopause. J Steroid Biochem Mol Biol 69:31–35

Dagan Y, Yovel I, Hallis D, Eisenstein M, Raicik I 1998 Evaluating the role of melatonin in the long-term treatment of delayed sleep phase syndrome (DSPS). Chronobiol Int 15:181–190

Evans WS, Sollenberger MJ, Booth RA Jr et al 1992 Contemporary aspects of discrete peak detection algorithms. II. The paradigm of the luteinizing hormone pulse signal in women. Endocr Rev 13:81–104

Genazzani AR, Petraglia F, Purdy RH 1996 The brain: source and target for sex steroid hormones. Parthenon, New York

Giustina A, Veldhuis JD 1998 Pathophysiology of the neuroregulation of growth hormone secretion in experimental animals and the human. Endocr Rev 19:717–797

Kalra SP, Sahu A, Kalra PS 1993 Ageing of the neuropeptidergic signals in rats. J Reprod Fertil Suppl 46:11–19

Loprinzi CL, Kugler JW, Sloan JA et al 2000 Venlafaxine in management of hot flashes in survivors of breast cancer: a randomized controlled trial. Lancet 356:2059–2063

Matt DW, Kauma SW, Pincus SM, Veldhuis JD, Evans WS 1998a Characteristics of luteinizing hormone secretion in younger versus older premenopausal women. Am J Obstet Gynecol 178:504–510

Matt DW, Gilson MP, Sales TE et al 1998b Characterization of attenuated proestrous luteinizing hormone surges in middle-aged rats by deconvolution analysis. Biol Reprod 59:1477–1482

Pincus SM, Mulligan T, Iranmanesh A, Gheorghiu S, Godschalk M, Veldhuis JD 1996 Older males secrete luteinizing hormone and testosterone more irregularly, and jointly more asynchronously, than younger males. Proc Natl Acad Sci USA 93:14100–14105

Pincus SM, Veldhuis JD, Mulligan T, Iranmanesh A, Evans WS 1997 Effects of age on the irregularity of LH and FSH serum concentrations in women and men. Am J Physiol 273:E989–E995

Prior JC 1998 Perimenopause: the complex endocrinology of the menopausal transition. Endocr Rev 19:397–428

Swartzman LC, Edelberg R, Kemmann E 1990 Impact of stress on objectively recorded menopausal hot flushes and on flush report bias. Health Psychol 9:529–545

Thorner MO, Vance ML, Hartman ML et al 1990 Physiological role of somatostatin on growth hormone regulation in humans. Metabolism 39:40–42

Veldhuis JD, Iranmanesh A, Mulligan T, Pincus SM 1999a Disruption of the young-adult synchrony between luteinizing hormone release and oscillations in follicle-stimulating hormone, prolactin, and nocturnal penile tumescence (NPT) in healthy older men. J Clin Endocrinol Metab 84:3498–3505

Veldhuis JD, Iranmanesh A, Demers LM, Mulligan T 1999b Joint basal and pulsatile hypersecretory mechanisms drive the monotropic follicle stimulating hormone (FSH) elevation in healthy older men: concurrent preservation of the orderliness of the FSH release process: a general chemical research center study. J Clin Endocrinol Metab 84:3506–3514

Veldhuis JD, Iranmanesh A, Godschalk M, Mulligan T 2000 Older men manifest multifold synchrony disruption of reproductive neurohormone outflow. J Clin Endocrinol Metab 85:1477–1486

Endocrine Facets of Ageing.
Novartis 242

Print ISBN 0-471-48636-1 Online ISBN 0-470-84654-2

The thyroid axis in ageing

Holger Leitolf, Jens Behrends and Georg Brabant[1]

Department of Clinical Endocrinology, Center for Internal Medicine and Dermatology, Medizinische Hochschule Hannover (MHH), 30625 Hannover, Germany

Abstract. The hypothalmo–pituitary–thyroid axis, among various endocrine systems, undergoes physiological alterations associated with the ageing process. Directly age-related changes have to be distinguished from indirect modifications which are caused by simultaneous thyroidal or non-thyroidal illness or other physiological or pathophysiological states whose incidence increases with age. In summary, direct changes of the hypothalmo–pituitary–thyroid axis seem to be subtle and suggestive of a decreased hypothalamic stimulation of thyroid function. In parallel, disease-specific alterations such as the development of thyroid autonomy or changes in energy intake or sleep lead to pronounced alterations of thyroid function with age which may dominate the underlying ageing of the hypothalmo–pituitary–thyroid axis itself. The following article attempts to delineate some aspects of the interplay of the regulation of thyroid function and the ageing process.

2002 Endocrine facets of ageing. Wiley, Chichester (Novartis Foundation Symposium 242) p 193–204

Ageing is associated with physiological alterations in various endocrine systems including the hypothalamo–pituitary–thyroid axis. A number of studies have attempted to delineate alterations in thyroid physiology due to the ageing process (Felicetta 1988, Levy 1991, Mooradian & Wong 1994). When focusing on age-dependent changes, alterations of the hypothalamo–pituitary–thyroid axis have to be considered which are not directly age-related but rather indirectly caused by simultaneous thyroidal or non-thyroidal illness or other physiological and pathophysiological modifications whose incidence increases with age. Nevertheless, these events have important influences on thyroid physiology and function.

Physiological regulation of the hypothalamo–pituitary–thyroid axis is characterized by pulsatile stimulation of pituitary thyrotropin (TSH) secretion resulting from an interplay of stimulatory hypothalamic influences mediated by

[1]This chapter was presented at the symposium by Georg Brabant, to whom correspondence should be addressed.

thyrotropin-releasing-hormone (TRH) pulses as well as inhibitory hypothalamic influences mediated by dopamine and somatostatin release. After being converted from the prohormone tetraiodothyronine (or thyroxine, T4) by specific deiodinases, triiodothyronine (T3) inhibits synthesis and secretion of TRH (Segerson et al 1987) and stimulates the release of inhibitory factors such as dopamine and somatostatin from the respective hypothalamic centres. Within the thyrotropic cells of the pituitary gland, T3 directly inhibits the synthesis of the TSH β-subunit and thereby TSH secretion by interaction with specific T3 receptors (Gurr & Kourides 1985). Thyrotropin is released from the pituitary gland in a pulsatile pattern with approximately one pulse every two hours. However, these pulses are not uniformly distributed but rather cluster within the evening hours. Fusion of TSH pulses with increasing pulse amplitude, leads to the nightly increase in mean TSH serum levels with a maximum between 0200 h and 0400 h (Brabant et al 1986, Greenspan et al 1986, Clark et al 1987, Table 1).

Many factors have been described which influence the secretory pattern of TSH. Pulse amplitude is rapidly dampened by increasing circulating T4 levels in healthy subjects. A similarly rapid effect can be observed after bolus application of glucocorticoids but, in contrast to thyroid hormones, this effect appears to be specifically exerted on the hypothalamic level whereas the responsiveness of the pituitary to TRH remains unaltered (Re et al 1976, Brabant et al 1987, 1989). Physiological alterations such as sleep and energy supply also exert profound effects on the hypothalamo–pituitary–thyroid system. In healthy volunteers, 36 hours of fasting significantly decreases TSH levels and almost completely suppresses the normal TSH increase during the night. Despite this profound effect, the pattern of TSH pulses and their frequency remains constant, indicating a selective effect on TSH pulse amplitude (Romijn et al 1990). As it is known that leptin plasma levels decrease with fasting, these may also serve as potential mediators. When starving rodents received intraperitoneal leptin injections twice daily, the fasting-dependent decrease in hypothalamic–pituitary–thyroid axis was reversed (Seoane et al 2000). Experimental sleep alterations have been shown to modify TSH secretion and change hypothalamic and pituitary responsiveness. Acute sleep withdrawal in healthy young subjects induces an activation of the nightly increase in TSH secretion whereas a longer lasting sleep deprivation period and sleep fragmentation induces a dramatic decrease in the mean 24 h TSH secretion (Brabant et al 1990, Behrends et al 1998, Spiegel et al 1999).

The examples described above are important as they all may affect the hypothalamo–pituitary–thyroid axis in ageing and may thus obscure physiological alterations. Moreover, in iodine-deficient regions such as Germany, the prevalence of goitre is estimated to range between 30–50% with an increasing frequency with age (Berghout et al 1990, Hintze et al 1991a, Hampel et al 1995). Alterations in iodine supply are known to change thyroid function. Most likely due

TABLE 1 Age-related changes of the thyroidal axis and the peripheral responsiveness to thyroid hormones

Parameter	*Change*
TSH response to TRH	= or decreased
Nocturnal TSH peak	Decreased
Thyroid response to TSH	= or decreased
Radioactive iodine uptake	Decreased
T4 production	= or decreased
5′ deiodinase activity	Decreased
T3 production	= or decreased
T4 / T3 degradation	= or decreased
Serum thyroid hormone binding	=
Total or free T4 serum concentration	= or decreased
Total or free T3 serum concentration	=
Reverse T3 serum concentration	= or increased
Metabolic rate	Decreased
Lipid peroxidation	= or decreased
Malic enzyme, Na/K-ATPase, S14	Decreased

=, stays the same.
Adapted from Levy (1991).

to a higher sensitivity of the thyroid gland to TSH, at least in short-term experiments, circulating TSH levels drop by approximately 50% without any effect on the frequency of TSH pulses when iodine supply is experimentally decreased (Brabant et al 1992). Prolonged iodine deficiency results in a high number of patients with nodular goitre estimated to account for more than 15% of the population in Germany beyond 60 years of age. This percentage is even further increased if only hospitalized subjects are considered (Felicetta 1988). Overt or occult thyroid diseases have to be considered when assessing the physiological alterations of the hypothalamo–pituitary–thyroid axis associated with ageing, and their clinical presentation, at least in part, may differ substantially from the symptoms and signs found in younger patients (Köbberling et al 1981, Hennemann & Krenning 1987, Nordyke et al 1988, Trivalle et al 1996). In many studies, thyroid autonomy and autoimmune thyroid disease have been identified as the leading causes for hyperthyroidism in ageing (Trzepacz et al 1989, van Coevorden et al 1989, Brabant et al 1991, Greenspan et al 1991, Levy 1991, Mariotti et al 1993, Mooradian & Wong 1994, Samuels 1998). A large study of 583 healthy subjects including 34 centenarians revealed an

age-dependent increase in thyroid-specific antibodies with a significant peak between 70 and 85 years, accompanied by a decrease in total and $CD5^+$ B cells (Mariotti et al 1992).

The predominant cause of hyperthyroidism in old age, however, is related to the widespread therapeutic use of thyroid hormones. Data from the Framingham study demonstrated in an unselected population of 2575 adults beyond the age of 60 (mean age 68.6 years) that 6.9% (2.3% of men and 10.0% of women) were receiving medical treatment with thyroid hormones. It is interesting to note that apart from the therapeutic use in goitre patients and as substitution therapy for hypothyroidism, approximately 20% of the subjects studied were using thyroid hormones without appropriate identifiable cause. Insufficient treatment could be demonstrated in 37% of the hypothyroid patients using thyroid hormones by elevated TSH serum levels (Sawin et al 1989). Approximately 6% of the group with lowered TSH serum levels in the Framingham study were hyperthyroid when all laboratory and clinical indicators were combined. This suggests that lowered TSH serum levels in the elderly may be under a dominant influence of other factors than the thyroid status alone (Sawin et al 1991). The ratio of 'truly' hyperthyroid subjects as compared to other causes of lowered thyrotropin serum levels changes even more dramatically when the study is focused on hospitalized patients. Our group recently investigated patients of a rehabilitation clinic after treatment of acute illness (Brabant et al 1996). Restricting this prospective analysis to those 619 patients with no previous suspicion of active thyroid disease during the primary hospital stay, 5 subjects (0.8%) were identified to have elevated TSH serum levels indicating hypothyroidism. As expected for a region with deficient dietary iodine supply, the prevalence of suppressed TSH serum concentrations was much higher (22.6%). In 19% of the subjects from this group (i.e. 4% of the total group), overt hyperthyroidism was diagnosed supporting the insidious clinical signs of thyroid dysfunction in elderly subjects (Stolte et al 1998, Sawin et al 1994). This fits to recent data in hospitalized patients where a frequency of 0.8% overt clinical (4.2% subclinical) hypothyroidism was found in an iodine-deficient region, whereas 1.5% of the patients (10.4%) in a region with sufficient dietary iodine supply and 7.6% of the patients (23.9%) with high dietary iodine supply could be shown to be clinically (subclinically) hypothyroid. In contrast, clinical and subclinical hyperthyroidism was observed in 3.4% of the patients in a region with iodine deficiency, 3.0% in an area with sufficient dietary iodine and 0% in a region with high dietary iodine supply (Szabolcs et al 1997).

In large population studies, the incidence of hypothyroidism in elderly patients varies from less than 1% to 17% depending on the iodine supply. Women are more commonly affected than men, and subclinical hypothyroidism is more frequent than overt hypothyroidism. Virtually all the cases of hypothyroidism found are related to autoimmune thyroid disease (Hintze et al 1991b).

Apart from the iodine supply, thyroid function may be altered by the availability of other micronutrients such as selenium in the thyroid gland itself as well as in major target tissues for thyroid hormones such as the liver. Ravaglia et al (2000) demonstrated lower serum selenium levels in a group of healthy subjects beyond 90 years of age as compared to younger subjects. This was accompanied by other changes such as lower zinc serum concentrations. Many studies underline the importance of selenium for normal bioactivity of the type II deiodinase (Beckett et al 1993, Meinhold et al 1993, Mitchell et al 1997, Hotz et al 1997) at least in serious iodine deficiency.

Total energy intake exerts a profound influence on thyroid function, mainly on the hypothalamic level but also on the pituitary and thyroid level. The importance of inadequate energy intake in ageing has been discussed in large population studies. In the Euronut–Seneca study (de Groot et al 1992) on 2600 subjects born between 1913 and 1918 in 18 European communities, body mass index was below 20 kg/m^2 in up to 15% of elderly men and 17% of elderly women, in addition, the daily energy intake in these subjects was found to be lower than their respective requirements.

Similarly, in severe non-thyroidal illness (NTI) the decrease in mean TSH secretion (Custro et al 1994) follows a comparable pattern to fasting with significantly reduced TSH pulse amplitude but an unchanged pulse frequency (Romijn et al 1990). Malnutrition may at least play a partial role in the explanation of disturbed thyroid function in NTI. The prevalence of chronic diseases in the Euronut–Seneca study varied between 59% and 92% (Danforth & Burger 1989, de Groot et al 1992), thereby indicating that NTI may introduce a significant bias when assessing age-dependent changes of the thyroid axis. Under these pathophysiological conditions, the glucocorticoid axis may be activated. This may suppress, to a variable degree, TSH secretion, an effect potentially important in physiological ageing where an activation of glucocorticoid secretion has been previously reported (van Cauter et al 1996, Harper et al 1999, Bergendahl et al 2000).

Finally, sleep may play an important role. It is well known that the sleep pattern changes with age. With increasing age, total sleep duration decreases and sleep fragmentation increases. In addition the relative time intervals in slow wave sleep are decreased as less deep sleep stages and REM sleep dominate (van Cauter et al 2000). When allowed to sleep only four hours every 24 h over one week, thereby mimicking the sleep pattern of elderly subjects, a profound effect on TSH secretion with very low circulating TSH levels at the end of the experiment could be demonstrated in young volunteers (Spiegel et al 1999). This indicates that sleep-dependent processes not directly related to ageing may change TSH secretion and the functional activity of the thyroid axis.

Most of the epidemiological studies on thyroid function in ageing are based on population studies including subjects where the above mentioned mechanisms may very well result in a significant study bias due to disease-related alterations or independent age-related problems (Mariotti et al 1995). Reliable studies focusing on healthy subjects are scarce. A careful evaluation of healthy centenarians selected according to criteria of the Eurage Senieur protocol (Mariotti et al 1993) revealed subtle changes of the thyroid axis. Free triiodothyronine and TSH serum concentrations were slightly decreased in parallel to a small but significant increase in reverse triiodothyronine serum concentrations. Free thyroxine serum levels were unchanged when comparing a group of 41 healthy centenarians with 98 healthy subjects aged 20–64 years or with 33 subjects aged 65–80 years, respectively. The changes observed were so subtle that they would not have been evident if only subjects up to 80 years of age had been included. These results are compatible with a decline in hypothalamic–pituitary activity during ageing resulting in a decreased TSH secretion. Studies on the 24 h rhythm of TSH in young and elderly subjects showed that the nocturnal TSH peak was blunted in the elderly (Barreca et al 1985, van Coevorden et al 1989, Greenspan et al 1991). Analysis of the pulsatile release pattern of thyrotropin revealed that this change selectively rests on a decreased TSH pulse amplitude whereas the temporal structure of the circadian TSH secretion with a nadir in the late afternoon and a peak around midnight in conjunction with an increased frequency of TSH pulses appears to be preserved (Brabant et al 1991). Thyrotropin response to a defined TRH challenge in the study group, male subjects from 67–84 years of age, was attenuated, pointing to a decreased pituitary TSH secretory reserve despite comparable triiodothyronine and thyroxine serum (van Coevorden et al 1989), which would be compatible with a hypothalamic or pituitary mechanism.

Animal studies in male Fisher rats suggest an age-related decrease in hypothalamic TRH synthesis followed by a decreased formation of thyrotropin β subunit within the pituitary gland (Cizza et al 1992). Studies in humans using the dopamine antagonist metoclopramide indicated a decreased responsiveness of pituitary TSH secretion to TRH (Targum et al 1989). This may be explained by a change in the nocturnal increase of TSH secretion due to an increased nightly dopamine tone (Greenspan et al 1991). As dopamine has direct inhibitory effects on pituitary TSH synthesis (Shupnik et al 1986), the attenuated response to TRH may fit into this concept. As an alternative or additional mechanism, an altered set point for the feedback action of thyroxine in elderly subjects was proposed, due to a putatively increased pituitary T4 to T3 conversion (Lewis et al 1991) and a selective decrease of free serum T3 levels (Mariotti et al 1993). Such age-related alterations in the activity of T4 to T3 conversion have not only been described for the activity of the 5′ deiodinase in the pituitary, but also in the liver (Donda & Lemarchand-

Beraud 1989). On the contrary, TSH appears to stimulate deiodinase activity and may thus counteract these effects (Köhrle 1990).

In summary, changes of the hypothalamic–pituitary–thyroid axis with age seem to be subtle and suggest a decreased hypothalamic stimulation of thyrotropin release. In parallel, disease-specific alterations such as the development of thyroid autonomy, or changes in energy intake or sleep may dominate these small physiological changes and lead to pronounced alterations of thyroid function with age, which are only indirectly related to ageing of the hypothalamo–pituitary–thyroid axis itself.

References

Barreca T, Franceschini R, Messina V, Bottaro L, Rolandi E 1985 24 hour thyroid-stimulating hormone secretory pattern in elderly men. Gerontology 31:119–123

Beckett G J, Nicol F, Rae PW, Beech S Guo Y, Arthur JR 1993 Effects of combined iodine and selenium deficiency on thyroid hormone metabolism in rats. Am J Clin Nutr 57:240S–243S

Behrends J, Prank K, Dogu E, Brabant G 1998 Central nervous system control of thyrotropin secretion during sleep and wakefulness. Horm Res 49:173–177

Bergendahl M, Iranmanesh A, Mulligan T, Veldhuis JD 2000 Impact of age on cortisol secretory dynamics basally and as driven by nutrient-withdrawal stress. J Clin Endocrinol Metab 85:2203–2214

Berghout A, Wiersinga WM, Smits NJ, Touber JL 1990 Interrelationships between age, thyroid volume, thyroid nodularity, thyroid function in patients with sporadic nontoxic goitre. Am J Med 89:602–608

Brabant A, Brabant G, Schürmeyer T et al 1989 The role of glucocorticoids in the regulation of thyrotropin. Acta Endocrinol 121:95–100

Brabant G, Ranft U, Ocran K, Hesch RD, von zur Mühlen A 1986 Thyrotropin — an episodically secreted hormone. Acta Endocrinol 112:315–322

Brabant G, Brabant A, Ranft U et al 1987 Circadian and pulsatile thyrotropin secretion in euthyroid man under the influence of thyroid hormone and glucocorticoid administration. J Clin Endocrinol Metab 65:83–88

Brabant G, Prank K, Ranft U et a 1990 Physiological regulation of circadian and pulsatile thyrotropin secretion in normal man and woman. J Clin Endocrinol Metab 70:403–409

Brabant G, Prank K, Hoang-Vu C, Hesch RD, von zur Mühlen A 1991 Hypothalamic regulation of pulsatile thyrotopin secretion. J Clin Endocrinol Metab 72:145–150

Brabant G, Bergmann P, Kirsch CM, Köhrle J, Hesch RD, von zur Mühlen A 1992 Early adaptation of thyrotropin and thyroglobulin secretion to experimentally decreased iodine supply in man. Metabolism 41:1093–1096

Brabant G, Mayr B, Lucke C 1996 Klinische und laborchemische Aspekte der latenten und manifesten Hyperthyreose im höheren Lebensalter. Schilddrüse 1995, deGruyter, Berlin S241–S251

Cizza G, Brady LS, Calogero AE et al 1992 Central hypothyroidism is associated with advanced age in male Fischer 344/N rats: in vivo and in vitro studies. Endocrinology 131:2672–2680

Clark PMS, Clark JDA, Holder R, Raggatt PR, Wheatley T 1987 Pulsatile secretion of TSH in healthy subjects. Ann Clin Biochem 24:470–476

Custro N, Scafidi V, Gallo S, Notarbartolo A 1994 Deficient pulsatile thyrotropin secretion in the low-thyroid-hormone state of severe non-thyroidal illness. Eur J Endocrinol 130:132–136

Danforth E Jr, Burger AG 1989 The impact of nutrition on thyroid hormone physiology and action. Ann Rev Nutr 9:201–227

de Groot LC, Hautvast JG, van Staveren WA 1992 Nutrition and health of elderly people in Europe: the Euronut-Seneca study. Nutr Rev 50:185–194

Donda A, Lemarchand-Beraud T 1989 Aging alters the activity of 5′-deiodinase in the adenohypophysis, thyroid gland, and liver of the male rat. Endocrinology 124:1305–1309

Felicetta JV 1988 The Thyroid and Aging. In: Sowers JR, Felicetta JV (eds) The endocrinology of aging. Raven Press, New York, p 15–39

Greenspan SL, Klibanski A, Schoenfeld D, Ridgway EC 1986 Pulsatile secretion of thyrotropin in man. J Clin Endocrinol Metab 63:661–668

Greenspan SL, Klibanski A, Rowe JW, Elahi D 1991 Age-related alterations in pulsatile secretion of TSH: role of dopaminergic regulation. Am J Physiol 260:E486–E491

Gurr JA, Kourides IA 1985 Thyroid hormone regulation of thyrotropin alpha- and beta-subunit gene transcription. DNA 4:301–307

Hampel R, Külberg T, Klein K et al 1995 [Goiter incidence in Germany is greater than previously suspected.] Med Klin 90:324–329

Harper AJ, Buster JE, Casson PR 1999 Changes in adrenocortical function with aging and therapeutic implications. Semin Reprod Endocrinol 17:327–338

Hennemann G, Krenning EP 1987 Pitfalls in the interpretation of thyroid function tests in old age and non-thyroidal illness. Horm Res 26:100–104

Hintze G, Windeler J, Baumert J, Stein H, Köbberling J 1991a Thyroid volume and goitre prevalence in the elderly as determined by ultrasound and their relationships to laboratory indices. Acta Endocrinol 124:12–18

Hintze G, Burghardt U, Baumert J, Windeler J, Köbberling J 1991b Prevalence of thyroid dysfunction in elderly subjects from the general population in an iodine deficiency area. Aging 3:325–331

Hotz CS, Fitzpatrick DW, Trick KD, L'Abbé MR 1997 Dietary iodine and selenium interact to affect thyroid hormone metabolism of rats. J Nutr 127:1214–1218

Köbberling J, Hintze G, Blossey HC 1981 Diagnostische Probleme der Hyperthyreose im höheren Lebensalter. Dtsch med Wschr 106:973–978

Köhrle J 1990 Thyrotropin (TSH) action on thyroid hormone deiodination and secretion: one aspect of thyrotropin regulation of thyroid cell biology. Horm Metab Res Suppl 23: 18–28

Levy EG 1991 Thyroid disease in the elderly. Med Clin North Am 75:151–167

Lewis GF, Alessi CA, Imperial JG, Refetoff S 1991 Low serum free thyroxine index in ambulating elderly is due to a resetting of the threshold of thyrotropin feedback suppression. J Clin Endocrinol Metab 73:843–849

Mariotti S, Sansoni P, Barbesino G et al 1992 Thyroid and other organ-specific autoantibodies in healthy centenarians. Lancet 339:1506–1508

Mariotti S, Barbesino G, Caturegli P et al 1993 Complex alteration of thyroid function in healthy centenarians. J Clin Endocrinol Metab 77:1130–1134

Mariotti S, Franchesi C, Cossarizza A, Pinchera A 1995 The aging thyroid. Endocr Rev 16: 686–715

Meinhold H, Campos-Barros A, Walzog B, Köhler R, Müller F, Behne D 1993 Effects of selenium and iodine deficiency on type I, type II and type III iodothyronine deiodinases and circulating thyroid hormones in the rat. Exp Clin Endocrinol 101:87–93

Mitchell JH, Nicol F, Beckett GJ, Arthur JR 1997 Selenium and iodine deficiencies: effects on brain and brown adipose tissue selenoenzyme activity and expression. J Endocrinol 155: 255–263

Mooradian AD, Wong NC 1994 Age-related changes in thyroid hormone action. Eur J Endocrinol 131:451–461

Nordyke RA, Gilbert FI Jr, Harada AS 1988 Graves' disease. Influence of age on clinical findings. Arch Intern Med 148:626–631
Ravaglia G, Forti P, Maioli F et al 2000 Blood micronutrient and thyroid hormone concentrations in the oldest-old. J Clin Endocrinol Metab 85:2260–2265
Re RN, Kourides IA, Ridgway EC, Weintraub BD, Maloof F 1976 The effect of glucocorticoid administration on human pituitary secretion of thyrotropin and prolactin. J Clin Endocrinol Metab 43:338–346
Romijn JA, Adriaanse R, Brabant G, Prank K, Endert E, Wiersinga WM 1990 Pulsatile secretion of thyrotropin during fasting: a decrease of thyrotropin pulse amplitude. J Clin Endocrinol Metab 70:1631–1636
Samuels MH 1998 Subclinical thyroid disease in the elderly. Thyroid 8:803–813
Sawin CT, Geller A, Hershman JM, Castelli W, Bacharach P 1989 The aging thyroid. The use of thyroid hormone in older persons. JAMA 261:2653–2655
Sawin CT, Geller A, Kaplan MM, Bacharach P, Wilson PW, Hershman JM 1991 Low serum thyrotropin (thyroid-stimulating hormone) in older persons without hyperthyroidism. Arch Intern Med 151:165–168
Sawin CT, Geller A, Wolf PA et al 1994 Low serum thyrotropin concentrations as a risk factor for atrial fibrillation in older persons. N Engl J Med 331:1249–1252
Segerson TP, Kauer J, Wolfe HC et al 1987 Thyroid hormone regulates TRH biosynthesis in the paraventricular nucleus of the rat hypothalamus. Science 238:78–80
Seoane LM, Carro E, Tovar S, Casanueva FF, Dieguez C 2000 Regulation of in vivo TSH secretion by leptin. Regul Pept 92:25–29
Shupnik MA, Greenspan SL, Ridgway EC 1986 Transcriptional regulation of thyrotropin subunit genes by thyrotropin-releasing hormone and dopamine in pituitary cell culture. J Biol Chem 261:12675–12679
Spiegel K, Leproult R, van Cauter E 1999 Impact of sleep debt on metabolic and endocrine function. Lancet 354:1435–1439
Stolte H, von zur Mühlen A, Lucke C, Brabant G 1998 Atrial fibrillation and hyperthyroidism in geriatric patients — a prospective study in acute disease. J Endocrinol Invest 21:45
Szabolcs I, Podoba J, Feldkamp J et al 1997 Comparative screening for thyroid disorders in old age in areas of iodine deficiency, long-term iodine prophylaxis and abundant iodine intake. Clin Endocrinol 47:87–92
Targum SD, Marshall LE, Magac-Harris K, Martin D 1989 TRH tests in a healthy elderly population. Demonstration of gender differences. J Am Geriatr Soc 37:533–536
Trivalle C, Doucet J, Chassagne P et al 1996 Differences in the signs and symptoms of hyperthyroidism in older and younger patients. J Am Geriatr Soc 44:50–53
Trzepacz PT, Klein I, Roberts M, Greenhouse J, Levey GS 1989 Graves' disease: an analysis of thyroid hormone levels and hyperthyroid signs and symptoms. Am J Med 87:558–561
van Cauter E, Leproult R, Kupfer DJ 1996 Effects of gender and age on the levels and circadian rhythmicity of plasma cortisol. J Clin Endocrinol Metab 81:2468–2473
van Cauter E, Leproult R, Plat L 2000 Age-related changes in slow wave sleep and REM sleep and relationship with growth hormone and cortisol levels in healthy men. JAMA 284:861–868
van Coevorden A, Laurent E, Decoster C et al 1989 Decreased basal and stimulated thyrotropin secretion in healthy elderly men. J Clin Endocrinol Metab 69:177–185

DISCUSSION

Carroll: Your wonderful data bring us back to some of the points made earlier, about nocturnal cortisol in ageing and stress. Can you tell us what the end of the line functional status of the thyroid is in the elderly in terms of basal metabolic rate

(BMR)? Is there decreased BMR or is there a change in set point of body temperature?

Brabant: I don't have good data on body temperature. For BMR, you have to bear in mind that it changes with body weight. You have to stratify for similar weight and thyroid status, and to my knowledge this is not normally done. But it adds another level of control and regulation. For example, leptin changes BMR, as shown in rodent models. As you can rescue the thyroid axis in fasting with leptin, there is an evident integration of systems. But to date, the sequence of events has not been determined.

Veldhuis: The other confounder is that both growth hormone (GH) and androgens increase BMR. In the male, both of these are going to be falling with age.

Handelsman: There are always issues about how one constitutes a sample of healthy elderly. If you looked at the original data, and if the disease wasn't so rigorously excluded, how much of an effect is there? Is it a very marked effect of disease on thyroid function? Related to this, have there been any studies with thyroxine supplementation in just the elderly?

Brabant: We need to discuss separately whether we have thyroid pathology or hypothyroidism (as is common in the USA in population studies with rates of up to 10%) or we have hyperthyroidism (as we have in Germany). There we have definite data to show that correction is important. There are some German data, recently generated by our group, showing 8–10% hyperthyroidism. If you include the subclinical forms, there is a 1–2% rate of hypothyroidism.

Handelsman: I was thinking more of non-thyroidal hormone exclusions.

Brabant: The exclusion of non-thyroidal illness is not easy. It is estimated in large population studies to add up to 15–20%.

Laron: We know that hypothyroidism reduces secretion of GH. If we don't see obvious hypothyroidism in old people, can we conclude that the reduction in GH/insulin-like growth factor (IGF)1 is not thyroid dependent?

Brabant: The observation that population studies show constant thyroid hormone levels up to age 80 but a prominent decrease in GH/IGF1 argues against such interrelations. However, treating this large population of hypothyroidal subjects may have effects but I am not aware of data where this has been formally investigated, and I do not know which change in thyroid hormone concentrations will elicit alterations in GH/IGF1.

Shalet: I don't think anyone has ever defined the threshold level of thyroid hormone that has a significant effect on GH secretion.

Brabant: There is a huge range of normal thyroid hormone concentrations. In our lab 4.5–12 μg/dl, a threefold variation, is considered to be normal. There are individual set points that differ between individuals. It is difficult to define the thyroid status we would like to aim for.

Ruiz-Torres: Two thyroid parameters are more or less dependent on age. Both TSH and T3 decrease with age. But what role does the thyroid have in ageing?

Brabant: The data are conflicting. In the studies I referred to in my paper we find TSH first goes up and then decreases. If TSH is increasing you have a more prominent effect from the thyroid itself. You may get primary hypothyroidism which is sensed by the hypothalamic–pituitary complex, and responds by increasing in TSH. In contrast, in Marrioti's study there is a decrease in TSH by concentrations of peripheral thyroid hormones, which means there is a dominant defect in the hypothalamic–pituitary axis. But this doesn't exclude the possibility that there might be some additional problems with the thyroid. I deliberately put in the data on thyroid autonomy. It takes so little in a country that is as iodine deficient as Italy and Germany to really get some subclinical thyroid autonomy, with a perfectly normal situation if you do the readings for T3 and T4, but a slightly lowered TSH serum concentration. Is this already clinical hyperthyroidism? Probably not, but there is certainly some problem in the hypothalamo–pituitary interaction, with a primary change in the thyroid.

Ruiz-Torres: In old age, there is remarkable tendency to get hypothyroidism, which shows the same manifestations as ageing itself.

Brabant: From the data up to the 90s there isn't profound hypothyroidism. There is an intensive discussion about whether subclinical hypothyroidism should be treated. For subclinical hyperthyroidism the situation is less controversial, because atrial fibrillation increases threefold and cardiovascular consequences develop. Anyway, I don't think mild hypothyroidism explains ageing, especially as we only see the condition in centenarians.

Haus: We have studied 194 clinically healthy children living in the endemic goitre area of Romania and standardized in their daily routine by their school activity. 60% of the children had endemic goitre, but were clinically and chemically euthyroid. We compared these children with a group of 284 elderly subjects between 60 and 90+ years of age also living in Romania. The circadian mean of plasma TSH was significantly higher in the elderly than in the children due to higher circadian trough values, while the circadian peak values were essentially the same (Nicolau & Haus 1992). The circulating thyroid hormones also showed low-amplitude circadian rhythms with a difference between the children and the elderly in the levels as well as in timing. The TSH peak in the children usually occurred during the night, and the peak of the circulating thyroid hormones followed in the morning. In contrast, in the elderly, although the peak in TSH occurred also during night hours there was a delay of several hours of the peak times (acrophases) of the thyroid hormones. It appears that in the elderly higher levels of TSH lead to a lower output of thyroid hormones than in children and after the nightly peak in TSH the thyroid responds slower with a phase delay in the thyroid hormone output.

Another finding was a difference in the seasonal variation of the pituitary–thyroid axis between children and the elderly. In the elderly, the seasonal amplitude was substantially smaller and a seasonal (circannual) variation was present only up to the eighth decade (Haus et al 1988). Beyond this it was absent.

The elderly also showed a difference in the mix of the circulating thyroid hormones with a statistically significantly lower T3:T4 ratio in the elderly than in young adults or children (Dumitriu et al 1996a,b).

References

Dumitriu L, Bogdan C, Nicolau GY et al 1996a Seasonal variations in TSH, TT3, TT4, log TSH/TT4 and T3/T4 ratio in elderly subjects. Proceedings of the 10th International Congress of Endocrinology, June 12–15, San Francisco, CA, Abstract P2-1059:669

Dumitriu L, Plinga L, Nicolau GY et al 1996b Seasonal variations in iodine excretion, plasma TSH, TT3, TT4, long TSH/TT4 and T3/T4 ratio in children with and without endemic goiter. Proceedings of the 10th International Congress of Endocrinology, June 12–15, San Francisco, CA, Abstract P2-1058:669

Haus E, Nicolau GY, Lakatua D, Sackett-Lundeen L 1988 Reference values for chronopharmacology. Annu Rev Chronopharmacol 4:333–424

Nicolau GY, Haus E 1992 Chronobiology of the hypothalamic-pituitary-thyroid (HRT) axis. In: Touitou Y, Haus E (eds) Biological rhythms in clinical and laboratory medicine. Springer-Verlag, Heidelberg p 330–347

Endocrine Facets of Ageing.
Novartis 242

Print ISBN 0-471-48636-1 Online ISBN 0-470-84654-2

Critical illness as a model of hypothalamic ageing

Greet Van den Berghe and Stephen M. Shalet*[1]

*Department of Intensive Care Medicine, University Hospital, Gasthuisberg, Catholic University of Leuven, Belgium and *Department of Endocrinology, Christie Hospital, Manchester, UK*

Abstract. In this review we shall consider the endocrine changes seen in the various hypothalamic pituitary-target gland axes at different stages of critical illness and conclude by comparing these changes with those seen in the normal ageing process.

2002 Endocrine facets of ageing. Wiley, Chichester (Novartis Foundation Symposium 242) p 205–221

By definition, 'critical illness' is any condition requiring support for failing vital organ functions, either with mechanical aids or with pharmacological agents, without which death would ensue — it is the ultimate example of acute severe physical stress. If onset of recovery does not follow within several days of intensive medical care, critical illness often becomes prolonged and intensive care must be continued for weeks or even months.

Patients previously died from these severe challenges, which include septic shock, multiple trauma or extensive burns. However, the survival mediated by intensive care is also associated with negative aspects. The highly technological intervention in the natural course of the dying process has unmasked previously unknown conditions, including a non-specific wasting syndrome: despite feeding, protein continues to be lost from vital organs and tissues due to increased proteolysis and decreased protein synthesis, whereas re-esterification of free fatty acids (FFAs) allows fat stores to build up (Streat et al 1987, Gamrin et al 1996). Moreover the muscle wasting is accompanied by hyperglycaemia, insulin resistance, hypoproteinaemia, hypercalcaemia, intracellular water and potassium depletion, and hypertriglyceridaemia, which often prompt symptomatic treatment.

[1]This paper was presented at the symposium by Stephen Shalet, to whom correspondence should be addressed.

Growth hormone axis

In the acute phase circulating levels of growth hormone (GH) are elevated. The peak GH levels as well as interpulse concentrations are high (Ross et al 1991, Voerman et al 1992) and the GH pulse frequency is increased. Serum insulin-like growth factor (IGF)1 concentrations are low, (Ross et al 1991). The combination of high GH levels and low IGF1 levels has been interpreted as resistance to GH, which may be related to decreased GH receptor expression (Hermansson et al 1997). The other GH-dependent peptides IGF binding protein (IGFBP)3 and acid labile subunit (ALS) are also decreased in the circulation (Baxter 1997, Timmins et al 1996), preceded by a drop in the GH binding protein (GHBP). Circulating levels of the small IGFBPs such as IGFBP1, IGFBP2 and IGFBP6 are elevated (Baxter et al 1998, Rodrígues-Arnao et al 1996). It has been suggested that these changes are brought about by the effects of cytokines such as tumour necrosis factor (TNF)α, interleukin (IL)1 and IL6. The hypothesis is that reduced GH receptor expression and thus low IGF1 levels are the primary events (cytokine-induced) which, in turn, through reduced negative feedback inhibition, induce the abundant release of GH during acute stress, exerting direct lipolytic, insulin-antagonizing and immune-stimulating actions, while the indirect IGF1-mediated effects of GH are attenuated. This explanation is plausible in that such changes would prioritize essential substrates such as glucose, FFAs and amino acids (glutamine) towards survival rather than anabolism. Increased IGFBP3 protease activity in plasma has also been reported, however, and is thought to result in increased dissociation of IGF1 from the ternary complex, thereby shortening the IGF1 half-life in the circulation (Baxter 1997, Gibson & Hinds 1997).

In prolonged critical illness (PCI) the pattern of GH secretion is very chaotic and the quantity of GH released in pulses is much reduced compared with the acute phase (Van den Berghe et al 1997a, 1998, 1999). Furthermore, although the non-pulsatile fraction is still elevated and the number of pulses is still frequent, mean nocturnal GH concentrations are scarcely elevated compared with the healthy non-stressed condition. The mean nocturnal GH level is about 1 μg/l, though levels are easily detectable and peak GH levels hardly ever exceed 2 μg/l; these results are surprisingly, independent of age, gender, body composition and type of underlying disease (Fig. 1).

The pulsatile component of GH secretion, which is substantially reduced, correlates positively with circulating levels of IGF1, IGFBP3 and ALS, all of which are low (Van den Berghe et al 1997a, 1998, 1999). Thus the more pulsatile GH secretion is suppressed, the lower circulating levels of the GH-dependent IGF1 and ternary complex binding proteins become. This clearly no longer represents a state of GH resistance! The elevated serum levels of GHBP, assumed

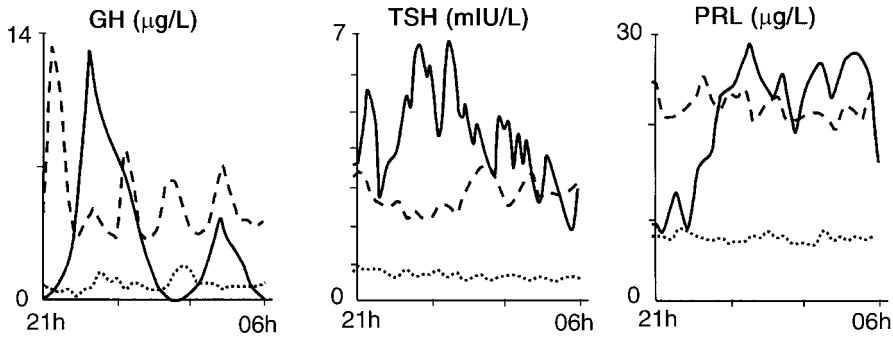


FIG. 1. Nocturnal serum concentration profiles of GH, TSH and PRL illustrating the differences between the initial phase (interrupted line) and the chronic phase (dotted line) of critical illness within an intensive care setting. The continuous lines illustrate normal patterns.

to reflect GH receptor expression in peripheral tissues, in PCI patients are in line with recovery of GH-responsiveness with time during severe illness. The low levels of IGF1, IGFBP3, ALS and IGFBP5 are tightly related to biochemical markers of impaired anabolism such as low serum osteocalcin and leptin (Van den Berghe et al 1999). These findings suggest that relative GH deficiency, epitomised by reduced pulsatile GH secretion participates in the pathogenesis of the 'wasting syndrome' especially in the chronic phase of critical illness. Furthermore there is a gender dissociation in that men show a greater loss of pulsatility and regularity within the GH secretory pattern than women (despite indistinguishable total GH output) and concomitantly lower IGF1 and ALS levels (Van den Berghe et al 2000). It remains unknown whether the sexual dimorphism within the GH–IGF1 axis and the fact that males seem to be at higher risk for an adverse outcome from PCI than females is a casual or causal association.

The pathogenesis of the secretory pattern of GH in PCI is probably complex. One of the possibilities is a deficiency of the endogenous GH releasing peptide (GHRP)-like ligand together with reduced somatostatin tone and maintenance of some GH releasing hormone (GHRH) effect. In reality the GH responses to a bolus injection of GHRP are exuberant in long-stay intensive care patients and several-fold higher than the response to GHRH, the latter being normal or often subnormal. GHRH and GHRP evoke a clear synergistic response under these circumstances (Van den Berghe et al 1996), revealing the highest GH response ever reported in a human study (Fig. 2). The pronounced GH responses to secretagogues exclude the possibility that the blunted GH secretion during the chronic phase of critical illness is due either to a lack of pituitary capacity to synthesise GH or to exaggerated somatostatin-induced suppression of GH

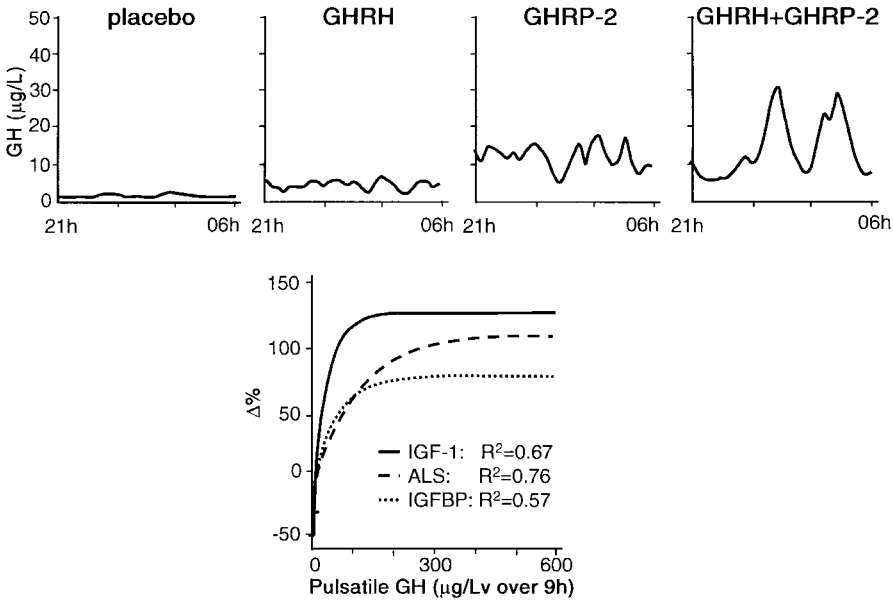


FIG. 2. (Upper part) Nocturnal serum GH profiles in the prolonged phase of illness illustrating the effects of continuous infusion of placebo, GHRH (1 μg/kg per h), GHRP2 (1 μg/kg per h) or GHRH plus GHRP2 (1+1 μg/kg per h). The age range of the patients was 62–85 yrs; the duration of illness was between 13–48 days: infusions were started 12 h before the onset of the respective profile. (Lower part) Exponential regression lines have been reported between pulsatile GH secretion and the changes in circulating IGF1, ALS and IGFBP3 obtained with a 45 h infusion of either placebo, GHRP2 or GHRH plus GHRP2. They indicate that the parameters of GH responsiveness increase in proportion to GH secretion up to a point beyond which a further increase in GH secretion has apparently little or no additional effect. In the chronic non-thriving phase of critical illness, GH sensitivity is clearly present in contrast to the acute phase of illness, which is thought to be primarily a condition of GH resistance (Van den Berghe et al 1998).

release. The combination of reduced somatostatin tone and deficiency of an endogenous GHRP-like ligand would explain the reduced GH burst amplitude, the increased frequency of spontaneous GH secretory bursts, and the elevated interpulse levels as well as the striking responsiveness to GHRP alone or in combination with GHRH. Females with PCI have a markedly greater GH response to a bolus of GHRP compared with males, a difference which is eliminated when GHRH is injected together with GHRP. Lower endogenous GHRH action in men with PCI possibly due to the concomitant profound testosterone deficiency (Van den Berghe et al 2000) accompanying loss of action of an endogenous GHRP-like ligand with prolonged stress in both genders, may explain this finding.

TSH–thyroid axis

Acute illness or trauma induces alterations in thyroid hormone equilibrium within hours. Although serum TSH usually remains normal, circulating T3 rapidly drops, partly due to reduced conversion of T4 to T3 and/or increased turnover of thyroid hormones. The extent of the T3 drop within 24 hours reflects the severity of illness (Schlienger et al 1991, Rothwell & Lawler 1995). Serum reverse T3 (rT3) levels increase partly due to reduced rT3 degradation. In animal models hepatic nuclear T3 receptors appear to decrease in number and occupancy. The absence of a TSH elevation in the presence of low circulating T3 levels suggests that there is also an altered feedback setting at the hypothalamic–pituitary level. Experimental data indicate that reduced TRH gene expression as well as enhanced nuclear T3 receptor occupancy within the thyrotrophs may be involved (Kakucska et al 1994, St Germain & Galton 1985).

The mechanism responsible for the low T3 syndrome remains speculative. Increased levels of cytokines TNF-α, IL1 and IL6, increased endogenous thyroid hormone analogues resulting from alternative deamination and decarboxylation, and low concentrations of binding proteins and inhibition of hormone binding, transport and metabolism by elevated levels of FFA and bilirubin, have all been proposed as factors contributing to the low T3 syndrome at tissue level, although definitive proof is awaited.

In the prolonged phase of critical illness, the situation changes; pulsatile TSH secretion is diminished (Fig. 1) and positively related to the low serum T3 levels (Van den Berghe et al 1997b, 1998), suggesting that the reduced production of thyroid hormone at this time may reflect neuroendocrine dysfunction. Consistent with this suggestion, hypothalamic TRH gene expression is positively related to serum T3 and, in addition, an increase in serum TSH is a marker of the onset of recovery from severe illness. The exact mechanisms underlying the neuroendocrine dysfunction responsible for low thyroid hormone levels in PCI remain unknown, but the low thyroid hormone levels, *per se*, cannot be interpreted as a beneficial response; low thyroid hormone levels have been found to correlate inversely with urea production and bone degradation and therefore do not reflect an adaptive protective mechanism against hypercatabolism. Thus the restoration of physiological levels of thyroid hormones by continuously infusing TRH, together with a GH secretagogue, reduces hypercatabolism, an effect related only to changes in the thyroid hormone levels. During TRH infusion, the negative feedback exerted by thyroid hormones upon the thyrotrophs was maintained, thus avoiding overstimulation of the thyroid axis, which would inadvertently aggravate catabolism. The co-infusion of TRH and GH-releasing factors appears to be a better strategy than the infusion of TRH alone, since the combination but not TRH alone, increases the pulsatile fraction of TSH release and avoids a rise in

circulating rT3 (Van den Berghe et al 1998). The latter observation may reflect the well-known effect of GH on type 1 deiodinase activity. It remains unknown however if the normalization of circulating and tissue T3 concentrations has any beneficial clinical consequences. Thus far pioneering studies with T4 administration have failed to demonstrate clinical benefits within an intensive care setting but in view of the impaired conversation of T4 to T3 this is not really surprising. In this context a recent report on thyroid hormone treatment during the entire stay on the intensive care unit involving substitution doses of T3 in paediatric patients after correction of congenital heart defects revealed improvement in post-operative cardiac function. In contrast to treatment with thyroid hormones, infusing TRH encourages appropriate changes in thyroid hormone metabolism thereby normalizing thyroid hormone levels in the circulation and tissues (Van den Berghe et al 1998). Outcome benefit of TRH infusion alone or in combination with GH secretagogues in PCI has yet to be studied.

Prolactin

In response to acute physical or psychological stress the circulating prolactin level rises (Noel et al 1972), a response that may be mediated by vasoactive intestinal peptide, oxytocin, dopaminergic pathways and/or other still uncharacterized factors; cytokines may also play a signalling role. Although prolactin appears to have immunostimulatory properties in animal models as well as in humans, it remains unclear whether the relative hyperprolactinaemia during the initial phase of critical illness or post-trauma contributes to the initial activation of the inflammatory cascade.

In the chronic phase of critical illness, serum prolactin levels are no longer as high as in the acute phase and the secretory pattern is characterised by a reduced pulsatile fraction (Fig. 1) (Van den Berghe et al 1998, Gardner et al 1979). A role for endogenous dopamine has been suggested. It is unknown whether the blunted prolactin secretion in the chronic phase plays a role in the anergic immune dysfunction or in the increased susceptibility for infections characterising the chronically ill (Meakins et al 1977). However, exogenous dopamine often infused as an inotropic drug in intensive care-dependent patients has been shown to further suppress prolactin secretion and was found to aggravate concomitantly both T lymphocyte dysfunction and impaired neutrophil chemotaxis (Van den Berghe 1994a).

Luteinizing hormone–testosterone axis

The low serum testosterone concentrations despite elevated luteinizing hormone (LH) levels documented during the acute stress of surgery or myocardial infarction

suggest an immediate stress-induced Leydig cell suppression (Wang et al 1978a,b, Dong et al 1992), the exact cause of which remains obscure. A role for inflammatory cytokines (IL1 and IL2) is possible, as suggested by experimental studies. It may be considered appropriate that the secretion of anabolic androgens be switched off in circumstances of acute stress, in order to reduce the consumption of energy and substrates at such a critical life-threatening time.

As critical illness becomes prolonged, hypogonadotropic hypogonadism ensues (Vogel et al 1985, Woolf et al 1985). Circulating levels of testosterone become extremely low, often undetectable in men whereas oestradiol concentrations are reduced to a lesser degree, thereby increasing the oestradiol:testosterone molar ratio. The progressive decline in serum gonadotropin levels, however, appears to lag behind the rapid decline in serum testosterone. In men with PCI, a high LH pulse frequency with an abnormally low LH pulse amplitude is seen and this has been interpreted as an impaired LH response to very low circulating testosterone levels (Van den Berghe et al 1994b). Endogenous dopamine, opiates IL1, and the 'relatively spared' oestradiol level may be involved in the pathogenesis of the gonadotropin deficiency. Androgen treatment in men affected by prolonged critical illness failed to induce conclusive clinical benefit (Tweedle et al 1972). An alternative approach with exogenous gonadotropin-releasing hormone (GnRH) has proved a little more promising in that the gonadotropin deficiency could be partially reversed with pulsatile GnRH administered intravenously (Van den Berghe et al 2001). The inability to completely reverse the profound hypogonadotropic hypogonadal state may be due to relative pituitary desensitization to GnRH or enhanced-feedback inhibition from the markedly elevated oestradiol:testosterone molar ratio. Peripheral tissues were sensitive to transient changes in sex steroids, as reflected by anabolic and inflammatory responses (Van den Berghe et al 2001).

Pituitary–adrenal axis

It has been known for a long time that the vital stress-induced hypercortisolism induced by surgery, trauma or sepsis is associated with augmented adrenocorticotropic hormone (ACTH) release, driven presumably by corticotropin-releasing hormone (CRH), cytokines and the noradrenergic system. Concomitantly, circulating aldosterone rises markedly probably under the control of an activated renin-angiotensin system. Hypercortisolism acutely shifts carbohydrate, fat and protein metabolism, so that energy is instantly and selectively available to vital organs such as the brain and so that anabolism is delayed.

In PCI serum ACTH levels are low, while cortisol levels remain elevated, indicating that cortisol release may in this phase be driven through an alternative

pathway possibly involving endothelin (Vermes et al 1995). The mechanism responsible for low ACTH levels in PCI is unknown although a role for atrial natriuretic peptide or substance P has been suggested. In contrast, circulating levels of adrenal androgens such as dehydroepiandrosterone sulfate (DHEAS) (Van den Berghe et al 1995) and the mineralocorticoid, aldosterone, are reduced, despite increased renin activity (Zipser et al 1981) in PCI. This steroid profile suggests a shift of pregnenolone metabolism away from both mineralocorticoid and adrenal androgen pathways towards the glucocorticoid pathway. The mechanism responsible for this change in steroid secretory pattern is unknown but ultimately it may fail thereby accounting for the 20-fold higher incidence of adrenal insufficiency in critically ill patients over the age of 50 years and being treated on the intensive care unit for more than 14 days. The fact that this type of relative adrenal failure coincides with adverse outcomes suggests that high levels of glucocorticoids remain essential for haemodynamic stability. Whether hypercortisolium in the chronic phase of critical illness is exclusively beneficial remains uncertain.

Protracted critical illness versus ageing

Pattern of abnormality

In PCI anterior pituitary functional status is impaired with decreased secretion of GH, ACTH, LH, TSH and prolactin. The low normal serum TSH and prolactin levels are generated mainly through tonically released TSH and prolactin. Pulsatile TSH and prolactin release is significantly reduced quantitatively with absence of the nocturnal hormone surges independent of concomitant sleep, although pulse frequency is normal. Peripheral thyroid hormone levels are low and related to the reduced nocturnal pulsatile TSH secretion. In ageing there are significant similarities in that the balance of opinion suggests a blunted nocturnal TSH rise, decreased TRH synthesis and a reduction in TSH levels; the most pronounced change in this axis being the gradual age-dependent decline in serum T3 concentration as a consequence of decreased peripheral degradation of T4.

There are significant differences in the LH–testosterone–oestradiol profile seen in men with PCI compared with the normal elderly male. In the former the testosterone level is grossly reduced in the presence of only a modest reduction in the oestradiol level, whereas the LH pulse frequency is increased although the pulses are greatly reduced in amplitude. In contrast with advancing age normal males show a slight decline in the serum testosterone level with an increased proportion showing a testosterone level in the hypogonadal range. Basal LH levels increase with age and there is a reduction in the amount and pulsatile frequency of LH secretion by the pituitary in response to GnRH stimulation,

whereas oestradiol levels remain constant with increasing age. Nonetheless, despite the quantitative difference in degree of testosterone reduction, there are qualitative similarities between PCI and ageing, primarily based on the suppressive action of oestradiol on LH secretion, a feedback phenomenon which is facilitated by the increase in aromatase activity observed in both situations.

In a similar fashion to TSH, prolactin and LH, the amount of GH secreted over 24 hours in PCI is reduced, with substantial reduction in the pulsatile component but a non-pulsatile fraction that remains elevated. In normal subjects, after the age of 40 years, GH production decreases gradually — at a rate of about 14% per decade, primarily because of a decrease in the amplitude of nocturnal GH pulses. At 65 years of age daily spontaneous secretion of GH is reduced by about 50–70%.

Thus there are considerable similarities between the pituitary functional changes seen in PCI and normal ageing; major differences include the intensity of the testosterone deficiency, the elevated non-pulsatile fraction of GH secretion and the reduction in ACTH secretion seen in PCI compared with normal ageing.

Despite the increased incidence of adrenal insufficiency and the recognized decrease in ACTH secretion, cortisol levels remain elevated in the majority of patients with PCI, whereas in both humans and experimental animals there are no major alterations of the hypothalamic–pituitary–adrenal axis, in terms of ACTH and cortisol levels, during ageing. Interestingly adrenal androgen production is significantly reduced both with ageing and PCI.

Site of defect

In both PCI and normal ageing the relatively normal and sometimes exuberant pituitary hormone responses to a number of secretagogues indicate that any degree of hypopituitarism is not a consequence of pituitary failure *per se* (Fig. 2). Equally, the consistent relationships between pulsatile GH and TSH levels with IGF1 and T3 levels respectively in PCI suggests that failure of peripheral endocrine gland responsiveness is not contributing significantly to the hypofunctional state. Similar conclusions have been drawn in the normal elderly in whom the IGF1 response to an acute bolus of GH remains undiminished compared with that seen in young adults.

These observations imply that the relative hypopituitarism in both PCI and normal ageing is primarily hypothalamic in origin with the caveat that in certain systems (i.e. pituitary–gonadal) there is some degree of end organ failure (i.e. testis).

Thus impaired secretion of endogenous TRH, GnRH and hypothalamic factors controlling GH secretion appear to be common to both PCI and normal ageing. Reduced hypothalamic GHRH and probably decreased hypothalamic GH secretagogue (i.e. ghrelin) are likely to be found in both ‘pathologies’. As for

hypothalamic somatostatin, this appears reduced in PCI but the exact nature of any change with ageing remains contentious.

In conclusion, PCI is a reasonable paradigm for studying the relative hypopituitarism associated with normal ageing — more so for certain axes, such as TSH and GH, but less so for others, such as gonadotropin secretion.

References

Baxter RC 1997 Acquired growth hormone insensitivity and insulin-like growth factor bioavailability. Endocrinol Metab 4:65-69

Baxter RC, Hawker FH, To C, Stewart PM, Holman SR 1998 Thirty day monitoring of insulin-like growth factors and their binding proteins in intensive care unit patients. Growth Horm IGF Res 8:455–463

Dong Q, Hawker F, McWilliam D, Bangah M, Burger H, Handelsman DJ 1992 Circulating immunoreactive inhibin and testosterone levels in men with critical illness. Clin Endocrinol 36:399–404

Gamrin L, Essén P, Forsberg AM, Hultman E, Wernerman J 1996 A descriptive study of skeletal muscle metabolism in critically ill patients free amino acids, energy-rich phosphates, protein, nucleic acids, fat, water, and electrolytes. Crit Care Med 24:575–583

Gardner DF, Kaplan MM, Stanley CA, Utiger RD 1979 Effect of triiodothyronine replacement on the metabolic and pituitary responses to starvation. N Eng J Med 300:579–584

Gibson FA, Hinds CJ 1997 Growth hormone and insulin-like growth factors in critical illness. Intensive Care Med 23:369–378

Hermansson M, Wickelgren RB, Hammerqvist F et al 1997 Measurement of human growth hormone receptor messenger ribonucleic acid by a quantitative polymerise chain reaction-based assay: demonstration of reduced expression after elective surgery. J Clin Endocrinol Metab 82:421–428

Kakucska I, Romero LI, Clark BD et al 1994 Suppression of thyrotrophin-releasing hormone gene expression by interleukin-l-beta in the rat: implications for nonthyroidal illness. Neuroendocrinology 59:129–137

Meakins JI, Pietsch JB, Bubenick O et al 1977 Delayed hypersensitivity: indicator of acquired failure of host defenses in sepsis and trauma. Ann Surg 188:241–250

Noel GL, Suh HK, Stone SJG, Frantz AE 1972 Human prolactin and growth hormone release during surgery and other conditions of stress. J Clin Endocrinol Metab 35:840–851

Rodrígues-Arnao J, Yarwood Y, Ferguson C, Miell J, Hinds CJ, Ross RJ 1996 Reduction in circulating IGF-I and hepatic IGF-I mRNA levels after caecal ligation and puncture are associated with differential regulation of hepatic IGF-binding protein-1, -2 and -3 mRNA levels. J Endocrinol 151:287–292

Ross R, Miell J, Freeman E et al 1991 Critically ill patients have high basal growth hormone levels with attenuated oscillatory activity associated with low levels of insulin-like growth factor-1. J Clin Endocrinol 35:47–54

Rothwell PM, Lawler PG 1995 Prediction of outcome in intensive care patients using endocrine parameters. Crit Care Med 23:78–83

Schlienger H, Sapin R, Capgras T et al 1991 Evaluation of thyroid function after myocardial infarction. Ann Endocrinol (Paris) 52:283–288

St Germain DL, Galton VA 1985 Comparative study of pituitary–thyroid economy in fasting and hypothyroid rats. J Clin Invest 75:679–688

Streat SJ, Beddoe AH, Hill GL 1987 Aggressive nutritional support does not prevent protein loss despite fat gain in septic intensive care patients. J Trauma 27:262–266

Timmins AC, Cotterill AM, Cwyfan-Hughes SC et al 1996 Critical illness is associated with low circulating concentrations of insulin-like growth factors-I and -II, alterations in insulin-like growth factor binding protein and induction of an insulin-like growth factor binding protein 3 protease. Crit Care Med 24:1460–1466

Tweedle D, Walton C, Johnston DA 1972 The effect of an anabolic steroid on postoperative nitrogen balance. Br J Clin Pract 27:130–132.

Van den Berghe G, de Zegher F, Lauwers P 1994a Dopamine and the euthyroid sick syndrome in critical illness. Clin Endocrinol 41:731–737

Van den Berghe G, de Zegher F, Lauwers P, Veldhuis JD 1994b Luteinizing hormone secretion and hypoandrogenemia in critically ill men: effect of dopamine. Clin Endocrinol 41:563–569

Van den Berghe G, de Zegher F, Schetz M, Verwaest C, Ferdinande P, Lauwers P 1995 Dehydroepiandrosterone sulphate in critical illness: effect of dopamine. Clin Endocrinol 43:457–463

Van den Berghe G, de Zegher F, Bowers CY et al 1996 Pituitary responsiveness to growth hormone releasing hormone, GH-releasing peptide-2 and thyrotropin releasing hormone in critical illness. Clin Endocrinol 45:341–351

Van den Berghe G, de Zegher F, Velduis JD et al 1997a The somatotropic axis in critical illness: effect of continuous growth hormone-releasing hormone and GH-releasing peptide-2 infusion. J Clin Endocrinol Metab 82:590–599

Van den Berghe, de Zegher F, Veldhuis JD et al 1997b Thyrotrophin and prolactin release in prolonged critical illness: dynamics of spontaneous secretion and effects of growth hormone secretagogues. Clin Endocrinol 47:599–612

Van den Berge G, de Zegher F, Baxter RC et al 1998 Neuroendocrinology of prolonged critical illness: effects of exogenous thyrotropin-releasing hormone and its combination with growth hormone secretagogues. J Clin Endocrinol Metab 83:309–319

Van den Berghe G, Wouters P, Weekers F et al 1999 Reactivation of pituitary hormone release and metabolic improvement by infusion of growth hormone-releasing peptide and thyrotrophin-releasing hormone in patients with protracted critical illness. J Clin Endocrin Metab 84:1311–1323

Van den Berghe G, Baxter RC, Weekers F et al 2000 A paradoxical gender dissociation within the growth hormone/insulin-like growth factor I axis during protracted critical illness. J Clin Endocrinol Metab 85:183–192

Van den Berghe G, Weekers F, Baxter RC 2001 Five days pulsatile GnRH administration unveils combined hypothalamic-pituitary-gonadal defects underlying profound hypoandrogenism in men with prolonged critical illness. J Clin Endocrinol Metab 86:3217–3226

Vermes I, Bieshuizen A, Hampsink RM, Haanen C 1995 Dissociation of plasma adrenocorticotropin and cortisol levels in critically ill patients: possible role of endothelin and atrial natriuretic hormone. J Clin Endocrinol Metab 80:1238–1242

Voerman HJ, Strack van Schijindel RJM, de Boer H, van der Veen EA, Thijs IG 1992 Growth hormone: secretion and administration in catabolic adult patients, with emphasis on the critically ill patient. Neth J Med 41:229–244

Vogel AV, Peake GT, Rada RT 1985 Pituitary–testicular axis dysfunction in burned men. J Clin Endocrinol Metab 60:658–665

Wang C, Chan V, Tse TF, Yeung RT 1978a Effect of surgical stress on pituitary–testicular function. Clin Endocrinol 9:255–2266

Wang C, Chan V, Tse TF, Yeung RT 1978b Effect of acute myocardial infarction on pituitary–testicular function. Clin Endocrinol 9:249–253

Woolf PD, Hamill RW, McDonald JV, Lee LA, Kelly M 1985 Transient hypogonadotrophic hypogonadism caused by critical illness. J Clin Endocrinol Metab 60:444–450

Zipser RD, Davenport MW, Martin KI et al 1981 Hyperreninemic hypoaldosteronism in the critically ill: a new entity. J Clin Endocrinol Metab 53:867–873

DISCUSSION

Veldhuis: You have tackled an interesting problem that few groups have studied, partly because of the complex confounding by concurrent drugs. Dr Greet Van den Berghe has managed to find a fairly homogeneous population that has convinced many of us that there is excellent pituitary responsiveness to a host of releasing factors, particularly given in combination (Van den Berghe et al 1997a,b, 1998, 1999a,b, 2000, 2001a,b).

Bowers: One of the surprises of the GHRP2 is that as one gives it continuously, it would be expected to down-regulate the effect. But it is only partially down-regulated because there is a persistent increase of pulsatile secretion. We have done this study in older individuals up to 30 days, and in one individual for 90 days. The increased pulsatile secretion of GH remains sustained. When we give acute bolus injections of GHRP2, the GH response is markedly desensitized. However, during chronic infusion pulsatile GH secretion is augmented. In the elderly studies there was a difference between males and females. In some of the men, GHRP2 did not work very well, so we gave it in combination with GHRH. My interpretation of these data is that the women do not have as much endogenous GHRH deficiency. GHRP2 is ineffective unless one has endogenous GHRH. I assume that these women have more GHRH reserve. Men have more of a deficiency of both the natural GHRP hormone, ghrelin, and GHRH.

Laron: I have had discussions with several of our colleagues about the fact that to achieve the anabolic effects of GH, pulsatile secretion is not necessary. What is the benefit of using secretagogues rather than IGF1 or GH?

Bowers: That is not a simple question. When one gives GH itself, there is a sustained level and there will be more side effects. By stimulating pulsatile secretion one can get the same effects as with GH. This means that there is much less GH secreted and indicates how efficacious the effects of pulsatile GH are on peripheral targets. Comparatively, when one administers GH the levels are high. Another issue is the difficulty of determining the dose of GH that should be given if GH is to be given exogenously. By the GHRP approach, GH levels are totally determined by the feedback system. On each patient the effect is individualized internally by the patient's feedback response.

Laron: You are saying that by using this technique, one would be able to reduce the very high sensitivity to GH in the aged population, while we still don't know the lowest (minimal) effective dose.

Bowers: With regard to the total quantity of GH needed, one can obtain the same effect with a small quantity of pulsatile GH secretion as with administration of GH at larger doses.

Veldhuis: The IGF feedback on the GHRP2 system is so effective that if one happens to overdrive GH output and IGF comes up, it will restrain the GH

(Giustina & Veldhuis 1998). I was under the impression that GH levels rise promptly under continued infusions, and as the IGF1 levels rise the GH levels fall about 50%. The system re-sets at a higher level of IGF.

Müller: I was impressed by the findings shown by Dr Shalet that the pulsatile secretion of GH changes so dramatically from the acute phase to the chronic phase, in spite of similar serum concentrations of peripheral IGF1.

Carroll: I want to raise a philosophical question. We are used to thinking of stress responses as adaptive. There is a long tradition of this viewpoint for acute stress responses. We never evolved to survive in intensive care units: there was no opportunity for selection on this success! Are we to look at these hypothalamic changes as adaptive stress responses, or as allostatic changes? Are we to look on them, therefore, as pathological and in need of treatment?

Shalet: I think these are unnatural changes, because this is a very abnormal set of circumstances.

Bowers: But there is an under-stress response in that second phase, it is not a full stress response. Since these patients are still under stress during the second phase, the full capacity of normal stress responses appears to be impaired and the stress response is inappropriately low.

Carroll: It is some kind of adaptive response, but whether it will promote survival is another matter.

Shalet: It is a failing system.

Carroll: You moved very quickly over the cortisol data. There are very good data, for example, from burns units, showing sustained elevation of cortisol going on for weeks, and a severe drop in production of cortisol binding globulin. How much of the other axis changes are attributable to a primary change in cortisol?

Shalet: That is a plausible suggestion. You could argue the case in terms of changes in gonadotrophin or GH secretion. There are a number of possible hypotheses to explain the endocrine findings in protracted critical illness, and this would be one. We hardly touched at all on the use of other drugs in these patients. In critical care, even if we exclude dopaminergic drugs, we haven't mentioned opiates.

Handelsman: We have done a study similar to this. We don't see testosterone levels of as low as 1 nM in men unless there is something more even than this huge stress response. For example, hypercortisolism alone does not seem to produce that. What does is opiates. Burns patients in particular, are on opiates for long periods of time. I don't know the data that you present very well, but I suspect that there is an opiate effect there that we should take into account.

Shalet: In fairness, in some of the studies Dr Van den Berghe has tried to look at statistical differences in terms of opiate use or not. This is presumably a fairly crude overview of opiate use.

Handelsman: With regard to the aromatase, I don't doubt the observations, but the interpretation of whether a change in the oestradiol:testosterone ratio in peripheral blood indicates a change in aromatase is suspect. Only 0.2% of the body's production of testosterone is converted to oestradiol on a whole body basis. Just this very fact alone should necessitate caution. In addition, clearance rate differences in oestradiol will give all sorts of changes, especially in this setting. I would be hesitant to take this interpretation further.

Shalet: Dr Van den Berghe raises the issue of the increased fat mass that occurs in protracted critical illness. This may well contribute to the 37-fold increase in aromatase activity. Changes in catecholamines and TNFα levels may also be contributory factors.

Elahi: I have been discussing some work with respect to hypoglycaemia that persists during this time. The therapy is glucose, potassium and insulin. There are other modes of therapy currently under investigation, which are less risky. Would the therapies you mention exaggerate the glucose excursion?

Shalet: I don't think Dr Van den Berghe's data touch on this. I would say that she has moved on, and another key hormone she is focusing on at the moment is insulin. This will be a key measure in her future studies.

Ruiz-Torres: I am interested in the relationship between the hormonal changes in critical illness and those which appear during ageing. I have some doubts regarding the value of this model for studies on ageing. For instance, each critical illness has high dysproteinaemia as a common manifestation. The consequence is a decrease of plasma protein transport and an increase of the distribution volume. For this reason, the blood levels of hormones, substances, or drugs are decreased. What happens afterwards is dependent not only on feedback mechanisms but also on a wide range of other factors produced by toxins and intermediary products.

Shalet: Let me answer you simply. I hope I portrayed in my presentation that I didn't see this as a marvellous model for ageing. I tried to be careful there. But there were some similarities in the behaviour of certain axes. Even if what you said is true (and I'm sure that much of it is), you still have to explain things like the gender variation in GH secretion which is very striking.

Veldhuis: A 30% drop in blood volume puts an adult into shock. If you are talking about any hormone changes of several-fold, you don't have to worry that distribution volume is the sole explanation: it won't be for changes of that magnitude.

Prior: In a study of women post-premenopausal ovariectomy, their initial fasting HDL levels were −1.5 standard deviations compared to the normal age range. These were not women with cardiovascular risk factors and their HDL levels subsequently came into the normal range over the next three months. When I looked at this I found that there are reports in the literature of low HDL

in association with acute trauma or illness. What do people think the mechanisms might be?

Veldhuis: This is a protein that is under negative control by insulin, like others such as IGFBP1. Beyond this, and the high insulin output that occurs during hyperalimentation regimens, it is not clear to me what would lower HDL in these women. The half-life is presumably several weeks.

Prior: These women were not on hyperalimentation: they were just fasting post-operatively.

Laron: Stephen Shalet, considering the Scandinavian data you have presented in both acute phase and in chronic illness, what is your advice concerning GH therapy?

Shalet: The simple answer is that I don't know. The dose of GH used in the intensive care study was something like 20 units per day, whereas a standard GH replacement dose is of the order of 1.2 units daily. This was a very big dose of GH. Considering what we have heard, I am attracted to the approach that Dr Van den Berghe and her colleagues are following, which is to begin to look at combinations of pituitary hormone secretagogues. I can't say more about the insulin side. I know Dr Van den Berghe feels that insulin is going to be a key factor in terms of outcome. Do I personally see a big place in this discussion for GH? 'Not particularly' would be my immediate reaction. Other strategies look more attractive to me. The one area where I think the data are most promising for GH therapeutically would be the burns patients. The data look reasonable but are limited.

Giustina: You mentioned that the combination of GHRP2 and TRH may be effective in modifying some target organ effects. You explain this by implying that GHRP has an effect on the GH axis, and TRH has an effect on the thyroid axis. If I remember correctly, in critical illness, as occurs in other situations such as acromegaly and type 1 diabetes, TRH may have some GH-stimulating action. Might this be a combination acting mainly via the GH axis?

Shalet: Individual pituitary hormone secretagogues contribute significantly to the effect on other pituitary hormone axes when used in combination.

Bowers: Van den Berghe found that TRH by itself had very little effect; it was only when she put it together with GHRP2 that she got an additional effect.

Carroll: In terms of intervention, in the near future we will have CRH antagonists for human use. These have already been looked at in animal models of delirium tremens, alcohol withdrawal and drug withdrawal. Drugs like CRH antagonists will probably show great promise for managing the centrally driven hyperstimulated hypothalamic–pituitary–adrenal states. Critical illness might well be a clinical context where they will find application.

Björntorp: I have a vague memory of having heard about studies in which GH has been used to treat heart failure. What is the current situation?

Shalet: The first paper, as I recollect, was by Fazio et al (1996). There were seven patients with dilated cardiomyopathy, and the results were encouraging in terms of improvement in cardiac function. To my knowledge, at least two subsequent controlled studies from Sweden (Isgaard et al 1998) and Germany (Osterziel et al 1998) did not show benefit.

Giustina: The issue is very complex. The selection of patients is probably critical in understanding the results of giving GH to patients with heart failure. There are probably some patients with a certain degree of GH resistance. These are the patients who are not likely to benefit from standard doses of GH.

Elahi: I presume you were talking about the paper by Fazio et al (1996). We were very stimulated by this study. So far we have looked at four patients with congestive heart failure. We gave them 21 d of GHRH therapy. Their IGF1 levels rose following therapy. We administered GHRH 1 mg qd and a month later, 2 mg qd. We now have switched to $\sim$2 mg/day given in four equal pulses, every 2 h from 23:00 to 05:00 h. The total dose of 30 mg/kg is split into four pulses. Whereas with a 1 mg dose, nothing happened, with 2 mg we saw a substantial increase in IGF1 levels. Having said this, it is only one-third of the response seen in normal age-matched low-IGF volunteers. We are doing positron emission tomography (PET) scans to see whether we get any changes in protein synthesis in the heart.

References

Fazio S, Sabatini D, Capaldo B et al 1996 A preliminary study of growth hormone in the treatment of dilated cardiomyopathy. N Engl J Med 334:809–814

Giustina A, Veldhuis JD 1998 Pathophysiology of the neuroregulation of growth hormone secretion in experimental animals and the human. Endocr Rev 19:717–797

Isgaard J, Bergh CH, Caidahl K, Lomsky M, Hjalmarson A, Bengtsson BA 1998 A placebo-controlled study of growth hormone in patients with congestive heart failure. Eur Heart J 19:1704–1711

Osterziel KJ, Strohm O, Schuler J et al 1998 Randomised, double-blind, placebo-controlled trial of human recombinant growth hormone in patients with chronic heart failure due to dilated cardiomyopathy. Lancet 351:1233–1237

Van den Berghe G, de Zegher F, Veldhuis JD et al 1997a The somatotropic axis in critical illness: effect of continuous growth hormone (GH)-releasing hormone and GH-releasing peptide-2 infusion. J Clin Endocrinol Metab 82:590–599

Van den Berghe, de Zegher F, Veldhuis JD et al 1997b Thyrotrophin and prolactin release in prolonged critical illness: dynamics of spontaneous secretion and effects of growth hormone secretagogues. Clin Endocrinol 47:599–612

Van den Berghe G, de Zegher F, Baxter RC et al 1998 Neuroendocrinology of prolonged critical illness: effects of exogenous thyrotropin-releasing hormone and its combination with growth hormone secretagogues. J Clin Endocrinol Metab 83:309–319

Van den Berghe G, Wouters P, Bowers CY, de Zegher F, Bouillon R, Veldhuis JD 1999a Growth hormone-releasing peptide-2 infusion synchronizes growth hormone, thyrotropin and prolactin release in prolonged critical illness. Eur J Endocrinol 140:17–22

Van den Berghe G, Wouters P, Weekers F et al 1999b Reactivation of pituitary hormone release and metabolic improvement by infusion of growth hormone-releasing peptide and thyrotrophin-releasing hormone in patients with protracted critical illness. J Clin Endocrinol Metab 84:1311–1323

Van den Berghe G, Baxter RC, Weekers F, Wouters P, Bowers CY, Veldhuis JD 2000 A paradoxical gender dissociation within the growth hormone/insulin-like growth factor I axis during protracted critical illness. J Clin Endocrinol Metab 85:183–192

Van den Berghe G, Wouters P, Weekers F et al 2001a Combined administration of growth hormone-, thryotropin- and gonadotropin secretagogues to prolonged critically ill men: endocrine and metabolic effects. J Clin Endocrinol Metab

Van den Berghe G, Weekers F, Baxter RC 2001b Five-day pulsatile gonadotrophin-releasing hormone administration unveils combined hypothalamic-pituitary-gonadal defects underlying profound hypoandrogenism in men with prolonged critical illness. J Clin Endocrinol Metab 86:3217–3226

Endocrine Facets of Ageing.
Novartis 242

Print ISBN 0-471-48636-1 Online ISBN 0-470-84654-2

Glucose tolerance, glucose utilization and insulin secretion in ageing

Dariush Elahi, Denis C. Muller*, Josephine M. Egan*, Reubin Andres*, Johannes Veldhuis† and Graydon S. Meneilly‡

*Department of Medicine, Massachusetts General Hospital, Harvard Medical School, Boston, MA 02114, *Laboratory of Clinical Physiology, Gerontology Research Center, National Institute on Aging, National Institutes of Health, Baltimore 21224, MD, †University of Virginia, Division of Endocrinology and Metabolism, Charlottesville, VA 22908, USA, and ‡Department of Medicine, University of British Columbia, Vancouver, BC V6T 1Z3, Canada*

Abstract. Ageing is associated with an increased incidence of hypertension, macrovascular disease and type 2 diabetes (non-insulin-dependent diabetes). It has been suggested that a common mechanism may be responsible for all of these pathological states since all of these conditions often cluster in the same individual. Epidemiological and clinical data have consistently demonstrated an association between insulin resistance and/or hyperinsulinaemia and glucose intolerance, dyslipidaemia and elevated systolic blood pressures. Therefore, insulin resistance and hyperinsulinaemia have been proposed as the causal link among the elements of the clusters. The elderly are more glucose intolerant and insulin resistant, but it remains controversial whether this decrease in function is due to an inevitable consequence of 'biological ageing' or due to environmental or lifestyle variables, noticeably increased adiposity/altered fat distribution and physical inactivity. An increase of these modifiable factors has been shown to result in increases in insulin resistance and hyperinsulinaemia and vice versa. However, insulin secretion appears to decrease with age even after adjustments for differences in adiposity, fat distribution and physical activity. The glucose intolerance of ageing may be due, in part, to decreased insulin sensitivity of pancreatic β cells to insulinotropic gut hormones (GLP1/GIP) and in part to alterations of hepatic glucose production.

2002 Endocrine facets of ageing. Wiley, Chichester (Novartis Foundation Symposium 242) p 222–246

Ageing is associated with a decline in function in many, if not all, human physiological systems. Cardiovascular, haemodynamic, metabolic and renal functions generally decrease with advancing age. Insulin has been causally related in the aetiology of many of these decrements (Reaven 1988, Kaplan 1989, Stout 1990, DeFronzo & Ferrannini 1991). The reduction in whole-body carbohydrate metabolism in the elderly is one of the hallmarks of the ageing process and substantial evidence shows that increasing age is associated with decreased

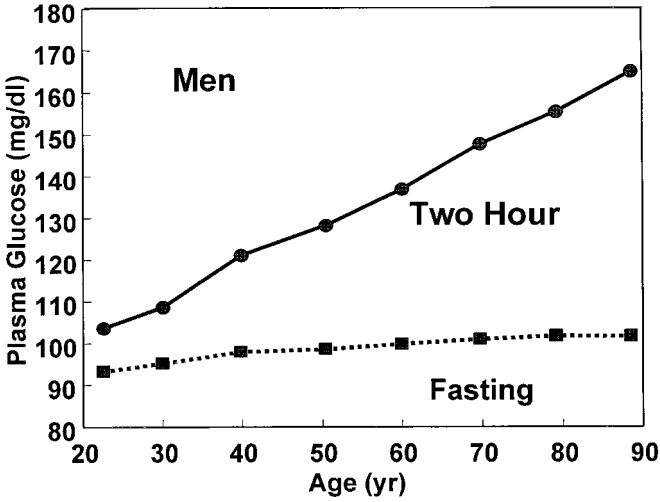


FIG. 1. Effect of age on fasting and 2 h plasma glucose levels in men from the Baltimore Longitudinal Study of Aging.

glucose tolerance (Andres 1971, Broughton & Taylor 1991, Davidson 1979, DeFronzo 1981, Reaven et al 1989). Oral glucose tolerance tests (OGTTs), conducted in healthy non-diabetic individuals across the age range clearly demonstrate a decline in glucose tolerance, as judged from the fasting, intermediate and the 2 h levels, in each age decade from the third decade (20–29 year old) to the ninth decade (80–89 year old) (Elahi & Muller 2000). This decline is easily appreciated from the relationship of age and either fasting or 2 h level, as shown in Fig. 1, and is observed both in men and women. The two-hour plasma glucose level during an OGTT rises on average, 5.3 mg/dl per decade and the fasting plasma glucose rises on average, 1 mg/dl per decade (Davidson 1979). The decline in glucose tolerance is also reflected in NHANES III survey on the prevalence of diabetes and impaired fasting glucose and impaired glucose tolerance in US adults (Harris et al 1998). Comparison of the percentage of physician-diagnosed diabetes in middle-aged adults (40–49 years) and elderly adults ($\geqslant$75 years) reveals an increase from 3.9% to 13.2%. The percentage of adults with (a) undiagnosed diabetes (fasting plasma glucose [FPG] $\geqslant$126 mg/dl) increased from 2.5 to 5.7%; and (b) impaired FPG (110–125 mg/dl) increased from 7.1% to 14.1%. Thus, approximately a third of the elderly adults in the USA have abnormal glucose metabolism as defined by the revised 1997 criteria of the American Diabetes Association (The Expert Committee on the Diagnosis and Classification of Diabetes Mellitus 1997). A detailed review of the effects of ageing on glucose homeostasis, in both humans and animals, has recently been

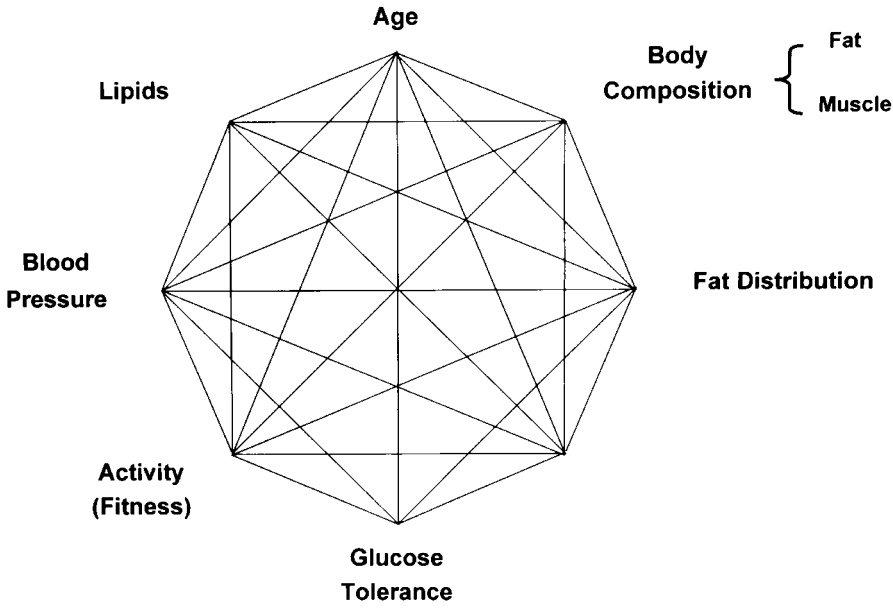


FIG. 2. Risk factors of the metabolic syndrome. Each line represents a statistically significant association between each risk factor that is found in most populations.

published in a number of relevant articles (Evans & Farrell 2001). The reduction of glucose tolerance associated with ageing is also accompanied with dyslipidaemia and hypertension. The clustering of these conditions is commonly referred to as syndrome X or the metabolic syndrome. These pathological conditions are more prevalent in the elderly and are complex, interrelated and multifunctional. The complexity of these inter-relationships is depicted in Fig. 2. The lines connecting the different factors represent the statistically significant associations that exist in the cross-sectional analysis of most populations. For example, a significant decrease in glucose tolerance with increasing age can be demonstrated from all epidemiological studies. However, this relationship is confounded by an increase in adiposity and a decrease in physical activity with age and each of these factors are also associated with a decrease in glucose tolerance. It is difficult to demonstrate how much of the glucose intolerance can be attributed solely to ageing, decreased activity or increased adiposity; the combination of these certainly leads to glucose intolerance. Similarly, the relationships of age, hyperinsulinaemia, dyslipidaemia and insulin resistance are interwoven. This review focuses on the clinical evidence of a change with age in insulin resistance and insulin secretion that is independent of changes in other known factors.

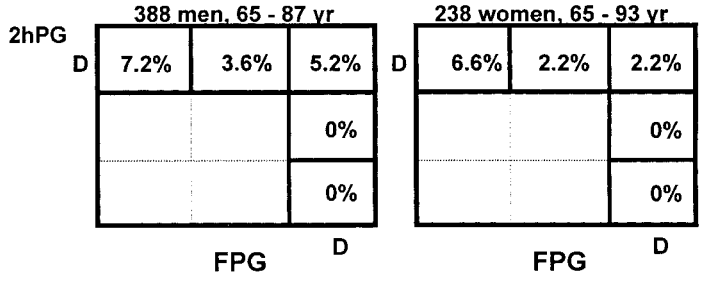
Shimokata et al (1991) examined the relationship between age, obesity, physical activity and glucose tolerance in a community dwelling with men and women ranging in age from 17 to 92, from the Baltimore Longitudinal Study of Aging (BLSA). The independent effect of age on glucose tolerance was examined after statistical adjustment for the confounding effects of obesity, fat distribution and physical activity. They found that the decline in glucose tolerance from early adult (17–39 year) to middle age (40–59 year) is entirely explained by the secondary influences of fatness and fitness. However, the decline from mid-life to old age (60–92 year) was still influenced by chronological age. Other population studies also attribute the decline in glucose tolerance to age-related environmental factors (obesity, physical activity, dietary habits, diabetogenic drug use) (Zavaroni et al 1986, Maneatis et al 1982, Zamboni et al 1997). However, it should be noted that anthropometric determination of body composition and questionnaire-derived assessment of impaired activity have their limitations. Furthermore, when statistical adjustment is made, in addition to the variability in the precision of the measured confounders, linear relationships between the confounders are assumed, interactions between them are ignored, and sample size may not be sufficiently large. Thus, in a complex statistical model, true cause–effect relationships may not be detectable due to under/over adjustment or imprecision.

Diagnostic criteria and the elderly

It is desirable to have a screening test for detection of glucose intolerance which is both sensitive (has a high probability of being positive when there is glucose intolerance) and specific (has a high probability of being negative when there is no glucose intolerance). Often, there is compromise between the two. Increasing sensitivity reduces specificity and vice versa. The screening tests should be reliable and reproducible. The expert committee on the diagnosis and classification of diabetes mellitus of the American Diabetes Association (ADA) provided new criteria that simplified the method of detection of glucose intolerance and diabetes (The Expert Committee on the Diagnosis and Classification of Diabetes Mellitus 1997). For clinical diagnosis in asymptomatic individuals the ADA recommends that diabetes be defined as an FPG value $\geqslant 7.0$ mmol/l (126 mg/dl). This recommendation revises the 1985 World Health Organization (WHO) diagnostic criteria (WHO 1985) and the National Diabetes Data Group (NDDG)'s 1979 criteria for diabetes (National Diabetes Data Group 1979), which relied on both fasting glucose and the 2 h oral glucose tolerance test. An FPG $\geqslant 7.8$ mmol/l (140 mg/dl) and/or a 2 h plasma glucose value $\geqslant 11.1$ mmol/l (200 mg/dl) was diagnostic of diabetes by the previous (1985/1979) criteria. The 1997 ADA criteria also provided recommendations for an additional

diagnostic class. Impaired fasting glucose (IFG) is defined as an FPG level $\geqslant$6.1 mmol/l (110 mg/dl) and $<$7.0 mmol/l (126 mg/dl); normal fasting glucose is changed to an FPG $<$6.1 mmol/l. The OGTT is not recommended for routine diagnosis of glucose intolerance or diabetes. The 1999 WHO report on 'Definition, diagnosis and classification of diabetes mellitus and its complications' essentially endorses the ADA's 1997 recommendation with the exception that they strongly advocate the use of the OGTT (WHO 1999). The 1985 WHO criteria had defined impaired glucose tolerance (IGT) as a fasting glucose $<$7.8 mmol/l and a two-hour FPG $\geqslant$7.8 mmol/l and $<$11.1 mmol/l. Numerous reports (at least 25) have examined the new ADA 1997 criteria in order to assess their impact on prevalence of diabetes by age, sex and ethnicity. An example, is the report by Harris et al (1998), who used the FPG and the 2 h glucose value from an OGTT from the data obtained from the NHANES III survey to compare the percentage of the population meeting the 1997 ADA and the 1985 WHO diagnostic criteria by age, sex and ethnic groups.

It is not entirely clear how the various cut-off points were agreed upon by the various expert committees. It is absolutely evident from all the population data available, almost no one with a fasting value of 140 mg/dl (7.8 mmol/l), has a 2 h plasma value $\leqslant$200 mg/dl (11.1 mmol/l). However, many individuals with a fasting value of $<$140 mg/dl will have a 2 H value $\geqslant$200 mg/dl. It is also not clear how exactly the new cut-off fasting value of 126 mg/dl was agreed upon. This new value is reported to be more in agreement for the diagnosis of diabetes with the 2 h plasma glucose value of 200 mg/dl following the OGTT, than the previous fasting value of 140 mg/dl. However, comparisons of the results of FPG and the 2 h plasma glucose level following an OGTT from the NHANES III survey using the 1985 WHO and the 1997 ADA criteria are still often more than 50% discrepant (Harris et al 1998). The ADA's 1997 report has stated that the justification for the cut-off point for the 2 h plasma glucose of the OGTT of 200 mg/dl is derived from the evidence that the prevalence of microvascular complications increases dramatically at this point. Another justification has to do with the fact that the 2 h plasma glucose value following an OGTT from several populations has a bimodal distribution. The nadir intersection of the two modes is known as the antimode and it shifts to the right with advancing age (Bennett et al 1976). The 200 mg/dl level, purportedly, represents the average level of the antimodes from several large population studies (Pima Indians, Naruans, Samoans, Mexican-Americans, and East Indians). However, the antimodes from these populations range widely from about 150 to over 300 mg/dl (Bennett et al 1976, Zimmet et al 1978, Loo et al 1993, Dowse et al 1994, Rosenthal et al 1985) and do not support the average 200 mg/dl level. They do, however, increase with age. Nevertheless, the 200 mg/dl level remains the standard.



	Men	Women
Diabetic by FPG + GTT	16.0%	11.0%
Diabetic by FPG	5.2%	2.2%
Diabetics missed if no GTT	68%	80%

FIG. 3. Distributions for diagnosis of diabetes (D) made from FPG ($\geqslant$ 7.0 mmol/l; x-axis) and from two-hour plasma glucose level following OGTT ($\geqslant$ 11.1 mmol/l; y-axis) in the BLSA population.

We summarized 25 reports of studies that have computed the prevalence of diabetes in a population when both fasting glucose and the glucose tolerance test (GTT) were used (the WHO recommendation) in contrast to the prevalence when only the fasting level is considered (the ADA recommendation). A very high percentage (ranging from 11 to 80%) of the diagnosable diabetics are missed if the GTT is not considered.

When both tests are used, there are nine possible diagnostic categories since each test results in a classification of normal, impaired or diabetic. Figure 3 illustrates the distribution of test results from our BLSA population in men and women over 65 years. The percentage of subjects classified as diabetic when both tests are used is remarkably reduced when the fasting glucose alone is used: 65% of the men and 80% of the women would have been missed.

In other analyses, since the BLSA subjects are followed over many years with repeated testing, we could estimate that, on average, the diagnosis of diabetes can be established some 7 to 9 years earlier by glucose tolerance testing than by fasting glucose testing alone.

The result of our study is in agreement with many of the reported studies which show that the 1997 ADA diagnosis standards do not result in equal sensitivity for fasting and 2 h glucose levels, especially in older individuals. A disturbingly high percentage of older men and women will have to be informed either that they have diabetic or impaired test results. Although the use of fasting plasma glucose alone for diabetes diagnosis simplifies testing, the WHO criteria identify a much greater percentage of elderly subjects with diabetes or impaired glucose metabolism.

We will now provide a short summary on the effect of age on hepatic glucose production, glucose uptake and insulin secretion. This topic has been recently reviewed in detail, as previously noted, by Evans & Farrell (2001) and we will focus mainly on our own work using the clamp technique. The methodology of the technique has been previously reported (DeFronzo et al 1979) and its various uses reviewed (Elahi 1996). We will additionally review our data with respect to the insulinotropic effect of incretins and our preliminary data using these agents to stimulate insulin secretion and to regulate glucose homeostasis in type 2 diabetic individuals.

Effect of age on hepatic glucose production

During the post-prandial state stable plasma glucose levels are maintained by a coordinated balance between hepatic glucose production (HGP) and glucose uptake by peripheral tissues (primarily muscle). The role of the liver in the maintenance of plasma glucose level has been best evaluated with the euglycaemic clamp. Dose–response relationships between plasma insulin level and HGP clearly demonstrate that in normal tolerant individuals, the liver is exquisitely sensitive to insulin and HGP is completely suppressed at insulin levels well below the commonly used insulin dose (240 $pmol \cdot m^{-2} \cdot min^{-1}$). Additionally, as demonstrated in Fig. 4, there is no difference in either the basal HGP or in the dose–response curve of its suppression by insulin between young

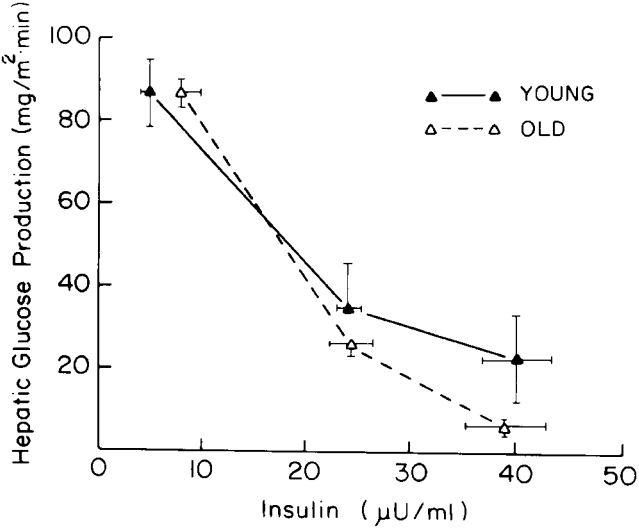


FIG. 4. Dose–response curves for suppression of hepatic glucose output by insulin in young and old participants.

and old individuals. HGP is suppressed over 90% in aged individuals with insulin levels of 240 pmol/l (40 mU/ml) (Meneilly et al 1987). The half-maximal effect is approximately 150 pmol/l (25 μU/ml). This finding is in general agreement with findings of other investigators in both young and elderly volunteers (DeFronzo 1979, Fink et al 1983, Rizza et al 1981). In our studies (Meneilly et al 1987), we have also observed that with low dose insulin infusions, HGP is more rapidly suppressed in the elderly. We have attributed this to a delayed suppression of endogenous insulin release in the elderly individuals (as assessed with C-peptide levels). Thus, early in the study, the liver is exposed to a greater insulin level in the old than in the young. The liver is exquisitely sensitive to portal insulin concentration and an increase of 30–60 pmol/l ($\sim$5 μU/ml) reduces HGP by 50% (DeFronzo et al 1983). Thus, although total suppression of HGP does not differ with age, reduction of HGP by insulin occurs more rapidly in the elderly at physiological levels. The European Group for the Study of Insulin Resistance (EGIR, described in more detail below) has reported their analysis of HGP in 344 non-diabetic subjects (212 men and 132 women) during a euglycaemic clamp (240 pmol$\cdot$m$^{-2}\cdot$min^{-1}) (Natali et al 2000). They ranged in age from 18–85 years and in BMI from 15–55 kg/m^2. Basal HGP showed a large variability and was strongly related to lean body mass (LBM) ($r = 0.63$). HGP was 23% higher in men than in women, which is entirely due to higher LBM in men and no longer different when HPG is adjusted for LBM. Similarly HGP was also positively related to BMI and percentage fat and again these associations were no longer different when adjusted for LBM. There was a tendency for HGP to decline with increasing age (-1.1 0.7 μmol$\cdot$m^{-1} per year) which was not significant ($P = 0.10$) and any significance was completely lost when adjusted for LBM (0.002 μmolm$^{-1}\cdot$kg LBM^{-1} per year). Therefore the variability of basal HGP is due to differences of LBM which also explains the differences of HGP due to age, sex and BMI. Additionally, independent of LBM and fasting plasma insulin, peripheral insulin resistance is associated with higher HGP. The latter suggests that regulation of peripheral glucose uptake and HGP are coupled.

Effect of age on peripheral tissue glucose utilization

Muscle is the primary sink for glucose uptake as adipose tissue is relatively inert and accounts for only 2–3% of glucose uptake (DeFronzo 1981). Brain and the splanchnic bed account for another 20–55% (DeFronzo 1988). The latter two sinks for glucose uptake are insulin independent. The majority of reports examining the role of age on glucose uptake, utilizing a variety of techniques (clamp, MinMod, local forearm perfusion, glucose–insulin–somatostatin infusion) have demonstrated decreased insulin sensitivity during hyperinsulinaemia as summarized in the report by EGIR (Ferrannini et al 1996).

However, this decreased insulin sensitivity is not demonstrated for fasting insulin levels (Coon et al 1989). The EGIR report is an analysis of euglycaemic clamps, at a single dose of 240 $pmol \cdot m^{-2} \cdot min^{-1}$, from 20 European clinical research centres. In this study a total of 1146 clamps were performed (776 men and 380 women) for 2 h in individuals with normal glucose tolerance and arterial blood pressure. In addition, anthropometric data were available. Glucose utilization, *M*, was calculated during the last 40 min of the study. In univariate analysis, age was associated with a significant decrease in insulin action of ($P = 0.0002$) 0.9 $\mu mol \cdot kg^{-1}$ per decade of life, ($\sim 0.2\ mg \cdot kg^{-1}$ per decade of life) which was no longer significant when adjusted for BMI ($P = 0.08$). However, BMI was strongly associated with a decrease in insulin action (5 $\mu mol \cdot kg^{-1} \cdot 10$ kg of body weight, 0.9 $mg \cdot kg^{-1} \cdot 10$ kg of body weight, $P < 0.001$), which remained significant, even after adjustment for age. Furthermore, when the relationship of glucose utilization, *M*, was examined as a function of gender and obesity (BMI $\leqslant 25$ or > 25), age-related decrease in insulin action was demonstrated only in lean women (cf. Fig. 3 of Ferrannini et al 1996), without a slope change after age 50 years (i.e. no menopause effect). Thus, this large study demonstrates that glucose uptake is not altered as a function of age *per se* except in lean women at this hyperinsulinaemic level.

Despite the strength of the EGIR study, the issue of age-related insulin resistance is still controversial, because it has been argued that complete dose-response curves are necessary to resolve the issue. Several groups have conducted dose-response studies as a function of age. The studies are rather small ($n \sim 50$), mainly in men, without a significant difference in BMI between young and old. One study (Rowe et al 1983) has examined the effect of age on glucose utilization over the insulin range of 60–6000 pmol/l (10–1000 μU/ml). An age-associated decrease in glucose utilization was demonstrated with preservation of maximal glucose uptake (i.e. a shift to the right). The half-maximal glucose uptake occurred when plasma insulin levels were 324 pmol/l (54 μU/ml) in the young and 678 pmol/l (113 μU/ml) in the old. When the glucose utilization was plotted per kilogram of lean body mass, the relationship remained. This study is in agreement with other studies where several insulin doses were employed (DeFronzo 1979, Fink et al 1983). In addition, studies using various techniques, including the hyperglycaemic clamp (DeFronzo 1979, Elahi et al 1993), the forearm glucose uptake technique (Jackson et al 1982) and Minimal Model (Min Mod) (Chen et al 1985) have revealed resistance to insulin-induced glucose disposal in aged volunteers. It is obvious, that in addition to age, various factors influence insulin sensitivity including fat mass, fat distribution, physical fitness, dietary composition and genetic factors. When these factors are taken into account, it is not clear that age *per se* still has an independent effect on peripheral glucose uptake.

The effect of age on pancreatic β cell sensitivity

A major component of glucose homeostasis is the balance between insulin secretion and tissue sensitivity to insulin. Again there are many reports that either support or refute a decline in pancreatic β cell response to glucose as a function of age. However, very few investigators have examined β cell secretion in a dose–response manner. We have performed 230 hyperglycaemic clamps for 2 h at four doses (3.0, 5.4, 7.9 and 12.8 mmol/l above basal glucose levels) in healthy young, middle-aged and old volunteers (Elahi et al 1993). Deconvolution analysis, which provides an analysis of insulin secretion from the plasma insulin concentration curves, showed that insulin secretion is characterized by (1) a spike lasting about one minute (first phase), (2) cessation of secretion from 0–15 min, (3) a step increase at 15 min to a level above basal, and (4) a gradual increase in secretion from 15–120 min. In these dose–response studies, as the hyperglycaemic level increased, there was a gradual increase in insulin response within each age group. Furthermore, with each group, comparisons of insulin responses between doses were statistically significant both for the early phase insulin response and for each of the succeeding 30 min periods (30–60, 60–90 and 90–120 min) of the late-phase insulin response. However, in neither the early phase, nor the later-phase insulin responses were there any statistically significant differences among the age groups (Fig. 5). These results are consistent with the observation of DeFronzo (1979) where hyperglycaemic clamps were performed at 6.9 mmol/l above basal in young, middle-aged and old. Using the Min Mod technique, two studies (Chen et al 1985, Pacini et al 1988) have also shown that neither first phase nor second phase insulin response differs significantly between young and old. Thus, the redundant, unnecessary consensus is that insulin secretion does not differ in ageing.

As previously discussed, comparisons between groups (e.g. young vs. old) have inherent difficulties due to the differences between them other than age. In our own studies, we have tried to 'match' for differences using several approaches. In the clamp studies reported above (Elahi et al 1993), we allowed a higher BMI entry criterion in the young than the old in order to match for loss of LBM and increased adiposity in the older group. In a study of insulin secretion of women master athletes across the age span, we carefully restricted entry criteria for both VO_2 max and percentage in order to examine the effect of ageing in very active individuals who maintained their LBM. We found, again, that insulin secretion does not differ in ageing (Ryan et al 2001). However, despite our strict entry criteria for similarity for percentage fat, older female athletes had higher amount of visceral fat than younger female athletes ($\sim 30\,cm^2$) (Ryan & Elahi 1996). An elegant cross sectional study (Pimenta et al 1995) enrolled 100 volunteers of European ancestry with normal OGTT according to the 1985 WHO criteria. There were two groups ($n = 50$ in each), one of which had at least one first-degree

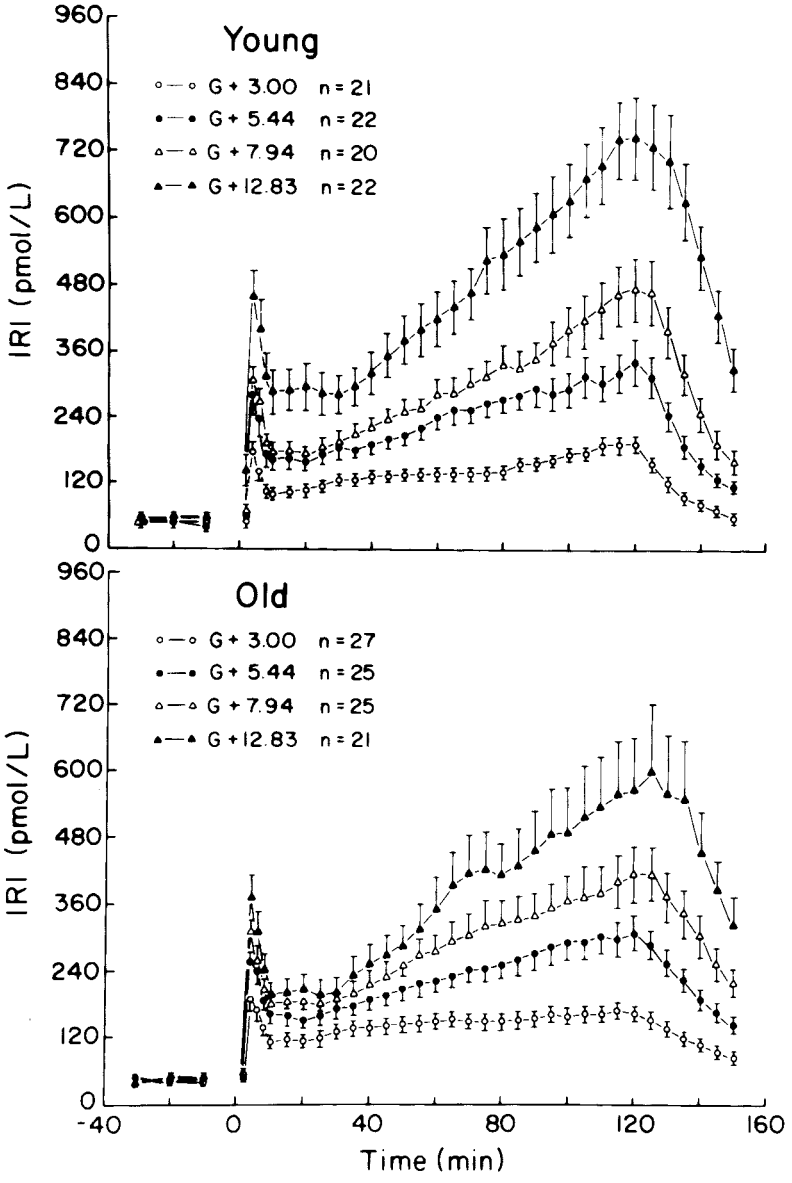


FIG. 5. Bimodal time course of the plasma insulin response to fixed hyperglycaemia. There is a graded increase in insulin as hyperglycaemia increases, and a delay in the fall of plasma insulin in response to the instantaneous fall in glucose at the end of the clamp.

type 2 relative. The two groups were matched for gender, age, weight, height, waist hip ratio, HbA_{1C}, and fasting glucose and insulin levels. None were being treated for hypertension and none were engaged in habitual exercise. A hyperglycaemic clamp at a plasma glucose level 180 mg/dl (10 mmol/l) was performed for three hours. In 62 individuals (26 from the type 2 group and 36 from the normal group) an additional hyperinsulinaemic–euglycaemic clamp ($240\,m \cdot Um^{-2} \cdot min^{-1}$) was also performed. Again, the two groups were carefully matched for all the confounding variables listed above. The group with a first-degree type 2 relative had reduced first and second phase insulin response (cf. Fig. 2 of Pimenta et al 1995). However, their peripheral tissue sensitivity to insulin (from both clamps) was not significantly different. This study demonstrates that middle-aged (~ 40 2 years) individuals of European ancestry with normal OGTT but with a first-degree type 2 diabetic relative have impaired β cell function without a significant reduction in peripheral tissue sensitivity to insulin as compared with normal individuals without a type 2 diabetic relative. Furthermore, β cell defects were not uniform. These results support the hypothesis that a defect in insulin secretion precedes a defect in insulin sensitivity in the development of type 2 diabetes.

During a hyperglycaemic clamp in normal and type 2 diabetic individuals, second phase insulin response is seen to progressively increase throughout the 180 or 240 min duration of the clamp. Does the second phase response ever plateau? We have performed a 10 h hyperglycaemic clamp at ~ 190 mg/dl (10.6 mmol/l) in young (age = 23 1 years, BMI = 23 0.6, WHR = 0.8, VO_2 max = $44\,ml \cdot kg^{-1} \cdot min^{-1}$, 5 males and 5 females) and old (age = 80 2, BMI = 24 0.5, WHR = 0.89, VO_2 = max $21\,ml \cdot kg^{-1} \cdot min^{-1}$, 5 males and 5 females) with normal OGTTs (Meneilly et al 1999). First phase insulin responses were not different between the two groups (Fig. 6). Second phase insulin responses increased progressively until ~ 120 to 150 min in the old after which it reached a plateau. In the young a plateau was not reached until ~ 300 min. The second phase insulin responses were significantly different after 120 min. Glucose infusion rates necessary to maintain the stable hyperglycaemia reached a plateau at ~ 180–240 min in both groups, but at significantly different rates. We also obtained one-minute samples for determination of plasma insulin levels for 150 min during both the basal period and after 1 h of achievement of a plateau in the rate of glucose infusion. Insulin release was evaluated by cluster analysis (Porksen et al 1995, Engdahl et al 1977). Disorderly insulin release, a reduction in the amplitude and mass of rapid insulin pulses and decreased frequency of ultradian pulses is characteristic of normal ageing in the basal state (Meneilly et al 1997). In the stimulated state, a reduction in mass and amplitude of rapid pulses, decrease in frequency and regularity of ultradian pulses occurs with ageing (Meneilly et al 1999).

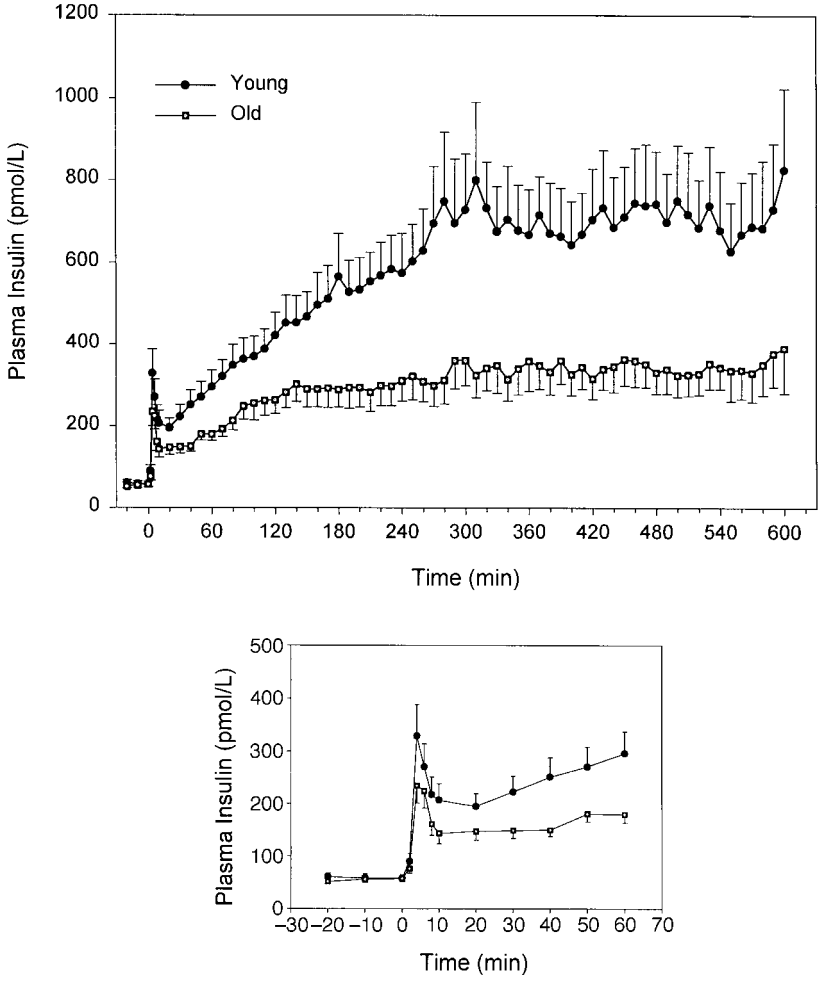


FIG. 6. Plasma insulin response during a 10 h hyperglycaemic clamp at ~11 mmol/l. The insert on the bottom shows the first phase insulin response with an adjusted scale.

The incretin effect in ageing and in type 2 diabetes

Incretin is the umbrella term to cover the multiple gut factors (now known to be hormones) which augment insulin response above that which can be attributed to glucose alone. This insulinotropic effect has been demonstrated by matching the time course of the plasma glucose excursion following an OGTT with both an i.v. infusion of glucose (Perley & Kipnis 1967) and during a hyperglycaemic clamp. In the hyperglycaemic clamp, plasma glucose was increased to ~11 mmol/l for 2 h on

two different occasions. In one, only glucose was infused, while in the second, an OGTT was administered at 60 min and the exogenous glucose infusion rate was adjusted during the second hour as the ingested glucose was being absorbed; in this manner the plasma glucose level remained at the same plateau level in the second hour as in the first hour (Andersen et al 1978). During the second hour in the study, despite the constancy of the plasma glucose concentration, a marked potentiation of insulin secretion was observed. The increase in insulin level is preceded by an increase in plasma concentration of a measured gut hormone, glucose-dependent insulinotropic polypeptide (GIP) by 5 min, with a subsequent time course very similar to the potentiation of insulin response. It was logical to attribute the increase in insulin to this hormone. To test this further, we subsequently infused GIP during the second hour of a hyperglycaemic clamp and we showed that GIP can indeed potentiate insulin response in normal individuals in a similar fashion to that observed following ingestion of glucose (Elahi et al 1979). We then examined β cell sensitivity to endogenously released GIP as a function of age (Elahi et al 1984) and β cell sensitivity to exogenously administered GIP as a function of age and hyperglycaemia (Meneilly et al 1998) during hyperglycaemic clamps. β cell sensitivity to endogenously released GIP was analysed from the association of the ratio of insulin response after OGTT to that which would occur had OGTT not been administered, divided by the ratio of GIP response after administration of OGTT to GIP levels before administration of OGTT:

[IRI (90 – 120 min+GIP) / IRI (90 – 120 min – GIP)] / [GIP (90–120 min) / (GIP (0–60 min)]

There was a significant negative age relationship, indicating that β cell sensitivity to GIP is reduced with advancing age (Elahi et al 1984). In studies with exogenous infusion of GIP, two hyperglycaemic clamps were performed at a level of $\sim$11 mmol/l and at $\sim$18 mmol/l in young (19–26 years) and old (67–79 years) volunteers (Meneilly et al 1998). A total of 93 clamps were performed. During each clamp, GIP was infused for 60 min at a dose of 2 pmol$\cdot$kg$^{-1}\cdot$min^{-1} and 4 pmol$\cdot$kg$^{-1}\cdot$min^{-1}. A clamp was also performed, at each glycaemic level, without GIP administration. The GIP levels during the basal state, before the infusion of GIP at hyperglycaemia and after infusion, were similar between groups and between hyperglycaemic plateaus during the 2 and 4 pmol$\cdot$kg$^{-1}\cdot$min^{-1} infusions (60–120 min levels = $\sim$350 and 580 pmol/l, respectively). In response to GIP infusions, significant increases in insulin occurred in young and old at both glucose levels. The potentiation of the insulin response caused by GIP was greater in the young subjects than the old in the 11 mmol/l glucose hyperglycaemic study. However, the insulin response to GIP was similar in both young and old during the 18 mmol/l glucose hyperglycaemic

clamps. The insulinotropic effect of this incretin was greater in both the young and the old in the 18 mmol/l clamps than in the 11 mmol/l clamps. We concluded that normal ageing is characterized by a decrease in β cell sensitivity to GIP during modest hyperglycaemia. The age-related impairment in response to GIP may be an important cause of the glucose intolerance of ageing, a precursor for diabetes in this age group. The insulinotropic effect of GIP is increased with increasing levels of glycaemia, and the defect in β cell response to GIP disappears when plasma glucose is increased to higher levels.

While we were examining the effect of GIP on insulin secretion, Habener and colleagues (Mojsov et al 1986) reported the identification of another gut hormone, glucagon-like peptide 1 (GLP1), and subsequently showed it to have a potent insulinotropic effect in rats (Weir et al 1989). Infusions of GLP1 were also subsequently shown to lower fasting plasma glucose levels in type 2 diabetic patients (Nathan et al 1992). We examined the insulinotropic effect of GLP1 in normal glucose-tolerant and in type 2 diabetic volunteers during hyperglycaemic clamp ($\sim$11 mol/l) and compared its effect to that of GIP (Elahi et al 1994). We demonstrated that GLP1 is indeed a more potent insulinotropic hormone than GIP. Furthermore, there was an additive effect of GIP and GLP1 on β cell stimulation. Most importantly, we showed that while GLP1 has a potent insulinotropic effect in type 2 individuals, albeit less than in normal glucose tolerant individuals, the GIP insulinotropic effect is totally absent. Thus GLP1 is being investigated as a potential therapeutic agent for the normalization of glucose homeostasis in type 2 diabetes.

Recently, it has been shown during a hyperglycaemic clamp ($\sim$11 mmol/l) GLP1 significantly potentiates insulin release in elderly type 2 diabetic volunteers (age $\geqslant$70 years). The potentiation is clinically relevant and at least threefold greater than insulin release with glucose alone (Fig. 7) (Meneilly et al 2001a). The same volunteers were also examined with a hyperinsulinaemic–euglycaemic clamp during infusion of somatostatin in the presence and absence of GLP1 (Meneilly et al 2001b). The plasma glucose was allowed to fall to a normal level in both euglycaemic clamps ($\sim$5.3 mmol/l). During these two clamp studies both plasma insulin and glucose levels were similar. We showed that peripheral tissue sensitivity to insulin was significantly greater when GLP1 was infused. This study demonstrates that in states of glucose intolerance, such as type 2 diabetes, GLP1 has insulinomimetic, or at least insulin-augmenting, properties in peripheral tissues which can not be attributed to the well known delayed gastric emptying properties of GLP1. It was previously reported that while there was a small tendency for GLP1 to augment peripheral tissue sensitivity to insulin in young normal glucose tolerant men, the increase was not statistically significant (Ryan et al 1998).

We are currently examining the role of continuous subcutaneously administered GLP1 for 12 weeks in type 2 diabetic volunteers. These volunteers were previously

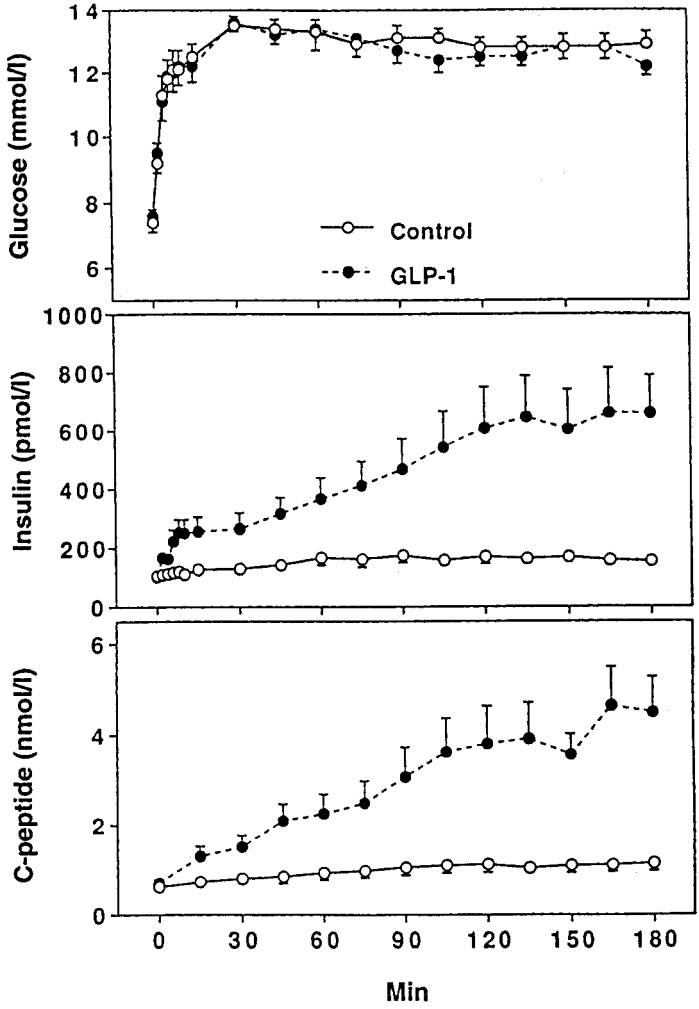


FIG. 7. Plasma glucose, insulin and C-peptide levels during the hyperglycaemic clamp studies.

being treated with oral hypoglycaemic agents and none had received insulin for control of blood glucose. Hyperglycaemic clamps (5.4 mmol/l above basal glucose level) were performed before and at 12 weeks of GLP1 treatment. Our preliminary data have shown that GLP1 is at least as good as the usual hypoglycaemic agents in the control of glucose homeostasis, as demonstrated by the amount of insulin released during the clamp and by a lowering of HbA_{1C} (Meneilly et al 2001c). More importantly, we obtained 2 min samples before and

6 weeks after treatment with GLP1 during a hyperglycaemic clamp in two volunteers. Insulin release was evaluated by cluster analysis (Porksen et al 1995, Engdahl et al 1977). The results show that GLP1 restores insulin burst amplitude in the diabetic patients from a low level ($\sim$8 pmol/l) to those normally observed in elderly individuals with a normal glucose tolerance ($\sim$30 pmol/l) (Meneilly et al 2001c). Thus, this hormone appears to be an excellent candidate for the treatment of type 2 diabetes and studies from multiple research centres are currently evaluating the long-term efficacy of this hormone.

It should be noted that GLP1 has a relatively short half-life ($\sim$2 min). Therefore, continuous infusion will most likely be necessary for its use as a monotherapeutic agent. This will probably not be well tolerated by the patient. However, its use will probably be most efficacious as a secondary agent and this use has several advantages which will become obvious as clinical trials continue. We also note that several investigators/pharmaceutical companies are making substitutions in the amino acid sequence of GLP1, which increase its half-life substantially. However, human clinical trials with these agents have not been reported other than for very acute administrations. Finally, there is a naturally occurring analogue of GLP1, exendin 4, which is found in the salivary gland of the Gila monster. This peptide has at least 10 times the potency of GLP1 and at least 150-fold longer half-life. Acute administration of this peptide augments insulin release markedly (Egan et al 1999). Additionally, limited experience in type 2 diabetic patients receiving twice daily administration of this peptide for a month has shown excellent control of glucose levels (J. M. Egan, G. S. Meneilly & D. Elahi, unpublished results 2001). There are no data from humans on the efficacy of this peptide as a function of age, to our knowledge.

We re-affirm that ageing is associated with a deterioration of glucose tolerance. The deterioration in large part can be attributed to insulin resistance and not insulin secretion. The cause of this deterioration is mainly attributable to lifestyle changes (increased adiposity, loss of LBM, reduced activity, changes in diet) or to genetic factors. That insulin resistance is not an obligatory result of ageing is best exemplified by the demonstration of maintained insulin secretion and action across the age-span in women athletes (Ryan et al 2001) and by preserved insulin action in healthy centenarians (102 0.8 years) compared to octogenarians (78 0.7 years) and middle-aged volunteers (44 1.8 years) (Barbieri et al 2001, Paolisso et al 1996).

Acknowledgements

We express our appreciation to all the volunteers who participated in our studies. We also thank the staff of the clinical research centre at our various medical centres and the excellent technical support of our staff. We thank Restoragen, Inc. and Amylin Pharmaceutical, Inc. for the generous donation of drugs and their support. The studies were also supported in part by NIH

grant AG-00599, the intramural research program of the NIA, a grant from the Canadian Diabetes Association, and the Medical Research Council of Canada.

References

Andersen DK, Elahi D, Brown JC, Tobin JD, Andres R 1978 Oral glucose augmentation of insulin secretion. Interactions of gastric inhibitory polypeptide with ambient glucose and insulin levels. J Clin Invest 62:152–161

Andres R 1971 Aging and diabetes. Med Clin North Am 55:835–846

Barbieri M, Rizzo MR, Manzella D, Paolisso G 2001 Age-related insulin resistance: is it and obligatory finding? The lesson from healthy centenarians. Diabetes Metab Res Rev 17:19–26

Bennett PH, Rushfroth NB, Miller M, Lecompte PM 1976 Epidemiologic studies of diabetes in the Pima Indians. Recent Prog Horm Res 32:333–376

Broughton DL, Taylor R 1991 Review: deterioration of glucose tolerance with age: the role of insulin resistance. Age Ageing 20:221–225

Chen M, Bergman R, Pacini G, Porte D J 1985 Pathogenesis of age-related glucose intolerance in man: insulin resistance and decreased beta-cell function. J Clin Endocrinol Metab 60:13–20

Coon PJ, Bleecker ER, Drinkwater DT, Meyers DA, Goldberg AD 1989 Effects of body composition and exercise capacity on glucose tolerance, insulin, and lipoprotein lipids in healthy older men: a cross-sectional and longitudinal intervention study. Metabolism 38:1201–1209

Davidson MB 1979 The effect of aging on carbohydrate metabolism: a review of the English literature and a practical approach to the diagnosis of diabetes mellitus in the elderly. Metabolism 28:688–705

DeFronzo RA 1979 Glucose intolerance and aging: evidence for tissue insensitivity to insulin. Diabetes 28:1095–1101

DeFronzo RA 1981 Glucose intolerance and aging. Diabetes Care 4:493–501

DeFronzo RA 1988 Lilly lecture 1987. The triumvirate: beta-cell, muscle, liver. A collusion responsible for NIDDM. Diabetes 37:667–687

DeFronzo R, Ferrannini E 1991 Insulin resistance. A multifaceted syndrome responsible for NIDDM, obesity, hypertension, dyslipidemia, and atherosclerotic cardiovascular disease. Diabetes Care 14:173–194

DeFronzo R, Tobin JD, Andres R 1979 Glucose clamp technique: a method for quantifying insulin secretion and resistance. Am J Physiol 237:E214–E223

DeFronzo RA, Ferrannini E, Hendler R, Felig P, Wahren J 1983 Regulation of splanchnic and peripheral glucose uptake by insulin and hyperglycemia in man. Diabetes 32:35–45

Dowse G, Spark R, Mavo B et al 1994 Extraordinary prevalence of non-insulin-dependent diabetes mellitus and bimodal plasma glucose distribution in the Wanigela people of Papua New Guinea. Med J Aust 160:767–774

Egan J, Clocquet A, Elahi D 1999 Insulinotropic effect of exendin-4 in humans. Diabetologia 42:148A

Elahi D 1996 In praise of the hyperglycemic clamp. A method of assessment of beta-cell sensitivity and insulin resistance. Diabetes Care 19:278–286

Elahi D, Muller DC 2000 Carbohydrate metabolism in the elderly. Eur J Clin Nutr 54: S112–S120

Elahi D, Andersen DC, Brown J et al 1979 Pancreatic alpha- and beta-cell responses to GIP infusion in normal man. Am J Physiol 237:E185–E191

Elahi D, Andersen DC, Muller DC, Tobin JD, Brown JC, Andres R 1984 The enteric enhancement of glucose-stimulated insulin release. The role of GIP in aging, obesity and non-insulin-dependent diabetes mellitus. Diabetes 33:950–957

Elahi D, Muller DC, McAloon-Dyke M, Tobin JD, Andres R 1993 The effect of age on insulin response and glucose utilization during four hyperglycemic plateaus. Exp Gerontol 28:393–409

Elahi D, McAloon-Dyke M, Fukagawa N et al 1994 The insulinotropic actions of glucose-dependent insulinotropic polypeptide (GIP) and glucagon-like peptide-1 (7-37) in normal and diabetic subjects. Regul Pept 51:63–74

Engdahl J, Veldhuis J, Farrell P 1977 Altered pulsatile insulin secretion associated with endurance training. J Appl Physiol 79:1977–1985

Evans W, Farrell P 2001 The aging pancreas: effects of aging on insulin secretion action. In: Jefferson LS, Cherrington AD (eds) Handbook of Physiology. Section 7: The endocrine system. Vol 2: The endocrine pancreas and regulation of metabolism. Oxford University Press, Oxford, p 969–998

Ferrannini E, Vichi S, Beck-Nielsen H, Laakso M, Paolisso G, Smith U 1996 Insulin action and age. European group for the study of insulin resistance (EGIR). Diabetes 45:947–953

Fink RI, Kolterman OG, Griffin J, Olefsky JM 1983 Mechanisms of insulin resistance in aging. J Clin Invest 71:1523–1535

Harris M, Flegal K, Cowie C et al 1998 Prevalence of diabetes, impaired fasting glucose, and impaired glucose tolerance in US adults. The third national health and nutrition examination survey, 1988–1994. Diabetes Care 21:518–524

Jackson RA, Blix PM, Matthews JA et al 1982 Influence of ageing on glucose homeostasis. J Clin Endocrinol Metab 55:840–848

Kaplan NM 1989 The deadly quartet. Upper-body obesity, glucose intolerance, hypertriglyceridemia, and hypertension. Arch Intern Med 149:1514–1520

Loo SG, Dowse GK, Finch C, Zimmet P 1993 Bimodality analysis of frequency distributions of 2-hour plasma glucose concentrations in the urban Micronesian population of Kiribati. J Diabetes Complicat 7:73–80

Maneatis T, Condie R, Reaven G 1982 Effect of age on plasma glucose and insulin responses to a test mixed meal. J Am Geriatr Soc 30:178–182

Meneilly G, Minaker K, Elahi D, Rowe J 1987 Insulin action in aging man: evidence for tissue-specific differences at low physiologic insulin levels. J Gerontol 42:196–201

Meneilly GS, Ryan AS, Veldhuis JD, Elahi D 1997 Increased disorderliness of basal insulin release, attenuated insulin secretory burst mass, and reduced ultradian rhythmicity of insulin secretion in older individuals. J Clin Endocrinol Metab 82:4088–4093

Meneilly GS, Ryan AS, Minaker KL, Elahi D 1998 The effect of age and glycemic level on the response of the β-cell to glucose-dependent insulinotropic polypeptide and peripheral tissue sensitivity to endogenously released insulin. J Clin Endocrinol Metab 83:2925–2932

Meneilly G, Veldhuis J, Elahi D 1999 Disruption of the pulsatile and entropic modes of insulin release during an unvarying glucose stimulus in elderly individuals. J Clin Endocrinol Metab 84:1938–1943

Meneilly G, McIntosh CHS, Pederson R et al 2001a Glucagon-like peptide-1 (7-37) augments insulin release in elderly patients with diabetes. Diabetes Care 24:964–965

Meneilly GS, McIntosh CHS, Pederson RA et al 2001b Effect of glucagon-like peptide-1 on non-insulin-mediated glucose uptake in the elderly patient with diabetes. Diabetes Care, in press

Meneilly G, Egan J, Greig N, Veldhuis J, Habener J, Elahi D 2001c Twelve Weeks of Continuous Administration of GLP-1 in Elderly Diabetic Patients. Diabetologia 44:910A

Mojsov S, Heinrich G, Wilson I, Ravazzola M, Orci L, Habener J 1986 Preproglucagon gene expression in pancreas and intestine diversifies at the level of post-translational processing. J Biol Chem 261:11880–11889

Natali A, Toschi E, Camastra S, Gastaldelli A, Groop L, Ferrannini E 2000 Determinants of postabsorptive endogenous glucose output in non-diabetic subjects. European group for the study of insulin resistance (EGIR). Diabetologia 43:1266–1272

Nathan D, Schreiber E, Fogel H, Mojsov S, Habener J 1992 Insulinotropic action of glucagonlike peptide-I (7-37) in diabetic and nondiabetic subjects. Diabetes Care 15: 270–276

National Diabetes Data Group 1979 Classification and diagnosis of diabetes mellitus and other categories of glucose intolerance. Diabetes 28:1039–1057

Pacini G, Valerio A, Beccaro F, Nosadini R, Cobelli C, Crepaldi G 1988 Insulin sensitivity and beta-cell responsivity are not decreased in elderly subjects with normal OGTT. J Am Geriatr Soc 36:317–323

Paolisso G, Gambardella A, Ammendola S et al 1996 Glucose tolerance and insulin action in healthy centenarians. Am J Physiol 33:E890–E894

Perley M, Kipnis D 1967 Plasma insulin responses to oral and intravenous glucose: studies in normal and diabetic subjects. J Clin Invest 46:1954–1962

Pimenta W, Korytkowski M, Mitrakou A et al 1995 Pancreatic beta-cell dysfunction as the primary genetic lesion in NIDDM. Evidence from studies in normal glucose tolerant individuals with a first-degree NIDDM relative. JAMA 273: 1855–1861

Porksen N, Munn S, Steer J, Vore S, Veldhuis J, Butler P 1995 Pulsatile insulin secretion accounts for 70% of total insulin secretion during fasting. Am J Physiol 269:E478–E488

Reaven G 1988 Banting Lecture 1988. Role of insulin resistance in human disease. Diabetes 37:1595–1607

Reaven G, Chen N, Hollenbeck C, Chen YD 1989 Effect of age on glucose tolerance and glucose uptake in healthy individuals. J Am Geriatr Soc 37:735–740

Rizza RA, Mandarino LJ, Gerich JE 1981 Dose-response characteristics for effects of insulin on production and utilization of glucose in man. Am J Physiol 240:E630–E639

Rosenthal M, McMahan CA, Stern MP et al 1985 Evidence of bimodality of two hour plasma glucose concentrations in Mexican Americans: results from the San Antonio Heart Study. J Chronic Dis 38:5–16

Rowe J, Minaker K, Pallota J, Flier J 1983 Characterization of the insulin resistance of aging. J Clin Invest 71:1581–1587

Ryan A, Elahi D 1996 The effects of acute hyperglycemia and hyperinsulinemia on plasma leptin levels: its relationships with body fat, visceral adiposity, and age in women. J Clin Endocrinol Metab 81:4433–4438

Ryan AS, Egan JM, Habener JF, Elahi D 1998 Insulinotropic hormone glucagon-like peptide-1-(7-37) appears not to augment insulin-mediated glucose uptake in young men during euglycemia. J Clin Endocrinol Metab 83:2399–2404

Ryan A, Muller D, Elahi D 2001 Sequential hyperglycemic-euglycemic clamp to assess β-cell and peripheral tissue: studies in female athletes. J Appl Physiol 91:872–881

Shimokata H, Muller DC, Fleg JL, Sorkin J, Ziemba AW, Andres R 1991 Age as an independent determinant of glucose tolerance. Diabetes 40:44–51

Stout RW 1990 Insulin and atheroma. Diabetes Care 13:631–654

The Expert Committee on the Diagnosis and Classification of Diabetes Mellitus 1997 Report of the expert committee on the diagnosis and classification of diabetes mellitus. Diabetes Care 20:1183–1197

Weir GC, Mojsov S, Hendrick GK, Habener JF 1989 Glucagonlike peptide I (7-37) actions on endocrine pancreas. Diabetes 38:338–342

WHO 1985 Diabetes mellitus. World Health Organization, Geneva (Technical Report Series 727)

WHO 1999 Definition, diagnosis and classification of diabetes mellitus and its complications. Report of a WHO consultation, part 1: Diagnosis and classification of diabetes mellitus. World Health Organization, Geneva

Zamboni M, Armellini F, Harris T et al 1997 Effects of age on body fat distribution and cardiovascular risk factors in women. Am J Clin Nutr 66:111–115

Zavaroni I, Dall'Aglio E, Bruschi F et al 1986 Effect of age and environmental factors on glucose tolerance and insulin secretion in a worker population. J Am Geriatr Soc 34:271–275

Zimmet P, Whitehouse S 1978 Bimodality of fasting and two-hour glucose tolerance distributions in a Micronesian population. Diabetes 27:793–800

DISCUSSION

Veldhuis: The growth hormone (GH) axis is of course much more closely related to modulators produced by the gut, based on recent studies. For example, ghrelin, a major GH-releasing hormone, is produced in the fundus of the stomach, except it is inversely controlled by nutrition. It goes up with fasting and drives GH in that circumstance.

One of the things that struck me about your paper, and which I have missed as an endocrinologist over the years, is that the diabetic loses the first phase, but the ageing individual appears to dominantly lose the second phase. Did I understand this correctly?

Elahi: In the non-diabetic individual, using the hyperglycaemic clamp technique and large numbers of patients (restricting the obesity to BMI of $\leqslant 25$ we enrol older people with even lower BMI), we do not see a discrepancy between first phase and second phase in patients lower than 75 years of age. However, if we go above age 75 and don't control for obesity we begin to see real discrepancies in the second phase.

Veldhuis: I had always supposed that ageing was a representation of an increased probability of an non-insulin-dependent diabetes mellitus (NIDDM) phenotype emerging on some genetic base, probably driven by increased visceral fat. Therefore, one would have an insulin-release profile matching NIDDM. Your results suggest a different pathophysiology. On the other hand, a terribly messy confound is the visceral obesity/changes in body composition with ageing. I thought that it was interesting that the only study population that unmasks an age effect specifically on insulin action in the periphery is the lean female (Ferrannini et al 1996).

Elahi: That same group subsequently published a paper that looked at hepatic glucose output as a function of this (Natali et al 2000). Total endogenous glucose output is largely explained by the amount of lean mass, which explains differences due to obesity, sex, and age.

Veldhuis: This is somewhat reminiscent of the GH axis in adiposity studies (Vahl et al 1997). If you take an obese individual, his GH levels are so low even in young adulthood that one cannot see any age effect at all. GH is already reduced to 20% of normal. If however one considers the leanest cohort, the lowest quintile of percentage body fat, there is a strong negative age effect even in men (Veldhuis et al 1995, Iranmanesh et al 1994, 1998), it isn't restricted to women in this case (Weltman et al 1994). This shows a strong confound with visceral obesity, again.

Elahi: We have just completed a study in which we examined approximately 65 females who are considered master athletes. These are Olympic level athletes who because of their age can no longer compete with younger people. They are still very active. We advertised, and got 65 people across the country. I can tell you emphatically, despite the fact that the BMIs and percentage fat is the same, the insulin release in the older group, aged 50–69, is only giving hints of not being equal to the young.

Veldhuis: There is some visceral obesity even in well conditioned individuals. It is a nasty confound for all of us working in this field.

Handelsman: Euglycaemic clamps essentially work on the idea of the body as a black box. Most of the black box, as far as insulin sensitivity is concerned, is in muscle. Are there ways to look at other tissues and their sensitivity? When we think of sex steroids, there is increasing focus on the idea that each tissue may have different threshold effects, or at least sensitivities. Can this be done with clamps, or are we forever having to think of the body as one big black box? When you measure sensitivities you are talking about are essentially muscle sensitivity, and mainly striated muscle sensitivity. Can you work out ways of looking at the sensitivity of fat or skin, for example?

Elahi: Usually, the doses of insulin used are so high that it suppresses the hepatic glucose output right away. In order to look at the liver you have to use very low dose insulin clamps. We have done this, and it is possible to tease out changes in liver sensitivity. The other way is during the insulin clamp itself. We have taken muscle and fat biopsies and looked at incorporation of protein into muscle, for example, before and after treatment.

Björntorp: One can measure glucose uptake in adipose tissue by using small amounts of radioactive glucose. It is very small: less than 5%.

Laron: Would you say that the normal progression of ageing, during which the muscle mass is decreased and adipose tissue mass is increased, plays a role in the development of the insulin resistance?

Elahi: We have consecutive two year OGTTs for diagnoses of diabetes for ~10–25 years. If you look at the fasting glucose and decide when there is a diagnosis of diabetes, and compare this with the 2 h value, do you have any idea what the discrepancy is with respect to time, i.e. when you can detect diabetes with the 2 h glucose level and the other one with fasting level? Nine years. Similarly, you can pick up impaired tolerance seven years earlier if you use the 2 h glucose level from the OGTT rather than the fasting glucose level. No one knows when someone becomes diabetic; if you have a test you are either normal or diabetic. We can also look at how long it takes to go from impaired to diabetic. Let us imagine that you are impaired, as diagnosed either from fasting levels or the 2 h OGTT levels. How long does it take to go from impaired to diabetic? Again, on the average seven years. If you put the two together, i.e. both

fasting and OGTT, as well as impaired and diabetic diagnosis, one can have an early warning something like 7–16 years before an individual progresses from a normal test to an impaired and finally a diabetic diagnosis. Imagine what we could do in terms of interventions if we have this much time.

Prior: I remember the first criteria for diabetes that were created with data in young people. These individuals weren't carbohydrate-loaded so they had a starved liver. How reproducible are the OGTTs? Does the reproducibility decrease as you get older?

Elahi: We have looked at that. There is movement over two years. It is difficult to control because the doctors write reports to the participants telling them that they are impaired or diabetic, and there is no way of knowing whether these people have made any life function adjustments in the light of this. In general, there is approximately 80% concurrence of the diagnosis and 30% who move back and forth.

Prior: Has anyone studied this at weekly intervals over an extended period?

Elahi: Not that I know of. In general the reproducibility is terrible. This test is essentially for diagnosis of diabetes.

Prior: It is a tremendous social and financial burden if you are diagnosed with diabetes. It changes your health insurance and your concept of yourself. What I see in clinical practice is people being diagnosed who are elderly who probably don't have diabetes. We need to be sure.

Veldhuis: This brings up the problem that I am still struggling with, namely the notion of what is defined as 'normal'. This comes up in the bone field. Because of the high predictive risk of z scores against the young population, we are willing to intervene based on a comparison between older and young skeletal mass values. In many endocrine areas we haven't reached this predictive capability yet. With IGF we clearly say that the age-related norms are appropriate because IGF collapses over decades. This is an interesting issue. By one of your classifications, 80% of the elderly would have impaired glucose tolerance or diabetes. In a sense, this is a philosophical issue of when to use a young adult range. For the thyroid it is clear because the range looks fixed. We are struggling with testosterone.

Handelsman: This is an important issue. In the end, a lot of what we do is geared to ill health-treatment and prevention of disease. In a sense the hard clinical endpoints are an important reference point we mustn't lose sight of. In the bone field, we are talking about fractures, disability and death. But the concept of a surrogate variable is very important. Bone density is a well-accepted surrogate variable. We have other surrogate variables that work well, such as total cholesterol for heart disease. A lot of other things that we think of as intermediary factors are not accepted because they are not sensitive or specific enough as surrogate variables. What we are really talking about is defining convenient surrogate variables. The question here is what is the right surrogate

variable for disability and death from diabetes in a 70 year old? Does it make sense to talk about long-range micro- and macrovascular complications? What is the predictive value?

Carroll: Of all the competing definitions of diabetes, the only practical thing to do is to look at them with respect to outcome. This is how one validates criteria.

Veldhuis: I think David Handelsman's remark on this is compelling for clinical issues at least. If you can find me a determinant that predicts adverse consequence, for which I have either a putative intervention or a known intervention, I become interested in that marker. It is embarrassing to be hung up at such a basic practical level, and not to go a little bit deeper into what is the normal. We were wise to have the Rotterdam group begin by asking how does a healthy older body behave? Give me a few hundred such observations to compare with a subset in the general population of similar age, who manifest measurable differences in a body parameter and are complaining about it clinically, and I will begin to construe a possible relationship. I am beginning to think we need more normative data in understanding ageing and normal physiology. Thus, as Dariush Elahi's talk illustrates, we need to know the predictive value of biochemical anomalies.

Prior: One of the things that may be of value in the elderly is standardizing glycosylated haemoglobin levels. It gives a broader picture. My understanding is that it hasn't become a tool because the laboratory diagnosis is not secure.

Elahi: You are correct; that is the problem. I imagine that in the next 10 years it will become the standard marker for diagnosis of diabetes. Having said this, I currently know of at least three publications that cross-sectionally examine haemoglobin as a function of age and BMI. There is an increase in haemoglobin A_{1C} (HbA_{1C}) as a function of age both in the lean and the obese, and it is obesity independent. The values differ between the populations because of the non-standardization, but the shape of the curve doesn't change.

Prior: Are there any HbA_{1C} data in the Rotterdam study?

Haus: In some populations the HbA_{1C} values may be less reliable than the measurement of glycated haemoglobin due to the higher percentages of abnormal haemoglobins. Which determination method would be preferable for general use?

Elahi: Whichever one can be normalized across the world the fastest.

Laron: Ageing is a normal biological process. It includes an adaptation of the body to the progressive changes; if we do not establish norms along each phase of ageing, but rather take one point as the normal, we must conclude that ageing is a disease and not a normal state.

Elahi: There is a nice paper that has a normogram that gives you a recommendation for diagnosis diabetes not only as a function of age but also for each gender. This was before the 1979 ADA criteria. Before 1979 the criterion for diagnosis was a 2 h glucose level $\geqslant 140$ mg/dl. This is why this normogram was

good because it really shifted as you got older. The 1979 ADA raised the 2 h level to 200 mg/dl, so there was no more age adjustment necessary.

Laron: The basic question is whether we should adapt subjects to 'normality' along the ageing process?

Elahi: I think we should, but it would be hard to implement this in the general practitioners' offices across the world.

Handelsman: This is an important and familiar discussion, but it goes round in a circle because it lacks the reference point of prevention of disability and death. This breaks that vicious circle. We can easily say that all parameters should be age adjusted or not, but in the end the criteria for which of these is better is going to be which prevents disease and disability best. Without this framework, no meaningful answer is possible; it is an internal vicious circle.

References

Ferrannini E, Vichi S, Beck-Nielsen H, Laakso M, Paolisso G, Smith U 1996 Insulin action and age. European Group for the Study of Insulin Resistance (EGIR). Diabetes 45:947–953

Iranmanesh A, Grisso B, Veldhuis JD 1994 Low basal and persistent pulsatile growth hormone secretion are revealed in normal and hyposomatotropic men studied with a new ultrasensitive chemiluminescence assay. J Clin Endocrinol Metab 78:526–535

Iranmanesh A, South S, Liem AY et al 1998 Unequal impact of age, percentage body fat, and serum testosterone concentrations on the somatotropic, IGF-I, and IGF-binding protein responses to a three-day intravenous growth-hormone-releasing hormone pulsatile infusion in men. Eur J Endocrinol 139:59–71

Natali A, Toschi E, Camastra S, Gastaldelli A, Groop L, Ferrannini E 2000 Determinants of postabsorptive endogenous glucose output in non-diabetic subjects. European Group for the Study of Insulin Resistance (EGIR). Diabetologia 43:1266–1272

Vahl N, Jorgensen JO, Skjaerbaek C, Veldhuis JD, Orskov H, Christiansen J 1997 Abdominal adiposity rather than age and sex predicts the mass and regularity of GH secretion in healthy adults. Am J Physiol 272:E1108–E1116

Veldhuis JD, Liem AY, South S et al 1995 Differential impact of age, sex-steroid hormones, and obesity on basal versus pulsatile growth hormone secretion in men as assessed in an ultrasensitive chemiluminescence assay. J Clin Endocrinol Metab 80:3209–3222

Weltman A, Weltman JY, Hartman ML et al 1994 Relationship between age, percentage body fat, fitness and 24 hour growth hormone release in healthy young adults: effects of gender. J Clin Endocrinol Metab 78:543–548

Endocrine Facets of Ageing.
Novartis 242

Print ISBN 0-471-48636-1 Online ISBN 0-470-84654-2

Endocrine causes of age-related bone loss and osteoporosis

B. Lawrence Riggs

Endocrine Research Unit, Mayo Clinic and Mayo Foundation, North 6 Plummer, Rochester, MN 55905, USA

Abstract. Women have an early postmenopausal phase of rapid bone loss that lasts for 5–10 years after menopause, whereas both ageing women and men have a slow continuous phase of bone loss that lasts indefinitely. In women, the rapid phase is mediated mainly by loss of the direct restraining effect of oestrogen on bone cell function, whereas the slow phase is mediated mainly by the loss of oestrogen action on extraskeletal calcium homeostasis leading to net calcium wasting and secondary hyperparathyroidism. Because elderly men have low serum bioavailable oestrogen and testosterone levels, and because recent data suggest that oestrogen is the main sex steroid regulating bone metabolism in men, oestrogen deficiency may also be the principal cause of bone loss in elderly men. Decreased bone formation contributes to bone loss in both genders and may be caused by a decreased production of growth hormone and IGF1 as well as oestrogen and testosterone deficiency. Other changes in endocrine secretion, although present in the elderly, seem less important in the pathophysiology of age-related bone loss and osteoporosis.

2002 Endocrine facets of ageing. Wiley, Chichester (Novartis Foundation Symposium 242) p 247–264

The problem of osteoporosis

Osteoporosis is one of the major problems facing ageing women and men. In the USA, osteoporosis annually causes ~1.5 million fractures and costs ~US$15 billion to the healthcare system. The lifetime risk of fractures of the spine (symptomatic), hip and distal radius is 40% for white women and 13% for white men (Melton et al 1988 and Table 1).

Patterns of age-related bone loss

The pattern of age-related bone loss varies between genders. Women undergo two phases of bone loss — an early, accelerated transient phase followed by a slow continuous phase — whereas men undergo only a slow continuous phase (Riggs et al 1998). The accelerated bone loss in the early postmenopausal phase decreases

TABLE 1 Lifetime risk of major fractures due to osteoporosis for white men and women at age 50

	Women (%)	*Men (%)*
Hip fracture	17.5	6.0
Vertebral fracture	15.6	5.0
Forearm fracture	16.0	2.5
Any of the three	39.7	13.1

exponentially over 5–10 years to merge asymptotically with the subsequent slow phase that continues indefinitely. This early phase involves predominantly cancellous bone loss; it accounts for losses of 20–30% of cancellous bone but only of 5–10% of cortical bone. It is associated with high bone turnover and the increase in bone resorption is greater than the increase in bone formation. The reason for the slowing and eventual cessation of the rapid phase of bone loss is unclear. However, it is likely that with the rapid depletion of the cancellous bone, biomechanical forces act to limit further loss.

The late slow phase in women involves equal losses ($\sim$20–25% each) of cortical and cancellous bone over life. The slow continuous phase in men is similar to the late slow phase in postmenopausal women, both with respect to its rate and time course and with respect to the type and amount of bone loss. The slow phases of bone loss in both genders are associated with high bone turnover. These patterns are shown schematically in Fig. 1.

Endocrine abnormalities and bone loss in women

Early accelerated phase

This phase begins at menopause, can be prevented by oestrogen replacement, and almost certainly results from the cessation of ovarian function. Oestrogen acts through high affinity oestrogen receptors in osteoblasts and osteoclasts to restrain bone turnover, and when this restraint is lost at menopause, overall bone turnover increases and resorption increases more than formation. In addition, the increased activity of osteoclasts and their prolonged lifespan lead to trabecular plate perforation and to loss of structural elements, thus weakening bone out of proportion to the loss of bone density. The high rate of bone resorption increases skeletal calcium outflow, which leads to a partial suppression of parathyroid hormone (PTH) secretion and compensatory increases in urinary calcium excretion (Riggs et al 1998). The reason for the cessation of the rapid phase of

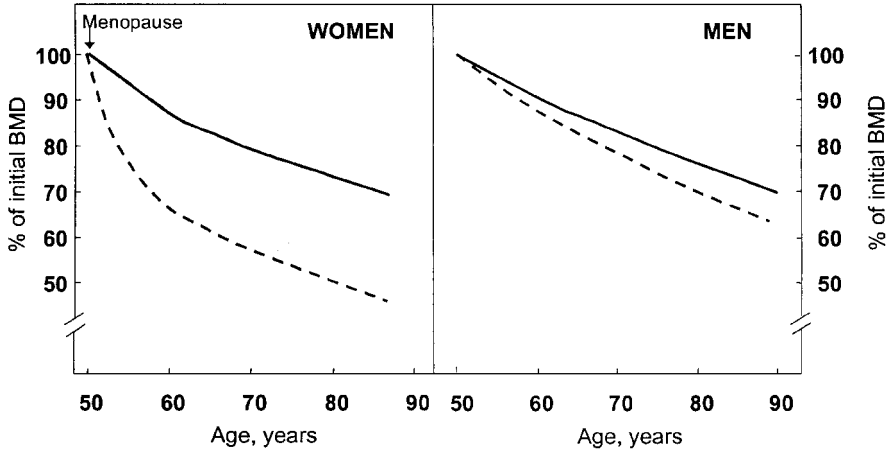


FIG. 1. Schematic representation of changes in bone mass over life in cancellous (broken line) and cortical (solid line) bone in women (left panel) and men (right panel) from age 50 onward. Note that men have only one phase of continuous bone loss but women have two—an early accelerated phase and a late slow phase. Note also that the accelerated phase, but not the slow phase, involves disproportionate loss of cancellous bone. (With permission from Riggs et al 1998.)

bone loss is unclear but may relate to the activation of biomechanical forces that limit the rate of further bone loss when the amount of cancellous bone falls below some critical level.

Late slow phase

As the rapid bone loss phase subsides, serum levels of PTH increase progressively throughout the remainder of life. Markers for bone turnover also increase and these increases are directly correlated with the increase in serum PTH (Table 2). Suppression of PTH secretion by intravenous calcium infusion abolishes the differences in bone resorption markers between young and elderly women (Ledger et al 1994), strongly suggesting that the increase in bone resorption in ageing women is PTH-dependent. Also, a chronic, high calcium intake will also reduce elevated values for serum PTH in elderly women into the premenopausal range (McKane et al 1995). Thus, a considerable body of data implicates secondary hyperparathyroidism as the cause of the late slow phase of bone loss in elderly women. The secondary hyperparathyroidism has traditionally been assumed to be secondary to age-related factors that impair calcium absorption and renal Ca^{2+} homeostasis. However, the recent findings that the increases in both serum PTH and bone resorption in late postmenopausal women can be normalized by oestrogen replacement suggests that oestrogen deficiency may play a causal role (McKane et al 1997, Khosla et al 1998; Table 3).

TABLE 2 Changes in serum PTH and in biochemical markers for bone turnover in 304 women residents of Rochester, MN from the third into the tenth decade of life

		Spearman correlation coefficients	
Variable	*Increase with Δ (%)*	*vs. age*	*vs. PTH*
PTH	54%	0.354**	1.000
BSAP	38%	0.329**	0.192*
OC	64%	0.392**	0.206*
fPYD	76%	0.505%**	0.203*
NTx	86%	0.344**	0.190*

Samples are age-stratified and population-based. Postmenopausal women receiving oestrogen replacement are not included. There are age-related increases in serum PTH and in markers for bone formation (serum bone specific alkaline phosphatase [BSAP] and osteocalcin [OC]) and bone resorption (urine free pyridinoline [fPYD], and cross-linked N-terminal telopeptide of type I collagen [NTx]). Furthermore, the increases in biochemical markers are directly correlated with the increases in serum PTH. (Data are from re-analysis of study of Khosla et al 1997.)
$^{*}P < 0.0001$, $^{**}P < 0.001$.

Both the early accelerated and the late slow phases of bone loss in women are associated with low levels of serum oestrogen, and oestrogen treatment is effective in preventing further bone loss in both phases (McKane et al 1997, Ettinger et al 1985). However, in the early phase of bone loss there is a trend to decreased serum PTH and these levels increase following oestrogen treatment. By

TABLE 3 Comparative effects of age and oestrogen status in women as described by McKane et al (1997)

Variable	*Premenopausal*	*Postmenopausal untreated*	*Postmenopausal treated*
N	30	30	30
Age (years)	32.0 0.5	74.2 0.6	73.8 0.6
Serum:			
PTH (pmol/l)	2.7 0.2	3.6 0.3*	2.5 0.2
Urine:			
NTx (nmol/mmol Cr)	28.8 2.3	42.9 3.5**	24.6 2.3
PYD (nmol/mmol Cr)	45.6 1.6	61.2 3.2**	40.7 1.6
DPD (nmol/mmol Cr)	11.9 0.5	16.2 1.0**	9.4 0.5

Serum intact PTH was fasting morning value. Bone resorption markers were measured by ELISA kit for N-telopeptide of type I collagen (NTx) and by fluorometric detection after HPLC for pyridinoline (PYD) and deoxypyridinoline (DPD). All results are mean SEM.
For difference from premenopausal groups: $^{*}P < 0.05$, $^{**}P < 0.005$.

contrast, serum PTH levels increase progressively in the late slow phase of bone loss and are decreased by oestrogen treatment. We have attempted to resolve this paradox by hypothesizing that oestrogen has two major effects on bone — a direct action on bone cells and an action on external calcium homeostasis that increases PTH secretion and indirectly increases bone loss (Riggs et al 1998). The loss of the direct effect of oestrogen on bone cells is responsible for the early rapid phase of bone loss. This direct effect is initiated by the large and relatively rapid fall in serum oestrogen levels at menopause, and it becomes less important after the rapid accelerated phase of bone loss subsides. In the late slow phase, the indirect effect of oestrogen deficiency on extra-skeletal calcium metabolism leads to the secondary hyperparathyroidism that is the major cause of the bone loss.

The indirect effect is initiated by loss of oestrogen action on the intestine and kidney (Riggs et al 1998). The intestine contains oestrogen receptors and oestrogen stimulates calcium absorption (Gennari et al 1990). Oestrogen also increases renal calcium conservation (McKane et al 1995) through a PTH-independent enhancement of tubular reabsorption of calcium. During oestrogen deficiency, the loss of the intestinal and renal actions of oestrogen leads to external losses of calcium and negative calcium balance. Unless these losses of calcium are offset by large increases in dietary calcium (McKane et al 1996), they will lead to secondary hyperparathyroidism and continued bone loss.

Decreased bone formation

Both the early accelerated and the late slow phases of bone loss are associated with an absolute increase in bone resorption and a relative decrease in bone formation. Normally, there is a tight coupling of bone formation to bone resorption. During oestrogen deficiency, however, there is a failure of a compensatory increase in bone formation to offset the increase in bone resorption and this leads to continued bone loss. This abnormality is demonstrable soon after menopause suggesting that it is caused by oestrogen deficiency. Oestrogen has been shown to increase collagen synthesis in skin fibroblasts and to increase production of insulin-like growth factor (IGF)1 (Ernst et al 1989) and transforming growth factor (TGF)β (Ashcroft et al 1997), growth factors that are anabolic for osteoblasts. Also, oestrogen prolongs the lifespan of mature osteoblasts by decreasing apoptosis (Manolagas 2000). Oestrogen deficiency could also account for the impaired osteoblastic function in older women, although it is possible that age-related abnormalities in hormones or growth factors regulating osteoblast function also contribute.

The probable mechanisms by which oestrogen deficiency produces bone loss in ageing women are shown in Fig. 2A.

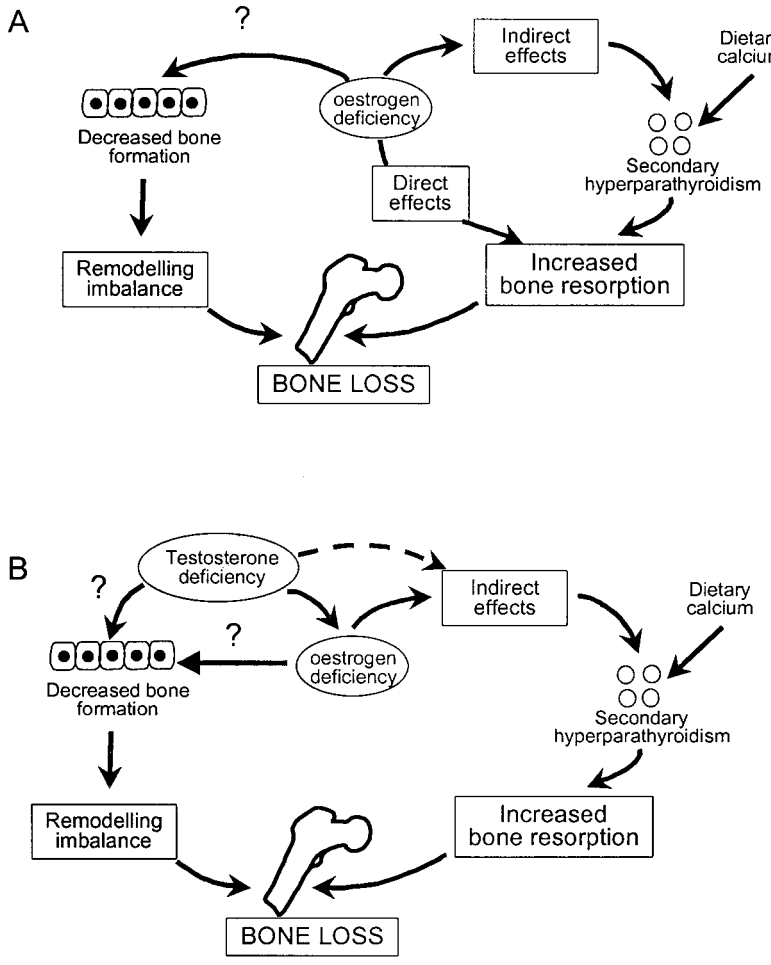


FIG. 2. Schematic representation of unitary model for bone loss in postmenopausal women (A) and in ageing men (B). See text for details. (With permission from Riggs et al 1998.)

Endocrine abnormalities and bone loss in ageing men

Except after orchiectomy, men do not have an equivalent of the rapid phase of bone loss that women experience following menopause. After accounting for the absence of this phase, the patterns of late bone loss and of the increases in serum PTH and bone resorption markers in ageing men are virtually superimposable upon those occurring in women (Riggs et al 1998). In the past, it has been difficult to attribute male bone loss to sex steroid deficiency because men do not have an equivalent of menopause, and because serum total testosterone levels

TABLE 4 Gender differences in changes over life in sex steroids

	Men % change	*Women % change*
Lateral spine BMD	−27**	−45**
Serum:		
Bio E	−47**	−83**
Bio T	−64**	−28*
SHBG	+124**	−1
LH	+285**	+731**
FSH	+505**	+1805**

*$P < 0.05$, **$P < 0.005$.
Data are adapted from Khosla et al (1998).

decrease only marginally with age except for a small subset of elderly men who develop clinical hypogonadism. However, in the last 5 years, thinking on this issue has undergone a sea change.

First, in population studies, we (Khosla et al 1998) and others have shown that although levels of serum total testosterone and oestrogen decrease only slightly in men with ageing, there are major decreases in biologically available levels of both sex steroids (Table 4, Figs 3 and 4). This disassociation is due to a progressive increase in men in levels of the fraction of sex steroids bound to serum sex hormone binding globulin (SHBG) (Fig. 5) which is not available to tissues. The

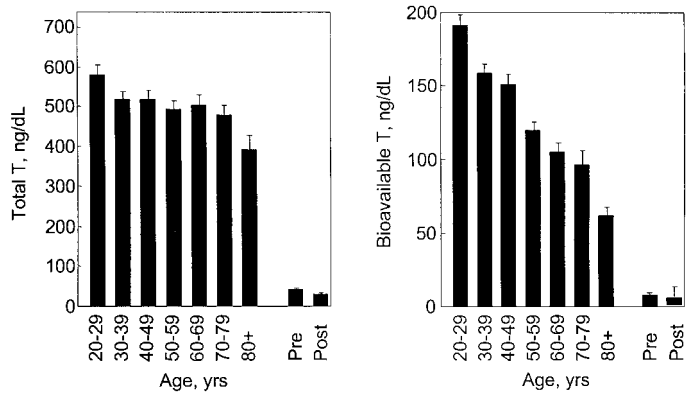


FIG. 3. Changes in serum testosterone (T) in ageing men. The left panel shows that serum total testosterone decreases only slightly in ageing men. Total T ($P < 0.001$), bioavailable T ($P < 0.001$). The right panel shows the changes in serum bioavailable testosterone, which decreased progressively with ageing. Values for premenopausal (Pre) and postmenopausal (Post) women are given for comparison. Error bars represent SEM. (With permission from Riggs et al 2000.)

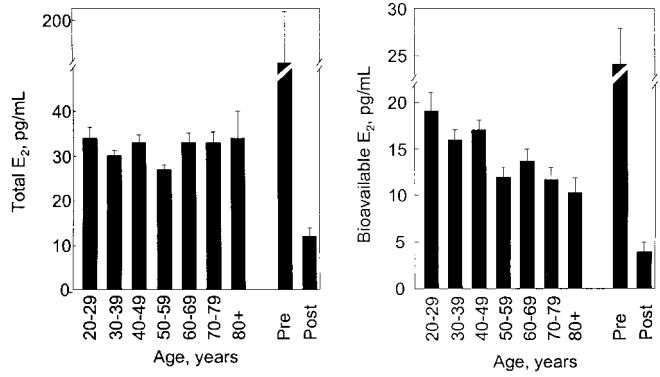


FIG. 4. Changes in serum oestrogen (E) in ageing men. The left panel shows that serum total oestrogen decreases only slightly in ageing men. Total E (not significant), bioavailable E ($P < 0.001$). The right panel shows the changes in serum bioavailable oestrogen, which decreased progressively with ageing. Values for premenopausal (Pre) and postmenopausal (Post) women are given for comparison. Error bars represent SEM. (With permission from Riggs et al 2000.)

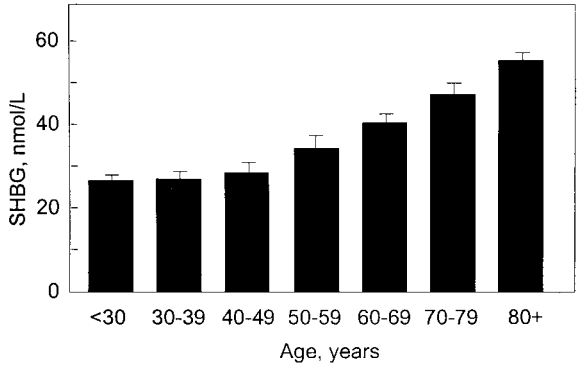


FIG. 5. Changes in sex hormone binding globulin (SHBG) in ageing men. Note the progressive increase in SHBG which binds to approximately 50% of total serum oestrogen or serum testosterone, rendering it largely unavailable to target tissues. The remaining 50%, which is bound to albumin or is free, is bioavailable. Thus, the progressive increases in serum SHBG with ageing are a major reason for the progressive deficiency in bioavailable testosterone and oestrogen in ageing men. Error bars represent SEM. (With permission from Riggs et al 2000.)

mechanisms for the increase in serum SHBG are complex but are probably due, in part, to decreases in production of growth hormone and IGF1 coupled with an impaired gondal secretory reserve (Table 5). Thus, as assessed by their free or bioavailable levels, about half of elderly males have a substantial deficiency of oestrogen and testosterone, and, in general, these are the ones that are losing bone (Khosla et al 2000).

TABLE 5 Differences between genders: mechanisms of sex steroid deficiency

	Females	*Males*
Onset	Begins acutely at menopause	Gradual and progressive
Oestrogen deficiency	++++	++
Testosterone deficiency	++	++++
Δ, SHBG	0	+++
Mechanism	Ovarian failure	(a) Inactivation by SHBG (b) Impaired hypothalamic, pituitary, gonad axis

Data from several recent 'experiments of nature' are consistent with the concept that oestrogen plays a major role in maintaining bone mass in men. A young adult who was unable to respond to oestrogen because of homozygous mutations of oestrogen receptor α genes (Smith et al 1994) and two young adults who were unable to synthesize oestrogen because of homozygous mutations of the aromatase genes (Carani et al 1997, Morishima et al 1997) had osteopenia despite normal or elevated levels of testosterone. Moreover, Vanderschueren et al (1997) found no differences in the effects of orchiectomy or treatment with aromatase inhibitor on decreasing bone density in aged male rats, suggesting that the aromatization of androgens to oestrogens was playing a major role in skeletal maintenance.

Four recent population-based, observational studies (Khosla et al 1998, Slemenda et al 1997, Center et al 1997, Greendale et al 1997) involving an aggregate total of 1410 men from young adulthood to old age found by multivariate analysis that free serum oestrogen rather than free serum testosterone was the main predictor of bone mass at all measured sites except some cortical bone sites in the appendicular skeleton. Khosla et al (2000) have also demonstrated that this also applies to the rate of bone loss in elderly men. Finally, our group (Falahati-Nini et al 2000) has recently shown that when a group of elderly men were pharmacologically rendered hypogonadal and their aromatase activity was blocked, oestrogen, but not testosterone, prevented an increase in bone resorption markers. Collectively, these studies provide convincing evidence that a deficiency in oestrogen is a major cause of bone loss in ageing men. Interestingly, Bernecker et al (1995) found that mean levels of serum oestrogen but not testosterone were significantly reduced in 56 men with established idiopathic osteoporosis.

Collectively, these data support the hypothesis that oestrogen deficiency plays a major role in involutional bone loss in men as well as in women. However, testosterone clearly accounts for the sexual dimorphism of the skeleton that

develops following puberty and probably also stimulates the subsequent periosteal growth of cortical bone (Seeman 1997). In addition, we (Khosla et al 1998) have found that testosterone deficiency is the main determinant of the predominantly cortical bone mass of the appendicular skeleton. More studies must be made to define the additional effects of testosterone on the male skeleton and to determine the relative contributions of deficiencies of oestrogen and testosterone in causation of the slow phase of bone loss in ageing men.

The probable mechanisms by which sex steroid deficiency produces bone loss in ageing men are shown in Fig. 2B.

Other age-related endocrine abnormalities

Although decreases in serum sex steroids and increases in serum PTH are by far the most important endocrine abnormalities causing age-related bone loss, there are other abnormalities that contribute variably. The two most important of these are those of the vitamin D–endocrine system and the growth hormone–IGF1 system. Reduced serum concentrations of both the active vitamin D metabolites — 25-hydroxyvitamin D (25[OH]D and 1,25-dihydroxyvitamin D ($1,25[OH]_2D$)—have been demonstrated in both genders with ageing. Serum 25(OH)D is an indicator of vitamin D nutrition. Several population based studies have shown that 25(OH)D decreases by 30–60% with ageing in both genders (Tsai et al 1987). This may contribute to the secondary hyperparathyroidism of ageing because these decreases correlate inversely with serum PTH levels (Khosla et al 1998). Elderly, housebound persons with inadequate exposure to ultraviolet radiation and poor nutrition are particularly prone to vitamin D deficiency which, unless severe, is manifested by osteoporosis, rather than osteomalacia. This is particularly likely in populations living in higher latitudes, such as Great Britain and France, that do not fortify milk products with vitamin D. Indeed, Chapuy et al (1992) have demonstrated that supplementing the diet of elderly, house-bound women from Lyon, France with 800 U/day of vitamin D and 1000 mg/day of calcium for 18 months decreased the incidence of hip fracture by 43% over the following 18 months. Also, serum levels of the physiologically active vitamin D metabolite, $1,25(OH)_2D$ decrease with ageing (Manolagas et al 1983), at least relative to the concomitant increases in serum PTH (Eastell et al 1991). Because infusions with the trophic hormone, PTH, result in a blunting of the increase in serum $1,25(OH)_2D$ levels relative to young adults (Tsai et al 1984), ageing may result in a primary deficiency in the renal enzyme, 25(OH)D 1α-hydroxylase, that is responsible for the conversion of 25(OH)D to $1,25(OH)_2D$, and this may also contribute to the secondary hyperparathyroidism and increased bone resorption with ageing.

There is indisputable evidence that ageing decreases the amplitude and frequency of growth hormone secretion (Thorner & Vance 1988). There is also a 60% decrease in serum IGF1 levels with ageing and a smaller decrease in serum IGF2 levels (Bennett et al 1984). Whether the decreased serum IGF1 levels are due to a decreased production of hepatic or skeletal IGF1 production or increased plasma clearance is still unclear. Nonetheless, these abnormalities may contribute to the decreases in osteoblastic function in elderly men and women. They may also contribute to the progressive, age-related increases in serum SHBG levels in men which is a major cause of their decreases in serum bioavailable sex steroids and, thus, to their slow phase of bone loss.

Other changes in endocrine function with ageing appear to make smaller contributions to bone loss. There are decreases of almost 70% in serum dehydroepiandrosterone (DHEA) and DHEA sulfate in elderly women and men (Meikle et al 1991). However, this is a relatively weak adrenal androgen and, its importance, if any, in bone loss is problematic. In contrast to the decrease in bioavailable anticatabolic sex steroids with ageing, the concentration of plasma-free cortisol, a catabolic steroid, increases by 20–50% (Van Cauter et al 1996). Thus, the increased catabolic/anti-catabolic ratio of circulating steroid hormones with ageing could contribute to bone loss.

Non-endocrine age-related abnormalities

Although endocrine factors appear to be the major cause of age-related bone loss, there are important non-endocrine factors that also contribute. The level of bone mass present prior to the onset of age-related bone loss is clearly important: those persons who have high levels are relatively protected against osteoporosis whereas those with low levels are clearly at a greater risk. As has been long recognized, there are a number of episodic factors that increase bone loss in some, but not other, members of the ageing population. These include use of certain drugs such as corticosteroids, diseases such as malabsorption, anorexia nervosa and renal hypercalciuria, and behavioural factors such as smoking, alcohol abuse and inactivity — to enumerate but a few. These may make major contributions to fractures in about 40% of men and 20% of women (Riggs et al 1986).

Conclusion

In women, oestrogen deficiency due to the menopause is the major cause of both the early rapid and late slow phases of age-related bone loss. In ageing men, oestrogen deficiency also appears to be the dominant cause and is due to age-related increases in serum SHBG and to impaired gonadal production of sex steroids. The role played by decreases in bioavailable testosterone in bone loss in

men is presently unclear. Thus, although other factors contribute, age-related osteoporosis appears to be primarily an endocrine deficiency disease in both genders.

Acknowledgement

This work was supported by National Institutes of Health grants AR27065 and AG04875.

References

Ashcroft GS, Dodsworth J, van Boxtel E et al 1997 Estrogen accelerates cutaneous wound healing associated with an increase in TGF-β1 levels. Nat Med 3:1209–1215

Bennett A, Wahner HW, Riggs BL, Hintz RL 1984 Insulin-like growth factors I and II: aging and bone density in women. J Clin Endocrinol Metab 59:701–704

Bernecker PM, Willvonseder R, Resch H 1995 Decreased estrogen levels in male patients with primary osteoporosis. J Bone Miner Res 10:S445

Carani C, Qin K, Simoni M et al 1997 Effect of testosterone and estradiol in a man with aromatase deficiency. N Engl J Med 337:91–95

Center JR, Nguyen TV, White CP, Eisman JA 1997 Male osteoporosis predictors: sex hormones and calcitropic hormones. J Bone Miner Res 12:S368

Chapuy MC, Arlot ME, Duboeuf F et al 1992 Vitamin D_3 and calcium to prevent hip fractures in elderly women. N Engl J Med 327:1637–1642

Eastell R, Yergey AL, Vieira N, Cedel SL, Kumar R, Riggs BL 1991 Interrelationship among vitamin D metabolism, true calcium absorption, parathyroid function and age in women: evidence of an age-related intestinal resistance to 1,25-dihydroxyvitamin D action. J Bone Miner Res 6:125–132

Ernst M, Heath JK, Rodan GA 1989 Estradiol effects on proliferation, messenger ribonucleic acid for collagen and insulin-like growth factor-I, and parathyroid hormone-stimulated adenylate cyclase activity in osteoblastic cells from calvariae and long bones. Endocrinology 125:825–833

Ettinger B, Genant HK, Cann CE 1985 Long-term estrogen replacement therapy prevents bone loss and fractures. Ann Intern Med 102:319–324

Falahati-Nini A, Riggs BL, Atkinson EJ, O'Fallon WM, Eastell R, Khosla S 2000 Relative contributions of testosterone versus estrogen in regulating bone resorption and formation in normal elderly men. J Clin Invest 106:1553-1560

Gennari C, Agnusdei D, Nardi P, Civitelli R 1990 Estrogen preserves a normal intestinal responsiveness to 1,25-dihydroxyvitamin D3 in oophorectomized women. J Clin Endocrinol Metab 71:1288–1293

Greendale GA, Edelstein S, Barrett-Connor E 1997 Endogenous sex steroids and bone mineral density in older women and men: the Rancho Bernardo Study. J Bone Miner Res 12:1833–1843

Khosla S, Melton LJ III, Atkinson EJ, O'Fallon WM, Klee GG, Riggs BL 1998 Relationship of serum sex steroid levels and bone turnover markers with bone mineral density in men and women: a key role for bioavailable estrogen. J Clin Endocrinol Metab 83:2266–2274

Khosla S, Atkinson EJ, Melton LJ III, Riggs BL 1997 Effects of age and estrogen status on serum parathyroid hormone levels and biochemical markers of bone turnover in women: a population-based study. J Clin Endocrinol Metab 82:1522–1527

Khosla S, Melton LJ III, Atkinson, EJ, O'Fallon WM, Riggs BL 2000 Relationship of sex steroids to longitudinal changes in bone mineral density and bone resorption in young

versus elderly men: effects of estrogen on peak bone mass and on age-related bone loss. J Bone Miner Res 15:S159
Ledger GA, Burritt MF, Kao PC et al 1994 Abnormalities of parathyroid hormone secretion in elderly women that are reversible by short term therapy with 1,25-dihydroxyvitamin D3. J Clin Endocrinol Metab 79:211–216
Manolagas SC 2000 Birth and death of bone cells: basic regulatory mechanisms and implications for the pathogenesis and treatment of osteoporosis. Endocr Rev 21:115–137
Manolagas SC, Culler FL, Howard JE, Brickman AS, Deftos LJ 1983 The cytoreceptor assay for 1,25-dihydroxyvitamin D and its application to clinical studies. J Clin Endocrinol Metab 56:751–760
McKane WR, Khosla S, Burritt MF et al 1995 Mechanism of renal calcium conservation with estrogen replacement therapy in women in early postmenopause — a clinical research center study. J Clin Endocrinol Metab 80:3458–3464
McKane WR, Khosla S, Egan KS, Robins SP, Burritt MF, Riggs BL 1996 Role of calcium intake in modulating age-related increases in parathyroid function and bone resorption. J Clin Endocrinol Metab 81:1699–1703
McKane WR, Khosla S, Risteli J, Robins SP, Muhs JM, Riggs BL 1997 Role of estrogen deficiency in pathogenesis of secondary hyperparathyroidism and increased bone resorption in elderly women. Proc Assoc Am Physicians 109:174–180
Meikle AW, Daynes RA, Araneo BA 1991 Adrenal androgen secretion and biologic effects. Endocrinol Metab Clin North Am 20:381–400
Melton LJ III, Kan SH, Wahner HW, Riggs BL 1988 Lifetime fracture risk: An approach to hip fracture risk assessment based on bone mineral density and age. J Clin Epidemiol 41:985–994
Morishima A, Grumbach MM, Bilezikian JP 1997 Estrogen markedly increases bone mass in an estrogen deficient young man with aromatase deficiency. J Bone Miner Res 12:S126
Riggs BL, Melton LJ 1986 Involutional osteoporosis. N Engl J Med 314:1676–1686
Riggs BL, Khosla S, Melton LJ III 1998 A unitary model for involutional osteoporosis: estrogen deficiency causes both type I and type II osteoporosis in postmenopausal women and contributes to bone loss in aging men. J Bone Miner Res 13:763–773
Riggs BL, Khosla S, Melton LJ III 2000 Primary osteoporosis in men: role of sex steroid deficiency. Mayo Clin Proc 75:S46–S50
Seeman E 1997 From density to structure: growing up and growing old on the surfaces of bone. J Bone Miner Res 12:509–521
Slemenda CW, Longcope C, Zhou L, Hui SL, Peacock M, Johnston C 1997 Sex steroids and bone mass in older men. Positive associations with serum estrogens and negative associations with androgens. J Clin Invest 100:1755–1759
Smith EP, Boyd J, Frank GR et al 1994 Estrogen resistance caused by a mutation in the estrogen-receptor gene in a man. N Engl J Med 331:1056–1061 (erratum: 1995 N Engl J Med 332:131)
Thorner MO, Vance ML 1988 Growth hormone, 1988. J Clin Invest 82:745–747
Tsai KS, Heath H III, Kumar R, Riggs BL 1984 Impaired vitamin D metabolism with aging in women. Possible role in pathogenesis of senile osteoporosis. J Clin Invest 73: 1668–1672
Tsai KS, Wahner HW, Offord KP, Melton LJ III, Kumar R, Riggs BL 1987 Effect of aging on vitamin D stores and bone density in women. Calcif Tissue Int 40:241–243
Van Cauter E, Leproult R, Kupfer DJ 1996 Effects of gender and age on the levels and circadian rhythmicity of plasma cortisol. J Clin Endocrinol Metab 81:2468–2473
Vanderschueren D, Van Herck E, Nijs J, Ederveen AGH, De Coster R, Bouillon R 1997 Aromatase inhibition impairs skeletal modelling and decreases bone mineral density in growing male rats. Endocrinology 138:2301–2307

DISCUSSION

Veldhuis: Even allowing for this marvellously interesting and prominent effect of oestrogen, the regressions don't account for 100% of the variability in bone mass. Is there room for non-genetic ageing-related factors independently of the endocrine axes on bone? Would bone cells age in a perfectly normal milieu?

Riggs: Obviously, there is. It is interesting that sex steroid deficiency appears to be much more important than I thought some five to 10 years ago. It does seem that sex steroid deficiency is mostly responsible. Clearly, there are other non-endocrine non-ageing factors involved. There are secondary causes of osteoporosis and behavioural changes such as smoking and alcohol abuse that lead to bone loss. There are genetic factors, but most of these appear to act in the development of peak bone mass. They are much less important in the rate of bone loss.

Prior: I would like to raise a somewhat philosophical but important issue. The concept of 'oestrogen deficiency' related to menopause is wrong. Menopause is not a choice for women. We are born with a programmed set of ovarian signals, and it is normal for every woman to go through menopause. If it is normal, how does that make it 'deficiency'?

Riggs: I think it is usual, but not 'normal'. Except for advanced primates, other animals do not have the equivalent of the menopause. But whether it is normal or not, if it causes disease then it has to be treated, and I think it causes disease.

Prior: The model breaks down because there is good evidence now that continually oestrogen-treated menopausal women do not preserve their bone density (Prior et al 1997). They also lose bone density at a rapid rate when oestrogen replacement is stopped. In addition, there is also good evidence from many centres that the bone loss begins several years before the final menstrual period and certainly before menopause (defined as one year after the final menstrual period) (Recker et al 2000, Okano et al 1998). During this time statistically, oestradiol levels are not low (Prior 1998). Finally, if you are going to talk about '*oestrogen* deficiency', you also have to talk about its partner female steroid hormone, progesterone, which is equally deficient and for which low levels begin even earlier. The concept of oestrogen as a solo deficiency is wrong. As a woman who has no choice about going through this process of life, it feels prejudicial to continue to use 'oestrogen deficiency' rhetoric.

Riggs: I think you are mixing in social issues with medical issues.

Prior: Can you separate them?

Riggs: Yes, I think they can be separated. The key issue here is medical. Does this deficiency state cause osteoporosis, and can this be prevented? The evidence is overwhelming that this is so. Oestrogen replacement at the menopause is certainly not going to prevent 100% of osteoporotic fractures, but the evidence is strong that it will prevent the majority of them. You can then make the case that

premenopausal loss, except possibly for the proximal femur, is related to decreases in sex steroid production in the early pre-menopause. The major question here between the physician and his or her patient is whether the patient will benefit from treatment. Of course, there is the issue with risk and benefit with oestrogen replacement therapy. If there wasn't, it would be as easy to give hormone-replacement therapy (HRT) as it would to give thyroid hormone replacement. No one would dispute that.

Prior: We have a problem here of a concept that has become a paradigm. There is only one small, one-year randomized placebo-controlled trial that sort of shows that hormone therapy after menopause prevents fractures (Lufkin et al 1992).

Handelsman: Larry Riggs, I thought your paper was an elegant presentation of the case for the importance of sex steroids, but I have a couple of issues I'd like to raise. Dirk Vanderschueren has done a study in the Tfm (testicular feminized) rat, which has completely non-functional androgen receptors (Vanderschueren et al 1994). On the basis of what you said, you would predict that there would be no difference between the Tfm genetic males and females. This is not the case, and suggests that androgen effects are not all due to aromatization. Although periosteal bone growth due to androgen is an interesting alternative, it doesn't resolve the issue of these androgen receptors.

Riggs: Please don't come away from here with the impression that we think that testosterone has no effect on bone. Clearly, it does. It is just difficult to show. Regarding the animal models, mice are very different from primates. Even the ER^{-}/ER^{-} double knockout mice don't approach the degree of osteopenia observed in women after ovariectomy.

Handelsman: I have real doubts about what is called the free hormone hypothesis. It is a superficially attractive concept, but I think it is also vague and misleading. If a 'free hormone' is not bound to a protein, it may well be more accessible to tissues, but it is also more accessible to metabolism. There is no real reason why such a hormone should be more active rather than more rapidly metabolized. It is impossible to predict a priori. This issue has never really been fully resolved. Widely used measures of 'free' hormones are just manipulations of the data. In particular, you showed nicely the SHBG levels rise: those bio testosterone and oestrogen levels you measured are really an inverted measure of SHBG. If you offered that to the regression equations I wouldn't be surprised if you find that the reciprocal of SHBG is as good or better predictor than the supposedly manipulated steroid levels.

Riggs: When we measure the total hormone we get weak correlations, but when we measure the so called bioavailable T and E we get strong correlations. But I agree with you: the sex hormone binding component is the main predictor.

Ruiz-Torres: The difference between pathology and ageing is difficult to work out. Many years ago you published an important paper in which you gave the

normal values of bone density in women from age 20 to age 80+ (Riggs et al 1981). If you put your data on a semilogarithmic scale you get a half-life of around 50 years. This means that those young women whose bone density values are clearly below the mean, according with the half-life mentioned, will become osteoporotic at the age (but without the influence) of the menopause. Moreover, during the life of a rat, from adulthood onwards there is normally a continuous decrease of both bone DNA and collagen, which is not reversible with oestrogens or testosterone. Furthermore, using the model based on the chronic application of aminoacetonitrile to rats, it is possible to detect the effects of drugs, because the osteoporosis produced is reversible when stopping this type of intoxication. Oestrogen does not influence the recovery: the collagen or cell content of the bones remain similar to the controls, as opposed to androgen treatment which clearly improves the bone collagen deficiency. Finally, another point which underlines the doubts of the efficiency of oestrogen treatments against osteoporosis, is the fact that osteoblasts — the cells producing bone collagen — don't have oestrogen receptors but those for IGF1. In summary, the reduction of the main contents of the bone is a normal manifestation of ageing and is therefore not reversible. I consequently think that the best procedure against osteoporosis which becomes manifest around the age of the menopause is to ensure that females end adolescence reaching optimal bone values.

Riggs: I agree that the effect of oestrogen on stimulating bone formation is the weakest part of the hypothesis. This is why I think the paper recently published by Khastgir et al (2001) is so interesting. Nonetheless, I agree that oestrogen is weaker than testosterone with respect to stimulating collagen synthesis. I wouldn't agree that oesteoblasts don't have oestrogen receptors. We reported this back in 1988 (Eriksen et al 1988). They clearly have ERs and respond to oestrogen in a number of ways. With regard to the rats, to make a rat or mouse responsive to oestrogen, you need to ovariectomize them. If you do this, there is a response.

Müller: You have clearly shown that oestrogen deficiency also plays a role in male osteoporosis. Thus, it would be the rationale for administering oestrogens to males also, but this is not feasible. Are there any trials involving SERM (specific oestrogen receptor modulator) administration, e.g. raloxifene, to males?

Riggs: To my knowledge the only study that has been done so far is the one that I referred to. We identified a threshold level below which the markers decreased, and above which they increased, so the net effect was of no difference. This is probably additional evidence that this is the threshold between oestrogen deficiency and sufficiency. Raloxifene is a much weaker bone agonist than oestrogen, so you are displacing a stronger agonist with a weaker agonist and you will make an oestrogen-deficient male worse. We were hoping for a better result, and there are third generation SERMs in the pipeline that appear to have the same potency as

oestradiol. This may produce better results in elderly men who are partially oestrogen deficient.

Carroll: You mentioned the cortisol effect. Actually it is trough cortisol that correlates with rate of bone loss (Dennison et al 1999). This gets back to the point I was making on Tuesday. What are the steps in your model where you would see cortisol interacting with those other variables?

Riggs: We haven't really worked this out. We are in a process of actually measuring free cortisol excretion in our on-going studies. My guess is that at least there will be additional factors that will be additive to the oestrogen deficiency. Whether or not they interact with oestrogen deficiency in some synergistic way is unclear.

Handelsman: With regard to the issue of predictions from your concept, if you use a non-aromatizable androgen in men, it should cause bone loss. We have done a placebo-controlled three month study with DHT, which suppresses endogenous testosterone quite profoundly. We saw no effect on bone alkaline phosphatase or osteocalcin.

Wang: It suppresses oestradiol too.

Handelsman: We can find evidence in a more compelling model. If you take a post-menopausal women who is oestrogen deficient and then use a non-aromatizable androgen, such as nandrolone, this has profound effects on bone. These must be androgen receptor mediated.

Riggs: The osteoblasts and osteoclasts both have androgen receptors. However, we believe that oestrogen deficiency is the dominant cause of bone loss in ageing men, but that testosterone deficiency contributes also.

Laron: You mentioned the sex hormones. We know more these days about the gastrointestinal hormones which certainly regulate Ca^{2+} intake or response to hormones. What is the state of the art now?

Riggs: 1,25-dihydroxyvitamin D plays a key role, and this is regulatable by oestrogen. Thus part of the effect of oestrogen is through this mechanism. As for the other gut hormones, I am not clear whether these have major effects.

Haus: As pathologist, I am interested in the hyperparathyroidism you mentioned. Is this a transient phenomenon at the time of the accelerated phase? The reason I am asking is that morphologically the functional parathyroid parenchyma undergoes a very marked atrophy with advancing age. At the age when osteoporosis is most prevalent the parathyroid has lost a large percentage of its epithelial cells which in this age group have been replaced by adipose tissue.

Riggs: There is a suppression at the beginning and then an age-related increase that continues indefinitely. One autopsy study from Sweden showed an increase in the size in the parathyroid glands with ageing. The level of the hypertrophy of the parathyroid glands is certainly not to the extent that you see with secondary hyperparathyroidism in chronic renal failure.

Haus: The gland may be of similar size, but it is composed largely of fat in older people. Quite often there are just small sheets and clusters of active parathyroid tissue left.

Ruiz-Torres: I have two explanations for why PTH increases with age. The first is that ageing decreases the catabolic rate of hormones, as is well known in the case of thyroxin. The second is related to the regulatory mechanism that would appear when the mineral content of the bone decreases with age.

Riggs: We have looked at the secretory dynamics of the parathyroid gland, and there is clearly an increase in secretion. It is compensatory. If you give enough Ca^{2+}, you can bring the PTH back to normal.

References

Dennison E, Hindmarsh P, Fall C et al 1999 Profiles of endogenous circulating cortisol and bone mineral density in healthy elderly men. J Clin Endocrinol Metab 84:3058–3063

Eriksen EF, Colvard DS, Berg NJ et al 1988 Evidence of estrogen receptors in normal human osteoblast-like cells. Science 241:84–86

Khastgir G, Studd J, Holland N, Alaghband-Zadeh J, Fox S, Chow J 2001 Anabolic effect of estrogen replacement on bone in postmenopausal women with osteoporosis: histomorphometric evidence in a longitudinal study. J Clin Endocrin Metab 86:289–295

Lufkin EG, Wahner HW, O'Fallon WM et al 1992 Treatment of postmenopausal osteoporosis with transdermal estrogen. Ann Int Med 117:1–9

Okano H, Mizunuma H, Soda M et al 1998 The long-term effect of menopause on postmenopausal bone loss in Japanese women: results from a prospective study. J Bone Miner Res 13:303–309

Prior JC 1998 Perimenopause: the complex endocrinology of the menopausal transition. Endocr Rev 19:397–428

Prior JC, Vigna YM, Wark JD et al 1997 Premenopausal ovariectomy-related bone loss: a randomized, double-blind one year trial of conjugated estrogen or medroxyprogesterone acetate. J Bone Miner Res 12:1851–1863

Recker R, Lappe J, Davies K, Heaney R 2000 Characterization of perimenopausal bone loss: a prospective study. J Bone Miner Res 15:1965–1973

Riggs BL, Wahner HW, Dunn WL, Mazess RB, Offord KP, Melton LJ 1981 Differential changes in bone mineral density of the appendicular and axial skeleton with aging: relationship to spinal osteoporosis. J Clin Invest 67:328–335

Vanderschueren D, Van Herck E, Geusens P et al 1994 Androgen resistance and deficiency have different effects on the growing skeleton of the rat. Calcif Tissue Int 55:198–203

Endocrine Facets of Ageing.
Novartis 242

Print ISBN 0-471-48636-1 Online ISBN 0-470-84654-2

Ageing and water homeostasis

David Robertson, Jens Jordan*, Giris Jacob†, Terry Ketch, John R. Shannon and Italo Biaggioni

*Autonomic Dysfunction Center, Departments of Medicine, Pharmacology, and Neurology Vanderbilt University, Nashville TN 37232–2195, USA, *Clinical Research Center, Franz Volhard Institut, Berlin, Germany and †Recanati Autonomic Dysfunction Center, Rambam Medical Center, Haifa, Israel*

Abstract. This review outlines current knowledge concerning fluid intake and volume homeostasis in ageing. The physiology of vasopressin is summarized. Studies have been carried out to determine orthostatic changes in plasma volume and to assess the effect of water ingestion in normal subjects, elderly subjects, and patients with dysautonomias. About 14% of plasma volume shifts out of the vasculature within 30 minutes of upright posture. Oral ingestion of water raises blood pressure in individuals with impaired autonomic reflexes and is an important source of noise in blood pressure trials in the elderly. On the average, oral ingestion of 16 ounces (473 ml) of water raises blood pressure 11 mmHg in elderly normal subjects. In patients with autonomic impairment, such as multiple system atrophy, strikingly exaggerated pressor effects of water have been seen with blood pressure elevations greater than 75 mmHg not at all uncommon. Ingestion of water is a major determinant of blood pressure in the elderly population. Volume homeostasis is importantly affected by posture and large changes in plasma volume may occur within 30 minutes when upright posture is assumed.

2002 Endocrine facets of ageing. Wiley, Chichester (Novartis Foundation Symposium 242) p 265–278

Water is the major constituent of living beings. It engenders and facilitates homeostasis by virtue of its effects as a solvent, its ionizing potential, its high thermal conductivity, its high specific heat content, and its high latent heat of evaporation. Its plasma proteins (via oncotic pressure) and its electrolytes (via osmotic pressure) maintain body fluid homeostasis. (Greenleaf & Morimoto 1996.)

Total body water accounts for 50–70% of body weight (Fig. 1). The cell membranes demarcate an intracellular compartment comprising about 50% of body weight and the extracellular compartment comprising about 20% of body weight. Extracellular fluid is further subdivided by the capillary endothelium into interstitial fluid (~15%) and plasma volume (~5%).

The crucial cation in the extracellular fluid is Na^+ (~150 mEq/l) and its associated anions are Cl^- (~110 mEq/l) and bicarbonate (~28 mEq/l). The

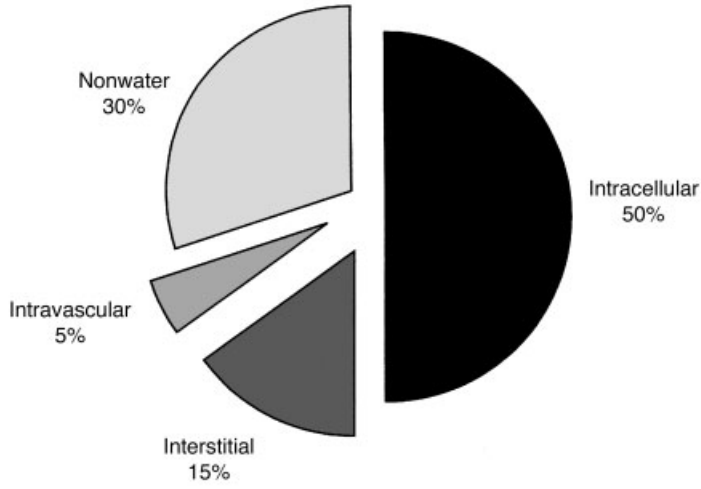


FIG. 1. The distribution of body water.

osmotic environment of the extracellular fluid is largely defined by these ions, and its osmolality is usually about double the Na^+ concentration.

Thirst is a subjective quality defined as 'a desire to drink potable liquids'. Thirst appears to have several different causes, and its fundamental mechanisms remain uncertain (Gordon et al 1997). Cellular, extracellular and volume factors all seem to be involved. Osmotic stimuli account for about 70% and volume factors for about 25% of dehydration-induced drinking, but under circumstances where volume loss is large, for example with haemorrhage or phlebotomy, the contribution of volume factors increases considerably.

Greenleaf et al (1966) developed a linear regression equation for predicting actual water intake in 87 young military trainees in field conditions which reflects ($r = 0.79$; $P < 0.01$) the complexity of this functional expression of thirst:

> Water intake (ml/day) = −11502+45.8 (serum osmolality)+1.2 (mean daily urine output) − 18.9 (mean daily urinary potassium)+4.4 (mean daily urinary chloride) − 18.7 (lying heart rate) + 1.8 (daily sweat rate).

Ageing has a significant effect on body water compartments. With increasing age beyond 30 years, there is a gradual fall in the fraction of body weight that consists of water. This is accompanied by a less dramatic increase in the fraction of water in the fat-free body. These changes are primarily due to loss of skeletal muscle tissue, a relatively water-rich bodily component, with ageing.

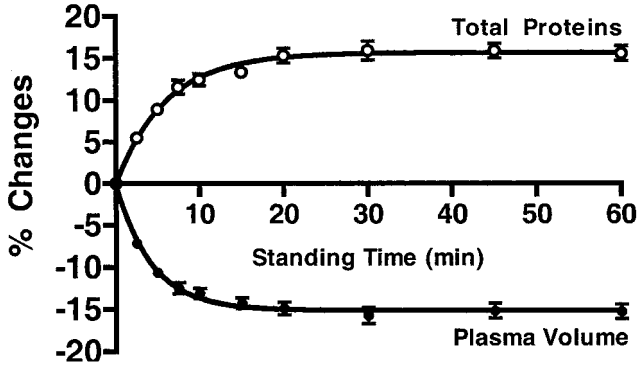


FIG. 2. On assumption of upright posture after 60 minutes of supine posture, there is a rapid loss of plasma volume from the vasculature into the interstitial tissue. This is reflected in the increased concentration of total protein. In this figure, the per cent change in plasma volume in the lower part of the figure is calculated on change in haematocrit.

Postural plasma volume shift

Although only about 5% of the body weight is intravascular fluid, this is obviously a very important 5% in terms of haemodynamic variables. The fluid component of the blood is in equilibrium with the interstitial fluid. With changes in posture, the amount of fluid in the vasculature versus the interstitial space may be altered by gravity-induced effects. The most important implication of this relates to changes from supine to upright posture. Under normal circumstances, there is pooling of blood in the vasculature of the lower part of the body within a few seconds of assumption of upright posture. Normally, about 700 ml of blood moves to the lower abdomen, pelvis, and lower extremities on standing. Compensatory neurohumoral adjustments rapidly occur leading to increases in sympathetic nerve traffic, vasopressin, and the activation of the renin–angiotensin–aldosterone axis.

Much less appreciated but nevertheless very significant is the loss following the assumption of upright posture of 12–18% of plasma volume into the interstitial fluid compartment over the succeeding 20–30 minutes (Fig. 2) (Jacob et al 1996, 1998). This fluid shift translates into loss of about a unit of blood within 30 minutes of standing. This occurs in addition to the well-known intravascular pooling effect in the venous capacitance bed. The concurrence of these two effects render the period from 10 minutes after standing particularly vulnerable for maintenance of adequate blood flow to the brain and other important organs. This is when most individuals who have a tendency to experience syncope due to low blood pressure will have this become manifest.

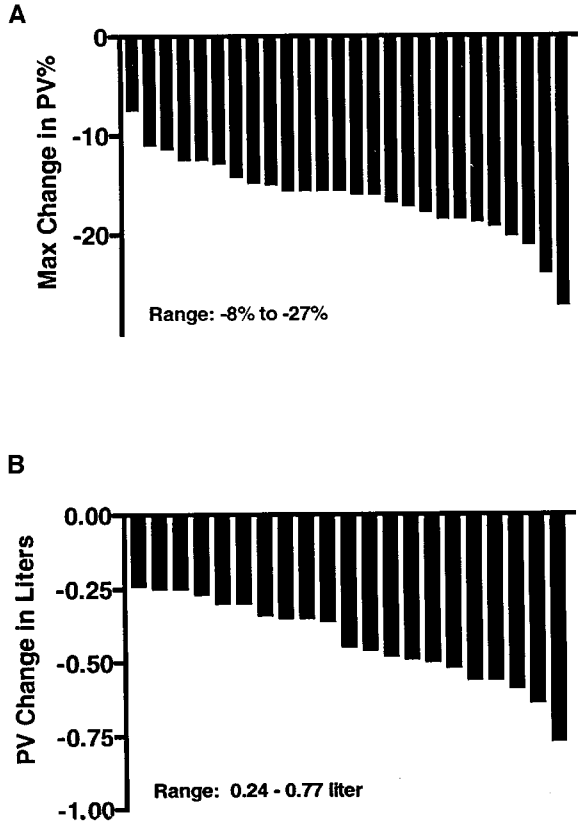


FIG. 3. In this figure, the enormous interindividual variability in per cent change in plasma volume (upper register) and absolute value of volume change (lower register) is seen. The volume loss in this population ranged from 240 to 770 ml within a period of 30 minutes.

Perhaps more important than the average shift in plasma volume from the intravascular to the interstitial space is the substantial interindividual variability, which in a recent study of 25 individuals ranged from a change in plasma volume of 7% to a change of 28% (Fig. 3). The latter resulted in a shift in haematocrit from 42% to 34% within 30 minutes of lying down, even though no bleeding had actually occurred.

An important future direction for research is the understanding of interindividual variation in plasma volume shift with changes in posture and how pathophysiological conditions and drugs may alter this function. The determinants of and the effect of ageing on this shift, remain unknown.

Vasopressin pathophysiology

Vasopressin (AVP, ADH) is the principal hormone responsible for the body's narrow control of plasma osmolality (Baylis 2001). Vasopressin is synthesized in the supraoptic (SON) and paraventricular (PVN) nuclei in the hypothalamus. The synthesized hormone is released from the neurohypophysis (posterior pituitary gland). It is a nonapeptide derived from a 155 amino acid precursor encoded in chromosome 20 about 11 kilobases from the gene for oxytocin. Vasopressin is excreted in approximately equimolar amounts with its hypophysin and then circulates in the bloodstream with a half-life of 5–15 minutes.

Receptors for vasopressin have been identified (Baylis 2001, Preisser et al 2000). A vasopressin 1A receptor utilizes phospholipase C/G protein, inositol phosphates and diacylglycerol as intracellular messengers for vasopressin functions in smooth muscle, platelets, liver and some sites in the central nervous system (CNS). A vasopressin 1B receptor utilizes similar intracellular mechanisms and is primarily located in the pituitary corticotroph. A vasopressin 2 receptor utilizes adenylate cyclase and G_s protein through cAMP and protein kinase A to activate aquaporin 2 channel insertion in renal tubular cells.

Vasopressin release is powerfully stimulated by high osmolality, reduced blood pressure (stretch), by nausea/vomiting, and by a variety of visceral traction stimuli, but the correlation between plasma vasopressin and osmolality of blood is tightly and directly linked over the range 285–305 mOsmol/kg (Helderman et al 1978, Moses et al 1976). When osmolality is maintained, the effect of changes in blood pressure on plasma arginine vasopressin is also quite tight over the range 0–40 mm Hg fall in blood pressure.

A number of studies have been undertaken to explore abnormalities in vasopressin in ageing and in Alzheimer's disease (Robertson & Rose 1980, Hoogendijk et al 1985, Faull et al 1993, Frolkis et al 1999, Liu et al 2000). Not all these studies are in agreement, but the following changes seem to be reasonably well established. In ageing, the basal arginine vasopressin level in blood is normal or increased, the plasma arginine vasopressin level following stimulation is increased, the cerebrospinal fluid (CSF) arginine vasopressin level is normal and the renal response to arginine vasopressin is diminished.

In Alzheimer's disease, basal arginine vasopressin is normal or decreased, stimulated arginine vasopressin is decreased and CSF levels of arginine vasopressin are decreased. There are also changes with ageing in other hormones of relevance to volume homeostasis (Lesser et al 1963, Shoeller 1989). Plasma noradrenaline tends to increase with ageing, particularly in men, whereas there is a lesser change in plasma adrenaline changes. Plasma renin activity and plasma aldosterone decrease with age and plasma atrial natriuretic hormone is increased in ageing.

End-organ changes also occur. Anatomic changes in the ageing kidney include reduced size of the kidney, fewer glomeruli, a reduced tubular mass, and sclerosis of pre- and post-glomerular arterioles. At the functional level, there is decreased glomerular filtration rate, decreased renal blood flow, and a reduction in the maximum urine concentration capability and the maximum urine dilution capability. There are also sluggish responses to Na^+ deprivation and to an acid load.

It is not uncommon for elderly subjects to have changes in fluid homeostasis related to low fluid intake (Miller 1995, Roth et al 2001). There are many reasons for this lower fluid intake, including limited access to fluids due to immobility, visual problems, or in some cases, restraints; fluid restriction (therapeutic, procedural, or preventive); altered mental status (CNS disease, infections, drugs); gastrointestinal disorders (swallowing problems, obstruction, drugs); and altered thirst mechanisms (impaired thirst, CNS disease, drugs). These situations may account for much of the hypernatraemia in the elderly. In one study, febrile illness was present in 70% of elderly subjects presenting with hypernatraemia, infirmity in 40%, surgery in 21%, nutritional supplementation in 20%, intravenous solutions in 18%, diabetes mellitus in 15%, diarrhoea in 11%, gastrointestinal bleed in 9% and diuretic use in 9%. Only 7% of such patients actually had diabetes insipidus as the cause of the hypernatraemia.

The age-dependent pressor effect of water

While individual patients with orthostatic hypotension due to autonomic failure had described improvement in upright blood pressure and functional capacity following ingestion of water, there was limited support for this concept based on current understanding of human physiology. We examined the effect of oral ingestion of water in patients with two forms of autonomic failure, pure autonomic failure (PAF) and multiple system atrophy (MSA) (Jordan et al 1999, 2000a,b). PAF and MSA patients often have severe autonomic dysfunction, but the site of pathophysiology is distinct. In PAF, there is loss of peripheral autonomic neuronal fibres, so that the intact CNS control mechanisms lack the peripheral 'wiring' needed to effect changes in autonomic outflow. In contrast, in patients with MSA, the autonomic failure is not so much characterized by destruction of peripheral sympathetic and parasympathetic nerves, but rather by a failure of CNS mechanisms to appropriately engage the largely intact peripheral autonomic innervation.

The testimony of patients about water had been surprising. Traditional physiology did not provide a rationale for a pressor effect of water drinking in individuals who were not dehydrated. Indeed most attention of blood pressure investigators has been aimed at examining and understanding the long-term

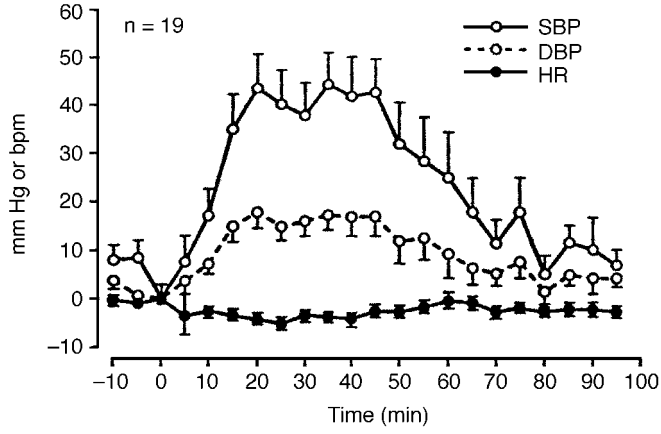


FIG. 4. In a population of patients with autonomic impairment, the ingestion of water (16 ounces; 473 ml) elicited an increase in systolic blood pressure of about 40 mmHg within 20 minutes. The diastolic pressure increment was about half that. Note that most of the effects of the water ingestion are dissipated within 90 minutes.

effects of dietary Na^+ on arterial pressure, and even this remained controversial. It was therefore unexpected when under controlled conditions we did indeed observe a substantial effect of water on blood pressure in autonomic failure patients.

Water was given to PAF and MSA patients in a dosage of 16 ounces (473 ml), ingested fairly rapidly over a period of 2–3 minutes (Fig. 4). In response to this, blood pressure became detectably higher within 10 minutes and climbed to a maximal pressure increase of about 40–50 mmHg at approximately 25 minutes following ingestion (Robertson et al 2001). After that pressor effect, a gradual decline occurred that brought blood pressure back to baseline over the next 45 minutes. While some patients had responses smaller than 30 mmHg, in others blood pressure rose more than 90 mmHg. The magnitude of this response was astonishing. Indeed, it was greater than that observed with commonly used doses of any pressor drug in autonomic failure. The effect was most dramatic in the supine posture but was also present in the seated and upright postures and translated into a significant increase also in the standing blood pressure and functional capacity.

Autonomic failure often increased the amplitude of effects that were also present albeit less prominently in normal subjects. Thus from a modelling standpoint, autonomic dysfunction frequently made it possible to detect pressor and depressor stimuli in small numbers of subjects that would be very difficult to detect in larger numbers of normal subjects. After observing such a dramatic response in MSA patients, it was important to determine if these effects could be

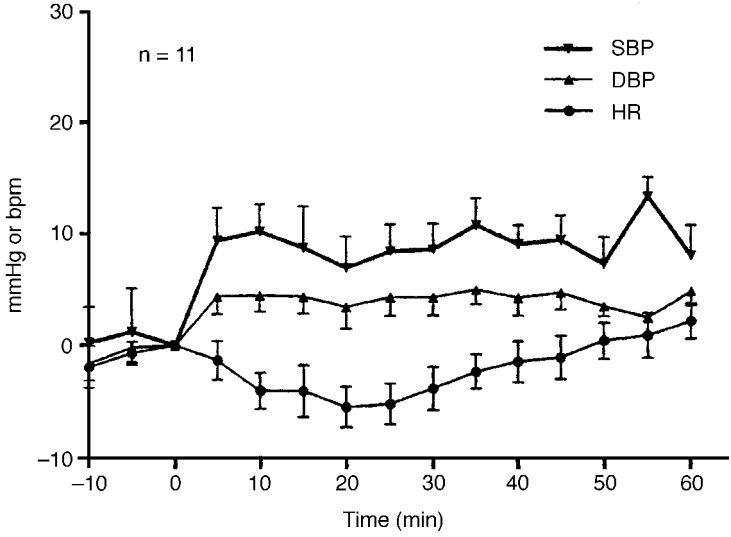


FIG. 5. Effect of oral water (480 ml) on blood pressure and heart rate in healthy older controls. A mean increment of 10 mmHg in systolic blood pressure is noted.

replicated in normal individuals. Using a similar protocol in healthy young subjects the pressor effect of water was not observed, although a significant increase in plasma noradrenaline was seen. In contrast, water administration in older normal subjects elicited a significant and reproducible mean increase in blood pressure of 11 mmHg (Fig. 5). In the older patients this pressor effect was accompanied by a trend toward reduced heart rate, but this did not reach statistical significance.

To address the mechanism of this unexpected effect of water, trimetaphan was administered to determine if the effect was dependent on the integrity of autonomic function. In the presence of trimetaphan, no effect of water on blood pressure was observed. This suggests that the pressor effect is dependent on a degree of integrity of the autonomic nervous system for expression (Jordan et al 2000b, Rossi et al 1998). In this regard it is noteworthy that even in severe disorders of autonomic dysfunction, some level of autonomic function remains characteristic, and, in the absence of the buffering capacity of a fully functioning baroreflex, autonomic pressor and depressor reflexes can be surprisingly strong.

To determine whether oral administration was crucial, we gave a comparable volume of 5% dextrose solution intravenously. This agent was selected because the hypotonicity of water itself would be harmful if administered parenterally, and use of physiological saline would add the confounding variable of Na^+ to the study. The 5% dextrose did not replicate the pressor effect of oral water.

The temperature of oral water might be related to its pressor effect. To test this possibility, administration of cold (4 °C) and warm (37 °C) were compared. They were effective to the same degree as water at room temperature. These data suggest that sympathetic activation is somehow elicited by oral water but that this is most dramatically manifest with impairment in central autonomic control as in the case of MSA.

It is remarkable that this effect of water had been missed by physicians for so many years, but we were probably so influenced by our physiology training that we could not see this striking anomaly. However, once recognized, the effect of water has proven to be a significant therapeutic advance. Its value vis-à-vis other drugs lies in the potency of its action, the lack of major side effects, the rapidity of its onset and, since supine hypertension is often a limiting factor in therapy of patients with MSA, the relatively fast return of blood pressure to normal. The depressor effect of food, especially carbohydrate, has long been recognized in MSA. Now with recognition of the pressor effect of water, a simple and patient-controlled approach to blood pressure management is possible. Our experience so far suggests that many patients need no other therapy for control of blood pressure than judicious dosing of food and water. Careful further studies will be required in order to elucidate the precise mechanism underlying the effect of water, but an action through gastrointestinal stretch receptors or osmoreceptors must be addressed in future investigations. There remains the possibility that subtle blood volume increases in the hour after water ingestion, and before it is fully compensated by effects of the vasopressin/renal system, might contribute to this effect.

For the older normal subjects, the importance of the pressor effect of water is the noise it adds to the monitoring of blood pressure. Until now recent water intake has not been a variable controlled in studies of blood pressure and antihypertensive agents in the older age group. It would now appear that water ingestion may represent a major component of the noise inherent in blood pressure monitoring in this population. Recognition of this effect and control for it may reduce the number of subjects needed in future studies of antihypertensive drugs.

Conclusions

Fluid handling in ageing is altered in many ways. There is a reduced fraction of the body consisting of water in ageing. Postural plasma volume shifts occur although the precise role of ageing in interindividual variation in this variable remains unstudied. Oral water raises blood pressure in individuals with impaired autonomic reflexes and is an important source of noise in blood pressure trials in the elderly. On the average, oral ingestion of 16 ounces (473 ml) of water will raise blood pressure 11 mmHg in elderly normal subjects. In patients with autonomic

impairment such as multiple system atrophy, strikingly exaggerated pressor effects of water have been seen with blood pressure elevations greater than 75 mmHg not at all uncommonly. These responses tend to be maximal within 30 minutes of water ingestion and to be largely dissipated within 90 minutes.

References

Baylis PH 2001 Vasopressin, diabetes insipidus and syndrome of inappropriate antidiuresis. In: DeGroot LJ, Jameson JL (eds) Endocrinology. Saunders, Philadelphia, PA p 363–376

Faull CM, Holmes C, Baylis PH 1993 Water balance in elderly people: is there a deficiency of vasopressin? Age Ageing 22:114–120

Frolkis VV, Kritnitskaya-Ryzhora TY, Dubiley TA 1999 Vasopressin, hypothalame-neurohypophyseal system and ageing. Arch Gerontal Geriatr 29:193–214

Greenleaf JE, Averkin EG, Sargent F 1966 Water consumption by man in a warm environment: A Statistical Analysis. J Appl Physiol 54:414–419

Greenleaf JE, Morimoto T 1996 Mechanisms controlling fluid ingestion: Thirst and drinking. In: Buskirk ER, Puhl SM (eds) Body fluid balance: exercise and sport. CRC Press, Boca Raton, FL, p 3–16

Gordon MS, Majzoub JA, Williams GH, Gordon MB 1997 Sodium balance modulates thirst in normal man. Endocr Res 23:377–392

Helderman JH, Vestal RE, Rowe JW, Tobin JD, Andres R, Robertson GL 1978 The response of arginine vasopressin to intravenous ethanol and hypertonic saline in man: the impact of aging. J Gerontol 33:39–47

Hoogendijk JE, Fliers E, Swaab DF, Verwer RW 1985 Activation of vasopressin neurons in the human supraoptic and paraventiruclar nucleus in senescence and senile dementia. J Neurol Sci 69:291–299

Jacob G, Ertl AC, Shannon JR et al 1996 Idiopathic orthostatic tachycardia; the role of dynamic orthostatic hypovolemia and norepinephrine. Circulation 94:1–627

Jacob G, Ertl AC, Shannon JR, Robertson RM, Robertson D 1998 Effect of standing on neurohumoral responses and plasma volume in healthy subjects. J Appl Physiol 84:914–921

Jordan J, Shannon JR, Grogan E, Biaggioni I, Robertson D 1999 A potent pressor reflex elicited by drinking water. Lancet 353:723

Jordan J, Tank J, Diedrich A, Robertson D, Shannon JR 2000a Vasopressin and blood pressure in humans. Hypertension 36: E3–E4

Jordan J, Shannon JR, Black BK et al 2000b The pressor response to water drinking in humans: a sympathetic reflex? Circulation 101:504–509

Lesser GT, Kumar I, Steel JM 1963 Changes in body composition with age. Ann NY Acad Sci 110:576–588

Liu RY, Zhou JN, Hoogendijk WJ et al 2000 Decreased vasopressin gene expression in the biological clock of Alzheimer disease patients with and without depression. J Neuropathol Exp Neurol 59:314–322

Miller M 1995 Hormonal aspects of fluid and sodium balance in the elderly. Endocrinol Metab Clin North Am 24:233–253

Moses AM, Miller M, Streeten DH 1976 Pathophysiologic and pharmacologic alterations in the release and action of ADH. Metabolism 25:697–721

Preisser L, Teillet L, Aliotti S et al 2000 Downregulation of aquaporin-2 and -3 in aging kidney is independent of V(2) vasopressin receptor. Am J Physiol Renal Physiol 279:F144–152

Robertson D, Shannon JR, Jordan J et al 2001 Multiple system atrophy: New developments in pathophysiology and therapy. Parkinsonism Relat Disord 7:257–260

Robertson GL, Rose JW 1980 The effect of aging on neurophypophysial function. Peptides 1:159
Rossi P, Andriesse GI, Oey PL, Wienke GH, Roelofs JM, Akkermans JM 1998 Stomach distension increases efferent muscle sympathetic nerve activity and blood pressure in healthy humans. J Neurol Sci 161:148–155
Roth J, Koch CA, Rother KI 2001 Aging, endocrinology and the elderly patient. In: DeGroot LJ, Jameson JL (eds) Endocrinology. Saunders, Philadelphia, PA p 529–555
Schoeller DA 1989 Changes in total body water with age. Am J Clin Nutr 50:1176–1181

DISCUSSION

Handelsman: This is very interesting. A comment you made in passing about pharyngeal receptors prompted me to think of some experiments. It would be interesting to see in humans given fluid via a nasogastric tube whether you would see this phenomenon or not — in other words, whether you could localize the effect to the pharynx. The second issue is whether chemical sympathectomy via 6-hydroxydopamine would have effects like this in animals. It would also be interesting to deliver the water into the stomach, bypassing the pharynx, or actually allow the subject to drink the water, and then suck it out of the stomach as quickly as it is drunk.

Robertson: Studies on rats have been done. I believe they were not sympathectomized. There were some observations of increases in blood pressure with water ingestion. It was more prominent with water than with fluid with Na^+ in it. The question about pharyngeal receptors is very important, and I don't have an answer for that. It would be a wonderful study to do. Our current studies are focused on blowing up a balloon in the stomach to observe the effect of stretch. As you know, there are osmoreceptors in the liver also that might detect small changes in the diluteness of bloodflow from the liver. I don't know any other purpose for those osmoreceptors.

Veldhuis: It would have to involve more specific receptors, because just passage of food may not be relevant. I was thinking of the simple sugar experiment. It has been a clinical adage that bad hiccups can be sometimes suppressed by swallowing some granulated sugar irritating the glossopharangyeal nerve.

Laron: Your data on the orthostatic shift are extremely interesting. Elderly people drink less, so they are relatively dry. They are more prone to have arteriovascular incidents. If you take out so much fluid they could have more plugging of small arteries. What is the practical application of this? We know that if people don't move, they get more osteoporosis. How do you meet the needs of the population we are discussing here?

Robertson: That is a good point. There are data indicating there are more heart attacks in the early hours of the morning, soon after people have woken up. The main reason cited for this is stress, although other factors may be involved. But the

constituents of the blood are concentrated by standing. Although 12–15% increase in concentration might not seem much, it is certainly possible that a 12% increase in the platelet count might make blood clots in the heart more likely.

Laron: Would you say that elderly people should drink more before standing up?

Robertson: We view water as potentially a very dangerous drug! Seriously, though, we do ask people who have these rare autonomic disorders not to drink water after about 9 p.m. unless they are really thirsty. In some of these patients with dysautonomia, water can raise blood pressure by 90 mmHg, and if this occurs before they go to bed, the resulting hypertension could be deleterious.

Burger: Is the difference between oral versus intravenous fluid load in any way explicable by the speed at which the plasma compartment is expanded by the two routes?

Robertson: I wish I had a better way to do that experiment. In addition to the problem you have identified there is another: dextrose has its own tendency to lower blood pressure by calling out some of the gastrointestinal hormones that may themselves be vasodilatory. On the other hand, if we gave saline, this wouldn't be a fair test of water either. We tried to spread the saline out over an hour. We wish we could have just looked at volume.

Carroll: Could you do an isotope dilution study and look at the rate of entry of that water volume into the circulation?

Roberston: We need to do that. Although the haematocrit didn't change, the fluid could be distributed both intracellularly and extracellularly.

Carroll: It is probably not operating through blood volume changes, but rather through some sort of reflex.

Elahi: We have done some studies where we have infused hypertonic saline in young and old volunteers. There are great changes in atrial natriuretic peptide (ANP). It is delayed in the elderly. We did the opposite experiments in which we used half normal saline. There is very little change in the elderly. Do you have any information about brain ANP with respect to age?

Robertson: No, I don't.

Haus: In the elderly, there is a shift in urine excretion from daytime to night. This is not a function of the enlarging prostate because it occurs just as much in women as in men (Haus et al 1988). Do you have any explanation for this?

Robertson: It is in part circadian, because it isn't just supine posture that is responsible.

Haus: Other solutes in the urine such as adrenaline and noradrenaline do not shift. Their circadian rhythm remains unchanged in its timing and is dissociated from that in urinary volume excretion (Haus et al 1988).

Robertson: I don't have the answer, though there is a substantial literature about this.

Haus: The blood viscosity is higher in the morning, which may be one of the factors contributing to the morning incidence of myocardial infarction. The number of circulating granulocytes peaks in the late afternoon. Granulocytes and lymphocytes show high amplitude circadian rhythms. The number of circulating platelets peaks in the afternoon, but the amplitude of their rhythm is relatively small (Haus 1996). In contrast, platelet aggregation and adhesion peak in the morning (Haus et al 1990). This may be responsible for some of the cardiovascular and cerebrovascular accidents occurring at that time. Also, the plasminogen activator inhibitor (PAI), which determines the overall activity of the fibrinolytic system peaks in the morning decreasing the activity of the fibrinolytic component of the haemostatic system (Andreotti & Patti 1997).

Robertson: I don't know whether we really have data that will address the postural effects on platelets. For example, remember these changes in fluid occur over 10–15 minutes. It may be that acutely standing up in the morning could significantly increase the number of platelets within 10 minutes. This may be separate from the circadian variation.

Haus: The change in position may increase the function of platelets probably more than their number. Tofler et al (1987) studied platelet aggregation and found a rise with the change from recumbent to upright position which paralleled the rise in plasma catecholamines. We studied clinically healthy subjects in the morning while still lying down and during the day after 30 minutes in recumbent position and found the rise in platelet adhesion and aggregation (Haus et al 1990) a little earlier than Tofler et al (1987).

Robertson: The postural changes could induce additional changes on top of that.

Björntorp: As you mentioned, this work has practical clinical and research implications. Most blood sampling is done with the patient lying down, but some is when the patient is sitting. How long does it take before you reach equilibrium after lying down?

Robertson: I think it is 30 minutes to equilibrium in either change of posture. When I was an intern, patients would walk into the emergency room, get screening blood work and be admitted. Then they would become supine. In those days we didn't watch our testing too much, and the next day another blood test would show a 4% fall in the haematocrit, and it would appear that the patient had lost a unit of blood. As an intern, I was frequently pushed by my resident physician to find out where the patient was bleeding. We were probably observing this volume shift.

Veldhuis: We learned this in our General Clinical Research Center. If the nurses call you for a slightly low haematocrit, have the patient walk about for 30 minutes and repeat the blood count: it will typically be normal.

Björntorp: What about sitting?

Robertson: I don't have data on sitting.

Prior: We need to find out, because this is the posture in which most samples are obtained.

Müller: Would it be worthwhile testing this water role in Parkinsonian patients: many of them have a natural orthostatic hypotension. There are data showing that the noradrenergic innervation of the heart of these patients is decreased (Goldstein et al 2000). In your data you show that there is a blockade of dopamine β-hydroxylase which will involve an increase in the dopaminergic tone.

Robertson: We recorded our findings about water in the literature about 18 months ago. Then, investigators in France gave water to patients with Parkinson's disease and did not see any effect. I haven't yet gone back and looked at Tennessee Parkinson's disease patients.

References

Andreotti F, Patti G 1997 Chronobiology of the haemostatic system. In: Redfern PH, Lemmer B (eds) Handbook of experimental pharmacology: physiology and pharmacology of biologic rhythms. Springer-Verlag, Heidelberg, vol 125:533–555

Goldstein DS, Holmes CMT, Li S-T, Bruce S, Metman LV, Cannon RO III 2000 Cardiac sympathetic denervation in Parkinson disease. Ann Intern Med 133:338–347

Haus E 1996 Biologic rhythms in hematology. Path Biol 44:618–630

Haus E, Nicolau GY, Lakatua D, Sackett-Lundeen L 1988 Reference values for chronopharmacology. Annu Rev Chronopharmacol 4:333–424

Haus E, Cusulos M, Sackett-Lundeen L, Swoyer J 1990 Circadian variations in blood coagulation parameters, alpha-antitrypsin antigen and platelet aggregation and retention in clinically healthy subjects. Chronobiol Int 7:203–216

Tofler GH, Brenzinski D, Schafer AL et al 1987 Concurrent morning increase in platelet aggregability and the risk of myocardial infarction and sudden cardiac death. N Engl J Med 316:15141–518

Endocrine Facets of Ageing.
Novartis 242

Print ISBN 0-471-48636-1 Online ISBN 0-470-84654-2

Summing-up

Johannes Veldhuis

University of Virginia Health System, NIH General Clinical Research Center, Department of Internal Medicine, Division of Endocrinology and Metabolism, PO Box 800202, Charlottesville, Virginia 22908-0202, USA

Abraham Lincoln once said that it is better to remain silent and appear ignorant than to speak freely and remove all doubt! Thus, I will attempt briefly to highlight some of the discussions we have had over the last few days. Ageing can be viewed as an array of sequelae, some of which we interpret as undesirable, ranging from decreased bone mass to atrophic skin and hair greying; and from relative sarcopenia and variable cognitive defects to increased visceral fat and heightened risk of cardiovascular disease. This panoply is somehow directed by an ensemble of cellular and systemic factors. The resultant complexity of the ageing process is thus challenging. Causal endocrine and non-endocrine factors undoubtedly overlap, even in ways beyond those we recognize. From an endocrine perspective, there is a substantial decline of GH and sex-steroid input to target cells. A gradual reciprocal increase in integrated cortisol receptor drive throughout age seems to contribute to catabolic changes, particularly in a waning anabolic context of attenuated GH/IGF1 and sex-steroid production. Concomitantly, alterations in the insulin pathway condition cellular signalling in ageing, which strongly influences epidemiological risk. Operating on this fourfold hormonal background is the genetic endowment and any number of environmental factors. Only the thyroidal axis seems to be spared substantially. This symposium has tried to unravel some of the intersecting causes and consequences of these neurohormonal changes in ageing.

Endocrine Facets of Ageing.
Novartis 242

Print ISBN 0-471-48636-1 Online ISBN 0-470-84654-2

Index of contributors

Non-participating co-authors are indicated by asterisks. Entries in bold type indicate papers; other entries refer to discussion contributions.

Endocrine Facets of Ageing.
Novartis 242

Print ISBN 0-471-48636-1 Online ISBN 0-470-84654-2

Subject index

C

D