EARLY NUTRITION AND ITS LATER CONSEQUENCES: NEW OPPORTUNITIES

Perinatal Programming of Adult Health – EC Supported Research

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PREFACE

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Nutrition during pregnancy and infancy has powerful programming effects on long-term development and health of the child, extending well into adulthood and old age. Exploration and understanding of these fascinating interrelationships offer new opportunities for improving nutrition policy, public health, and nutritional products. Up-to-date information on this exciting area of research is presented in this volume. The contributions are based on presentations and discussions at a European Commission supported Scientific Conference held on 2-3 July 2004 at Paris, France, that preceded the 2nd World Congress on Pediatric Gastroenterology, Hepatology and Nutrition. The conference has been supported by a grant of the European Commission's Fifth Framework Programme "Quality of Life and Management of Living Resources" as an Accompanying Measure to the research project "Childhood Obesity – Programming by Infant Nutrition?", one of three large research projects of the "EU Infant Nutrition Cluster". The conference was organized by the University of Munich, Germany, in close collaboration with the Danone Institutes International, and was attended by some 430 participants from 57 countries, with backgrounds in research, public health, governmental organizations, food and dietetic industry, and health care. The plenary lectures, 86 poster presentations and focus group sessions allowed for intensive exchange of information, fruitful discussions, and the transfer of knowledge for new applications in research, policy and practice.

We are very grateful indeed to the European Commission and Danone Institute International for their generous financial support, and to the International Association of Infant Food Manufacturers and the University of Munich for ancillary funding. We also thank Prof. Hans

Akerblom, Dr. Peter Dodds, and the members of the Scientific Advisory Committee for active help in developing the scientific programme; Prof. Olivier Goulet, President of the 2nd World Congress on Pediatric Gastroenterology, Hepatology and Nutrition for the integration of the conference with the World Congress; Dr. Doris Oberle, Dr. Hans Demmelmair, Agnes Martin and Sandrine Piredda for their untiring work organizing the conference; Dr. Margaret Ashwell for her thoughtful and vigilant input in preparing and disseminating the conference information, and for editing these proceedings; Alkmini Katsada, Isabelle de Froidmont-Goertz, and Achim Boenke from the EC Directorate General Research for their sympathetic support; and the many other friends and colleagues who helped to make this project a success.

May this volume be useful to its readers and stimulate further progress in research and practice in the area of early nutrition.

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University of Munich

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Danone Institute International

WHAT IS THE EU INFANT NUTRITION CLUSTER?

The Infant Nutrition Cluster is an association of three research projects funded by the EU; all are concerned with the effects of early nutrition on the health and development of the newborn child.

These research projects all aim to assess the roles of early nutritional influences on the current and future well-being of the child and its mother as well as to determine the potential of nutritional interventions during pregnancy and infancy to modify health and well-being.

1. CHILDHOOD OBESITY: PROGRAMMING BY INFANT NUTRITION?

Childhood obesity is a major public health problem. Breastfed infants are less likely to become obese children than infants fed formula. The higher protein content of infant formulae, compared with breast milk, could be a causal factor.

The EU Childhood Obesity Programme, includes a one year multicentre intervention trial on new-born infants, to see whether feeding infant formulae, which differ in their level of milk proteins, can influence the risk of later childhood obesity.

The trial is taking place in five countries with different habitual total protein intakes to test the 'early protein hypothesis', namely that early protein intake predicts infant growth and later risk of childhood obesity.

Expected achievements include:
- Improved health and quality of life by preventing childhood obesity.
- Promotion of the benefits of breast-feeding.
- A better understanding of consumer (parental) attitudes to infant feeding.

Applications are:

• The potential for the development of new infant foods (formula and complementary foods).
• The provision of safety data for infant formula with adequate protein content.
• The provision of information for the training of health professionals to make it easier for them to advise consumers about infant feeding.

Contract Number: QLK1-CT-2001-00389 www.childhood-obesity.org

2. INFLUENCE OF DIETARY FATTY ACIDS ON THE PATHOPHYSIOLOGY OF INTRAUTERINE FOETAL GROWTH AND NEONATAL DEVELOPMENT

Nutrition during pregnancy and early life is known to affect the health and development of the new-born child. A foetus that suffers intra-uterine growth restriction (IUGR) is more likely to suffer from heart problems or diabetes in later life.

PERILIP adopts a multidisciplinary approach to the study of lipids in perinatal nutrition.

Six partners, from as many countries, combine expertise in obstetric monitoring of foetal development, nutrition of premature infants, nutritional studies using animal models and the structure and functioning of the placenta as well as a range of lipid analytical techniques.

Expected achievements includes:
• An improved understanding of the roles of different fatty acids in the diets of women at different stages of pregnancy and during lactation.
• An improved understanding of the roles played by antioxidant status in the perinatal nutrition.

Potential applications are:
• The refinement of dietary recommendations for specific times during pregnancy and lactation.

• Improvement of formulations for intravenous feeding of premature babies.
• Protocols to reduce neonatal mortality in piglets, a common welfare and economic problem in the pig industry.

Acronym : Perilip
Contract Number : QLK1-CT-2001-00138
www.wye.ic.ac.uk/Perilip

3. TRIAL TO REDUCE IDDM IN THE GENETICALLY AT RISK (NUTRITIONAL PRIMARY PREVENTION OF TYPE 1 DIABETES)

Type 1 diabetes in children is a major health problem in Europe, and the incidence of the disease is increasing. The disease develops in genetically susceptible individuals, if one or several environmental factors (of which cow milk proteins are one of the main candidates) lead to autoimmune destruction of the pancreatic beta-cells.

The objective of TRIGR is to determine whether denial of nutritional cow milk proteins for at least the first 6 m of life reduces the incidence of Type 1 diabetes in children with increased genetic risk of developing the disease and/or the appearance of diabetes associated auto-antibodies by the age of 6 and 10 years.

Expected Achievements are to find an answer to the important question of the incidence of Type 1 diabetes in children by dietary intervention in infancy in subjects with increased genetic risk.

Application is the potential to use modified formula after exclusive breastfeeding to decrease the risk of Type 1 diabetes in subjects with increased genetic risk.

Acronym: TRIGR or DIABETES PREVENTION
Contract Number: QLK1-CT-2002-00372
www.trigr.org

EARLY NUTRITION AND ITS LATER CONSEQUENCES: NEW OPPORTUNITIES

Perinatal nutrition programmes adult health

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Abstract: Some 30 years ago Dörner proposed that disease risk and body functions in human adults are programmed during critical early periods of development by hormones and metabolites. Indeed, dietary factors in pregnant and lactating women and in their children were shown to modulate growth and functional development of the organism and to exert life-long programming effects on health, disease and mortality risks in adulthood, neural function and behaviour, and the quality of life. Much of the available evidence on nutritional programming in humans has come from historical observational studies that cannot examine the association with diet directly, establish whether associations are causal, and identify appropriate dietary recommendations for pregnant women and infants. Also, open questions exist on the critical pre- and postnatal time periods during which nutritional exposures programme later health. Therefore, a new approach is required to study early programming of adult health that integrates evidence from randomised controlled trials in humans, prospective observational studies and animal experiments. Considering the far-reaching consequences for public health, policy and product development, major investments in research on early nutritional programming are justified.

Key words*:* metabolic programming, metabolic imprinting, developmental origins of adult disease risk, accelerated early growth, infant feeding

1. SCIENTIFIC EXPLORATION OF THE ROLE OF EARLY NUTRITION FOR LONG TERM HEALTH

Evidence accumulates that food supply and the metabolism of food ingredients in women during pregnancy and lactation and in their children have marked implications on child development and long-term health. Epidemiological evidence and intervention studies performed in pregnant women and in infants have highlighted the fact that maternal and intrauterine influences are of special importance during the development of the infant and child. Early nutrition modulates growth and functional development of the organism and appears to exert life-long programming effects that modulate health, disease and mortality risks in adulthood, neural function and behaviour, and quality of life. The scientific exploration of these relationships and their underlying mechanisms offer new windows of opportunity for preventive health concepts, the provision of sound nutritional advice, and the development of improved food products for mothers and children.

The latest knowledge on long-term programming effects of early nutrition, future trends and the potential for application was discussed by some 450 scientists at a European Commission supported Scientific Workshop held at Paris, France, in July 2004, as a satellite meeting to the 2nd World Congress on Paediatric Gastroenterology, Hepatology and Nutrition. This volume summarises the data and concepts discussed at this workshop. The meeting was organised on behalf of the "EU Infant Nutrition Cluster", a collaboration of three large research projects supported by the European Commission's Fifth Framework Programme that all investigate long-term consequences of nutrition and metabolism in early life:

- Childhood Obesity – Early Programming by Infant Nutrition (www.childhood-obesity.org; QLRT–2001–00389; Coordinator Prof. Berthold Koletzko, University of Munich, Germany)
- Influence of Dietary Fatty Acids on the Pathophysiology of Intrauterine Foetal Growth and Neonatal Development (www.wye.ie.ac.uk; QLRT – 2001 –00138; Coordinator Dr. Peter Dodds, Imperial College, Wye, UK)
- Nutritional Primary Prevention of Type 1 Diabetes (www.trigr.org; QLRT – 2001 – 00372; Coordinator Prof. Hans Akerblom, Univ. of Helsinki, Finland)

The main goals of this scientific workshop on long-term effects of early nutrition were a) to provide a platform for a critical review of current knowledge on early nutritional programming of adult health and well-being, b) to discuss new results and open questions, c) to explore evolving opportunities for research, public health, policy and product development, d) to strengthen networking and involvement of young researchers, scientists from Central & Eastern Europe, outside Europe, industry and small and medium size enterprises, and e) to publish the lectures and workshop summaries to inform EU policy makers, researchers and the public. The workshop has been organised by the Div. of Metabolic Diseases and Nutrition at the Dr von Hauner Children‘s Hospital, University of Munich, Germany, in collaboration with the International Danone Institutes.

2. THE 30TH ANNIVERSARY OF METABOLIC PROGRAMMING OF ADULT HEALTH

The workshop commemorated the 30th anniversary of the introduction of the term “Programming” into the scientific literature in 1974 by Professor Günter Dörner, former head of the Institute of Experimental Endocrinology at the Charité Hospital, Humboldt University at Berlin, Germany (1). In a visionary article reviewing a series of clinical and experimental data, Dörner concluded that the concentrations of hormones, metabolites and neurotransmitters during critical early periods of development are capable of pre-programming brain development, functional disturbances, diseases as well as syndromes of reproduction and metabolism in human adulthood. Dörner also proposed an interaction between the genetic material of the individual and environmental influences during early development to determine later function in adult life, a concept that only recently has been confirmed by experimental data (2,3). Over many more years, Dörner and coworkers continued to study programming effects of perinatal metabolic and endocrine factors on later risk of diabetes, obesity and cardiovascular risk in a series of systematic studies (4,5).

Even though the thoughts of Dörner were revolutionary at that time, developmental plasticity and thus long term imprinting effects of early events are widely known in biology. For example, gametic imprinting through epigenetic modification during gonadal passage with parent-

specific gene expression causes serious diseases, including the Silver-Russel-syndrome, the Beckwith-Wiedemann-syndrome, the Prader-Willi-syndrome and the Angelman-syndrome (6). Intrauterine exposure to sex hormones during sensitive time windows of development programmes gender identity and gender specific cerebral lateralization (7,8). A critical dependency on the timing of exposure during sensitive time windows of early development is also known for teratogenic effects of radiation, infectious agents, drugs such as thalidomide and intrauterine metabolic insults such as hyperphenylalanemia due to maternal phenylketonuria (9,10).

Programming effects of early nutrition were first studied by Widdowson and McCance in the 1960ies. Limited periods of undernutrition in rats during the early postnatal period led to permanent alterations of adult body weight and body composition in spite of free access to food after the intervention period (11). In contrast, later undernutrition had no lasting effects. Widdowson summarized their observations as follows: "The size that animals undernourished at different stages of development can be expected to attain when they are rehabilitated depends on the stage of development that they were at when they were undernourished."

In spite of this experimental support, Dörner's hypothesis of early programming of human adult health only received wider recognition when Alan Lucas from Cambridge, UK, rediscovered the concept and the term programming (12). Lucas and co-workers introduced systematic long-term follow-up first of preterm, then also of term infants fed different diets during early life, which allowed them to test the programming hypothesis. Wide popularity for the programming concept was achieved when David Barker and co-workers provided strong epidemiological evidence for a link between anthropometric measures at birth and later morbidity and mortality in adult life (13). Indeed, the concept that early nutrition and growth programmes adult disease risk is often called the "Barker hypothesis" (14,15), even though the hypothesis was created almost 20 years prior to Barker's insightful studies and thus might rather deserve the name "Dörner hypothesis" (1).

3. FETAL OR POSTNATAL ORIGINS OF ADULT DISEASE?

The observation that body weight at birth and at age 1 year, respectively, is inversely related to the risk of hypertension, diabetes and coronary heart disease in adulthood lead Barker to suspect that maternal malnutrition during pregnancy would lead both to foetal growth restriction and increased risk of later disease, the foetal origins of adult disease hypothesis (13). However, this interpretation has recently been challenged based on the observation that low birth weight is associated with catch-up growth after birth, and accelerated weight gain by itself might be a risk factor for later disease (16).

Cole substantiated the latter concept by multiple-regression analysis of blood pressure outcomes on weights at different ages. Data from cohort studies from Brazil and the Philippines relating blood pressure in adolescence to weight through childhood showed small inverse weight effects in infancy, but early weight proved to be less important than weight and weight gain during adolescence (16). Furthermore, Tu and co-workers raised the possibility that evidence for the foetal origins of adult disease hypothesis might be a statistical artefact, due in part to inappropriate statistical adjustment for variables on the causal pathway such as early weight gain and current body size, which may create an artefactual statistical effect known as the "reversal paradox" (17). They performed computer simulations for three hypothetical relations between birth weight and adult blood pressure. The effect of statistically adjusting for different correlations between current weight and birth weight and between current weight and adult blood pressure was examined to assess their impact on associations between birth weight and blood pressure. When there was no genuine relation between birth weight and blood pressure, adjustment for current weight created an inverse association whose size depended on the magnitude of the positive correlations between current weight and birth weight and between current weight and blood pressure. When there was a genuine inverse relation between birth weight and blood pressure, the association was exaggerated following adjustment for current weight, whereas a positive relation between birth weight and blood pressure could be reversed after adjusting for current weight. Thus, researchers must consider the reversal paradox when adjusting for variables that lie within causal pathways.

Further evidence accumulates that high early weight gain is associated with greater disease risk at later ages. We studied early

predictors of overweight at school age in a large cohort of 4235 children in Bavaria, Southern Germany (18). Weight, length, body mass index, and ponderal index differences between birth, 6 months, 12 months, and 24 months of age were compared by receiver operating characteristic curves and predictive values for later overweight. For all variables, the largest area under the receiver operating characteristic curve was observed for weight gain between birth and age 2 years (0.76 [95% confidence interval, 0.74-0.79]). Thus, high weight gain from birth to 24 months predisposes for later overweight. This relationship might be at least one of the reasons why infants breast fed after birth have a lower risk of overweight and obesity in later life than previously formula fed individuals (19-21), since breast feeding is associated with lesser weight gain in the first year of life than formula feeding (22). Rapid early growth has also been associated with higher later risks for dyslipidemia, markers of insulin resistance, endothelial dysfunction, and cardiovascular diseases (23). Thus, further research needs to elucidate the critical time periods during which nutritional exposures may programme later disease risk.

4. EXPLORING LONG TERM EFFECTS OF NUTRITIONAL PROGRAMMING AND UTILISING THE PREVENTIVE POTENTIAL

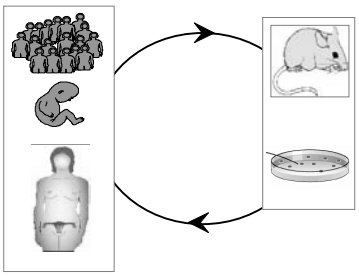
The available data from epidemiological studies, and to some extent also from prospective intervention studies, provide evidence for lasting effects of early nutrition during the pre- and postnatal period on later cardiovascular health, obesity, neural and brain function, immune function and allergy risk, diabetes type I and bone health. These data indicate the enormous potential for health prevention, improved performance and well-being by appropriate perinatal nutrition. However, the available evidence of nutritional programming in humans has, until recently, come largely from historical observational studies that have shown associations between small size in early life and adult disease risk. These cohorts have been constructed from available maternal or child health records and have necessarily relied on indirect measures of maternal and infant nutrition (rather than direct measures of maternal or infant diet) and have lacked detailed data on potential confounding variables. Many of these cohorts were born before the Second World

War and it is possible that the nature and size of the associations is different in contemporary European populations. While these studies have generated considerable interest they have been unable to:

- examine the association with diet directly,
- establish whether associations are causal (because of the observational nature of the data), and
- identify appropriate dietary recommendations for pregnant women, small babies and small infants

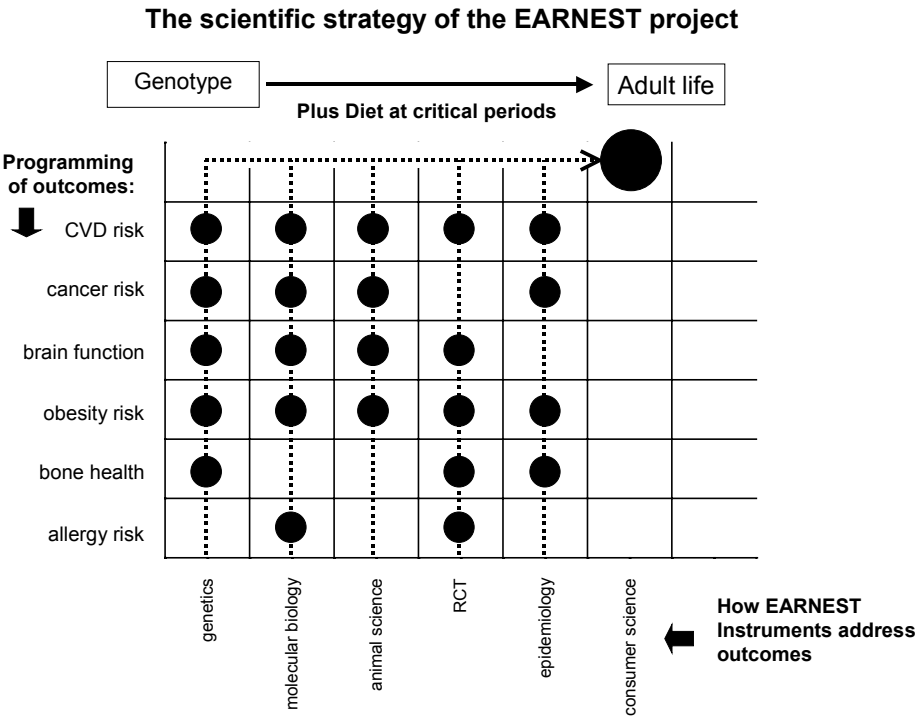
Therefore, a new approach is now required to study early programming of adult health that integrates evidence from randomised controlled trials in humans (RCTs), prospective observational studies and animal experiments. In the EU 6th Framework Programme, the project "*Early Nutrition Programming of Adult Health - Long term follow up of efficacy and safety trials and integrated epidemiological, genetic, animal, consumer and economic research* (*EARNEST)* will bring together a unique group of partners from 13 countries and of resources into just such an integrated programme of research (www.metabolic-programming.org). The project is planned to extend

The EARNEST project will integrate human, animal, molecular and cell studies to develop and test new hypotheses



from 2005 to 2010 with an allocated grant support by the European Commission of 13.4 million € and a total budget of approximately 20 million €. The scientific objectives of EARNEST will be achieved through the follow-up of informative randomized controlled trials in humans (conducted both in pregnant women and infants), through analyses of large contemporary prospective observational studies that have collected data on diet in pregnancy and in the first years of life, together with clearly defined animal studies aimed at defining the underlying mechanisms, including gene regulation and the integration and synthesis of knowledge from these three approaches. The expected achievements of this approach include:

- provision of a fully integrated approach to work on programming in humans,
- demonstration of causal associations in humans,
- establishment of the long-term *safety* as well as efficacy of early interventions,
- exploration of fundamental mechanisms to guide future intervention studies,
- estimation of the biological, social and economic importance of early nutritional programming,
- formulation of evidence-based policy and practice, and
- development of appropriate products and creation of wealth in Europe.



The approach taken by the EARNEST project is to use the best methodology and technology available to investigate the hypothesis and its implications. This consortium has assembled a majority of the key

relevant intervention trials conducted in infancy and pregnancy in this field for exploitation. Many of these trials were targeted at the outset to explore long-term health outcomes. Others were set up to examine shorter-term effects but the cohorts can now be exploited for long-term follow up. This emphasis on randomized trials and prospective observational trials means that the conclusions will be based on a sound scientific footing. In addition they will be of a sufficient quality to provide the level of detail required to quantify the size of the effects and thus their relative importance, economic impact and detect adverse effects.

The proposed strategy of utilizing *existing* trials and cohorts has (i) the economic benefit of exploiting vast prior expenditure on randomizing and maintaining these cohorts, and (ii) the scientific benefit of allowing us to study cohorts, randomly assigned to early nutrition, that have now reached an age when outcomes are relevant to adult health and morbidity. We recognize that in using randomized trials it is impractical to study some late adult endpoints such as stroke. However some outcomes have predictive value even from childhood (e.g. IQ). Moreover, changes in cardiovascular risk factors such as blood pressure and LDL cholesterol, which we shall measure in adults, have published predictive value for subsequent endpoint events. Indeed, demonstration in a randomized trial of a clear causal effect of an early nutritional intervention in reducing adult diastolic blood pressure (given the known association between each mm change in blood pressure and later risk of cardiovascular death), would be of much greater value in underpinning practice than the speculated causal significance of an observed relationship between birth weight and later ischaemic heart disease.

5. RELEVANCE OF PROGRAMMING FOR PUBLIC HEALTH

The general concept that early nutrition might programme long-term health has potentially far-reaching consequences. Recently published data give insight into the large potential effects sizes of early nutritional intervention and long-term health. The reported effect of early feeding with human milk or with formula providing long-chain polyunsaturated fatty acids, respectively, on lowering later mean or diastolic blood pressure by around 3-4mm Hg (24,25) is greater than all other non-

pharmacological means of reducing blood pressure such as weight loss, salt restriction, or exercise. These data must be viewed against the finding that lowering population-wide diastolic blood pressure by only 2 mm Hg would be expected to reduce the prevalence of hypertension by 17%, the risk of coronary heart disease by 6% and the risk of stroke/transient ischaemic attacks by 15% (23). Similarly, the 10% lowering of cholesterol shown in a recent randomised trial of early nutrition compares favourably with the effects of dietary interventions in adults, which lower cholesterol by only 3-6%. Such an effect on cholesterol concentration would be expected to reduce the incidence of cardiovascular disease by approximately 25% and mortality by 13-14%. These examples indicate that early nutrition may be one the most important influences on long term health that can be manipulated by public health practice and emphasises the immense importance of this field. Thus, further high quality research is needed to provide the critical data on the impact of a range of early nutritional interventions (whole diets and individual nutrients) in both healthy and high risk populations, and covering a range of programmed outcomes that include most of the main areas of adult morbidity in the West (hypertension, obesity, diabetes, vascular health, bone health, immune health and cancer). Such data should provide a strong scientific basis for the intelligent promotion of health in European populations.

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REFERENCES

1. Dörner G. Perinatal hormone levels and brain organization. In: Stumpf WE, Grant LD (eds) Anatomical neuroendocrinology. Basel, Karger 1975:245-52

2. Schmidt I., Schoelch C., Ziska T., Schneider D., Simon E., Plagemann A.. Interaction of genetic and environmental programming of the leptin system and of obesity disposition. Am J Physiol Physiol Genomics 2000;3:113-120.
3. Ozanne SE, Fernandez-Twinn D, Hales CN. Fetal growth and adult diseases. Semin Perinatol. 2004 Feb;28(1):81-7.
4. Dörner G, Plagemann A. Perinatal hyperinsulinism as possible predisposing factor for diabetes mellitus, obesity and enhanced cardiovascular risk in later life. Horm Metab Res 1994;26:213-21
5. Plagemann A. 'Fetal programming' and 'functional teratogenesis': on epigenetic mechanisms and prevention of perinatally acquired lasting health risks. J Perinat Med 2004; 32:297-305
6. Jiang YH, Bressler J, Beaudet AL. Epigenetics and human disease. Annu Rev Genomics Hum Genet. 2004;5:479-510
7. MacLaughlin DT, Donahoe PK. Sex determination and differentiation. N Engl J Med. 2004 Jan 22;350(4):367-78.
8. Cohen-Bendahan CC, Buitelaar JK, van Goozen SH, Cohen-Kettenis PT.Prenatal exposure to testosterone and functional cerebral lateralization: a study in same-sex and opposite-sex twin girls. Psychoneuroendocrinology. 2004 Aug;29(7):911-6.
9. Brent RL, Beckman DA. The contribution of environmental teratogens to embryonic and fetal loss. Clin Obstet Gynecol. 1994 Sep;37(3):646-70
10. Koch R, Hanley W, Levy H, Matalon K, Matalon R, Rouse B, Trefz F, Guttler F, Azen C, Platt L, Waisbren S, Widaman K, Ning J, Friedman EG, de la Cruz F. The Maternal Phenylketonuria International Study: 1984-2002. Pediatrics. 2003 Dec;112(6 Pt 2):1523-9
11. Widdowson EM, McCance RA, The effect of finite periods of undernutrition at different ages on the composition and subsequent development of the rat. *Proc. Roy. Soc., Lon.* 158, 329-342 (1963).
12. Lucas A. Programming by early nutrition in man: In: Bock GR, Whelan J Eds. The childhood environment and adult disease. (CIBA Foundation Symposium 156). Whiley, Chichester, UK. 1991: 38-55.
13. Barker D. Mothers, babies and diseases in later life. London, BMJ Publishing Group 1994
14. Khan IY, Lakasing L, Poston L, Nicolaides KH. Fetal programming for adult disease: where next? J Matern Fetal Neonatal Med. 2003 May;13(5):292-9.
15. Ellison PT. Evolutionary perspectives on the fetal origins hypothesis. Am J Hum Biol. 2005 Jan-Feb;17(1):113-8.
16. Cole TJ. Modeling postnatal exposures and their interactions with birth size. J Nutr. 2004 Jan;134(1):201-4.
17. Tu YK, West R, Ellison GT, Gilthorpe MS. Why Evidence for the Fetal Origins of Adult Disease Might Be a Statistical Artifact: The "Reversal Paradox" for the Relation between Birth Weight and Blood Pressure in Later Life. Am J Epidemiol. 2005 Jan 1;161(1):27-32.
18. Toschke AM, Grote V, Koletzko B, von Kries R. Identifying children at high risk for overweight at school entry by weight gain during the first 2 years. Arch Pediatr Adolesc Med. 2004 May;158(5):449-52.
19. von Kries R, Koletzko B, Sauerwald T, von Mutius E, Barnert D, Grunert V, von Voss H. Breastfeeding and obesity: cross sectional study. Brit Med J 1999;319:147-150

20. Toschke AM, Vignerova J, Lhotska L, Osancova K, Koletzko B, von Kries R. Overweight and obesity in 6- to 14- year-old Czech children in 1991: protective effect of breastfeeding. J Pediatrics 2002;141:764-769
21. Arenz S, Rückerl R, Koletzko B, von Kries R. Breast-feeding and childhood obesity. A systematic review. Int J Obesity 2004;28:1247-1256
22. Kramer MS, Guo T, Platt RW, Vanilovich I, Sevkovskaya Z, Dzikovich I, Michaelsen KF, Dewey K; Promotion of Breastfeeding Intervention Trials Study Group. Feeding effects on growth during infancy. J Pediatr. 2004 Nov;145(5):600-5.
23. Singhal A, Lucas A. Early origins of cardiovascular disease: is there a unifying hypothesis? Lancet. 2004 May 15;363(9421):1642-5.
24. Singhal A, Cole TJ, Lucas A. Early nutrition in preterm infants and later blood pressure: two cohorts after randomised trials. Lancet. 2001 Feb 10;357(9254):413-9.
25.Forsyth JS, Willatts P, Agostoni C, Bissenden J, Casaer P, Boehm G. Long chain polyunsaturated fatty acid supplementation in infant formula and blood pressure in later childhood: follow up of a randomised controlled trial. BMJ. 2003 May 3;326(7396):953.

THE DEVELOPMENTAL ORIGINS OF ADULT HEALTH AND WELL-BEING

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1. THE CONCEPT OF PROGRAMMING

Previously, nutritional scientists focused on meeting nutritional needs and preventing deficiencies. This focus has changed radically. Current interest lies in the biological effects that nutrition has on health, notably lifetime health.

That nutrition has lifetime effects raises a broader concept concerning the general importance of early life events. In this context, I popularised the term "programming" - the idea that "a stimulus or insult during a critical or sensitive period of development, can have long-term or life-time effects on the organism". Many short-lived internal 'signals' or environmental experiences, operating during brief critical periods, have lifetime effects.

2. EVIDENCE FROM ANIMALS

Evidence that early nutrition has such 'programming' effects in animals is overwhelming. Adult outcomes programmed by infant nutrition include lipid metabolism, blood pressure, obesity, diabetes, arteriosclerosis, behaviour and longevity. Such programming occurs in diverse species, including primates.

In humans, observational studies link adult disease with size or mode of nutrition in early life. Yet, it is difficult to use observational associations to prove causation, and hence underpin health policy. However, over 20 years ago we used the pharmaceutical intervention trial model to test prospectively long term effects of early diet, randomly assigned, on health and neurodevelopmental outcomes. Given the need for long-term follow up to detect emergence of programmed effects, the major impact of early nutrition has only recently emerged.

3. STUDIES ON PREMATURE BABIES

The longest-term experimental evidence for programming is based on studies of premature babies. We showed only 2-4 weeks of randomised dietary manipulation in neonates programmes in adolescence and beyond: (1) key components of the metabolic syndrome - blood pressure, tendency to obesity and diabetes and blood lipids, (ii) the first stages of the atherosclerotic process (determined by ultrasound), (iii) brain structure and function (iv) bone health, possibly relevant to degenerative bone disease, (v) atopy. Effect sizes are large. Thus early diet has a greater effect on later cardiovascular risk factors than lifestyle modification in adulthood.

4. STUDIES ON FULL TERM INFANTS

However, programming also occurs in healthy full-term infants, through effects of specific nutrients (e.g. iron, long-chain polyunsaturated fatty acids) or whole diets (e.g. breast milk).

Importantly for some outcomes, early nutrition may operate by influencing postnatal growth. Early growth acceleration in invertebrates and vertebrates carries long term health costs. Our new experimental evidence shows in humans faster postnatal growth is a major adverse influence on later cardiovascular risk.

5. POSTNATAL GROWTH ACCELERATION HYPOTHESIS

Relative effects of fetal versus postnatal growth need reappraisal. We suggest the postnatal period is particularly important and the risk for the small fetus may relate to deleterious postnatal growth acceleration ('catch-up') seen in this population. Thus the 'fetal origins hypothesis' may be largely explained in terms of the broader 'growth acceleration hypothesis'.

6. A BALANCE OF RISKS

Designing optimal early nutritional policies requires balance of risks. In premature babies, a high plane of nutrition benefits later brain development but adversely programmes cardiovascular health. Defining the corresponding balance of risks in healthy full-term infants is a critical priority.

7. CONCLUSIONS

In summary, 40 years of animal and human studies show early nutrition is a key factor for health with major biological and social implications.

LONG TERM EFFECTS OF BREASTFEEDING ON THE INFANT AND MOTHER

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Abstract: There is increasing evidence that breastfeeding has long term beneficial effects on the infant. The most important are improved cognitive development, reduced incidence of immune related diseases (e.g. Type-1 diabetes and inflammatory bowel disease), and childhood cancers. A reduced risk of breast cancer in the mother is another important benefit.

Key words: Breastfeeding; long-term effects; cognitive function, growth; cardiovascular effects; immune system; allergy; maternal effects.

1. INTRODUCTION

Breastfeeding (BF) has numerous advantages for the child and affects several physiological mechanisms/systems. Not only short-term effects, but also effects in later life have been reported. Many of these health benefits exhibit dose-response relationships; i.e. longer duration of BF is associated with greater degrees of benefit. The WHO recommended optimal duration of exclusive BF is 6 months (WHO, 2002). This is based on a systematic review of studies from developed and -developing countries comparing growth, development, morbidity, and mortality of infants with different durations of BF (Kramer and Kakuma, 2002). The aim of this paper is to briefly review the effects of BF on infant and maternal health with emphasis on long term effects. The effect of BF on childhood obesity is covered in another chapter.

2. METHODOLOGICAL CONSIDERATIONS

Studies examining the effects of BF are mainly based on observational studies, as it is unethical to randomise newborn infants to BF or formula. This presents methodological challenges due to possible sources of errors such as confounding, reverse causality and selection bias, e.g. social status and maternal education are strong predictors of the duration of BF and some of the outcomes studied. The quality of observational studies has, however, improved over the last 15 years through improved designs and better control for relevant confounders.

3. COGNITIVE DEVELOPMENT

Many studies have found an association between BF and cognitive development. A meta-analysis including studies of children between 6 months and 15 years reported an overall effect of 3.2 IQ points after controlling for potential confounders (Anderson et al., 1999). The effect was stronger in preterm infants. There was a significant dose-response relationship with the duration of BF and the effects seemed to be independent of the age at which the outcome was measured. One study has suggested that this effect persists into later life since a positive association between duration of BF and intelligence in a group of men (mean age 18.7) and in another group of both women and men (mean age 27.2) was found (Mortensen et al., 2002). Different IQ tests were, used for the two groups.

The most plausible explanation for the positive effects of BF on mental function is the higher level of long chain poly-unsaturated fatty acids (LCPUFA) especially the n-3 fatty acid docosahexaenoic acid (DHA.) in breast milk compared to infant formula, DHA accumulates in neural membranes during infancy (Lauritzen et al., 2001; Michaelsen et al., 2003; SanGiovanni, 2000). Preterm infants have a lower LCPUFA status which supports this mechanism. Although the effect on cognitive function is not large in the individual it can have an important impact at the population level.

4. GROWTH

Breast-fed infants generally exhibit a different growth pattern to that of formula-fed infants. The weight gain of breastfed infant is lower and, in some studies, the length gain is also affected. Furthermore, breast-fed infants are generally leaner then formula-fed infants by 12 months of age (Michaelsen et al., 1994; Dewey, 1998). There are no known adverse consequences related to the slower growth in breast-fed infants.

5. IMMUNE SYSTEM AND ITS DISORDERS

Breast milk contains many immune factors, which give the infant passive protection against infections. The most important immune related factors are leucocytes including B and T lymphocytes, macrophages, neutrophiles, secretory immunoglobulin A (SIgA), cytokines, bifidus factor, lyzosyme, oligosaccharides, and lactoferrin (Heinig & Dewey, 1996; Hanson et al., 2003). BF also stimulates the infant's own immune system: the thymic gland is larger in breast-fed infants (Hasselbalch et al., 1996), SIgA concentration in urine is higher in BF infants (Goldblum et al., 1996) who also respond with higher levels of antibodies after certain vaccines (Hahn-Zoric et al., 1990). It seems that this stimulation has long-term effects.

The incidence of **infections** is lower in breast-fed infants compared to formula-fed infants. BF provides the most significant protection against gastrointestinal infections but also the incidence of respiratory infections is reduced (Howie et al., 2002; Oddy et al., 2003). Protection against urinary tract infections and otitis media up to 3 years has also been reported (Heinig & Dewey, 1996; Léon-Cava et al., 2002).

The effect of BF on the development of **allergy** is commonly regarded as one of its most significant advantages. BF protects against cow's milk allergy (Halken, 2004), but the effect against other allergic diseases as atopic dermatitis and asthma is less conclusive. In a systematic review with meta-analysis of prospective studies of BF and the risk of bronchial asthma, BF was reported to reduce the incidence of wheezing and asthma by 30%. In case of family history for allergy the

effect was stronger (Gdalevich et al., 2001). In a recent review it was concluded that there seemed to be a positive effect of BF on atopic diseases and that the effect was stronger in families with atopic disease (van Odijk et al., 2003). However, two other studies report no overall effect of BF. If the children were stratified by family history of allergy, a protective effect was observed in children with parents with allergy, whereas there was a slightly increased risk of atopic dermatitis in children with parents without allergy (Laubereau et al., 2004; Stabell et al., 2004).

Coeliac disease, or permanent gluten-sensitive enteropathy, is an immunologic disease dependent on exposure to gluten. BF reduces the risk of coeliac disease if gluten-containing foods are introduced gradually into the diet of infants while they are still being breast-fed (Ivarsson et al., 2002).

Among **other immune related diseases,** BF has been reported to reduce the risk of Crohn's disease and colitis ulcerosa, but it is not know if BF is of major importance for development of these diseases (Davis, 2001; Klement et al., 2004). Also protection against development of multiple sclerosis, rheumatoid arthritis by BF has been reported (Hanson et al., 2001). The use of donor human milk versus formulas seems to reduce the risk of necrotising enterocolitis in preterm infants (McGuire & Anthony, 2003).

6. TYPE 1-DIABETES MELLITUS

Type 1-diabetes mellitus (DM) is caused by both genetic and environmental factors. A number of studies have shown a protective effect of BF, whereas introduction of formula milk and complementary food seems to increase the risk (Davis, 2001). In addition, increased early growth is also associated with type 1 diabetes risk and is independent of the other risk factor, early introduction to formula milk (Hyppönen et al., 1999, EURODIAB Group, 2002). In a multi centre study, BF was reported to reduce the risk of DM by 40% relative to children never being BF after adjusting for growth pattern (EURODIAB Group, 2002). It has been suggested that LCPUFA present in breast milk improves the resistance of the β-cells (Das, 2003).

7. CARDIOVASCULAR DISEASE

The serum cholesterol concentration is higher in breast-fed infants than in formula-fed infants since breast milk contains cholesterol but this is not seen once BF finishes. Serum cholesterol level is lower in adults who had been breast fed (Owen et al., 2002). In accordance with this a meta-analysis reported that BF was associated with lower systolic blood pressure in later life. However, the overall difference was only 1.1 mm Hg, which is of limited clinical importance (Owen et al., 2003). An adverse effect has also been reported in a single study, where BF was associated with reduced arterial distensibility (Leeson et al., 2001). In addition, two new studies find no conclusive evidence that BF influences the risk of developing cardiovascular disease mortality (Martin et al., 2004; Rich-Edwards et al., 2004)

8. CANCER

Two meta-analyses suggest that BF is associated with a small decrease in the risk for childhood leukaemia (Kwan et al., 2004) and other childhood cancer forms (UK CCS Investigators, 2001). The protective effect of BF seems similar for all cancer forms why a non-specific effect of BF or a systematic bias shared by most of the included studies cannot be excluded.

9. MATERNAL EFFECTS

Protection against breast cancer is the most significant effect of BF on maternal health. A meta-analysis showed that the relative risk of breast cancer decreased by 4.3% for every 12 month of BF, in addition to a decrease of 7% for each birth (Collaborative Group, 2002).

REFERENCES

Anderson, J.W., Johnstone, B.M., and Remley, D.T., 1999, Breast-feeding and cognitive development: a meta-analysis, Am J Clin. Nutr. 70:525-535.

Collaborative Group on Hormonal Factors in Breast Cancer, 2002, Breast cancer and breast feeding: collaborative reanalysis of individual data from 47 epidemiological studies in 30 countries, including 50302 women with breast cancer and 96973 women without the disease, Lancet, 360:187-95

Das, U.N., 2003, Can perinatal supplementation of long-chain polyunsaturated fatty acids prevent diabetes mellitus? Eur J Clin Nutr. 57:218-26.

Davis, M.K., 2001, Breastfeeding and chronic disease in childhood and adolescence. Pediatr Clin North Am, 48:125-41, ix.

Dewey, K.G., 1998, Growth characteristics of breast-fed compared to formula-fed infants. Biol Neonate, 74:94-105.

EURODIAB Substudy 2 Study Group, 2002, Rapid early growth is associated with increased risk of childhood type 1 diabetes in various European populations. Diabetes Care, 25:1755-60

Gdalevich, M., Mimouni, D., and Mimouni, M., 2001, Breast-feeding and the risk of bronchial asthma in childhood: a systematic review with meta-analysis of prospective studies. J Pediatr, 139:261-6.

Goldblum, R., Hanson, L., and Brandtzaeg, P., 1996, The mucosal defence system. In Immunological Disorders in Infants and Children. 3rd edit. E. Stiehm, Ed.: 159-199. Saunders. Philadelphia, PA.

Hahn-Zoric, M., Fulconis, F., Minoli, I., Moro, G., Carlsson, B., Bottiger, M., Raiha, N., and Hanson, L.A., 1990, Antibody responses to parenteral and oral vaccines are impaired by conventional and low protein formulas as compared to breast-feeding. Acta Paediatr Scand., 79 (12):1137-42

Halken S, 2004, Prevention of allergic disease in childhood: clinical and epidemiological aspects of primary and secondary allergy prevention. Pediatr Allergy Immunol. 16:4-5, 9-32.

Hanson, L., Silfverdal, S.A., and Stromback, L., 2001, The immunological role of breast feeding. Pediatr Allergy Immunol; 12 Suppl 14:15-9.

Hanson, L.A., Korotkova, M., Lundin, S., 2003, The transfer of immunity from mother to child. Ann N Y Acad Sci, 987:199-206.

Hasselbalch, H., Jeppesen, D.L., Engelmann, M.D., Michaelsen, K.F., and Nielsen, M.B., 1996, Decreased thymus size in formula-fed infants compared with breastfed infants. Acta Paediatr, 85:1029-32.

Heinig, M.J., and Dewey, K.G., 1996, The advantages of breast feeding infants: a critical review. Nutrition Research Reviews, 9:89-110.

Howie, P.W., 2002, Protective effect of breastfeeding against infection in the first and second six months of life, Adv Exp Med Biol 503 141-147

Hyppönen, E., Kenward, M.G., and Virtanen, S. M., 1999, Infant feeding, early weight gain, and risk of type 1 diabetes. Childhood Diabetes in Finland (DiMe) Study Group. Diabetes Care, 22:1961-5.

Ivarsson, A., Hernell, O., Stenlund, H., and Persson, L.A., 2002, Breast-feeding protects against celiac disease. Am J Clin Nutr 75:914-21.

Klement, E., Cohen, R.V., Boxman J., Joseph, A., and Reif S., 2004, Breastfeeding and risk of inflammatory bowel disease: a systematic review with meta-analysis. Am J Clin Nutr 80: 1342-52

Kramer, M.S., and Kakuma R., 2002, The optimal duration of exclusive breast feeding. A systematic review.

Laubereau, B., Brockow, I., and Zirngibl, A., 2004, Effect of breast-feeding on the development of atopic dermatitis during the first 3 years of life--results from the GINI-birth cohort study. J Pediatr, 144:602-7.

Kwan M.L., Buffler P.A., Abrams B., and Kiley V.A., 2004, Breastfeeding and the risk of childhood leukaemia: A meta-analysis. Public Health Rep. 119:521-535

Lauritzen, L., Hansen, H. S., Jorgensen, M.H., and Michaelsen, K. F., 2001, The essentiality of long chain n-3 fatty acids in relation to development and function of the brain and retina. Prog. Lipid Res. 40, 1-94.

Leeson, C.P., Kattenhorn, M., Deanfield, J.E., and Lucas, A., 2001, Duration of breast feeding and arterial distensibility in early adult life: population based study. BMJ, 322:643-7.

León-Cava, N., Lutter, C., Ross, J., and Martin, L., 2002, Quantifying the benefits of breast feeding: a summary of the evidence,
(www.linkagesproject.org/media/publications/Technical%20Reports/BOB.pdfLinkages.2004).

Martin, R.M., Davey, S.G., Mangtani, P., Tilling, K., Frankel, S., and Gunnell, D., 2004, Breastfeeding and cardiovascular mortality: the Boyd Orr cohort and a systematic review with meta-analysis. Eur Heart J 25:778-86.

McGuire, W., and Anthony, M.Y., 2003, Donor human milk versus formula for preventing necrotising enterocolitis in preterm infants: systematic review. Arch Dis Child Fetal Neonatal Ed, 88:F11-F14.

Michaelsen K.F., Petersen S., Greisen G., and Thomsen B.L., 1994, Weight, length, head circumference and growth velocity in a longitudinal study of Danish infants. Dan Med Bull 44:577-85.

Michaelsen K.F., Lauritzen, L., Jørgensen, M.H., and Mortensen, E.L., 2003, Breast-feeding and brain development, Scand. J. Nutr,. 47:147-151.

Mortensen, E.L., Michaelsen, K.F., Sanders, S.A., and Reinisch, J.M., 2002, The association between duration of breastfeeding and adult intelligence. JAMA, 287:2365-71.

Oddy, W.H., Sly, P.D., and de Klerk, N.H., 2003, Breast feeding and respiratory morbidity in infancy: a birth cohort study. Arch Dis Child 88 :224-8.

Owen, C.G., Whincup, P.H., Gilg, J.A., and Cook, D.G., 2003, Effect of breast feeding in infancy on blood pressure in later life: systematic review and meta-analysis. BMJ, 327:1189-95.

Owen, C.G., Whincup, P.H., Odoki, K., Gilg, J.A., and Cook, D.G., 2002, Infant feeding and blood cholesterol: a study in adolescents and a systematic review. Pediatrics, 110:597-608.

Rich-Edwards, J.W., Stampfer, M.J., and Manson, J.E., 2004, Breastfeeding during infancy and the risk of cardiovascular disease in adulthood. Epidemiology, 15:550-6.

SanGiovanni, J.P., Berkey, C.S., Dwyer, J.T., and Colditz, G.A., 2000, Dietary essential fatty acids, long-chain polyunsaturated fatty acids, and visual resolution acuity in healthy full term infants: a systematic review. Early Hum Dev, 57:165-88.

Stabell, B.C., Wohlfahrt. J., and Aaby, P., 2004, Breastfeeding and risk of atopic dermatitis, by parental history of allergy, during the first 18 months of life. Am J Epidemiol, 160:217-23.

UK Childhood Cancer Study Investigators, 2001, Breastfeeding and childhood cancer, Br J Cancer, 85

van Odijk, J., Kull, I., and Borres, M.P., 2003, Breastfeeding and allergic disease: a multidisciplinary review of the literature (1966-2001) on the mode of early feeding in infancy and its impact on later atopic manifestations. Allergy, 58:833-43.

WHO, 2002, The optimal duration of exclusive breastfeeding. Report of an expert consultation.

EXPERIMENTAL EVIDENCE FOR LONG-TERM PROGRAMMING EFFECTS OF EARLY DIET

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Abstract: Nutritional manipulation targeted at specific periods of embryo or placental development can result in substantial changes in fetal organ development despite no effects on fetal weight. In particular, kidney and fat mass are greater in nutrient restricted offspring in conjunction with higher mRNA abundance for leptin, insulin-like growth factors I/II and glucocorticoid receptors. As young adults, nutrient restricted offspring exhibit a blunting of the cardiovascular baroreflex. They also demonstrate increased plasma leptin following sympathetic stimulation, not observed in controls, indicating resetting of adipocyte sensitivity to stress. In conclusion, global nutrient restriction confined to periods of early development programmes adult physiology in a manner that may predispose to later disease given the appropriate environmental stimuli.

Key words: fetal development; mRNA; leptin; embryo, stress; kidney; fat

1. DEVELOPMENTAL PROGRAMMING OF ADULT DISEASE

Hypertension and obesity are major risk factors for coronary heart disease and represents a common cause of death in the population over the age of 50 years[1]. Wide ranging epidemiological evidence from different populations worldwide indicate that targeted changes in the nutritional and hormonal environment encountered by the fetus are strong determinants of later cardiovascular disease[2,3]. Epidemiological and animal studies both indicate that the timing of maternal nutritional manipulation is a key determinant of later outcomes[4-6]. Critically, these effects can occur in the absence of any change in birth weight. Indeed, it is striking that across a very wide range of caloric intakes there is very little change in birth weight[7]. The long term consequences of maternal

nutrient restriction in utero appear to be dependent on, or amplified by, the timing, magnitude and duration of nutritional rehabilitation[8,9]. Later, nutritional intake becomes increasingly important, particularly after birth when nutrient availability and physical constraints to growth are "unlimited" and an individual's full growth potential can be met. In the current review we will focus on the experimental evidence that kidney and fat development are programmed in utero given the strong link that compromised kidney function and excess fat mass have with adult hypertension[10]

2. ANIMAL MODELS OF FETAL PROGRAMMING

Studies from both small and large animal models have shown that maternal dietary manipulations, either throughout gestation or targeted to defined periods of pregnancy, can have long term health consequences[6,11]. The magnitude of response varies greatly between animal models which is likely to reflect the very different metabolic constraints imposed on the mother by the growing fetus. Rats appear to be particularly vulnerable to any nutritional imbalance during gestation, perhaps because they are litter bearing, have a short gestation and there is rapid growth of both the placenta and fetus over the final few days of pregnancy. The products of conception in the rat exhibit an exceptional rate of protein accretion during prenatal development (estimated at 23-fold that of the sheep and human fetus[12]) and have a large total weight relative to maternal weight at term (25-35% *vs.* 7-10% in the sheep and 3-5% in humans). Sheep, on the other hand, are similar to humans in that a rapid phase of placental growth precedes that of the fetus[13]. Depending on the breed of sheep, they are like humans and normally produce single offspring, of a similar body weight after a long gestation, with a mature hypothalamic-pituitary axis at birth.

3. NUTRITIONAL MANIPULATION AND FETAL PROGRAMMING

The full extent to which a deficiency or imbalance of macro or micronutrients directly act to adversely influence fetal development remains an area of debate. To date, the current consensus from

epidemiological and observational studies in the human support a primary role for macronutrients[4,14]. One of the best characterised animal models of fetal programming is the rat in which the effects of both a high and low protein diet have been examined[11,15]. These studies have repeatably shown that maternal consumption of a low protein diet either throughout gestation[11], or at specific time periods, results in offspring with raised blood pressure[16]. The magnitude of effect is partly dependent on the timing of exposure and in some[16], but not all, studies is gender specific. It is notable that a low protein diet does not have any initial stimulatory effects on fat deposition[17] which contrasts with the effects of a high protein diet[15]. However, any programming effects of high or low protein exposure in utero on later fat deposition appear to be dependent on the postnatal diet. Offspring of rats fed a high protein diet during fetal development only become obese when fed a standard diet after birth[15]. Whilst in mice, consumption of excess nutrition after birth, following exposure to a low protein diet is reported to result in obesity and reduce life span by almost one-third[18]. The extent to which these responses are the consequence of specific changes within the adipocyte, or related to centrally mediated effects on appetite regulation, are unclear.

4. TISSUE SPECIFIC RESPONSES TO NUTRITIONAL MANIPULATION THROUGH PREGNANCY

Offspring of pregnant rats that were globally nutrient restricted so as to cause intrauterine growth retardation in all offspring, become obese but only after puberty. In this model, the resulting offspring exhibit a range of adult complications including sedentary behaviour[19], hyperinsulinemia and hyperleptinemia[20,21]. Obesity is associated with hyperphagia but is observed on both a standard as well as a hypercaloric diet[20]. These rats are also hypertensive and the effects can be reversed by treatment with growth hormone[22]. No adverse responses in the kidney have been reported to date in this model.

In rats, the programming of higher blood pressure appears to be greatly amplified compared with human epidemiological and large animal studies. A 20-40 mmHg increase in systolic blood pressure occurs not only with low protein diet[11] but also with iron deficiency[23] and excess fat intake[24] through pregnancy. Impaired kidney development

with a low protein diet or iron deficiency is likely to cause this higher blood pressure[23,25]. The later outcomes in kidney function after feeding the mothers a high fat diet have not been reported, but an increase in offspring blood pressure was confined to the females despite plasma cortisone being raised in males and females[24]. Interestingly in this model, abnormalities of vascular endothelial function i.e. endothelium-dependent dilation were not gender specific and do not suggest this mechanism is involved in the progression of hypertension.

Adverse long term outcomes of consuming a low protein diet are not confined to blood pressure control but include abnormal pancreatic development, as β-cell mass and islet vascularisation are both reduced[26]. This defect can be overcome by taurine supplementation which has a number of beneficial effects including restoration of the normal volume and numerical density of blood vessels in fetal islets[27]. Taurine also prevented under expression of both vascular endothelial growth factor and its receptor fetal liver-kinase-1. It is not known if taurine similarly rectifies the adverse cardiovascular outcomes. Moreover, conversely, although pancreatic function is impaired, offspring born to taurine deficient dams do not develop obesity.

5. MECHANISMS OF FETAL PROGRAMMING AND LATER DISEASE

The kidney - is a primary organ implicated in fetal programming. In large animals, such as sheep, early kidney development is highly sensitive to excess corticosteroid exposure[28]. A key stage of fetal kidney maturation is the period in which the pronephros develops and then degenerates[29], coincident with the time of implantation/uterine attachment. Exposure to very high levels of glucocorticoids over this period has no effect on glucocorticoid receptor number but may have direct consequences for kidney function as assessed by a decline in the osmolality, sodium and chloride content of allantoic fluid together with a rise in potassium concentration[30]. These adaptations have been interpreted to be a consequence of the premature up-regulation of Na,K-ATPase activity within the mesonephroi which is similarly increased in offspring born to mothers fed a low protein diet[31]. Fetuses exposed to dexamethasone early in gestation exhibit increased urinary flow rate following three days of angiotensin II infusion in late gestation[32],

confirming persistent changes in kidney function. The offspring go on to have higher resting, but not stimulated, blood pressure[28].

In rat offspring, the higher blood pressure after low protein exposure in utero is likely to be a result of a reduction in kidney nephron number[33], possibly due to excess glucocorticoid exposure in utero[34] suppressing development of the renin-angiotensin system[35]. These findings are partly in accord with the effect of targeted global caloric restriction between early to mid gestation in sheep for which the offspring have kidneys with a greater mRNA abundance of glucocorticoid receptor and glucocorticoid responsive genes such as the angiotensinogen 2 type I receptor[36]. Nephron number is decreased in the offspring as is activity of the enzyme 11β-hydroxysteroid dehydrogenase (HSD) type 2[37,38], thereby potentially leading to increased sensitivity to subsequent stress. Interestingly, as a function of total body mass, the difference in kidney weight between nutrient restricted and control offspring reduces with age (Figure 1). At the same time blood pressure of nutrient restricted offspring switches from being lower than that of controls, to being higher. The transition to high blood pressure following in utero nutrient restriction thus appears to be an age dependent process. A prominent component of this adaptation resides in a resetting of the cardiovascular baroreflex that is essential in maintaining central pressure during ambulatory changes in blood pressure: if this is inadequate, then the risk of later hypertension is increased[39]. Sheep born to nutrient restricted mothers show a blunted baroreflex sensitivity during angiotensin II infusion, whereas the tachycardia following a reduction in central blood pressure is potentiated, relative to controls[39,40]. An increase in regional angiotensin II activity in the area postrema and nucleus tractus solitarius during this critical early phase of development is a likely candidate mechanism.

Fat - In sheep, fetal fat growth is under tight nutritional regulation and is highly sensitive to alterations in maternal nutrition through pregnancy[6]. Fetal fat deposition is, thus, enhanced by nutrient restriction commencing early in pregnancy but is reduced by maternal undernutrition in late gestation[41]. Fat mass is raised in the offspring of previously nutrient restricted mothers and remains so for as long as the offspring have been studied (Figure 1). At term, the increased adiposity is accompanied by higher mRNA abundance for leptin, plus insulin-like growth factors I/II and glucocorticoid receptors[36,37]. These adaptations

occur in conjunction with reduced maternal plasma cortisol, thyroid hormones and leptin concentrations over the period of nutrient restriction[36,42]. Then, as young adults, nutrient restricted offspring demonstrate increased plasma leptin following sympathetic stimulation, which is not observed in controls, indicating resetting of adipocyte sensitivity to stress[40]. It remains to be established whether increasing nutrient availability at specific stages in later life could exacerbate these symptoms, for example during lactation, when fat is the fastest growing organ of the body[43].

In conclusion, nutrient restriction, confined to the periods of embryonic and placental development, programmes adult physiologyogy[39]. This is predicted to enhance the offspring's predisposition to later disease given the appropriate environmental stimuli experienced as an adult.

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REFERENCES

1. C. M. Law and A. W. Shiell, Is blood pressure inversely related to birth weight? The strength of evidence from a systematic review of the literature. 14, 935-941 (1996).
2. D. J. P. Barker, In utero programming of chronic disease. *Clin Sci* 95, 115-128 (1998).
3. G. C. Curhan, W. C. Willett, E. B. Rimm, D. Spiegelman, A. L. Ascherio and M. J. Stampfer, Birth weight and adult hypertension, diabetes mellitus, and obesity in US men. *Circulation* 94, 3246-3250 (1996).
4. T. J. Roseboom, J. H. P. van der Meulen, C. Osmond, D. J. P. Barker, A. C. J. Ravelli and O. P. Blecker, Plasma lipid profile in adults after perinatal exposure to famine. *Am J Clin Nutr* 72, 1101-11106 (2000).
5. T. J. Roseboom, J. H. P. van der Meulen, C. Osmond, D. J. P. Barker, A. C. J. Ravelli, S.-T. von Montfrans, G.A., R. P. J. Michels and O. P. Blecker, Coronary heart disease in adults after perinatal exposure to famine. *Heart* 84, 595-598 (2000).
6. M. E. Symonds, S. Pearce, J. Bispham, D. S. Gardner and T. Stephenson, Timing of nutrient restriction and programming of fetal adipose tissue development. *Proc Nutr Soc* 63, (In press) (2004).

7. M. E. Symonds, D. S. Gardner, S. Pearce and T. Stephenson, in *Fetal Nutrition and Adult Disease - Programming of chronic disease through fetal exposure to undernutrition* ed. S. C. Langley-Evans 353-380 CAB International, Oxford, (2004).
8. J. Dandrea, V. Wilson, G. Gopalakrishnan, L. Heasman, H. Budge, T. Stephenson and M. E. Symonds, Maternal nutritional manipulation of placental growth and glucose transporter-1 abundance in sheep. *Reprod* 122, 793-800 (2001).
9. M. E. Symonds, H. Budge, T. Stephenson and I. C. McMillen, Fetal endocrinology and development - manipulation and adaptation to long term nutritional and environmental challenges. *Reprod* 121, 853-862 (2001).
10. J. E. Hall, The kidney, hypertension, and obesity. 41, 625-633 (2003).
11. S. C. Langley-Evans, Fetal programming of cardiovascular function through exposure to maternal undernutrition. *Proc Nutr Soc* 60, 505-513 (2001).
13. L. Heasman, L. Clarke, J. Dandrea, T. Stephenson and M. E. Symonds, Correlation of fetal number with placental mass in sheep. *Cont Rev Obs Gynecol* 10, 275-280 (1998).
14. K. Godfrey, S. Robinson, D. J. P. Barker, C. Osmond and V. Cox, Maternal nutrition in early and late pregnancy in relation to placental and fetal growth. *BMJ* 312, 410-414 (1996).
15. M. Daenzer, S. Ortmann, S. Klaus and C. C. Metges, Prenatal high protein exposure decreases energy expenditure and increases adiposity in young rats. 132, 142-144 (2002).
16. W. Y. Kwong, A. E. Wild, P. Roberts, A. C. Willis and T. P. Fleming, Maternal undernutrition during the preimplantation period of rat development causes blastocyst abnormalities and programming of postnatal hypertension. *Development* 127, 4195-4202 (2000).
17. S. E. Ozanne, B. T. Nave, C. L. Wang, P. R. Shepherd, J. Prins and G. D. Smith, Poor fetal growth causes long-term changes in expression of insulin signalling components in adipocytes. *Am J Physiol* 273, E46-E51 (1997).
18. S. E. Ozanne and C. N. Hales, Lifespan: Catch-up growth and obesity in male mice. 427, 411-412 (2004).
19. M. H. Vickers, B. H. Breier, D. McCarthy and P. D. Gluckman, Sedentary behavior during postnatal life is determined by the prenatal environment and exacerbated by postnatal hypercaloric nutrition 285, R271-3 (2003).
20. M. H. Vickers, B. H. Breier, W. S. Cutfield, P. L. Hofman and P. D. Gluckman, Fetal origins of hyperphagia, obesity, and hypertension and postnatal amplification by hypercaloric nutrition 279, E83-7 (2000).
21. M. H. Vickers, S. Reddy, I. B.A. and B. H. Breier, Dysregulation of the adipoinsular axis – a mechanism for the pathogenesis of hyperleptinemia and adipogenic diabetes induced by fetal programming. *J. Endocrinol.* 170, 323-332 (2001).
22. M. H. Vickers, B. A. Ikenasio and B. H. Breier, Adult growth hormone treatment reduces hypertension and obesity induced by an adverse prenatal environment 175, 615-23 (2002).
23. L. Gambling, S. Dunford, D. I. Wallace, G. Zuur, N. Solanky, K. S. Srai and H. J. McArdle, Iron deficiency during pregnancy affects postnatal blood pressure in the rat. 552.2, 603-610 (2003).

24. I. Y. Khan, P. D. Taylor, V. Dekou, P. Seed, L. Lakasing, D. Graham, A. F. Dominiczak, M. A. Hanson and L. Poston, Gender-linked hypertension in offspring of lard fed pregnant rats. 188, 454-460 (2003).
25. M. O. Nwagwu, A. Cook and S. C. Langley-Evans, Evidence of progressive deterioration of renal function in rats exposed to a maternal low-protein diet in utero. *Brit J Nutr* 83, 79-85 (2000).
26. A. Snoeck, C. Remacle, B. Reusens and J. J. Hoet, Effect of low protein diet during pregnancy on the fetal rat endocrine pancreas. 57, 107-118 (1990).
27. S. Boujendar, E. Arany, D. Hill, C. Remacle and Reusens. B, Taurine supplementation of a low protein diet fed to rat dams normalizes the vascularization of the fetal endocrine pancreas. 133, 2820-2825 (2003).
28. M. Dodic, V. Hantzis, J. Duncan, S. Rees, I. Koukoulas, K. Johnson, E. M. Wintour and K. Moritz, Programming effects of short prenatal exposure to cortisol. *FASEB J* 16, 1017-1026 (2002).
29. E. M. Wintour, D. Alcorn, A. Butkus, M. Congiu, L. Earnest, S. Pompolo and S. J. Potocnik, Ontogeny of hormonal and excretory function of the meso- and metanephros in the ovine fetus *Kidney Int.* 50, 1624-1633 (1996).
30. A. Peers, V. Hantzis, M. Dodic, I. Koukoulas, A. Gibson, R. Baird, R. Salemi and E. M. Wintour, Functional glucocorticoid recetpors in the mesonephros of the ovine fetus. *Kidney Int* 59, 425-433 (2001).
31. C. E. Bertram, A. R. Trowern, N. Copin, A. A. Jackson and C. B. Whorwood, The maternal diet during pregnancy programs altered expression of the glucocorticoid receptor and type 2 11β-hydroxysteroid dehydrogenase: Potential molecular mechanisms underlying the programming of hypertension in utero. *Endocrinology* 142, 2841-2853 (2001).
32. K. Moritz, K. Johnson, R. Douglas-Denton, E. M. Wintour and M. Dodic, Maternal glucocorticoid treatment programs alterations in the renin-angiotensin system ovine fetal kidney. *Endocrinology* 143, 4455-4463 (2002).
33. S. McMullen, D. S. Gardner and S. C. Langley-Evans, Prenatal programming of angiotensinogen type II receptor expression in the rat. 91, 133-140 (2004).
34. S. C. Langley-Evans, G. J. Phillips, R. Benediktsson, D. S. Gardner, C. R. W. Edwards, A. A. Jackson and J. R. Seckl, Protein intake in pregnancy, placental glucocorticoid metabolism and the programming of hypertension. *Placenta* 17, 169-172 (1996).
35. L. L. Woods, J. R. Ingelfinger, J. R. Nyengaard and R. Rasch, Maternal protein restriction suppresses the newborn renin-angiotensin system and programs adult hypertension in rats. 49, 460-467 (2001).
36. J. Bispham, G. S. Gopalakrishnan, J. Dandrea, V. Wilson, H. Budge, D. H. Keisler, F. Broughton Pipkin, T. Stephenson and M. E. Symonds, Maternal endocrine adaptation throughout pregnancy to nutritional manipulation: consequences for maternal plasma leptin and cortisol and the programming of fetal adipose tissue development *Endocrinology* 144, 3575-3585 (2003).
37. C. B. Whorwood, K. M. Firth, H. Budge and M. E. Symonds, Maternal undernutrition during early- to mid-gestation programmes tissue-specific alterations in the expression of the glucocorticoid receptor, 11β-hydroxysteroid dehydrogenase

isoforms and type 1 angiotensin II receptor in neonatal sheep. *Endocrinology* 142, 1778-1785 (2001).

38. L. Passingham, L. O. Kurlak, G. Gopalakrishnan, H. Budge, S. M. Rhind, M. T. Rae, C. E. Kyle, T. Stephenson and M. E. Symonds, The effect of maternal nutrient restriction during early to mid-gestation on the enzyme activity of 11 beta hydroxysteroid dehydrogenase type 2 in sheep kidneys of 3 year old offspring. *Early Hum Dev*, (In press) (2004).
39. D. S. Gardner, S. Pearce, J. Dandrea, R. M. Walker, M. M. Ramsey, T. Stephenson and M. E. Symonds, Peri-implantation undernutrition programs blunted angiotensin II evoked baroreflex responses in young adult sheep. 43, 1-7 (2004).
40. G. Gopalakrishnan, D. S. Gardner, S. M. Rhind, M. T. Rae, C. E. Kyle, A. N. Brooks, R. M. Walker, M. M. Ramsay, D. H. Keisler, T. Stephenson and M. E. Symonds, Programming of adult cardiovascular function after early maternal undernutrition in sheep. 287, R12-20 (2004).
41. H. Budge, L. J. Edwards, I. C. Mcmillen, A. Bryce, K. Warnes, S. Pearce, T. Stephenson and M. E. Symonds, Nutritional manipulation of fetal adipose tissue deposition and uncoupling protein 1 abundance in the fetal sheep; differential effects of timing and duration. *Biol Reprod*, (In press) (2004).
42. L. Clarke, L. Heasman, D. T. Juniper and M. E. Symonds, Maternal nutrition in early-mid gestation and placental size in sheep. *Brit J Nutr* 79, 359-364 (1998).
43. L. Clarke, D. S. Buss, D. S. Juniper, M. A. Lomax and M. E. Symonds, Adipose tissue development during early postnatal life in ewe-reared lambs. *Exp Physiol* 82, 1015-1017 (1997).

CANDIDATE GENES FOR OBESITY – HOW MIGHT THEY INTERACT WITH ENVIRONMENT AND DIET ?

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1. INTRODUCTION

Obesity is determined by genetic, environmental and behavioural factors acting through the physiological mediators of energy intake and energy expenditure. In the last few years, we and others have described five human disorders of energy balance that arise from genetic defects.

2. CONGENITAL LEPTIN DEFICIENCY

The first monogenic human obesity syndrome we reported was congenital leptin deficiency. Two severely obese cousins in a consanguineous family were found to have undetectable levels of serum leptin and were homozygous for a frameshift mutation in the ob gene (ΔG133), that results in a truncated protein that is not targeted normally for secretion.

These children were hyperphagic, developed severe disabling obesity, impaired T cell mediated immunity and hypogonadotropic hypogonadism. In a clinical trial of daily subcutaneous injections of recombinant human leptin, sustained, beneficial effects on appetite, fat mass, hyperinsulinaemia and hyperlipidaemia have been observed.

Leptin administration also permits the full progression of appropriately timed puberty and reverses impaired T cell mediated immunity.

3. LOSS OF FUNCTION MUTATIONS IN THE MELANOCORTIN 4 RECEPTOR

We have recruited over 1150 severely obese children to the Genetics of Obesity Study (GOOS). Using a candidate gene approach, we have identified several loss of function mutations in the melanocortin 4 receptor (MC4R), which cause a dominantly inherited syndrome that accounts for up to 5% of patients with severe, early-onset obesity. MC4R deficiency is characterised by hyperphagia, severe hyperinsulinaemia and increased linear growth and there is evidence for a genotype-phenotype correlation, as complete loss of function mutations result in a more severe phenotype.

4. CONCLUSIONS

These studies have highlighted the role of leptin and the melanocortin axis in humans and the characterization of these syndromes has shed light on the molecular and physiological mechanisms underlying the regulation of appetite and body weight.

RATE OF GROWTH IN EARLY LIFE: A PREDICTOR OF LATER HEALTH?

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Abstract: The purpose of this review is to describe the studies which investigate the association between early growth pattern and future metabolic risks. Childhood obesity is increasing but other growth parameters are also changing. There is a trend of earlier maturation and increasing height. The increase in height from one generation to the next occurs mainly in the first years of life. Rapid growth in early life (rapid weight and length gain, early adiposity rebound) is associated with various health risks in later life (obesity, cancer, cardiovascular diseases, diabetes). Pattern of growth rather than absolute level of fatness seams to be of most importance.

Key words: Child, growth, obesity, adiposity rebound, cancer, cardiovascular diseases, diabetes

1. INTRODUCTION

Childhood obesity is increasing worldwide, but at the same time, various growth parameters are also changing. Alterations in growth patterns, particularly accelerated growth for a few decades, suggest that environmental factors have acted early in life. For a long time, research focused on the consequences of poor growth. More recently, research focused on childhood obesity and its consequences. But various body characteristics have also changed substantially. Tall stature is often viewed as a favourable process, however, there is now increasing evidence that a rapid growth in infancy or early childhood can predispose to adult onset diseases. The different growth patterns, their determinants and their association with health risks will be described here.

2. SECULAR TRENDS IN OBESITY AND OTHER GROWTH PARAMETERS

In many countries, childhood obesity is increasing. Children now display more android or central body fat distribution and are taller (Eveleth and Tanner, 1990; Deheeger and Rolland-Cachera, 2004). Increased stature is mainly the result of increased leg length and, because this accounts for most growth before puberty, this trend reflects rapid growth in early life. Accelerated growth is also associated with an earlier age for adiposity rebound (AR). This is defined as the point of minimal body mass index (BMI) value (the nadir of the BMI curve) (Rolland-Cachera et al., 1984). As a rule, an early AR is associated with greater subsequent fatness. It is also associated with advanced bone age. Mean age at AR in children born in 1955 occurred at 6.2 years but at 5.6 years in those born in 1985 (Rolland-Cachera, 1999). These different observations are in accordance with the time trend of accelerated growth starting in early life.

3. ACCELERATED GROWTH AND FUTURE RISK OF OBESITY

Various studies have examined the association between early growth and the risk of later obesity. They considered either weight, length or BMI gains (Eid,1970; Ong et al., 2000; Cameron et al., 2003; Monteiro et al., 2003) and report that rapid growth after low birth weight is associated with large subsequent weight gain and a central body fat pattern. However, the prediction potentials differ according to the anthropometric indices. In a cohort of French children, large weight and length gain between birth and two years of age were associated with greater weight, BMI and waist circumference at age 14 (Rolland-Cachera et al., 2001). The association was stronger with weight gain than with length gain, but large length gain was associated with an android body fat distribution at 14 years and with an early AR, while large weight gain was not. The best anthropometric predictor for later overweight was the main focus in a retrospective cohort study of Bavarian children (Toschke et al., 2004). Weight, length, BMI and Ponderal index variations between birth and either 6, 12 or 24 months were compared. Weight gain from

birth to 24 months was the best overall predictor of later weight category, but the authors concluded that the predictability was poor in their population.

4. RISK OF ADULT DISEASES

Cancer: Several studies have found an association between stature and cancer (Albanes et al., 1998), particularly between leg length and sex hormone dependent cancers (Gunnell et al., 1998). Because most growth before puberty is due to increases in leg length, this can be used as a marker for exposures that generate the association between adult height and cancer. As rapid growth is associated with an earlier puberty, it has been suggested that the association between leg length and cancer can be explained by a longer exposure to adult concentrations of sex hormones. Another proposed hypothesis is that childhood diet may influence concentrations of Igf-1, subsequent growth and later risk of cancer.

Coronary heart disease: Growth acceleration in childhood increases the propensity to later cardiovascular disease. As rapid weight gain is often associated with low birth weight, some authors suggest an adverse effect of a poor fetal nutrition followed by improved postnatal nutrition (Erikson et al., 1999), while others favour the hypothesis of a direct adverse effect of accelerated postnatal growth (Singhal et al., 2004).

Diabetes*:* The risk of diabetes has been found to be associated with a rapid weight gain in the first 2 weeks of life (Singhal et al., 2003), and also with an early AR (Eriksson et al, 2003). Subjects with impaired glucose tolerance or diabetes typically had a low BMI up to the age of two years, followed by an early AR and an accelerated increase in BMI until adulthood (Bhargava et al., 2004). However, despite an increase in BMI between the ages of 2 and 12 years, none of these subjects were obese at the age of 12 years. This underlies the importance of growth pattern rather than the absolute BMI level.

While an early AR is known to predict future overweight and obesity, it is also significantly associated with a low fatness level in the first years of life (Rolland-Cachera et al., 1987; Williams and Dickson, 2002). The typical growth pattern associated with an early AR (low BMI followed by high BMI after the AR), similar to the BMI pattern of subjects with diabetes, has beeen reported in various circumstances (Rolland-Cachera,

1999). These include the growth pattern in children from industrialised vs developing countries and the pattern in children consuming high vs low protein diets.

5. CONCLUSION

Rapid growth in early life is associated with later health risks. Various anthropometric markers can predict future obesity (rapid weight and length gain, early AR). They have different predictive values and correspond to different growth patterns. They are likely to have different origins and may be associated with different health risks.

It is therefore important to continue research focussing on the identification of the early environmental factors which influence growth patterns and adult health.

REFERENCES

Albanes D, Jones DY, Schatzkin A, Micozzi MS, Taylor PR. Adult stature and risk of cancer *Cancer Res* 1988;48:1658-62.

Bhargava SK, Sachdev HS, Fall CH, Osmond C, Lakshmy R, Barker DJ, Biswas SK, Ramji S, Prabhakaran D, Reddy KS. Relation of serial changes in childhood body-mass index to impaired glucose tolerance in young adulthood. *N Engl J Med* 2004;350:865-75.

Cameron N, Pettifor J, De Wet T, Norris Shane. The relationship of rapid weight gain in infancy to obesity and skeletal maturity in childhood. *Obes Res* 2003 ;11 :457-60.

Deheeger M, Rolland-Cachera MF. Etude longitudinale de la croissance d'enfants parisiens suivis de l'âge de 10 mois à 18 ans. *Arch Pediatr* 2004;11:1139-44.

Eid EE. Follow-up study of physical growth of children who had excessive weight gain in first six months of life. *Br Med J* 1970;2:74-76.

Erikson JG, Forsen T, Tuomilehto J, Winter PD, Osmond C, Barker DJ. Catch-up growth in childhood and death from CHD: longitudinal study. *BMJ* 1999;318:427-31.

Eriksson JG, Forsen T, Tuomilehto J, Osmond C, Barker DJ. Early adiposity rebound in childhood and risk of Type 2 diabetes in adult life. *Diabetologia* 2003;46:190-4.

Eveleth P, Tanner JM. World wide variations in human growth 2nd ed. Cambridge: *Cambridge University Press* 1990 .

Gunnell DJ, Davey Smith G, Holly JMP, Frankel S. Leg length and risk of cancer in the Boyd Orr cohort. *BMJ* 1998;317:150-1.

Monteiro POA, Victora CG, Barros FC, Monteiro LMA. Birth size, early childhood growth and adolescent obesity in a Brazilian birth cohort. *Int J Obes* 2003;27:1274-82.

Ong KKL, Ahmed Ml, Emmet PM, Preece MA, Dunger DB. Association between postnatal catch-up growth and obesity in childhood: prospective cohort. *BMJ* 2000;320:967-71.

Rolland-Cachera MF, Deheeger M, Bellisle F, Sempé M, Guilloud-Bataille M, Patois E. Adiposity rebound in children: a simple indicator for predicting obesity. *Am J Clin Nutr* 1984;39:129-35.

Rolland-Cachera MF, Deheeger M, Avons P, Guilloud-Bataille M, Patois E, Sempé M. Tracking adiposity patterns from 1 month to adulthood. *Ann Hum Biol* 1987,14:219-22.

Rolland-Cachera MF. Obesity among adolescents: evidence for the importance of early nutrition in: Human growth in context. Eds FE Johnston, B Zemel, PB Eveleth *Smith-Gordon:* London, UK, 1999, pp 245-258.

Rolland-Cachera MF, Deheeger, M.Thibault H. Weight gain in infancy is associated with body fat but not fat pattern at age 14 years. *Ann Nutr Metab* 2001;45:332.

Singhal A, Fewtrell M, Cole TJ, Lucas A. Low nutrient intake and early growth for later insulin resistance in adolescents born preterm. *Lancet* 2003;361:1089-97.

Singhal A, Cole TJ, Fewtrell M, Deanfield J, Lucas A. Is slower early growth beneficial for long-term cardiovascular health? *Circulation* 2004;109:1108-13.

Toschke AM, Grote V, Koletzko B, von Kries R. Identifying children at risk of overweight at school entry by weight gain during the first 2 yrs. *Arch Pediatr Adolesc* 2004;158:449-52.

Williams S, Dickson N. Early growth, menarche and adiposity rebound. *Lancet* 2002;359:580-81.

PROTECTIVE EFFECT OF BREAST-FEEDING AGAINST OBESITY IN CHILDHOOD

Can a meta-analysis of observational studies help to validate the hypothesis?

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Abstract: The relationship between breast-feeding and childhood obesity is of great interest.. Since 2000, sixteen studies have been published with conflicting data regarding the potential protective effect of breast-feeding on childhood obesity. A narrative review of Dewey in 2003 suggested a protective effect of breast-feeding, but an editorial in the British Medical Journal later that year cited two more recent studies without such an effect and stated there was inconclusive evidence A recent meta-analysis, however, has suggested a small, but significant, protective effect of breast-feeding. This paper summarises this meta-analysis and discusses the strengths and limitations of the meta-analysis approach.

Key words: breast-feeding, overweight, obesity, childhood

1. INTRODUCTION

In the last few years the relationship between breast-feeding and childhood obesity has been a major focus of interest. Since 2000, sixteen studies on this issue have been published; some have found a protective effect, while others have not (figure 1).

The conclusions drawn from these data regarding the potential protective effect of breast-feeding on childhood obesity diverge. While a narrative review of Dewey suggested an effect of breast-feeding [1], an editorial in the British Medical Journal cited two recent studies without

such an effect and stated that there was inconclusive evidence [2]. A recent meta-analysis suggested a small but significant protective effect of breast-feeding [3]. The aim of this paper is to discuss the strengths and limitations of the meta-analysis approach and to summarize the results of the meta-analysis.

2. WHY DOES IT MAKE SENSE TO CONDUCT A META-ANALYSIS?

The assumption in a meta-analysis is that all studies are measuring the same exposure and effect. In this case, different odds ratios are explained by chance and are related to differences in the size of the study. The meta-analysis summarises the effects of the included studies and statistical power is therefore increased to allow for more precise estimates.

3. WHAT LIMITATIONS ARE THERE FOR A META-ANALYSIS ON BREAST-FEEDING AND CHILDHOOD OBESITY?

Different study characteristics

The published studies not only differed with respect to sample size, but also with regard to a number of other study characteristics. The included studies [4-12] used different approaches to measure the exposure "breast-feeding". Most of the studies compared children in the broad categories "never breastfed" with children "ever breastfed". But some studies use other more elaborate definitions of breast-feeding taking account of the exclusiveness and duration of breast-feeding.

Confounders

The assessment of potential confounders and the definition of the outcome were not consistent across the different studies. Overweight or obesity were defined by BMI percentiles $\geq$ 90, 95 or 97 with varying reference populations. Heterogeneity can be identified by appropriate tests; stratified analysis may indicate sources of heterogeneity.

Publication bias:

(Small) studies which do not show a significant effect are less likely to be published [13]. Publication bias can be detected by a funnel plot. A measure of the effect estimates of breast-feeding on childhood obesity (for example the log of the odds ratios) are plotted against a measure of precision reflecting the study size (for example the inverse of the standard error of the log odds ratio). It is assumed that the point estimates for more powerful studies will be closer to the pooled estimate. The plot therefore constitutes a funnel, the tip being formed by the more powerful studies (figure 2). The objective is to assess symmetry as an indicator of the absence of publication bias. A funnel plot regression analysis can also be conducted. This analysis is likely to have sufficient power if the number of included studies is 20 or more [13]. In this approach the degree of funnel plot asymmetry can be measured by the slope from a linear regression of the standardized effect sizes against precision. In absence of publication bias, this slope will be zero.

Inclusion criteria

Inclusion criteria for the meta-analysis can result in selection bias if influenced by prior knowledge of the results of the eligible studies leading to the exclusion of studies with negative findings. To minimize selection bias, inclusion criteria should be defined *a priori* in a study protocol. Eligibility should be assessed by at least two independent observers not familiar with the study results.

4. DOES A META-ANALYSIS OVERCOME CONFOUNDING AND TAKE US FURTHER TO CAUSALITY?

Meta-analyses can never be better than the primary studies included in them. If residual confounding is a problem of the observational studies there will also be potential residual confounding in the meta-analysis. Breast-feeding might be a surrogate for other factors that could not be assessed or adjusted for. Parental overweight, parental smoking and socioeconomic status of the parents is, at the same time, related to breast-feeding and to childhood obesity and this may account for confounding. Estimates of these factors are used in the meta-analysis to adjust for confounding. However the estimates of these potential confounders may not be precise enough for full adjustment (= residual confounding).

Residual confounding may also arise from unknown factors, associated both with the exposure and the outcome, for which data have not been collected.

5. METHODS AND MAIN RESULTS OF A RECENT META-ANALYSIS ON BREAST-FEEDING AND CHILDHOOD OBESITY [3]:

Cohort-, cross-sectional- or case-control studies were included in the meta-analysis. Only studies with adjustment for at least three potential confounding factors such as birth weight, dietary factors, physical activity, parental overweight, parental smoking and socio-economic status were included in the meta-analysis. Other inclusion criteria were: comparable risk estimates such as odds ratio or relative risk, ,age at the last follow-up had to be between 5 and 18 years; feeding-mode had to be assessed and reported and obesity as outcome had to be defined by BMI percentiles $\geq$ 90, 95 or 97. Inclusion criteria were defined *a priori* by someone not initially familiar with the study results.

A systematic computerised literature search of published studies for breast-feeding, obesity and children was conducted. Identification of additional studies was carried out by handsearching of original articles and reviews.

The pooled odds ratios of the eligible studies were calculated. Heterogeneity was tested to determine whether the studies were measuring the same effects. Then stratified analyses were carried out to detect potential sources of heterogeneity by testing the stability of the findings across different approaches in study design, exposure ascertainment and selection of study participants. Additionally the potential impact of inclusion of other studies not matching the inclusion criteria for the meta-analysis on the pooled estimates was assessed.

Nine studies, with more than 69000 participants in total, met the inclusion criteria. The adjusted odds ratio for breast-feeding on childhood obesity was 0.78, 95%CI (0.71, 0.85) in the fixed-effects model. The results of the included studies were homogeneous (Q-test for heterogeneity, $p>0.3$). Stratified analyses showed no significant differences regarding different study types, age groups, definition of breast-feeding or obesity and number of confounding factors adjusted for (table 1).

The funnel plot was asymmetrical due to one particular study. Funnel plot regression gave no indication of publication bias, however the statistical power might have been insufficient due to the small number of included studies.

Additionally it is difficult to definitely rule out publication bias. Some studies, which found no significant effect in a crude analysis, did not report adjusted estimates and therefore had to be excluded from the meta-analysis. Inclusion of these studies might reduce the protective effect of breast-feeding. However, most of the recently published studies with weak or absent effects in the crude analysis presented estimates with adjustment for confounding.

To assess potential selection bias a pooled estimate of all eligible studies which reported adjusted odds ratios with confidence intervals, including studies excluded from the original meta-analysis, was calculated with the two studies enrolling individuals either too young or too old to meet the original inclusion criteria [14,15]. In this analysis the AOR of 0.77 (95%CI: 0.72, 0.82) was similar to the base case.

In conclusion the meta-analysis indicates that breast-feeding has a small, but consistent, protective effect on obesity risk in childhood. Since it is difficult to rule out residual confounding and publication bias there remains some uncertainty. Regarding publication bias we felt reassured when we looked at unpublished data from the Bavarian school entry examinations 1999 and 2002 and compared the observed effect estimates for breast-feeding on childhood obesity to the original publication based on data from the 1997 school entry examinations [4] (table 2). While in 1999 no significant protective effect could be seen, in 2002 the effect became significant again.

6. HOW IS IT BIOLOGICALLY PLAUSIBLE FOR BREAST-FEEDING TO PREVENT CHILDHOOD OBESITY?

There are some hints for biological plausibility of a protective effect of breast-feeding including behavioural and hormonal mechanisms and differences in macronutrient intake. Formula-fed infants have higher plasma-insulin concentrations compared to breast-fed infants. This could stimulate fat deposition and lead to early development of adipocytes [16]. Bioactive factors in breast-milk might modulate growth factors which

inhibit adipocyte differentiation *i vitro* [17,18]. The amount of protein intake and energy metabolism is lower in breastfed than in formula-fed infants [19]. A longitudinal study showed a significant positive association between early protein intake and later BMI [20], suggesting that a higher amount of protein intake early in life might increase the risk of obesity in later life. In animal studies the availability of protein during fetal and early postnatal development was found to have a long term effect on the glucose metabolism and body composition [21,22].

7. PUBLIC HEALTH IMPACT

A protective relationship between breast-feeding and childhood obesity might be relevant on the population level. Even a small protective effect with an odds ratio near to one would have a large public health impact. This is reflected in the population attributable risk (PAR: reduction in the prevalence of childhood obesity by breast-feeding of all children) and the population attributable risk fraction (PARF: fraction of formula-fed children with obesity where obesity could have been prevented by breast-feeding of all children). Data from the Bavarian school entry examinations in 4916 children with an overweight prevalence of 10.4% showed a breast-feeding prevalence of 76.3% and an adjusted odds ratio for breast-feeding of 0.75 resulting in a potential reduction in the prevalence of overweight from 10.4 to 9.6% if 100% instead of 76% of the children had been breastfed (population attributable risk). In this situation, 7.3% of the risk for childhood overweight could be explained by not breast-feeding (population attributable risk fraction).

REFERENCES

1. Dewey KG. Is breastfeeding protective against child obesity? *J Hum Lact* 2003;**19**(1)**:**9-18.
2. Clifford TJ. Breast feeding and obesity. *BMJ* 2003;**327**(7420)**:**879-80.
3. Arenz S, Rückerl R, Koletzko B and von Kries R (2004). Breast-feeding and childhood obesity. A systematic review. Int J Obes Relat Metab Disord, 28, 1247-56.I
4. von Kries R, Koletzko B, Sauerwald T, et al. Breast feeding and obesity: cross sectional study. *BMJ* 1999;**319:**147-50.

5. Toschke AM, Vignerova J, Lhotska L, Osancova K, Koletzko B, Von Kries R. Overweight and obesity in 6- to 14-year-old Czech children in 1991: protective effect of breast-feeding. *J Pediatr* 2002;**141**(6)**:**764-9.
6. Poulton R, Williams S. Breastfeeding and risk of overweight. *Jama* 2001;**286**(12)**:**1449-50.
7. O'Callaghan MJ, Williams GM, Andersen MJ, Bor W, Najman JM. Prediction of obesity in children at 5 years: a cohort study. *J Paediatr Child Health* 1997;**33**(4)**:**311-6.
8. Liese AD, Hirsch T, von Mutius E, Keil U, Leupold W, Weiland SK. Inverse association of overweight and breast feeding in 9 to 10-y-old children in Germany. *Int J Obes Relat Metab Disord* 2001;**25**(11)**:**1644-50.
9. Li L, Parsons TJ, Power C. Breast feeding and obesity in childhood: cross sectional study. *BMJ* 2003;**327:**904-5.
10. Gillman MW, Rifas-Shiman SL, Camargo CA, Jr., et al. Risk of overweight among adolescents who were breastfed as infants. *Jama* 2001;**285**(19)**:**2461-7.
11. Bergmann KE, Bergmann RL, Von Kries R, et al. Early determinants of childhood overweight and adiposity in a birth cohort study: role of breast-feeding. *Int J Obes Relat Metab Disord* 2003;**27**(2)**:**162-72.
12. Hediger ML, Overpeck MD, Kuczmarski RJ, Ruan WJ. Association between infant breastfeeding and overweight in young children. *Jama* 2001;**285**(19)**:**2453-60.
13. Sterne JA, Egger M, Smith GD. Systematic reviews in health care: Investigating and dealing with publication and other biases in meta-analysis. *BMJ* 2001;**323:**101-5.
14. Armstrong J, Reilly JJ. Breastfeeding and lowering the risk of childhood obesity. *Lancet* 2002;**359:**2003-4.
15. Parsons TJ, Power C, Manor O. Infant feeding and obesity through the lifecourse. *Arch Dis Child* 2003;**88**(9)**:**793-4.
16. Lucas A, Sarson DL, Blackburn AM, Adrian TE, Aynsley-Green A, Bloom SR. Breast vs bottle: endocrine responses are different with formula feeding. *Lancet* 1980;**1:**1267-9.
17. Hauner H, Röhrig K, Petruschke T. Effects of epidermal growth factor (EGF), platelet-derived growth factor (PDGF) and fibroblast growth factor (FGF) on human adipocyte development and function. *Eur J Clin Invest* 1995;**25**(2)**:**90-6.
18. Petruschke T, Röhrig K, Hauner H. Transforming growth factor beta (TGF-beta) inhibits the differentiation of human adipocyte precursor cells in primary culture. *Int J Obes Relat Metab Disord* 1994;**18**(8)**:**532-6.
19. Whitehead RG. For how long is exclusive breast-feeding adequate to satisfy the dietary energy needs of the average young baby? *Pediatr Res* 1995;**37**(2)**:**239-43.
20. Rolland-Cachera MF, Deheeger M, Akrout M, Bellisle F. Influence of macronutrients on adiposity development: a follow up study of nutrition and growth from 10 months to 8 years of age. *Int J Obes Relat Metab Disord* 1995;**19**(8)**:**573-8.
21. Desai M, Hales CN. Role of fetal and infant growth in programming metabolism in later life. *Biol Rev Camb Philos Soc* 1997;**72**(2)**:**329-48.
22. Burns SP, Desai M, Cohen RD, et al. Gluconeogenesis, glucose handling, and structural changes in livers of the adult offspring of rats partially deprived of protein during pregnancy and lactation. *J Clin Invest* 1997;**100**(7)**:**1768-74.

23. Sutton AJ, Duval SJ, R.L. T, Abrams KR, Jones DR. Empirical assessment of effect of publication bias on meta-analyses. *BMJ* 2000;**320:**1574-77.

TABLES

Table 1. Stratified analyses of studies that met the inclusion criteria for the meta-analysis *

Component		Pooled odds ratio and 95%CI (fixed effects)
Study type	cohort study	0.73 (0.64, 0.85)
	cross-sectional study	0.76 (0.67, 0.86)
Age group[$]	up to 6 y.	0.75 (0.63, 0.90)
	older than 6 y.	0.76 (0.68, 0.85)
Definition of breast-feeding	never-ever	0.76 (0.67, 0.86)
	other definition	0.74 (0.64, 0.85)
No. of confounding factors adjusted for	<7	0.69 (0.59, 0.81)
	≥7	0.78 (0.70, 0.87)
Definition of obesity	≥95. Perc.	0.79 (0.68, 0.91)
	≥97. Perc.	0.76 (0.65, 0.89)

* table from: Arenz et al., in press [3]

Table 2. Adjusted odds ratios for breast-feeding and overweight or obestiy in school entrance examinations in bavaria

Year of school entrance examination	adjusted odds ratio and 95%CI for breast-feeding	
	overweight	obesity
1997	0.79 (0.68, 0.93)	0.75 (0.57, 0.98)
1999	0.84 (0.66, 1.06)	0.91 (0.60, 1.38)
2002	0.79 (0.63, 0.99)	0.70 (0.47, 1.04)

Figure 1: Publication period of studies on breast-feeding and childhood obesity and of studies which could show a protective effect of breast-feeding

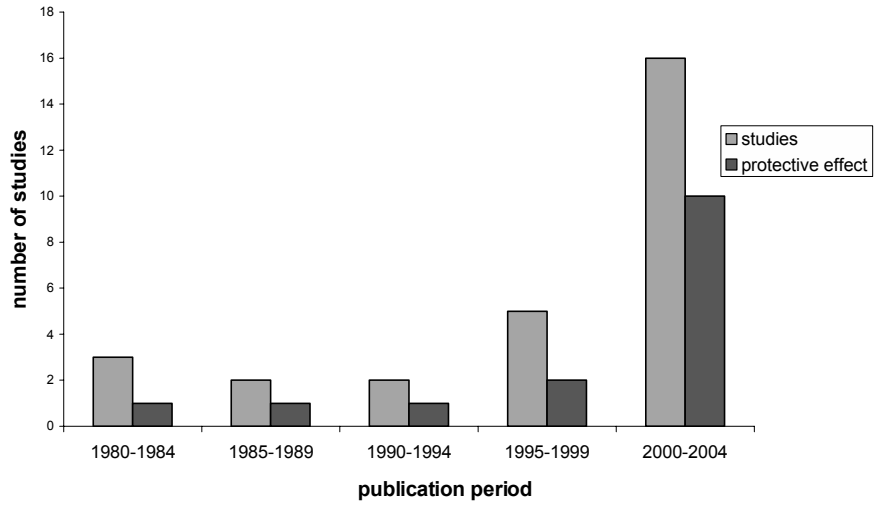
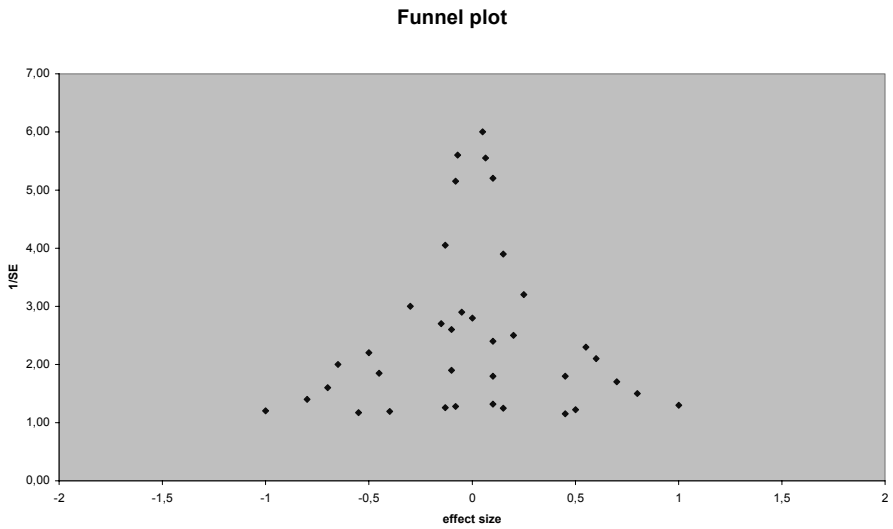


Figure 2: Symmetrical funnel plot generated from simulated studies



DISCUSSION FORUM: FROM INNOVATION TO IMPLEMENTATION

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Abstract: Requirements for the safety and nutritional adequacy of infant formula are set by legislation and aim for the best possible substitute for human milk with regard to growth, development and biological effects. This is, however, a continuous process and has to be supported by science-driven innovative activities of manufacturers and be confirmed by adequate clinical studies performed according to agreed standards.

Key words: infant formula - infant nutrition - human milk - study design - composition - biological effect - innovation - safety - scientific substantiation

1. INTRODUCTION

Innovations in the field of commercial infant formula are an ongoing process. Questions of hygiene and choice of ingredients which were paramount originally are increasingly supplanted by investigations into the composition and quality of ingredients and their technological modifications. While the composition of human milk used to be the model to mimic an infant formula, increases in knowledge of the many substances present in human milk and of their effects on development and health of the infant have changed the approach in the last two decades to aim for formulas which result in physiological, biochemical and functional outcomes comparable to those observed in breast-fed infants. Consequently, the need for clinical studies to assess not only the

safety and nutritional adequacy but also the biological effects of any newly developed formula has increased and is largely recognised by manufacturers, scientists and regulators.

2. EVALUATION OF INFANT FORMULA MODIFICATIONS

Core data for the evaluation of infant formula modifications have been defined by the scientific community and were presented by **Hernell** on behalf of ESPGHAN. The objectives for infant formulas have remained the same, namely the creation of the best substitute for breast-milk. While formerly the criteria for the evaluation of infant formula quality used to be the survival and linear growth of the infant during an observation period of one to three months in addition to analytical and biochemical data, there has been a switch towards the assessment of biological effects recently. ESPGHAN has published two position papers on the nutritional and safety assessment of breast-milk substitutes (1) and on core data required for nutritional trials in infants (2). These papers stress the necessity of identifying the biological effects as the main outcome, give guidelines for the conduction of clinical studies and underline the role and responsibilities of ethical committees. Data to be collected are proposed and their public availability is discussed. For that purpose a register of all clinical studies and a common data base should be considered. Such data bases would facilitate post-study surveillance indicated for the assessment of long-term effects. Although demanding, the requirements outlined in these position papers will serve the purpose to avoid the undertaking of meaningless studies with inconclusive results because of insufficient design, lack of statistical power and inappropriately defined study goals. Investigators who observe these guidelines can be reasonably sure that their results will not be rejected as insufficient. Study results should be evaluated by independent assessors.

3. COMPOSITIONAL STANDARDS

Regulations for infant formula concern the safety and nutritional adequacy but also their presentation and marketing. Compositional standards have been developed including the selection and quality of ingredients, hygiene requirements and contaminant limits, but also quantitative requirements for nutrients. **Turck** outlined the development of the scientific basis for the revised requirements for the composition of infant formula by the Scientific Committee on Food in 2003 (3). Compositional criteria are based on present knowledge and allow a range for the content of all nutrients. In some instances, e.g. for protein, vitamins and minerals, sources are specified, in other cases, e.g. fat, sources can be selected but the resultant mixture has to be in conformity with chemical specifications. Sources must be safe and suitable for the particular nutritional purposes of infant feeding and this is the responsibility of the manufacturer. Innovations in infant formula production with regard to the selection of new nutrient sources or not specified nutritional substances or with regard to deviations from specified nutritional substances or from specified nutrient amounts must be investigated clinically as to their suitability and nutritional adequacy before marketing. Again the need for the assessment of biological endpoints in addition to nutritional parameters was underlined. It was suggested that such innovations, their underlying rationale and the results of clinical studies performed should be subjected to an evaluation by independent experts prior to the marketing of the formula e.g. by the European Food Safety Authority (EFSA). The labelling of infant formula with regard to claims is restrictive but should continue to provide special compositional information, which can help in the selection of a formula . The justification of eventual health claims on potential benefits from the composition or the presence of an ingredient in a formula should also be subjected to an *a priori* scientific evaluation. Post-launch monitoring was a recommended as a tool to check for long-term effects.

4. BARRIERS FOR IMPLEMENTING INNOVATIONS FOR MANUFACTURERS

These were outlined by **Underwood** and **Secretin.** Any innovation resulting from the evolution of knowledge in the scientific community is a challenge for the manufacturer. This challenge can be purely technological and may require substantial investments. The next challenge is the necessity to prove the usefulness of innovations while observing the safety of the formula in both pre-clinical and clinical studies according to standardised protocols suitable for the demonstration of relevant outcomes. The time-frame necessary for fulfilling these tasks as well as the costs perspective may be viewed differently between academia and industry. Close co-operation between academia and industry is mandatory for the conversion of ideas into innovative products. Confidentiality of data during the process of development is an important issue because of competition between different manufacturers and their research activities. Manufacturers see a need for communication with the consumer in a meaningful manner and rules for such communication are required. It can be assumed that the observation of recognised guidelines for the conduction of clinical research and the evaluation of the dossier to support an innovation by a central scientific authority, who guarantees the preservation of confidentiality of submitted data, would result in a more transparent and faster procedure for implementing innovations.

5. CONCLUSIONS

From the discussion with the participants the following conclusions were reached:

- Safety, nutritional and functional assessments of innovative products should be made on the product containing a "new" ingredient and not on the ingredient alone.

- Guidelines for the assessment of innovative products are needed.

- An established filtering process to evaluate the validity of benefits and communication of innovations is considered desirable.

- Not all steps considered necessary or desirable need be made at once, but a pragmatic step-wise approach should be made.

REFERENCES

1. Aggett PJ, Agostoni C, Goulet O, Hernell O, Koletzko B, Lafeber HN, Michaelsen KF, Rigo J, Weaver LT (2001) The nutritional and safety assessment of breast milk substitutes and other dietary products for infants: a commentary by the ESPGHAN Committee on Nutrition. J Ped Gastroenterol Nutr 32: 256-258
2. Aggett P, Agostoni C, Axelsson I, Goulet O, Hernell O, Koletzko B, Lafeber HN, Michaelsen KF, Morley R, Rigo J, Szajewska H, Weaver LT (2003) Core data for nutritional trials in infants: a discussion document - A commentary by the ESPGHAN Committee on Nutrition. J Ped Gastroenterol Nutr 36: 338-342
3. Scientific Committee on Food (SCF) (2003) Report on the revision of essential requirements of infant formulae and follow-on formulae. *http://europa.eu.int/comm/fs/sc/scf/index_en.html*

CHALLENGES AND OPPORTUNITIES IN PAN-EUROPEAN COLLABORATION FOR RESEARCHERS FROM CENTRAL AND EASTERN EUROPE

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Abstract: Ten Central and Eastern [NLG4]European countries have recently joined the European Union. This historical enlargement provided a good opportunity to discuss the challenges and opportunities in Pan-European Research Collaboration for researchers from Central/Eastern Europe. This paper summarises examples of productive research collaboration between East and West, current challenges[NLG5], and ideas on how to facilitate better collaboration. A short overview of training, mobility and career development opportunities, covered by the Marie Curie actions, is also presented.

Key words: Eastern Europe; Central Europe; collaboration; Marie Curie; researchers. [NLG6]

1. INTRODUCTION

On the 1st May 2004, large parts of Central/Eastern and Western Europe were reunited. Ten new countries joined the European Union (EU) – Cyprus, the Czech Republic, Estonia, Hungary, Latvia, Lithuania, Malta, Poland, the Slovak Republic and Slovenia. This historical enlargement provided a good opportunity to discuss the challenges and opportunities in Pan-European Research Collaboration facing scientists from Central and Eastern Europe. Experts from different countries were

invited to present examples of productive research collaboration between Central/Eastern and Western Europe, current challenges, and ideas on how to facilitate better collaboration. Furthermore, an overview of the researchers' training network, mobility and career development, covered by the Marie Curie Fellowships, was presented. This paper summarises the reports presented during the Discussion Forum.

2. HUNGARIAN EXPERIENCE

National funding of medical research in Hungary is minimal. In 2004, the National Research Fund, the leading sponsor of research activities in Hungary, provided support for 28 research projects within non-surgical clinical sciences. However, the extent of the yearly allocation was between only 3,000 and 6,000 euros. Resources of this magnitude may represent a useful auxiliary tool, but they are clearly insufficient to serve as a basis for funding high quality medical research. International funding of research, primarily by the EU, may effectively fill the wide gap between actual needs and national resources. However, the experiences of Hungarian researchers in one completed EU study (NUHEAL, QLK1-1999-00888, 1999-2003) and in some project applications, have raised serious concerns about the long-term ability to apply successfully for funding at the European level. Serious drawbacks, both at the planning and completion stages of EU studies, may be faced. While the scientific essence of a project application might be sound, it might not be easy to fulfil the complex written and unwritten technical requirements of a successful project application.

3. SLOVENIAN EXPERIENCE

The research at the University Children's Hospital, Ljubljana, during the past four years has been based on several projects, supported by the Ministry of Education, Science and Sport. These activities have led to both bilateral and multinational research collaborations. Examples of bilateral collaboration include the Slovenian-German co-operation, sponsored by the Slovenian and German Ministry for Science, which enabled reciprocal visits of researchers from Ljubljana, and the *Deutscher Akademischer Ausatuschdienst* grant for laboratory equipment

for determination of fat content and fatty acid composition of human milk and plasma lipids. This is now in the Laboratory of the University Children's Hospital in Ljubljana. A good example of multinational co-operation is the Data Mining Tools and Services for Grid Computing Environments (DataMiningGrid) project, coordinated by W.Dubitzky from the University of Ulster. Computer experts from Germany, Ireland, Poland and Slovenia, will develop software for analysing distributed medical and nutritional databases. This new technology will be available to the Centre for Nutrition, University Children's Hospital, Ljubljana, for the purpose of technology demonstration and further studies. Additionally, funds will be received for several new computers and educational activities.

4. RUSSIAN EXPERIENCE

Although many problems in paediatric nutrition are the same as in other European countries, some might be specific to Russia. Therefore, there are various possibilities for collaborative European research projects, some of which have already been undertaken. Examples include studies on the effect of fruit and vegetable beikost on infant gut microflora, and the effect of traditional Russian acid-based milk product (kefir) on stool haemoglobin content (as index of intestinal bleeding).

The new journal *Problems of Paediatric Nutrition and Dietetics*, which is devoted to paediatric nutrition and publishes articles of Russian as well as European researchers, is another example of good co-operation between East and West.

Topics for future collaborative research include comparative studies of infant gut microflora in different European countries; nutritional status determined with stable isotopes; effects of some pre- and probiotic products; and the optimal time for the introduction of beikost foods into the diet.

However, the main problem for further collaborative research is lack of financial support, as well as limited instrumental and methodological possibilities of many Russian hospitals and laboratories. Special funds to support co-operative research, as well as training of Russian scientists in leading European hospitals and laboratories, would be of great assistance.

5. POLISH EXPERIENCE

Polish scientists have been, and are currently involved in, different Pan-European research activities, which are a good opportunity not only to perform well-designed and managed studies, but also to learn how to lead EU projects and how to deal with EU documentation. Three important examples of scientific collaboration include:

1.The EU Childhood Obesity Programme - this will investigate whether feeding infant formulas, which differ in their level of milk proteins, can influence the risk of subsequent childhood obesity. This important trial takes place in seven countries (Belgium, Germany, Italy, Poland, France, Great Britain, and Spain). Good co-operation and promising results of this and other projects concerning nutritional programmes have paved the way for a very large next-step EU research project, EARNEST, in which our Polish team will be more heavily involved.

2. The EU Project Paediatric Research Centre - Focusing on Effective Child Treatment (PERFECT) that acts on the basis of the Children's Memorial Health Institute (CMHI) in Warsaw. The aim of the PERFECT project is to establish a better position of CMHI in integrated Europe by networking, combining and constructing joint research projects with similar paediatric centres in the EU.

3. International Life Sciences Institute project - focusing on nutritional practices and recommendations for European children and adolescents.

6. MARIE CURIE ACTIONS

The Sixth Framework Programme's Human Resources and Mobility (HRM) activity has a budget of €1,580 million and is based largely on the financing of training and mobility activities for researchers. These activities, known as the **Marie Curie Actions**, are aimed at the development and transfer of research competencies, the consolidation and widening of researchers' career prospects, and the promotion of excellence in European research. The major actions are:

Marie Curie Research Training Network that provides training and research experience for researchers of any age or nationality by giving them the opportunity to spend between three months and three years in

another country as part of a collaborative international high-quality research project.

Marie Curie Early Stage Training in which funding is available for universities, research organisations, and businesses in the EU or Associated States to provide early-stage researchers of any nationality or age with structured scientific or technological training opportunities of between three months and three years.

Marie Curie Transfer of Knowledge in which universities, research institutions or enterprises in the EU Member or Associated States can apply to the Commission for funding to reinforce or develop new research competencies through the recruitment of experienced researchers.

Marie Curie Conferences and Training Courses in which funding is available to help universities, research centres and businesses in the EU or Associated States to organise conferences and training courses and to allow early-stage and more experienced researchers to take part.

Marie Curie Intra-European Fellowships are open to EU and Associated State researchers of all ages with at least four years' professional experience or a doctorate degree. The purpose is to give them the financial means to undertake advanced training through research or to acquire complementary skills at a European organisation most suited to their professional needs.

Marie Curie Incoming International Fellowships are targeted at experienced researchers from outside the EU and Associated States who want to move to Europe to take part in research training.

Marie Curie Outgoing International Fellowships are to allow experienced researchers from EU or Associated States to broaden their international research experience by spending time at a research centre outside the EU and Associated States for periods of between one and three years, including a compulsory return phase.

The Marie Curie Actions also focus on the promotion and recognition of excellence in European research. Three initiatives have been set up to increase the visibility and attractiveness of European research. The aim of the **Marie Curie Excellence Grants** is to create transnational research teams led by a researcher who has the potential to reach excellence in a particular scientific field. **Marie Curie Chairs** are to encourage world-class researchers working in fields of key importance for Europe to resume or further develop their careers in Europe, by providing support for a period of research and teaching of between one and three years. **Marie Curie Excellence Awards** are research prizes to

give recognition to the excellence achieved by researchers who have benefited from a Community mobility scheme for the training of researchers for a minimum of one year.

7. CONCLUSIONS

Research in Central and Eastern Europe is underfunded and lags behind that of Western Europe. However, these countries have great human potential and enthusiasm for growth. Collaboration of researchers across Europe could be encouraged – and given a much needed financial boost – through the support of the EU. In addition, a shortage of qualified technical personnel for the planning and completion of successful EU grant applications is a problem for researchers in many Central/Eastern European countries, and strategies to overcome this problem must be developed.

BEST PRACTICE IN COMMUNICATING THE RESULTS OF EUROPEAN RESEARCH TO THE PUBLIC

Communication of European Research

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Abstract: Dissemination of results is a contractual obligation of participation in research initiatives supported under the European Union's Framework Six Programme. The European Commission's Directorate-General for Research is heavily involved in communicating the results of EU-funded research to the media and the general public. In any research project it is important to start thinking about dissemination and exploitation right at the beginning. How can this be possible when the project has not yet yielded any results? Examples from two ongoing EU supported projects are shared and discussed.

Key words: EU; communication; dissemination; exploitation

1. WHY IS DISSEMINATION SO IMPORTANT?

The European Commission launches some 2,000 new research projects every year. An annual budget of more than EUR 4 billion is being allocated by the European Union for funding research projects.

In the Sixth Framework Programme 2002-2006 (FP6), the European Commission supports very large projects (50-100 partners). Against this background, dissemination of results is a contractual obligation of participation in research initiatives supported under the European Union's FP6. The specific aims of this provision are to promote knowledge sharing, greater public awareness, transparency and education. It also provides tangible proof that collaborative research not only exists, but also pays dividends in terms of academic excellence,

industrial competitiveness, employment opportunities, environmental improvements and enhanced quality of life for all.

At the same time, the communication of successes and the announcement of exploitable developments are of direct value to the participants themselves. Suitably framed messages can help by:

- Drawing the attention of national governments, regional authorities and other public and private funding sources to the needs and eventual benefits of the research;
- Encouraging talented students and scientists to join the partner institutes and enterprises;
- Enhancing the reputation of participants, at local, national and international level; etc.

The European Commission's Directorate-General for Research is heavily involved in communicating the results of EU-funded research to the media and the general public. Support and help are provided to assist project coordinators and team leaders to generate an effective flow of information and publicity about the objectives and results of their work.

The European Commission's communication strategy particularly addresses communications via the 'mass media' (TV, radio and the written press), the workings of which may be less familiar to scientific/academic partners. It also covers websites and other internally generated support such as print publications, CDs and video.

The European Commission draws in particular the attention of participants in FP6-funded projects on the fact that they can no longer ignore the 'public communication' dimension of their activity. As the 2001 and 2003 Eurobarometer surveys of European attitudes to science showed that Europe's citizens have a very positive perception of science and technology, we believe that exposing non-specialists to the results of research work helps to improve their understanding of scientific and technological developments and stimulate public debate on important issues.

However, research has shown that our acceptance or rejection of technological and scientific innovation is determined largely by our preconceived ideas. This means that we must therefore dispense with the widely held belief that high-quality scientific information can influence people's judgement. Many researchers continue to claim, for example, that opposition to genetically modified organisms is due to the fact that most of the population fail to understand the underlying scientific notions.

The workshop outlined the initiatives taken by the European Commission to improve communication, outreach and dissemination of results from EU-funded research projects, and to facilitate the work of project contractors in this respect. Guidelines and best practices to help project participants in communicating and disseminating their research results were presented and discussed.

2. DISSEMINATION AND EXPLOITATION IN PRACTICE

In any research project it is important to start thinking about dissemination and exploitation right at the beginning. How can this be possible when the project has not yet yielded any results? Examples from two ongoing EU funded projects were shared and discussed.

The *EU Childhood Obesity Programme* is a one year double blind randomised multi-centre intervention trial on new born infants, comparing isocaloric infant formulas with high and low protein contents within the limit of EU recommendations, balanced by fat. The trial is taking place in 5 EU countries, namely Belgium, Germany, Italy, Poland and Spain. A reference breast fed group will be studied at the same time.

The Programme will study, over the first two years of life, weight and height, body composition, hormonal status and protein metabolism. Important conclusions will be drawn at the age of 2 years on the relation between protein intake, growth and obesity risk as assessed by the length change over 2yrs. Additionally, children will be followed-up until age 8 years, in order to assess the long-term impact on the prevalence of obesity. Target audiences include: Scientists, Policymakers, Health professionals, and Consumers (parents). Dissemination materials include Printed materials (brochures, newsletters, leaflets), Web site (www.childhood-obesity.org) and Media briefings.

Food in Later Life (www.foodinlaterlife.org)will improve the nutritional well-being of older people and hence their independence and quality of life. The Project will benefit from the assistance of Dissemination and Exploitation Consensus Platforms (DECPs), both at a pan-European Level and at a National level. Some of the roles performed by these panels are as follows:

- Provide specific expertise and advice on industry, management issues and matters relating to health and social care services.

- Encourage the dissemination of European databases arising from *Food in Later Life* to research centres and industry.
- Assist the development of consumer-friendly tools to allow manufacturers and caterers to assess food choices and food provision preferences in older people and to allow health professionals to assess nutritional status in older people.
- Explore the potential for targeted ranges of food products and services for older people, according to identified preferences.

This pan-European platform will consist of a small group of stakeholder representatives from the manufacturing, retail and service industries, relevant charities and policy making departments of governments. This panel will meet three times during the project; the meetings taking place during a partners' meeting.

3. CONCLUSION

This workshop aimed to advise others how they can maximise their dissemination and exploitation plans in any research project.

REFERENCES

EU Childhood Obesity Programme website: www.childhood-obesity.org
Food in Later Life website: www.foodinlaterlife.org

LONGTERM EFFECTS OF PRE- AND POSTNATAL EXPOSURE TO LOW AND HIGH DIETARY PROTEIN LEVELS

Evidence From Epidemiological Studies And Controlled Animal Experiments

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Abstract: The purpose of this short review is to summarize the available evidence from observational studies and rodent models for an association between maternal protein intake, birth weight, pre- and post-weaning body mass gain and adult body fatness in the offspring.

Key words: observational studies; birth weight; body weight; obesity; maternal low protein model, maternal high protein model; rats.

1. INTRODUCTION

In many populations worldwide epidemiological evidence relates low birth weight to increased risk for syndrome X, coronary heart disease, and high blood pressure in adult age. On the basis of these observations it was suggested that low birth weight is causally related to fetal under- or malnutrition and subsequent fetal growth retardation which permanently affects adult health (Godfrey and Barker, 2000). Evidence is emerging that nutritional programming during fetal development might be involved in the development of obesity.

2. OBSERVATIONAL STUDIES IN HUMANS

Human studies on the relationship of maternal diet, birth weight and adult disease have generated inconclusive results. In one study low birth weight was related to either high carbohydrate intake during early

pregnancy, or to low dairy and meat protein intake during late pregnancy (Godfrey et al., 1996), while in another it was related to reduction of carbohydrate intake between early and late pregnancy (Shiell et al., 2001). No relation between macronutrient intake and birth weight was reported by Mathews et al., 1999. In two other studies an inverse relation between high maternal protein intake and birth weight was found (Langley-Evans et al., 2003; Campbell et al., 1996). Campbell et al., 1996, reported increased blood pressure in adult offspring with maternal animal protein intake below 50 g daily plus a higher carbohydrate intake, and with daily maternal animal protein intake above 50 g, and lower carbohydrate intake. Shiell et al., 2001, found a greater consumption of meat and fish in the second half of pregnancy to be related to higher systolic blood pressure in adult offspring.

An assessment of protein supplementation during pregnancy on protein intakes, gestational weight gain, and the outcome of pregnancy came to the conclusion that high-protein or balanced protein supplementation is not beneficial and may be harmful to the infant (Kramer, 1993; Kramer & Kakuma, 2003). Thus from these studies it appears that there is a tendency for association between high maternal protein intake and low birth weight.

In all studies mean maternal protein intake was 1.2-1.5 g/kg BW and thus above current recommendations. Thus it is likely that the individuals with 'lower' protein intakes are mostly in the recommended range, whereas those with 'higher' protein intakes were largely in excess of recommendations. It is impossible to derive from these studies an optimal range of protein intake in terms of birth weight or whether reported associations between low weight at birth and disease in later life are attributable to the effects of maternal nutrition at all. A further issue in these studies is the timing of acquisition of the maternal nutritional information. Because fetal growth trajectory is established very early in pregnancy it is not clear how relevant estimations of maternal diet in mid gestation are.

Although there is some evidence that birth weight and body fatness of children and young adults are inversely related (Metges, 2001; Jaquet et al., 2000), a meta-analysis of the relationship between birth weight and adult body fatness showed that the association between low birth weight

and high adult BMI is contradictory (Martorell et al., 2001). This is somewhat surprising because excess adipose tissue leads to reduced insulin sensitivity, which is frequently associated with a set of cardiovascular risk factors, including hyperinsulinemia, hypertension, and glucose intolerance. Possibly, obesity is either a less sensitive outcome of fetal growth retardation than other metabolic disorders, or postnatal factors such as overnutrition or sedentary life style mask possible prenatal effects.

3. CONTROLLED STUDIES WITH ANIMAL MODELS

Although the association between birth weight and adult disease has been recently challenged (Huxley et al., 2002), work with animal models suggests that nutritional programming during fetal life does affect adult health. A widely established model to study fetal programming is the maternal low protein (MLP) model in rodents. Usually an isocaloric casein-based semi-synthetic diet of about 40-50 % protein restriction during pregnancy is compared with an adequate maternal diet of 18-20% protein. However, there are subtle but possibly meaningful variations in experimental design. For example, casein-based diets are supplemented by methionine. In some studies the supplemental methionine: protein ratio is balanced (e.g. Bennis-Taleb et al., 1999), but in others methionine is supplemented independently of the dietary protein content (Rees et al., 1999; Gardner et al., 1997). Further, in some reports offspring was exposed to low protein diet in utero and during lactation (Desai et al., 1997), whereas in others the use of foster mothers on control diet throughout pregnancy and lactation allowed separation of dietary protein effects between prenatal and early postnatal phase (Bennis-Taleb et al., 1999). We found recently, that body mass gain pre-weaning differs between offspring exposed to low or high protein diet in utero and lactation, or in utero only (Daenzer, Petzke, Metges, Klaus, unpublished). This indicates that there are independent effects of milk quality during early lactation.

In numerous studies it was shown that a **low protein intake throughout pregnancy** in rats and mice results in low birth weight, altered hepatic glucose output, age-related loss of glucose tolerance and insulin resistance, and hypertension (e.g. Hales and Ozanne, 2003; Gardner et al., 1997). Also increased catch-up growth during lactation and post-weaning and higher body weight at age 10 wk was reported (Ozanne et al., 2004). Data on body fat were not reported. Also isocaloric maternal **high protein diet during pregnancy** was followed by a reduction of body weight at day of life 2 but a higher body weight than controls up to wk 6 in the offspring (Daenzer et al., 2002).

Exposure to high protein diets during pregnancy and lactation resulted in a decreased body weight of pups until weaning (Gambardella et al., 1987; Daenzer et al., 2002). Furthermore, the offspring from maternal high protein feeding had a higher body fatness and a reduced total energy expenditure at wk 9. In contrast, postnatal protein overnutrition only did not lead to an obese phenotype (Daenzer et al., 2002). This suggests that upon prenatal high protein exposure offspring overcompensated in terms of catch-up growth which was followed by increased body fat in young adults, which provides first evidence that in utero high protein exposure can predispose offspring to adult obesity.

REFERENCES

Bennis-Taleb N, Remacle C, Hoet JJ, Reusens B, 1999, A low-protein isocaloric diet during gestation affects brain development and alters permanently cerebral cortex blood vessels in rat offspring. *J Nutr.* **129**:1613-1619.

Campbell DM, Hall MH, Barker DJ, Cross J, Shiell AW, Godfrey KM, 1996,. Diet in pregnancy and the offspring's blood pressure 40 years later, *Br J Obstet Gynaecol.* **103**:273-280.

Desai M, Byrne CD, Meeran K, Martenz ND, Bloom SR, Hales CN, 1997, Regulation of hepatic enzymes and insulin levels in offspring of rat dams fed a reduced-protein diet. *Am J Physiol*. **273**(4 Pt 1):G899-904.

Daenzer M, Daenzer M, Ortmann S, Klaus S, Metges CC, 2002, Prenatal high protein exposure decreases energy expenditure and increases adiposity in young rats. *J Nutr;* **132**: 142-144.

Gambardella P, Sticchi R, Ferrante P, D'Aponte D, 1987, Hyper- and hypoproteic diet in growing rats: comparison between effects and evaluation of damages. *Int J Vitam Nutr Res*. **57**:441-445.

Gardner DS, Jackson AA, Langley-Evans SC, 1997, Maintenance of maternal diet-induced hypertension in the rat is dependent on glucocorticoids. *Hypertension* **30**:1525-1530.

Godfrey KM, Barker DJ. Fetal nutrition and adult disease, 2000, *Am J Clin Nutr.*; **71**:1344S-1352S.

Godfrey K, Robinson S, BarkerDJP, Osmond C, Cox V, 1996, Maternal nutrition in early and late pregnancy in relation to placental and fetal growth. *BMJ* **312**:410.

Hales CN, Ozanne SE, 2003, For debate: Fetal and early postnatal growth restriction lead to diabetes, the metabolic syndrome and renal failure, *Diabetologia* **46:**1013-1019.

Huxley R, Neil A, Collins R, 2002, Unravelling the fetal origins hypothesis: is there really an inverse association between birthweight and subsequent blood pressure? *Lancet* **31**:360(9334):659-665.

Jaquet D, Gaboriau A, Czernichow P, Levy-Marchal C, 2000, Insulin resistance early in adulthood in subjects born with intrauterine growth retardation. *J Clin Endocrinol Metab*. **85:**1401-1406.

Kramer MS, Kakuma R, 2003, Energy and protein intake in pregnancy. *Cochrane Database Syst Rev*. **4:**CD000032.

Kramer MS, 1993, Effects of energy and protein intakes on pregnancy outcome: an overview of the research evidence from controlled clinical trials. *Am J Clin Nutr.* **58:**627-635.

Langley-Evans SC, Langley-Evans AJ, Marchand MC, 2003, Nutritional programming of blood pressure and renal morphology. *Arch Physiol Biochem.* **111**:8-16.

Martorell R, Stein AD, Schroeder DG, 2001, Early nutrition and later adiposity. *J. Nutr.* **131**: 874S-880S.

Mathews F, Yudkin P, Neil A, 1999, Influence of maternal nutrition on outcome of pregnancy: prospective cohort study. *BMJ.* **319**(7206):339-343.

Metges CC, 2001, Does dietary protein in early life affect the development of adiposity in mammals? *J Nutr.* **131**:2062-2066.

Ozanne SE, Lewis R, Jennings BJ, Hales CN, 2004, Early programming of weight gain in mice prevents the induction of obesity by a highly palatable diet. *Clin Sci (Lond).* **106**:141-145.

Rees WD, Hay SM, Buchan V, Antipatis C, Palmer RM, 1999. The effects of maternal protein restriction on the growth of the rat fetus and its amino acid supply. *Br J Nutr.* **81**:243-250.

Shiell AW, Campbell-Brown M, Haselden S, Robinson S, Godfrey KM, Barker DJ, 2001, High-meat, low-carbohydrate diet in pregnancy: relation to adult blood pressure in the offspring. *Hypertension* **38:**1282-1288.

PROTEIN INTAKE IN THE FIRST YEAR OF LIFE: A RISK FACTOR FOR LATER OBESITY?

The EU Childhood Obesity Project

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Abstract: Effective strategies for primary prevention are urgently needed to combat the rapidly increasing prevalence of childhood obesity. Evidence accumulates that early nutrition programmes later obesity risk. Breast feeding reduces the odds ratio for obesity at school age, adjusted for biological and sociodemographic confounding variables, by some 20-25 %. We propose that the protective effect of breast feeding is related in part by the induction of a lower weight gain in infancy, which is related to differences in substrate intake. Protein intake per kg bodyweight is some 55-80 % higher in formula fed than in breast fed infants. We hypothesize that high early protein intakes in excess of metabolic requirements enhance weight gain in infancy and increase later obesity risk (the "early protein hypothesis"). The European Childhood Obesity Programme tests this hypothesis in a randomized double blind intervention trial in 1150 infants in five European centres. Infants that are not breast fed are randomized to formulae with higher or lower protein content and followed up to school age. If an effect of infant feeding habits on later obesity risk should be established, there is great potential for effective preventive intervention with a significant potential health benefit for the child and adult population.

Key words: Insulin; Insulin like growth factor I (IGF1); Metabolic Programming; Randomized clinical trial; European Commission Fifth Framework Programme;

1. THE NEED FOR OBESITY PREVENTION

Childhood obesity is now considered a global epidemic in view of the alarming increase of its prevalence and severity, not only in affluent but also in less privileged childhood populations worldwide (1,2). Serious short and long term consequences of childhood obesity arise in terms of damage to quality of life, performance, health and life expectancy. In addition, the size of the obesity epidemic is estimated to create huge costs for society due to loss of productivity and ensuing costs for health care and social security (1,2). Faced with the size of the problem, widely available and effective medical management of children that are already obese is needed, but at present the results of available treatments are far less than satisfactory, and costs are high (3). A recent Cochrane review on interventions for treating obesity in children found that no conclusions on the effects of treatment strategies and their components can be drawn with confidence (4).

Thus, in the present situation the emphasis must be put on development, evaluation and implementation of effective primary prevention of obesity. Some first evaluations are available on the efficacy of obesity prevention in children, even though the limited number of controlled trials available at this time allows only limited conclusions (5). Promising key strategies for prevention aim at modifying childhood behaviour to increase physical activity as a daily routine and to enhance health promoting dietary habits (1,2,5). In addition, new concepts evolve on prevention at a very early age. Already in the 1950s McCance and Widdowson observed that feeding conditions of animals during sensitive periods of early pre- and postnatal growth predetermined their weight in adulthood (6). This phenomenon, later called early nutritional or metabolic programming of adult health, has attracted renewed scientific attention. Today numerous experimental and epidemiological studies provide clear indications that metabolic events during critical time windows of pre- and postnatal development markedly modulate obesity risk in later life. Hence, modification of nutritional habits during early development may offer an opportunity for effective risk reduction of later obesity in populations. The potential practical relevance for obesity prevention is highlighted by a series of studies showing that breast fed individuals have a significantly lower obesity risk many years later than those who had been formula fed after

birth. Breast feeding reduced the odds ratio for obesity at school age, adjusted for a variety of biological and sociodemographic confounding variables, by some 20 to 25 % (8-11).

2. DOES BREAST FEEDING PROTECT AGAINST LATER OBESITY BY MODULATING CHILD BEHAVIOUR?

Elucidation of underlying mechanisms for the lesser obesity risk associated with breast feeding is important, because understanding of such mechanisms might offer opportunities for improvements of policy and practice of infant feeding regimens both for infants that are breast fed and for those that receive formula.

A number of hypotheses can be raised on potential causes. Even though the inverse relationship of both breast feeding and breast feeding duration with later obesity persists after adjustment for measurable confounding variables, residual confounding cannot be fully excluded. Since one cannot randomise healthy babies to feeding breast milk or formula for ethical and practical reasons, undisputable proof for a protective effect of breast feeding can hardly be obtained. However, the consistent results of many studies and the dose response effect between duration of breast feeding and later reduction of obesity risk observed in a number of studies make an effect of breast feeding highly likely (11).

Differences in feeding behaviour and mother-child-interaction between populations of breast and formula fed infants might have a role to play. Breast fed infants show a different suckling pattern and a higher suckling frequency (12,13). Breast fed infants seem to have greater degree of control on meal sizes and intervals than those fed formula. Sievers and co-workers monitored marked differences in feeding patterns, with a 20-30 % higher feeding volume of formula fed infants after 6 weeks of life as well as a smaller number of total meals and of nightly meals in bottle fed babies at 4 months of age (14). Such differences may modulate later body size. Agras and co-workers reported that early feeding patterns were predictive of body mass index at 3 years of age, with high-pressure sucking measured in the laboratory at 2 and 4 weeks of age (denoting a vigorous feeding style) associated with greater degree of adiposity in toddlers (15).

In contrast to infant formula, breast milk varies in taste and smell depending on maternal intake of diet and spices, and early taste experience in infancy has been shown to favour later consumption of foods with the same taste (16). Thereby, breast fed infants might be programmed to different food selection and dietary habits in alter life.

Moreover, breast feeding appears to enhance emotional bonding of the mother to her child, mediated in part by the stimulation of maternal oxytocin release by infant suckling, and breast feeding shown to lead to decreased neuroendocrine response to stressors and decreased negative mood in the mothers (17,18). These effects of breast feeding might well have repercussions on the interaction between mother and child and health related behaviours.

These and further behavioural hypotheses are plausible and attractive, but are difficult to test experimentally, thus for the time being they remain somewhat speculative.

3. ARE 'OBESITY PREVENTIVE' EFFECTS OF BREAST FEEDING RELATED TO EARLY GROWTH AND SUBSTRATE SUPPLY?

The mode of infant feeding at the breast cannot be copied with human milk substitutes, but if protective effects of breast feeding were related to compositional aspects of breast milk and to the nature of substrate supply, such benefits could potentially be extended also to formula fed populations by appropriate modifications of infant formula composition. Promising approaches can be deduced from studies evaluating physiological differences of breast and bottle fed infants.

Populations of formula fed infants show higher growth rates, with larger weight and length gains than infants fed formula (19). Based on a systematic review of 19 studies in affluent populations, Dewey concluded that by the age of 12 months, the cumulative difference in body weight amounts to approximately 400 g in infants breast-fed for 9 months and as much as 600-650 g in infants that are breast-fed for 12 months (20). This very large effect of the mode of feeding on weight gain must be expected to influence later obesity risk, based on our analysis of a large cohort study on 4235 children from Bavaria, southern Germany (21). We related overweight at school entry at school entry (age 5-7 years) to growth data obtained during the paediatric preventive

health checks at birth, 6 months, 12 months, and 24 months of age. High weight gain from birth to 24 months proved to be a strong predictor of later overweight. Children in the upper tertile of weight gain from birth to 2 years (weight gain over 24 months >9764 g) had an odds ratio of 5.7 (95% CI, 4.5-7.1) for overweight at school age relative to those with a

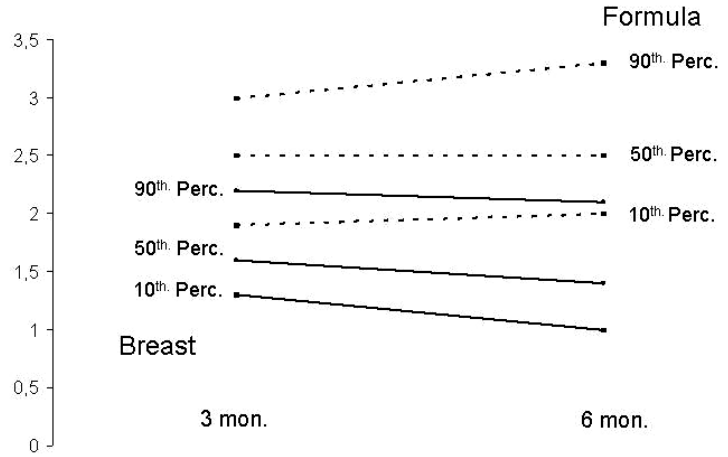

Fig. 1: Protein intake at ages 3 and 6 months (g/kg body weight, median and $90^{th}/10^{th}$ percentiles) in infants fed breast milk or infant formula (participants of the DONALD study [23])

lower early weight gain (21). Given this close relationship, we hypothesize that the protective effect of breast feeding against later obesity risk is based, at least in part, on its mediating effect on infant weight gain.

The degree of weight gain in infancy is influenced by genetic factors of the individual, birth weight, metabolic influences during pregnancy, health and disease (for example concurrent infections), and not the least dietary substrate supply. Infant formulae have a higher average caloric density (kcal/100 ml) than mean values for breast milk, and energy supplies per kg bodyweight to formula fed infants are 10-18 % higher than those to breast fed babies between 3 and 12 months of age (20). Even larger is the difference in protein intake per kg bodyweight, which is 55-80 % higher in formula than in breast fed infants (fig. 1) (22,23).

In animal studies, early nutrient supply was shown to programme later obesity risk. In rats, prenatal high protein exposure decreased

energy expenditure and increased later adiposity (24), and a high postnatal protein and nutrient supply led to higher adult body fat deposition (25) and increased adult weight by 10-40% (26).

A high protein intake in excess of metabolic requirements may enhance the secretion of insulin and insulin like growth factor 1 (IGF1). Indeed, infants fed formula had far greater postprandial levels of insulin on day six of life than infants fed cows' milk based formula (27). High insulin and IGF1 values can enhance both growth during the first 2 years of life (28) as well as adipogenic activity and adipocyte differentiation (29) (fig. 2). High protein intakes may also decrease human growth hormone (hGH) secretion and lipolysis.

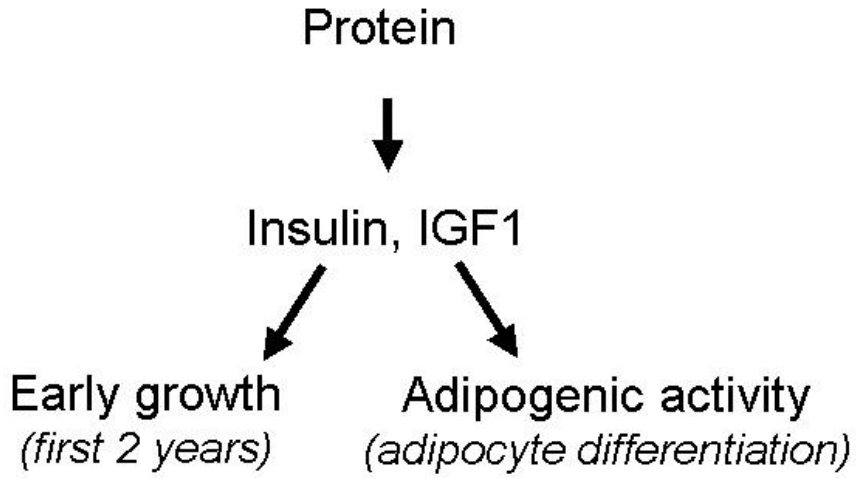

Fig. 2: High early protein intakes stimulate the secretion of insulin and insulin like growth factor 1 (IGF 1), which can enhance early growth and adipogenic activity.

Indeed, high protein intakes in early childhood, but not the intakes of energy, fat or carbohydrate, were significantly related to an early occurrence of adiposity rebound and to high childhood body mass index (BMI), corrected for parental BMI (30-32). Thus, we hypothesize that a high protein intake with infant formula, in excess of metabolic requirements, may predispose to an increased obesity risk in later life (early protein hypothesis) .

4. THE EUROPEAN CHILDHOOD OBESITY PROGRAMME

In addition to experimental approaches, human intervention studies are needed to test this "early protein hypothesis". The European Childhood Obesity Programme (www.childhood-obesity.org) funded by the European Commission's 5th Framework Research Programme has enrolled some 1150 infants after birth and aims at following them up through school age to test, in a randomized double blind intervention trial, whether variation in early protein intakes affects growth kinetics and later obesity risk. This trial is conducted in five European countries which differ substantially in their prevalence of adult obesity and also in the nutritional characteristics of the habitual diet of infants and children, in particular in protein supply with complementary feeding, i.e. Belgium (Co-ordinator Prof. Daniel Brasseur), Germany (Prof. Berthold Koletzko), Italy (Prof. Marcello Giovannini), Poland (Prof Jerzy Socha) and Spain (Dr. Ricardo Closa). Therefore the trial offers the opportunity to combine a multicentre intervention trial on infant formulae (kindly provided by Blédina SA, Steenvoorde, France) which differ in their balance of protein and fat, with an epidemiological observation study which can assess the balance of protein and fat in the overall early diet. This approach will enable us to assess the effect of variables which differ substantially within Europe, as well as allowing the intervention trial results to be analyzed within centres. The inclusion of a group of breast–fed infants in each centre will also allow an epidemiological comparison of the effects of breast feeding and formula feeding in the different countries. This approach will provide the opportunity for an external validation of the underlying hypothesis.

Growth from birth to age 2 years, a marker of later obesity risk, was chosen as the primary outcome variable. In addition, a variety of further variables are measured, including detailed data on diet, lifestyle and behaviour, biochemical and endocrine markers, markers of renal function, and others (Fig. 3). Randomisation and data collection are performed via the internet based on uniform electronic case report forms, using specially developed information technology architecture with a central database and 12 remote data entry stations as well as dedicated software that allows for secure data protection. Mechanisms for quality

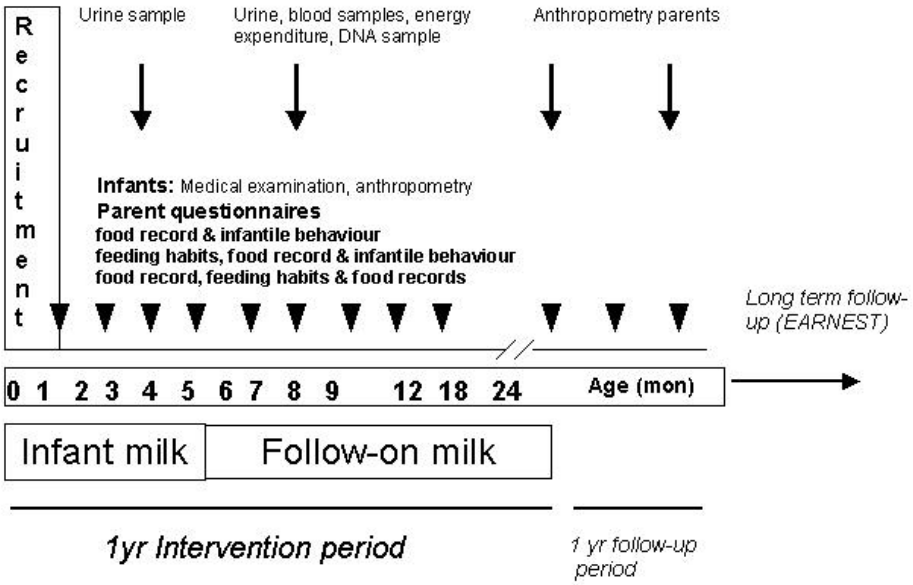

Fig. 3: Scheme of the study design of the European Childhood Obesity Programme. Infants are randomized to infant and follow-on formulae with higher or lower protein intakes from the neonatal period through the first year of life. Diet, behaviour, growth and a number of other variables are monitored during regular follow-up visits.

assurance have also been established. Data input and transfer to the central database are supervised by a contract research organization participating in the project.

The intervention trial started on 1st October 2002, and recruitment was completed on 30th June 2004. Following the study protocol and the requirements to report first results to the EU at the end of the first funding period, the study will be un-blinded in the second half of 2006 to allow for first data evaluations. However, participating children and their families will be invited for further follow-up in the project EARNEST (Early Nutrition programming of adult health, www.metabolic-programming.org) funded by the EU 6th Framework Programme.

In our view, the European Childhood Obesity Programme offers unique and exciting opportunities for evaluating the effects of early diet on long-term health in later life. If an effect of infant feeding habits on long-term growth, development of later body composition and obesity

risk is established, there is great potential for effective preventive intervention by modification of the composition and use of dietary products for infants. Thus, the expected results may have a very direct, simple application with a significant potential health benefit for the child and adult population.

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REFERENCES

1. Koletzko B, Girardet J P, Klish W, Tabacco O. Obesity in children and adolescents worldwide: current views and future directions. J Ped Gastroenterol Nutr 2002; 35:S205-12.
2. Fisberg M, Baur L, Chen W, Nelson T, Koletzko B, Moreno L, Uauy R, Hoppin R, Lau D, Strauss R. Childhood Obesity - a global perspective. J Pediatr Gastro Nutr 2004;39:S678-87.
3. Koletzko B. Childhood obesity: time for treatment or prevention? Eur J Lipid Sci Technology 2004;106:287-288
4. Summerbell CD, Ashton V, Campbell KJ, Edmunds L, Kelly S, Waters E. Interventions for treating obesity in children (Cochrane review). The Cochrane library 2004; Issue 1
5. Campbell KJ, Waters E, O'Meara S, Kelly S, Summerbell C. Interventions for preventing obesity in children (Cochrane review). The Cochrane library 2004; Issue 1
6. Koletzko B, de la Guéronnière V, Toschke AM, von Kries R. Nutrition in children and adolescents in Europe: what is the scientific basis? Introduction. Brit J Nutr 2004;92,suppl 2:S67-73

7. Ashwell M (ed.). McCance & Widdowson. A scientific partnership for 60 years. London, The British Nutrition Foundation 1993.
8.von Kries R, Koletzko B, Sauerwald T, von Mutius E, Barnert D, Grunert V, von Voss H. Breastfeeding and obesity: cross sectional study. Brit Med J 1999;319:147-150
9. Toschke AM, Vignerova J, Lhotska L, Osancova K, Koletzko B, von Kries R. Overweight and obesity in 6- to 14- year-old Czech children in 1991: protective effect of breastfeeding. J Pediatrics 2002;141:764-769
10. Koletzko B, von-Kries R. Are there long term protective effects of breast feeding against later obesity? Nutr Health. 2001; 15: 225-36.
11. Arenz S, Rückerl R, Koletzko B, von Kries R. Breast-feeding and childhood obesity. A systematic review. Int J Obesity 2004;28:1247-1256
12. Mathew OP, Bhatia J. Sucking and breathing patterns during breast- and bottle-feeding in term neonates. Effects of nutrient delivery and composition. Am J Dis Child. 1989 May;143(5):588-92.
13. Bosma JF, Hepburn LG, Josell SD, Baker K. Ultrasound demonstration of tongue motions during suckle feeding. Dev Med Child Neurol. 1990 Mar;32(3):223-9.
14. Sievers E, Oldigs HD, Santer R, Schaub J. Feeding patterns in breast-fed and formula-fed infants. Ann Nutr Metab. 2002;46(6):243-8.
15. Agras WS, Kraemer HC, Berkowitz RI, Hammer LD. Influence of early feeding style on adiposity at 6 years of age. J Pediatr. 1990 May;116(5):805-9.
16. Mennella JA, Jagnow CP, Beauchamp GK. Prenatal and postnatal flavor learning by human infants. Pediatrics. 2001 Jun;107(6):E88.
17.Klaus M. Mother and infant: early emotional ties. Pediatrics. 1998 Nov;102(5 Suppl E):1244-6.
18. Mezzacappa ES.Breastfeeding and maternal stress response and health. Nutr Rev. 2004 Jul;62(7 Pt 1):261-8.
19. Kramer MS, Guo T, Platt RW, Vanilovich I, Sevkovskaya Z, Dzikovich I, Michaelsen KF, Dewey K; Promotion of Breastfeeding Intervention Trials Study Group. Feeding effects on growth during infancy. J Pediatr. 2004 Nov;145(5):600-5.
20. Dewey KG. Growth characteristics of breast-fed compared to formula-fed infants. Biol Neonate. 1998;74(2):94-105.
21. Toschke AM, Grote V, Koletzko B, von Kries R. Identifying children at high risk for overweight at school entry by weight gain during the first 2 years. Arch Pediatr Adolesc Med. 2004 May;158(5):449-52.
22. Heinig MJ, Nommsen LA, Peerson JM, Lonnerdal B, Dewey KG. Energy and protein intakes of breast-fed and formula-fed infants during the first year of life and their association with growth velocity: the DARLING Study. Am J Clin Nutr. 1993 Aug;58(2):152-61.
23. Alexy U, Kersting M, Sichert-Hellert W, Manz F, Schöch G. Macronutrient intake of 3- to 36-month-old German infants and children: results of the DONALD Study. Dortmund Nutritional and Anthropometric Longitudinally Designed Study. Ann Nutr Metab. 1999;43(1):14-22.
24. Daenzer M, Ortmann S, Klaus S, Metges CC.Prenatal high protein exposure decreases energy expenditure and increases adiposity in young rats. J Nutr. 2002 Feb;132(2):142-4.

25. Kim S, Mauron J, Gleason R, Wurtman R. Selection of carbohydrate to protein ratio and correlations with weight gain and body fat in rats allowed three dietary choices. Internat J Vit Nutr Res 1991;61:166-179.
26. Jones A, Simson E, Friedman M. Gestational undernutrition and the development of obesity in rats. Journal of Nutrition 1984;114:1484-92.
27. Lucas A, Boyes S, Bloom SR, Aynsley-Green A. Metabolic and endocrine responses to a milk feed in six-day-old term infants: differences between breast and cow's milk formula feeding. Acta Paediatr Scand. 1981 Mar;70(2):195-200.
28. Karlberg J, Jalil F, Lam B, Low L, Yeung CY. Linear growth retardation in relation to the three phases of growth. Eur J Clin Nutr. 1994;48(Suppl 1):S25-S43.
29. Hauner H, Wabitsch M, Zwiauer K, Widhalm K, Pfeiffer EF. Adipogenic activity in sera from obese children before and after weight reduction. Am J Clin Nutr 1989;50(1):63-7.
30. Rolland-Cachera MF, Deheeger M, Akrout M, Bellisle F. Influence of macronutrients on adiposity development: a follow-up study of nutrition and growth from 10 months to 8 years of age. Int J Obes Metab Disord 1995;19(8):573-8.
31. Scaglioni S, Agostoni C, DeNotaris R, et al. Early macronutrient intake and overweight at five years of age. Int J Obesity 2000;24.
32. Parizkova J, Rolland-Cachera M. High proteins early in life as a predisposition for later obesity and further health risks. Nutrition 1997;13:818-9.
33. Akeston PMK, Axelsson IEM, Raiha NCR. Growth and nutrient intake in three to twelve month old infants fed human milk or formulas with varying protein concentrations. Journal of Pediatric Gastroenterology and Nutrition 1998;26:1-8.

THE ROLE OF LONG-CHAIN POLY-UNSATURATED FATTY ACIDS (LCPUFA) IN GROWTH AND DEVELOPMENT

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Abstract: It is debatable whether supplementation of infant formula with LCPUFA has an effect on infant growth and development. Up till now, there is little evidence of a negative effect on infant growth. A review of randomized controlled trials in term infants revealed that LCPUFA, in particularly supplementation with ≥ 0.30% DHA, seems to have a beneficial effect on neurodevelopmental outcome up to 4 months of age. The studies could not demonstrate a consistent positive effect beyond that age. However, in the majority of studies neurodevelopmental outcome was assessed between 6 to 24 months, i.e. at an age where there is a 'latency' in the expression of minor neurological dysfunction. Thus it is possible that LCPUFA might have a long lasting beneficial effect on neurodevelopmental outcome at school-age and beyond. This hypothesis urgently needs testing.

Keywords: LCPUFA; DHA; AA; growth; neurodevelopmental outcome; visual development; general movements

1. THE ESSENTIALITY OF LONG-CHAIN POLYUNSATURATED FATTY ACIDS (LCPUFA)

The long-chain polyunsaturated fatty acids doxosahexaenoic acid (DHA; 22:6ω3) and arachidonic acid (AA; 20:4ω6) are formed from the fatty acid precursors alpha-linolenic acid (ALA; 18:3ω3) and linoleic acid (LA;18:2ω6) respectively. ALA and LA are essential fatty acids, which means that these nutrients are needed for healthy survival and that they cannot be synthesized by the human.

DHA and AA are considered to be major functional LCPUFA during human ontogeny. They can be obtained directly from the diet or by endogenous conversion of the parent precursors ALA and LA by means of chain elongation and desaturation. The fetus and newborn infant are capable of these conversion processes, but the enzymatic systems involved seem to be unable to supply sufficient LCPUFA to meet the high requirements until 16 weeks beyond term.[1] This means that during early development, LCPUFA supply is largely dependent on dietary intake of DHA and AA. The fetus is dependent on maternal intake of LCPUFA; the young infant has to rely on LCPUFA in milk.[2] Breast milk does contain LCPUFA in levels which depend on maternal consumption, but the standard commercially available formulas for term infants do not contain these fatty acids.

LCPUFA are thought to play in role in growth and development. This concept is based on the finding that LCPUFA deficiency results in growth retardation and the observation that LCPUFA are abundant in the brain and retina.[1] In the brain LCPUFA accumulate mainly in the cortical gray matter; in particular in the synaptic membranes, and to a lesser extent in the white matter. In the retina LCPUFA are found preponderantly in the rod outer segment. Thus, it could be surmised that LCPUFA might have a general beneficial effect on brain development and brain function and a special positive effect on visual development.

2. LCPUFA SUPPLEMENTATION OF INFANT FORMULA

The observation that breastfed infants have a better developmental outcome than formula fed infants[3,4] and the idea that this effect may be mediated at least partially by the LCPUFA content of breast milk, inspired many scientists to evaluate the effect of LCPUFA supplementation of infant formula. In the following paragraphs I will first address the effect of LCPUFA supplementation on infant growth. Next I will review the outcomes of randomized controlled studies on the effect of LCPUFA supplementation on infant development. I will focus on studies in healthy term infants and not on studies in preterm infants, as the latter studies have the disadvantage of the many confounding and complex effect of problems associated with preterm birth on developmental outcome.[5]

2.1 LCPUFA and infant growth

Simmer, who conducted systematic reviews on the effect of LCPUFA supplementation of infant formula on growth and development in full-term and preterm infants, concluded that LCPUFA do not significantly affect parameters of infant growth such as weight, length and head circumference.[6,7] Nevertheless, caution is warranted with respect to a potential growth reducing effect of fatty acids of the ω3-series, as Lapillonne et al.,[8] who reviewed studies addressing the effect of LCPUFA supplementation on growth, indicated that in particular infants who were fed formulas enriched with ω3 fatty acids had a lower weight, length and/or head circumference than control infants.

2.2 LCPUFA and development in healthy term infants

The review on studies addressing the effect of LCPUFA supplementation on developmental outcome in term infants will be split into two parts. The first deals with the effect of LCPUFA on development up to the age of 4 months, the second with outcome after the age of 4 months. The 4 month cut-off point was chosen as this age is the end of a major transitional period in infant development. It is the age of emergence of goal directed motility of the arms,[9] the age of a significant change in postural control,[10] and the age at which functional activity in the basal ganglia, cerebellum and parietal, temporal and occipital cortices shows a substantial increase.[11]

2.2.1 LCPUFA and developmental outcome up to 4 months of age (Table 1)

Nine randomized controlled studies on LCPUFA supplementation in term infants have evaluated outcome during early infancy.[14-20] Six studies evaluated visual development,[12-14,17-19] one auditory processing[20], one general neurological condition[16] and one general developmental outcome.[15] They have indicated a positive relation between outcome and DHA-content. Three studies[12-14] used a formula with DHA < 0.20% and only one of them showed a temporary beneficial effect.[12] The other six studies used a formula with DHA ≥ 0.30%; five of them showed a

beneficial effect on infant development at 3 or 4 months of age.[15,16,18-20] The idea of a dose-effect relationship between DHA content and developmental outcome is in agreement with the outcome of a 'meta-regression' analysis of Uauy et al.[21] These authors provided evidence for the existence of a dose-effect relationship between DHA content of infant formula and visual acuity at 4 months of age.

2.2.2 LCPUFA and developmental outcome after 4 months of age (Table 2)

Twelve randomized controlled studies on LCPUFA supplementation in term infants have evaluated outcome between 6 and 39 months of age.[12-14,17,19,22-28] Only three have shown a positive effect of supplementation. Willats et al.[23] supplied study infants for four months with 0.20% DHA and 0.30% AA. Study infants performed significantly better on a problem solving task at 10 month than control infants. The other two papers[19,28] reported the outcome of a single study at two different ages. Study infants were supplied for four months with 0.35% DHA or with 0.36 DHA in combination with 0.72% AA. Both groups of supplemented infants had a better visual acuity as measured with sweep VEP at 12 months than control infants. At 18 months only the group who received DHA and AA in combination had a better mental developmental score than the group of control children. Eight studies could not establish a significant positive or negative effect of LCPUFA supplementation[12-14,17,24-27] and one study reported a negative association between supplementation with 0.20% DHA and language development at 14 months.[22]

The papers reviewed here include more recent studies than Simmer's meta-analysis.[8] Nevertheless, the conclusion is the same: there is no conclusive evidence that LCPUFA supplementation has a long-term beneficial effect on infant development. However, it should be realized that the majority of studies evaluated the infants between 6 and 24 months of age. During this age period it is difficult to find minor degrees of dysfunction of the nervous system. They only can be found when specific functions are evaluated with great care eg the study of Willats et al. [23] The fact that it is difficult to find the rather subtle effects of LCPUFA supplementation is illustrated by the fact that it is also difficult to demonstrate the well-known beneficial effect of breastfeeding during

this age period.[3,4] The finding of Bouwstra et al.[16] that LCPUFA supplementation was associated with a significant reduction in the occurrence of mildly abnormal general movements and the knowledge that mildly abnormal general movements during early infancy are associated with minor neurological dysfunction, attention problems and aggressive behaviour at school-age,[29] suggests that LCPUFA supplementation might have a beneficial effect on neurodevelopmental outcome at school-age and beyond.

3. CONCLUSION

The randomized controlled studies in healthy term infants did not provide consistent evidence that LCUPUFA supplementation affects infant growth. But they did indicate that LCPUFA supplementation, in particular supplementation with DHA $\geq$ 0.30% has a beneficial effect on infant neurodevelopmental condition up to the age of 4 months. Until now, studies have not demonstrated a consistent positive effect beyond that age. However, in the majority of studies neurodevelopmental outcome was assessed between 6 to 24 months. This is an age period where there is a 'latency' in the expression of minor neurological dysfunction. Thus it is possible that LCPUFA have a long lasting beneficial effect on neurodevelopmental outcome at school-age and beyond. This hypothesis urgently needs testing

Table 1. LCPUFA supplementation in term infants and outcome till 4 months

Author(s)	Groups[1]	Duration of supplementation	DHA content	AA content	Age at FU in mo	Attrition at last FU	Assessment at follow-up	Results[2]
Carlson et al. 1996[12]	E n = 19 C n = 20 BF n = 19	? BF ≥ 3 mo	0.10%	0.43%	2 and 4	38%	Teller visual acuity	2 mo: E > C; BF > C 4 mo: E = C; BF = C
Auestad et al. 1997[13]	E_1 n = 26 E_2 n = 28 C n = 28 BF n = 38	≥ 4 mo BF ≥ 4 mo	0.12% 0.20%	0.43%	2 and 4	39% (at 12 mo)	FPL Sweep VEP	E_1 = C; E_2 = C BF = C
Auestad	E_1 n =	12 mo	0.14%	0.45%	1, 2	27%	Teller visual	E_1 = C; E_2 = C

et al. 2001[14]	58 E_2 n = 60 C n = 56 BF n = 120	BF ≥ 3 mo	(egg) 0.13% (fish/fungal)	0.46%			BF = C
Agostoni et al. 1995[15]	E n = 27 C n = 29 BF n = 30	4 mo BF ≥ 4 mo	0.30%	0.44%	4 mo	4%	Brunet – Lezine DQ	E > C BF > C
Bouwstra et al. 2003[16]	E n = 131 C n = 119 BF n = 147	2 mo BF variable; median 9 wk	0.30%	0.45%	3 mo	16%	Quality of general movements	E > C BF > C
Makrides	E_1 n =	12 mo	0.34%	0.34%	4	18%	VEP	E_1 = C; E_2 = C

et al. 2000[17]	24 E_2 n = 23 C n = 21 BF n = 46	BF ≥ 3 mo	0.35%			(at 8 mo)		BF = C
Makrides et al. 1995[18]	E n = 13 C n = 19 BF n = 23	? BF ≥ 4 mo	0.36%		4	11%	VEP	E > C BF > C
Birch et al. 1998[19]	E_1 n = 23 E_2 n = 22 C n = 23 BF n =	4 mo BF ≥ 4 mo	0.36% 0.35%	0.72%	1.5 and 4	18%	FPL Sweep VEP	E = C; BF = C E_1 > C; E_2 > C BF > C

	21							
Ünay et al. 2004[20]	E n = 22 C n = 22 BF n = 23	4 mo BF ≥ 4 mo	0.50%		4	16%	BAEP	E > C BF > C

Table 2. LCPUFA supplementation in term infants and outcome beyond 4 months

Author(s)	Groups[1]	Duration of supplementation	DHA content	AA content	Age at FU in mo	Attrition at last FU	Assessment at follow-up	Results[2]
Carlson et al. 1996[12]	E n = 19 C n = 20 BF n = 19	? BF ≥ 3 mo	0.10%	0.43%	6 and 12	≥ 38%	Teller visual acuity	E = C BF = C

Table 2, continued. LCPUFA supplementation in term infants and outcome beyond 4 months

Author(s)	Groups[1]	Duration of supplementation	DHA content	AA content	Age at FU in mo	Attrition at last FU	Assessment at follow-up	Results[2]
Auestad et al. 1997[13]	E_1 n = 26 E_2 n = 28 C n = 28 BF n = 38	≥ 4 mo BF ≥ 4 mo	0.12% 0.20%	0.43%	6, 9 and 12	39%	FPL Sweep VEP	E = C BF = C
Scott et al. 1998[22]	E_1 n = 38 E_2 n = 33 C n = 42 BF n = 60	≥ 4 mo BF ≥ 3 mo	0.12% 0.20%	0.43%	12 14	37%	Bayley PDI / MDI McArthur language	E_1 = C; E_2 = C; BF = C E_1 = C; E_2 < C; BF = C
Auestad	E_1 n =	12 mo	0.14%	0.45%	6, 9,	27%	Teller vis.	E = C; BF = C

et al. 2001[14]	58 E_2 n = 60 C n = 56 BF n = 120	BF ≥ 3 mo	(egg) 0.13% (fish/fungal)	0.46%	12 6 and 9 6 and 12 6 and 12 9 and 14	(at 12 mo)	acuity Fagan Bayley PDI / MDI IBQ Language	E = C; BF = C E = C; BF = C E = C; BF = C E = C; BF = C
Willats et al. 1998[23]	E n = 21 C n = 23	4 mo	0.20	0.30	10	38%	Problem solving task	E > C
Auestad et al. 2003[24]	E_1 n = 35 E_2 n = 35 C n = 37 BF n =	12 mo BF ≥ 3 mo	0.12% 0.23%	0.43%	39	47%	Teller vis. acuity Beery VMI Stanford-Binet IQ Language	E = C; BF = C E = C; BF = C E = C; BF = C E = C; BF = C

	50							
Bouwstra et al. 2004[25]	E n = 135 C n = 157 BF n = 154	2 mo BF variable; median 9 wk	0.30%	0.45%	18	6%	Hempel neurological exam Bayley PDI / MDI	E = C; BF = C
Agostoni et al. 1997[26]	E n = 26 C n = 30 BF n = 25	4 mo BF ≥ 4 mo	0.30%	0.44%	24	10%	Brunet – Lezine DQ	E = C; BF = C
Lucas et al. 1999[27]	E n = 125 C n = 125 BF n = 104	6 mo BF ≥ 6 wk	0.32%	0.30%	9 18	21%	Knobloch DQ Bayley PDI / MDI	E = C; BF = C E = C; BF = C
Makrides	E_1 n =	12 mo	0.34%	0.34%	8	12%	VEP	E = C; BF = C

et al. 2000[17]	19 E_2 n = 22 C n = 19 BF n = 23	BF ≥ 3 mo	0.35%		12 and 24		PDI: E = C; BF = C 12 mo MDI: E = C; BF = C ; 24 mo MDI: E = C; BF > C
Birch et al. 1998[19]	E_1 n = 19 E_2 n = 22 C n = 20 BF n = 46	4 mo BF ≥ 4 mo	0.36% 0.35%	0.72%	6 and 12	26%	FPL Sweep VEP	E = C; BF = C 6 mo VEP: E = C; BF = C; 12 mo VEP: E_1 > C; E_2 > C; BF >C

REFERENCES

1. L. Lauritzen, H.S. Hansen, M.H. Jørgensen, K.F. Michaelsen, The essentiality of long chain n-3 fatty acids in relation to development and function of the brain and retina, *Progr. Lipid Res.* **40**, 1-94 (2001).
2. G. Hornstra, Essential fatty acids in mothers and their neonates, *Am. J. Clin. Nutr.* **71** (suppl 5), 1252S-1259S (2000).
3. J.W. Anderson, B.M. Johnstone, D.T. Remley, Breast-feeding and cognitive development: a meta-analysis, *Am. J Clin. Nutr.* **70**, 525-535 (1999).
4. A. Lucas, R. Morley, T.J. Cole, G. Lister, C. Leeson-Payne, Breast milk and subsequent intelligence quotient in children born preterm, *Lancet* **339**, 261-264 (1992).
5. M. Hadders-Algra, in: *Cambridge Encyclopeidia of Child Development*, edited by B. Hopkins (Cambridge University Press, Cambridge, 2004), in press.
6. K. Simmer, S. Patole, Longchain polyunsaturated fatty acid supplementation in preterm infants, *Cochrane Database Syst. Rev.* **1**, CD000375 (2004).
7. K. Simmer, Longchain polyunsaturated fatty acid supplementation in infants born term, *Cochrane Database Syst. Rev.* **4**, CD000376 (2001).
8. A. Lapillonne, S.D. Clarke, W.C. Heird, Plausible mechanisms for effects of long-chain polyunsaturated fatty acids on growth, *J. Pediatr.* **143**, S9-S16 (2003).
9. C. Von Hofsten, S. Fazel-Zandy, Development of visually guided hand orientation in reaching, *J. Exp. Child Psychol.* **38**, 208-219 (1984).
10. Å. Hedberg, E. Brogren Carlberg, H. Forssberg, M. Hadders-Algra, Development of postural adjustments in sitting position during the first half year of life, *Dev. Med. Child Neurol*, accepted for publication.
11. H.T. Chugani, M.E. Phelps, J.C. Mazziotta, 18-FDG Positrion Emission Tomography in human brain. Functional development, *Ann. Neurol.* **22**, 487-498 (1987).
12. S.E. Carlson, A.J. Ford, S.H. Werkman, J.M. Peeples, W.K.K. Koo, Visual acuity and fatty acid status of term infants fed human milk and formulas with and without docosahexaeoate and arachidonate from egg yolk lecithin. *Pediatr. Res.* **39**, 882-888 (1996).
13. N. Auestad, M.B. Montalto, R.T. Hall, K.M. Fitzgerald, R.E. Wheeler, W.E. Connor, M. Neuringer, S.L. Connor, J.A. Taylor, E.E. Hartmann, Visual acuity, erythrocyte fatty acid composition, and growth in term infants fed formulas with long chain polyunsaturated fatty acids for one year. *Pediatr. Res.* **341**, 1-10 (1997).
14. N. Auestad, R. Halter, R.T. Hall, M. Blatter, M.L. Bogle, W. Burks, J.R. Erickson, K.M. Fitzgerald, V. Dobson, S.M. Innis, L.T. Singer, N.B. Montalto, J.R. Jacobs, W. Qui, M.H. Bornstein, Growth and development in term infants fed long-chain polyunsaturated fatty acids: a double-masked randomized, parallel, prospective, multivariate study. *Pediatrics* **108**, 372-381 (2001).
15. C. Agostoni, T. Trojan, R. Bellu, E. Riva, M. Giovannini, Neurodevelopmental quotient of healhty term infants at 4 months and feeding practice: the role of long-chain polyunsaturated fatty acids. *Pediatr. Res.* **38**, 262-266 (1995).
16. H. Bouwstra, D.A.J. Dijck-Brouwer, J.A.L. Wildeman, H.M. Tjoonk, J.C. van der Heide, E.R. Boersma, F.A.J. Muskiet, M. Hadders-Algra, Long-chain polyunsaturated

fatty acids have a positive effect on the quality of general movements of healthy term infants. Am. J. Clin. Nutr. 78, 313-318 (2003).
17. M. Makrides, M.A. Neumann, K. Simmer, R.A. Gibson, A critical appraisal of the role of dietary long-chain polyunsaturated fatty acids on neural indices of term infants: a randomized, controlled trial. *Pediatrics* **105**, 32-38 (2000).
18. M. Makrides, M. Neumann, K. Simmer, J. Pater, R.A. Gibson, Are long-chain polyunsaturated fatty acids essential nutrients in infancy? *Lancet* **345**, 1463-1468 (1995).
19. E.E. Birch, D.R. Hofman, R. Uauy, D.G. Birch, C. Pastridge, Visual acuity and the essentiality of docosahexaenoic acid and arachidonic acid in the diet of term infants. *Pediatr. Res.* **44**, 201-209 (1998).
20. B. Ünay, Ü. Sarici, Ü.H. Ulas, R. Akin, F. Alpay, E. Gökçay E, Nutritional effects on auditory brainstem maturation in healthy term infants. *Arch. Dis. Child Fetal Neonatal Ed.* **89**, S177-S179 (2004).
21. R. Uauy, D.R. Hofman, P. Mena, A. Llano, E. Birch, Term infants studies of DHA and ARA supplementation on neurodevelopment: results of randomized controlled trials. *J. Pediatr.* **143**, S17-S25 (2003).
22. D.T. Scott, J.S. Janowsky, R.E. Carroll, J.A. Taylor, N. Auestad, M.B. Montalto, Formula supplementation with long-chain polyunsaturated fatty acids: are there developmental benefits? *Pediatrics* **102**, E59 (1998).
23. P. Willats, J.S. Forsyth, M.K. Dimodugno, S. Varma, M. Colvin, Effect of long-chain polyunsaturated fatty acids in infant formula on problem solving at 10 months of age. *Lancet* **352**, 688-691 (1998).
24. N. Auestad, D.T. Scott, J.S. Janowsky, C. Jacobsen, R.E. Caroll, M.B. Montalto, R. Halter, W. Qui, J.R. Jacobs, W.E. Connor, S.L. Connor, J.A. Taylor, M. Neuringer, K.M. Fitzgerald, R.T. Hall, Visual, cognitive and language assessments at 39 months: a follow-up study of children fed formulas containing long-chain polyunsaturated fatty acids to 1 year of age. *Pediatrics* **112**, e177-1183 (2003).
25. H. Bouwstra, D.A.J. Dijck-Brouwer, G. Boehm, E.R. Boersma, F.A.J. Muskiet, M. Hadders-Algra, Long-chain polyunsaturated fatty acids and neurological developmental outcome at 18 months in healthy term infants. *Acta Paediatr*, accepted for publication (2004).
26. C. Agostoni, S. Trojan, R. Bellu, E. Riva, M.G. Bruzzese, M. Giovannini, Developmental quotient at 24 months and fatty acid composition of diet in early infancy: a follow-up study. *Arch. Dis. Child* **76**, 421-424 (1997).
27. A. Lucas, M. Stafford, R. Morley, R. Abbott, T. Stephenson, U. MacFayden, A. Elias-Jones, H. Clements, Efficacy and safety of long-chain polyunsaturated fatty acid supplementation of infant-formula milk: a randomised trial. *Lancet* **354**, 1948-1954 (1999).
28. E.E. Birch, S. Garfield, D.R. Hofman, R. Uauy, D.G. Birch, A randomized controlled trial of early dietary supply of long-chain polyunsaturated fatty acids and mental development in term infants. *Dev. Med. Child Neurol.* **42**, 174-181 (2000).
29. M. Hadders-Algra, A.M.C. Groothuis, Quality of general movements in infancy is related to neurological dysfunction, ADHD, and aggressive behaviour. *Dev. Med. Child Neurol.* **41**, 381-391 (1999).

EXPERIMENTAL MODELS FOR STUDYING PERINATAL LIPID METABOLISM

Long-term effects of perinatal undernutrition

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Abstract: By using different experimental designs in the rat we have been able to answer several unanswered questions on the short- and long-term effects of alterations of lipid metabolism during the perinatal stage. The first was to demonstrate the importance of maternal body fat accumulation during the first half of pregnancy, since undernutrition in this critical period when fetal growth is slow, impedes fat depot accumulation and not only restrains intrauterine development but has long-term consequences, as shown by an impaired glucose tolerance when adults. Secondly, undernutrition during suckling has major long-term effect of decreasing body weight, even though food intake is kept normal from the weaning period. Our findings also show that a diet rich in n-3 fatty acids during pregnancy and lactation has adverse effects on offspring development, but cross fostered experiments showed that this effect was a consequence of the intake of these fatty acids during the lactation period rather than during pregnancy. Pups from dams that were fed a fish oil-rich diet during pregnancy and lactation were found to have altered glucose/insulin relationship at the age of 10 weeks. Since a n-3 fatty acid-rich diet decreases milk yield during lactation, additional experiments were carried out to determine whether decreased food intake or altered dietary fatty acid composition, or both, were responsible for the long-term effects on the glucose/insulin axis.

Results show that the decreased food intake caused by a n-3 fatty acid-rich diet rather than the change in milk composition during suckling was responsible for the reduced pancreatic glucose responsiveness to insulin release at 16 weeks of age.

In conclusion, present findings indicate that impaired maternal fat accumulation during early pregnancy and food intake during lactation, rather than a difference in dietary fatty acid composition, have major effects on postnatal development and affect glucose/insulin relationships in adult rats.

Key words: Pregnancy; Undernutrition; Rats; Fish oil diet; Olive oil diet; Suckling; Postnatal development; Insulin; Glucose.

1. INTRODUCTION

Impaired fetal and early postnatal growth confers an increased susceptibility for the development of adult chronic disease such as type 2 diabetes, obesity and cardiovascular disease (3; 15). Early nutrition influences development and can cause adaptive and permanent changes in structure, physiology and metabolism (14). During pregnancy, the availability of nutrients to the foetus depends on those crossing the placenta from maternal circulation, which depends on maternal nutrition. In order to determine how changes in maternal nutrition during pregnancy and lactation have short- and long-term consequences on offspring development and susceptibility for causing adult disease, appropriate experimental models are needed, due to ethical and methodological limitations. By using the rat, we applied different experimental designs to study the effects of alterations in maternal nutrition during the perinatal stage on lipid metabolism, and its consequences on postnatal development and susceptibility to alter the glucose/insulin relationships.

2. SHORT- AND LONG-TERM EFFECTS OF MATERNAL UNDERNUTRITION DURING THE FIRST HALF OF PREGNANCY

During pregnancy, the concept of maternal nutrition must be extended beyond a mother's diet to include her body composition and metabolism (6). Lipid metabolism plays a major role in maternal metabolic adaptations to warrant the availability of substrates to the foetus (7; 8). The accumulation of fat depots in maternal tissues is a constant characteristic feature in pregnancy (11; 19; 20), and takes place mainly during the first half of gestation, when opposite to the insulin resistant condition that occurs during late pregnancy, there is even an enhanced sensitivity of adipose tissue to insulin (17). A decrease in the capacity of the mother to accumulate fat depots during this early part of

gestation, as result of hypothyroidism, greatly compromises normal catabolic adaptations of late pregnancy and impairs fetal growth (4; 5). Thus, it is hypothesized that maternal accumulation of fat depots during early pregnancy may play a key role in the availability of nutrients to the foetus and in its subsequent growth and health. To investigate this possibility we determined the effect of undernutrition circumscribed to the first half of pregnancy in the rat, in order to avoid maternal fat depot accumulation in short and long-term effects in their offspring.

Age matched female rats were mated, and from the day of appearance of spermatozoids in vaginal smears (day 0 of pregnancy) they were divided into two groups. One group was maintained fed *ad libitum* (controls) whereas the other group was allowed to eat 60% of the amount of food consumed by controls (underfed). Animals were kept on this feeding conditions until day 12 of gestation, when the increase in maternal body weight from the onset of pregnancy was 68.9±1.5 g in controls whereas it was just 16.3±4.1 g in the underfed rats ($p<0.001$). This finding is interpreted in the sense of an incapacity of the underfed pregnant rat to increase her fat depots, since during this period of pregnancy the increase of fetal-placental structures ("conceptus") is very small (10), and most of the increase in maternal body weight during early pregnancy corresponds to her fat accumulation (9). From day 12 of pregnancy, all the animals were allowed to eat *ad libitum*, and maternal body weight increased in parallel in both groups, in such a way that at day 20 of pregnancy the increase in body weight during pregnancy of controls was 162.6±4.8 g, whereas it was 113.1±4.5 g in the rats that were underfed ($p<0.001$). Thus, impaired accumulation of fat depots, as a consequence of underfeeding during early pregnancy, is not overcome when a normal feeding condition is resumed during the second half of gestation.

At the time of delivery, both the number of alive newborns per litter and their body weight were significantly lower in the rats that were underfed during early pregnancy as compared to the controls. This finding shows that despite of the small fetal growth that takes place during the first half of pregnancy, an impairment of the mother to accumulate fat depots during this specific phase clearly damages the normal intrauterine development, with consequences that are seen at the time of birth.

During lactation, newborns from dams that were underfed during early pregnancy and those from control dams were allowed to suckle

from their respective mother fed *ad libitum*. It was found that at the time of weaning (21 days after delivery) pups from both groups had a similar body weight. This finding indicates that the negative effect caused by maternal undernutrition during early pregnancy on intrauterine development disappeared by allowing a free access of food during suckling.

In order of determining whether maternal undernutrition during early pregnancy caused any long-term effect on the insulin-glucose axis, pups were studied when they were 16 weeks old. They were subjected to an oral glucose tolerance test by giving 2 g glucose/Kg rat, and collecting blood from the tail at 0, 5, 10, 15, 20, 30, 45 and 60 min thereafter. The area under the curve of plasma insulin and glucose along the 60 min was calculated, and the corresponding ratios are shown in figure 1. It is clearly seen that both adult male and female pups from rats that were underfed during early pregnancy have an impaired response to the glucose load, as shown by a significant increase in the ratio of the area under the curve ratio for insulin and glucose.

Thus, present finding shows that maternal undernutrition circumscribed to just the first half of pregnancy has negative effects on intrauterine development and a long-term effect impairing glucose tolerance in adults.

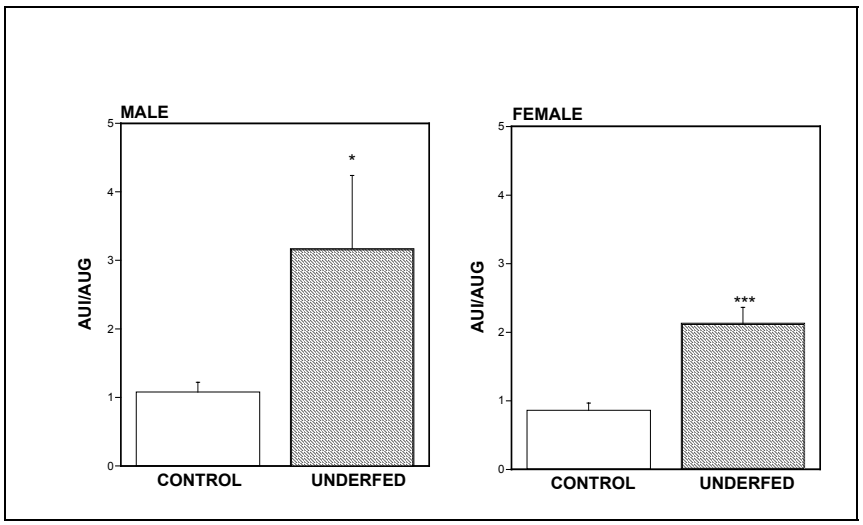

Figure 1. Ratio of the area under the curve of plasma insulin (AUI) and glucose (AUG) during an oral glucose tolerance test (2 g of oral glucose/Kg body weight) in 16 weeks old pups from dams that were either fed *ad libitum* throughout pregnancy (controls) or were underfed during the first half of pregnancy (available only 60% of the food eaten by the controls during just the first 12 days of pregnancy). Methodological details for the oral glucose tolerance tests as previously reported (13). Statistical comparison between underfed and control rats: *=$p<0.05$, ***=$p<0.001$.

3. LONG-TERM EFFECTS OF UNDERNUTRITION DURING EARLY POSTNATAL LIFE

Since negative effects on development and on glucose/insulin relationship could be also the result of an undernutrition condition just during suckling, we also used an animal model to test this possibility. In order of decreasing the amount of milk intake, litter size from untreated rats were adjusted to 16 pups per litter (underfed pups) whereas controls were adjusted to 9 pups per litter. At weaning (day 21 of age), underfed pups (i.e. those of litters having 16 pups) weighed less than controls ($p<0.001$), demonstrating a decreased milk intake in these pups. From this time on, all pups were allowed to eat *ad libitum,* but at 16 weeks of age, body weights remained lower in those pups that were underfed

during suckling than in their controls, showing that they were unable to catch up the body weight of the controls.

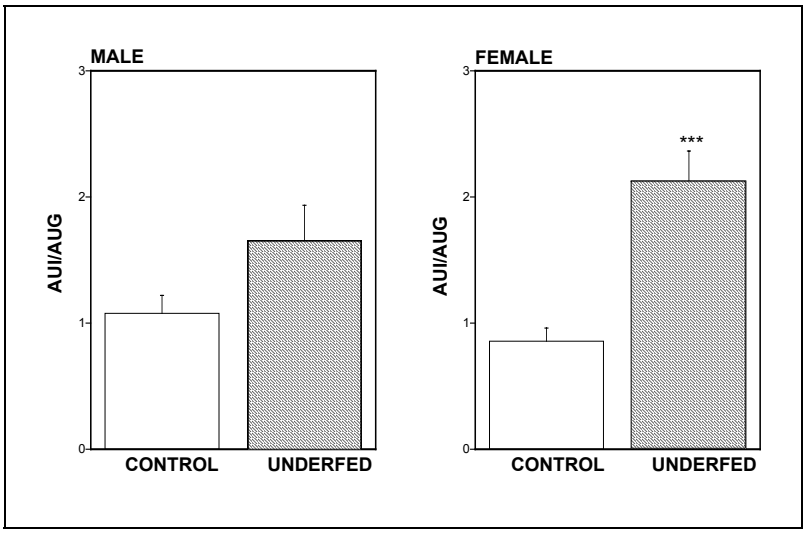

Figure 2. Ratio of the area under the curve ratio of plasma insulin (AUI) and glucose (AUI) during an oral glucose tolerance test (2 g of oral glucose/Kg body weight) in 16 weeks old pups born from rats fed normal chow diet that were either kept under control-(controls, 9 pups per litter) or underfed-conditions (underfed, 16 pups per litter) during suckling. All the rats were allowed to eat *ad libitum* from the time of weaning until the study. Methodological details for the glucose tolerance tests as previously reported (13). Statistical comparison between underfed and control rats: ***=$p<0.001$.

At 16 weeks of age, oral glucose tolerance tests were performed following the same protocol described above. As shown in figure 2, the ratio of the area under the curve for insulin versus the area under the curve of glucose was higher in the pups that were underfed during suckling, either males or females. It is interesting to notice that the effect of underfeeding during suckling impairing the oral glucose tolerance is smaller than that shown by those pups from mothers that were underfed during early pregnancy (figure 1).

4. SHORT-AND LONG-TERM EFFECT OF MODIFYING DIETARY FATTY ACID COMPOSITION DURING PREGNANCY AND LACTATION

Under the base of above results, we wanted to determine whether a change in dietary fatty acids during pregnancy and lactation in the rat affected the offspring outcome. With this aim, female rats were allowed to eat *ad libitum* from mating until the end of lactation a semisynthetic diet having a 10% of either fish oil (FOD) or olive oil (OOD) as the only non-vitamin fat component. The composition of the diets were as previously described, (1; 2) and their proportion of fatty acids is shown in figure 3, where it appears that the FOD had higher proportion of ω-3 fatty acids (eicosapentaenoic and docosahexaenoic acids, 20:5 ω-3 and 22:6 ω-3, respectively) but lower proportion of oleic acid (18:1) than the OOD.

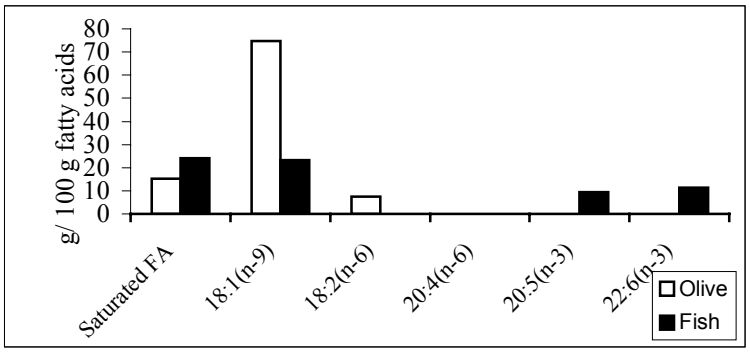

Figure 3. Fatty acid composition of semisynthetic diets containing 10% of either olive oil (OOD) or fish oil (FOD) as the only non-vitamin lipidic component. Methodological details as in (2).

At birth, newborns from dams fed FOD weighed less than those from dams fed OOD ($p<0.01$), and this difference was further enhanced along the suckling period despite that the litter size was always adjusted to 8 pups per litter. As already reported (2) the increase in body size and the acquisition of psychomotor reflexes during suckling were delayed in

pups suckled by dams fed FOD as compared to those of OOD. As shown in figure 4, the estimated milk yield of the dams fed FOD was lower than those fed OOD. Therefore, the delayed postnatal development of pups from dams fed FOD could be the result of both the different fatty acid composition as well as the decline in milk intake.

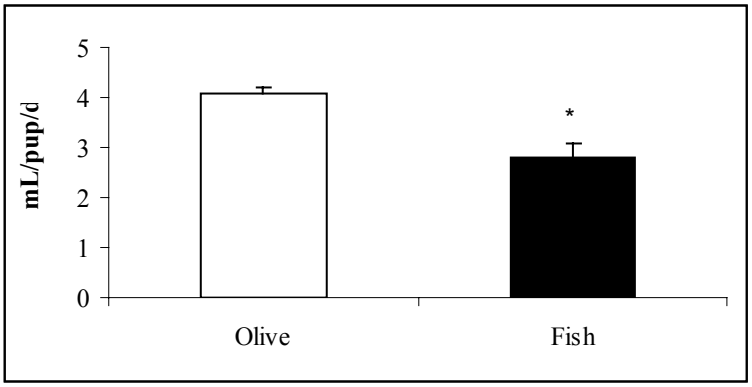

Figure 4. Estimated milk yield at day 10 of lactation, measured as described (18), in lactating rats fed with either olive oil diet (OOD) or fish oil diet (FOD). Statistical comparison: *=$p<0.05$.

We also wanted to determine whether these differences in the postnatal development of the pups from the two groups were due to the effects of the type of food eaten by the mother either during pregnancy or lactation. Thus, an experiment of cross-fostered was designed, where newborns from dams fed FOD during pregnancy were lactated by dams fed OOD (FOD-OOD) and vice versa (OOD-FOD). For comparison, we also studied pups that were suckled from dams that during lactation were fed the same diet that during pregnancy (OOD-OOD, and FOD-FOD). The indexes of acquisition of one representative psychomotor reflex, the air righting reflex, during suckling are shown in figure 5. It is seen that pups suckled by dams fed OOD during lactation but coming from dams fed FOD during pregnancy have the same value for the acquisition of the psychomotor reflexes than those whose mother was fed OOD for the whole time (OOD-OOD). However, when pups born from dams that were fed OOD during pregnancy were suckled by dams fed FOD, a

decreased capacity to acquire the psychomotor reflexes was observed, attaining the same level of pups from the FOD-FOD group.

These findings therefore show that a change in the composition of fatty acids in the diet during lactation affects more the postnatal development than when the change is circumscribed to pregnancy. Although during pregnancy the amount of food intake between dams fed FOD do not differ to that of OOD, there is a possibility that an additive effect of altered dietary fatty acid composition plus the undernutrition caused by decreased milk yield during lactation would be affecting the development of pups from dams fed FOD during lactation.

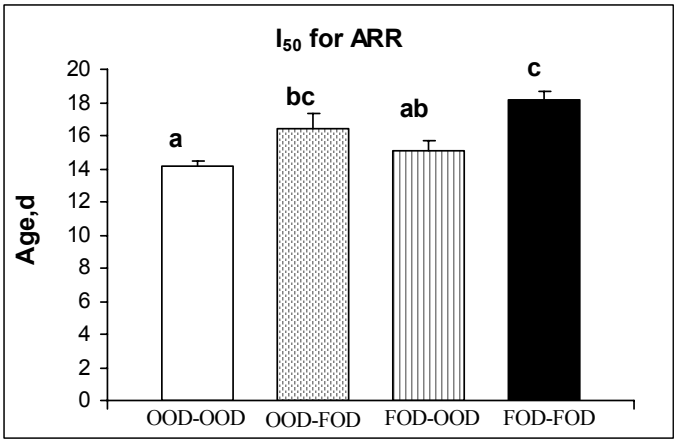

Figure 5. Acquisition of air righting reflex in suckling newborns (12), expressed as the day that 50% of the litter acquired the mature response (I_{50}), from rats fed fish oil diet (FOD) during pregnancy and lactation (FOD-FOD), olive oil diet (OOD) during pregnancy and FOD during lactation (OOD-FOD), FOD during pregnancy and OOD during lactation (FOD-OOD), or OOD during both pregnancy and lactation (OOD-OOD). Different letters indicate significant differences between the groups ($p<0.05$).

5. LONG-TERM EFFECTS OF CHANGES IN DIETARY FATTY ACIDS COMPOSITION DURING SUCKLING IN THE RAT

Under the findings summarized in the previous section, it was decided to determine whether the two factors together, an enhanced intake of ω-3 fatty acids and undernutrition during suckling, have any

long-term effect on the glucose/insulin axis. With this purpose, pups suckled by dams fed either FOD or OOD and kept in litters of 8 pups each, were allowed to eat *ad libitum* from the time of weaning. It was found that the decreased body weight of pups from FOD dams was maintained at 7 and 10 weeks of age in males and in females. At this later age, the oral glucose tolerance test showed a similar increase in plasma glucose levels but smaller increases in plasma insulin in those pups that were suckled by dams fed FOD than in those that suckled from dams fed OOD. Thus, whereas the area under the curve for plasma glucose was similar in the two groups, the area under the curve for the change of insulin was significantly lower in both females and males that were suckled by dams fed FOD as compared to those from OOD ($p<0.05$), indicating either an impaired pancreatic insulin release, an enhanced insulin sensitivity, or both.

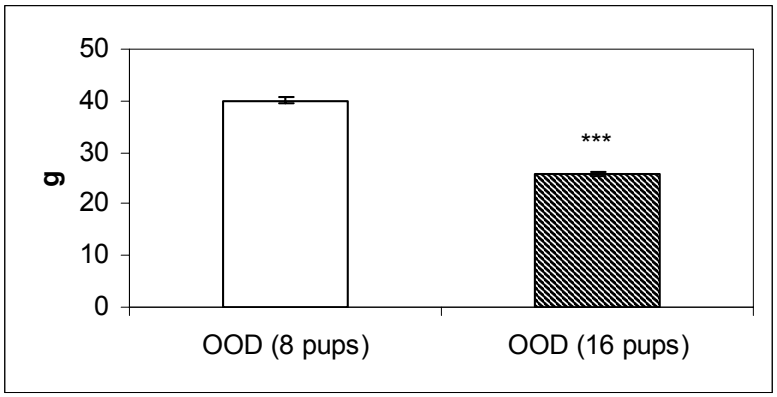

Figure 6. Body weight at the time of weaning (21 days) of pups from rats fed an olive oil diet (OOD) during pregnancy and lactation, that during lactation had litter size adjusted to either 8 (controls) or 16 pups (underfed)/dam. Statistical comparison: ***=$p<0.001$.

Since the altered oral glucose tolerance test in pups that were suckled by dams fed FOD could be the result of either the increased ω-3 fatty acids in maternal milk or the decreased food intake as result of the decreased milk yield (see above), two additional experiments were performed to determine between these two posibilities. In the first one, pups that were suckled by dams fed OOD that had 8 pups per litter during lactation (controls) were compared to those from dams that were

fed OOD but had 16 pups per litter during lactation (underfed). As shown in figure 6, at the time of weaning, the underfed pups weighed much less than their controls. At this time, pups from both groups were fed *ad libitum* regular chow diet, but still at 16 weeks of age pups that were underfed during suckling remained having a lower body weight than controls, the difference being statistically significant for males as well as for females. At this age (16 weeks old), oral glucose tolerance test showed a similar increase of plasma glucose in those pups that were underfed during suckling as compared to their controls but the increase in plasma insulin was lower in the former group (figure 7). This differential response between the two groups was similar in females as in male rats, and shows that decreased food intake during suckling contributes to the lower insulin release after the glucose load when adults. This finding agrees with those previously reported (21) showing a persistently reduced pancreatic glucose-responsiveness in rats subjected to large litters until weaning.

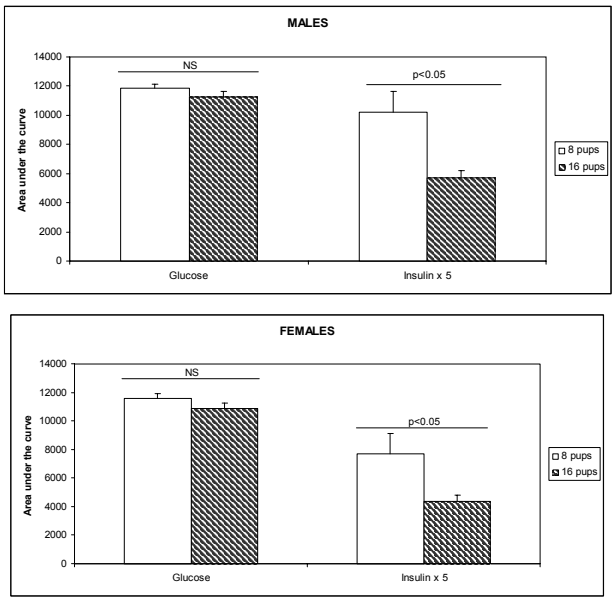

Figure 7. Ratio of the area under the curve of plasma insulin (AUI) and glucose (AUG) during an oral glucose tolerance test (2g /Kg) of 16 weeks old male and female pups that were underfed during suckling (16 pups/litter) as compared to controls (8 pups/litter,

during suckling). Methodological details for the glucose tolerance tests as previously reported (13).

The second experiment was addressed to determine whether an enhanced intake of ω-3 fatty acids during suckling but unchanged food intake could have long-term effects on the glucose/insulin axis. For this purpose, newborns from normally fed rats were suckled by dams that were fed a semisynthetic diet having 10% of olive oil as the only non-vitamin fat and had 8 pups per litter (OOD-8) were compared to others that were suckled by dams fed the same diet but having 10% fish oil instead of the olive oil and had 4 pups per litter (FOD-4). The estimated milk yield per pup at the peak of the lactation period (15 days) was similar in both groups, and the body weight at the time of weaning did not differ between them, showing a similar nutritional condition in both groups. As shown in figure 8, milk composition of dams fed FOD had higher proportion of ω-3 fatty acids but lower proportion of oleic acid (18:1) than in those fed OOD. Thus we had two rat groups with a similar food intake during suckling but with a different proportion of fatty acids in their diet. From weaning, both groups were allowed to eat *ad libitum* a regular rat chow and were studied at 16 weeks of age. At this time their body weight did not differ between the groups. After an oral glucose load (2g/Kg) the increase in plasma glucose and plasma insulin was similar in the two groups (OOD-8 and FOD-4), and this was so both in female and male rats. In fact the ratios of the area under the curve for insulin and glucose was similar in the OOD-8 and FOD-4 pups. Although as recently reviewed, changes in the glucose tolerance tests in pups as result of by maternal undernutrition during the perinatal stages could be transitory, ranging from enhanced glucose tolerance in early adult life (6-12 weeks), to unchanged at 44 weeks of age and even decreased at 15 months of age (16), present findings indicate that a change in the dietary fatty acid composition without affecting the amount of food intake during suckling in the rat does not have either short-or long-term effect in body weight nor affecting long-term glucose/insulin relationships.

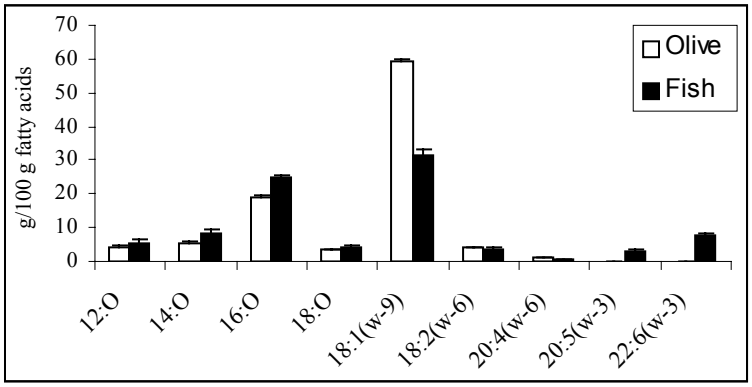

Figure 8. Fatty acid composition of milk at day 10 of lactation in rats fed either olive oil (OOD) or fish oil (FOD). Methodological details as previously described (2).

ACKNOWLEDGEMENTS

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REFERENCES

1. Amusquivar E and Herrera E. Influence of changes in dietary fatty acids during pregnancy on placental and fetal fatty acid profile in the rat. *Biol Neonate* 83: 136-145, 2003.

2. Amusquivar E, Rupérez FJ, Barbas C and Herrera E. Low arachidonic acid rather than α-tocopherol is responsible for the delayed postnatal development in offspring of rats fed fish oil instead of olive oil during pregnancy and lactation. *J Nutr* 130: 2855-2865, 2000.

3. Barker DJP. Fetal origin of adult disease. In: Fetal and Neonatal Physiology, edited by Polin RA, Fox WW and Abman SH. Philadelphia: Saunders, 2004, p. 160-165.

4. Bonet B and Herrera E. Different response to maternal hypothyroidism during the first and second half of gestation in the rat. *Endocrinology* 122: 450-455, 1988.
5. Bonet B and Herrera E. Maternal hypothyroidism during the first half of gestation compromises normal catabolic adaptations of late gestation in the rat. *Endocrinology* 129: 210-216, 1991.
6. Herrera E. Metabolic adaptations in pregnancy and their implications for the availability of substrates to the fetus. *Eur J Clin Nutr* 54, Suppl. 1: S47-S51, 2000.
7. Herrera E. Lipid metabolism in pregnancy and its consequences in the fetus and newborn. *Endocrine* 19: 43-55, 2002.
8. Herrera E and Lasunción MA. Maternal-fetal transfer of lipid metabolites. In: Fetal and neonatal physiology, edited by Polin RA, Fox WW and Abman SH. Philadelphia: W.B.Saunders Co., 2004, p. 375-388.
9. Herrera E, Lasunción MA, Gomez Coronado D, Aranda P, Lopez Luna P and Maier I. Role of lipoprotein lipase activity on lipoprotein metabolism and the fate of circulating triglycerides in pregnancy. *Am J Obstet Gynecol* 158: 1575-1583, 1988.
10. Herrera E, Muñoz C, Lopez-Luna P and Ramos P. Carbohydrate-lipid interactions during gestation and their control by insulin. *Brazilian J Med Biol Res* 27: 2499-2519, 1994.
11. Lopez Luna P, Maier I and Herrera E. Carcass and tissue fat content in the pregnant rat. *Biol Neonate* 60: 29-38, 1991.
12. Lopez Tejero D, Ferrer I, Llobera M and Herrera E. Effects of prenatal ethanol exposure on physical growth, sensory reflex maturation and brain development in the rat. *Neuropathol Appl Neurobiol* 12: 251-260, 1986.
13. López-Soldado, I. and Herrera, E. Different diabetigenic response to moderate doses of streptozotocin in pregnant rats, and long-term consequences in the offspring. Int.J.Exp.Diabetes Res. 4: 107-118, 2003.
14. Lucas A. Programming by early nutrition: an experimental approach. *J Nutr* 128: 401S-406S, 1998.
15. Lucas A, Fewtrell MS and Cole TJ. Fetal origins of adult disease - the hypothesis revisited. *Br Med J* 319: 245-249, 2000.
16. Ozanne SE and Hales CN. Early programming of glucose-insulin metabolism. *Trends Endocrinol Metab* 13: 368-373, 2002.
17. Ramos MP, Crespo-Solans MD, Del Campo S, Cacho J and Herrera E. Fat accumulation in the rat during early pregnancy is modulated by enhanced insulin responsiveness. *Am J Physiol Endocrinol Metab* 285: E318-E328, 2003.
18. Sampson DA and Jansen GR. Measurement of milk yield in the lactating rat from pup weight and weight gain. *J Pediatr Gastroent Nutr* 3: 613-617, 1984.
19. Sidebottom AC, Brown JE and Jacobs DR, Jr. Pregnancy-related changes in body fat. *Eur J Obstet Gynecol Reprod Biol* 94: 216-223, 2001.
20. Villar J, Cogswell M, Kestler E, Castillo P, Menendez R and Repke JT. Effect of fat and fat-free mass deposition during pregnancy on birth weight. *Am J Obstet Gynecol* 167: 1344-1352, 1992.
21. Waterland RA and Garza C. Early postnatal nutrition determines adult pancreatic glucose-responsive insulin secretion and islet gene expression in rats. *J Nutr* 132: 357-364, 2002.

EFFECT OF N-3 POLYUNSATURATED FATTY ACID SUPPLEMENTATION IN PREGNANCY: THE NUHEAL TRIAL

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Abstract: In this placebo controlled, randomised, double blind trial, pregnant women received from the 20th week of gestation onwards either 500 mg docosahexaenoic acid (DHA), 400 mg 5-methyl-tetra-hydro-folate (5-MTHF), or placebo, or a combination of 500 mg DHA and 400 mg 5-MTHF. The dietary supplements were well tolerated; the dropout rates did not differ significantly in the active arms of the study (10% to 19%) from that seen in the placebo group (13%). DHA supplementation resulted in significant enhancement of the contribution of DHA to maternal, placental and venous cord blood lipids.

Key words: Docosahexaenoic acid, fetal nutrition, long-chain polyunsaturated fatty acid, methyl-tetra-hydro-folate, pregnancy

1. INTRODUCTION

Docosahexaenoic acid (DHA), the principal n-3 long-chain polyunsaturated fatty (LC-PUFA) plays an important role in infantile neurodevelopment. Uauy et al. (1) have recently reviewed 14 controlled trials that included formula feeding with or without LC-PUFA and functional assessment of visual and other measures of neural development in full-term infants, and concluded that there was a significant relation between the total DHA equivalent provided and the effectiveness as defined by visual acuity measurements at 4 months of age. Moreover, the significant inverse correlation demonstrated in full-term infants between cord blood DHA contents and the decline in blood DHA contents by the age of 6 weeks (2) indicates that attempts to improve infantile DHA status might be initiated even before birth.

Supplementation of DHA to the diet of pregnant mothers represents an obvious tool in attempting to improve maternal and, consequently, fetal DHA status. However, there may be also indirect ways to enhance fetal LC-PUFA supply. The significant inverse correlation reported between total homocysteine concentrations in maternal plasma and DHA contents of erythrocyte phospholipids in the offspring (3) indicates that lowering plasma homocysteine concentrations in the mother may improve DHA status of the infant. Folate supplementation offers the possibility to reduce plasma homocysteine concentrations in the healthy organism.

2. SUBJECTS AND METHODS

On the basis of the above-mentioned considerations, we investigated the effects of DHA and 5-methyl-tetra-hydro-folate (5-MTHF) supplementation to the diet of expecting mothers living in Germany, Hungary and Spain. In this placebo controlled, randomized, double blind trial, expecting women received daily from the 20^{th} week of gestation onwards either 500 mg DHA, or 400 mg 5-MTHF, or placebo, or the combination of 500 mg DHA and 400 mg 5-MTHF. The four supplements were milk based and contained vitamins and minerals in amounts meeting the estimated additional requirements of expecting women during the second half of pregnancy(Laboratorios Ordesa, Barcelona, Spain). The DHA supplemented was of fish oil origin and was given to the supplement in microencapsulated form. All mothers were encouraged to breast-fed. Infants who were not fully breastfed were provided infant formula either with DHA, if the mother was supplemented with DHA, or without DHA, if the mother did not receive DHA supplementation.

The mothers were investigated at the 20^{th} and 30^{th} weeks of gestation and at delivery: physical examination was performed, dietary data were collected by using standardized food frequency questionnaires, and venous blood samples were taken for the analysis of fatty acid and folate statuses. The infants were investigated immediately following birth and at the ages of 8 and 26 weeks: physical examination was performed, development was assessed by anthropometrical methods and nutrient intakes were evaluated. Visual evoked potential techniques and Bayley's

Infantile Mental Developmental Index were used as major tools for the evaluation of neurodevelopment.

3. RESULTS

The dietary supplement was well tolerated by the mothers. The dropout rates were moderate and did not appear to differ considerably between the active and the placebo arms of the study (Table 1).

Table 1. Number of mothers participating in the randomized, double blind trial on docosahexaenoic acid (DHA) and 5-methyl-tetra-hydro-folate (5-MTHF) supplementation

	DHA	5-MTHF	Placebo	DHA+5-MTHF
No. at entry	77	80	80	75
No. at delivery	69	65	70	63
Dropout rate (%)	10	19	13	17

Fatty acid composition of plasma phospholipids and erythrocyte membrane phosphatidylcholine (PC) and phosphatydilethanolamine (PE) lipids was used for the evaluation of the effect of DHA supplementation on fatty acid status. Here we report preliminary data obtained at investigating fatty acid composition of PC and PE lipids obtained at delivery in 25-25-25-25 mother-infant pairs from each study group.

At delivery, contribution of DHA to the fatty acid composition of erythrocyte membrane PC and PE lipids was significantly higher in mothers who received DHA supplementation than in those receiving placebo or 5-MTHF alone (Table 2).

Table 2. Contribution of docosahexaenoic acid (DHA) to the fatty acids of erythrocyte phophatidylcholine (PC) and phosphatidylethanolamine (PE) lipids at delivery in mothers (n = 25 each) receiving DHA and 5-methyl-tetra-hydro-folate (5-MTHF) supplementation. Data are % wt/wt, medians (ranges from the 1st to the 3rd quartile); a = $P < 0.05$, b, c, d, = $P < 0.01$

	DHA	5-MTHF	Placebo	DHA+5-MTHF
PC	3.01 (2.50)[b]	2.94 (2.19)[a]	2.04 (1.05)[b,c]	3.43 (1.87)[a,c]
PE	8.81 (3.08)[a,b]	6.37 (2.56)[a,c]	5.74 (2.28)[b,d]	9.32 (2.70)[c,d]

DHA values were also significantly higher in erythrocyte PC and PE lipids in venous cord blood in infants whose mothers received DHA supplementation as compared to those who received placebo or 5-MTHF

alone. There were no significant differences between the 5-MTHF and the placebo groups (Table 2 and 3). Fatty acid composition of placental lipids showed differences similar to those seen in cord blood PC and PE lipids.

Supplementation of 5-MTHF resulted in significantly higher plasma folate and significantly lower plasma homocysteine concentrations in the mothers, whereas similar insignificant tendencies were seen in the infants. Visual evoked potential investigations showed significant difference in one subset of the test: the p1 latency measured at 1-degree angel was higher in the DHA+5-MTHF than in the placebo groups.

Table 3. Contribution of DHA to the fatty acids of erythrocyte PC and PE lipids at delivery in infants (n = 25 each) of mothers receiving DHA and 5-MTHF supplementation. For units, signs and abbreviations see Table 2

	DHA	5-MTHF	Placebo	DHA+5-MTHF
PC	3.62 (2.16)[b]	2.64 (2.04)[a,b]	3.00 (2.14)	3.42 (1.95)[a]
PE	7.43 (3.26)	6.43 (2.03)[b]	6.99 (2.61)[c]	8.13 (3.32)[b,c]

4. CONCLUSIONS

In the present study, supplementation of DHA in a dose of 400 mg/day from the 20th week of gestation onwards resulted in significant enhancement of the contribution of DHA to the fatty acid composition of maternal, placental and cord blood lipids. Recently Malcolm et al (4) supplemented expecting mothers with DHA in a dose of 200 mg/day and saw significant enhancement of DHA contents in maternal but not in cord blood lipids. Hence, our present results suggest that 400 mg/day might be an effective dosage of DHA supplementation during pregnancy. Neither in the present study, nor in the study of Malcolm et al (4) did visual evoked potentials investigated during the first months of life reveal robust effects of DHA supplementation. However, an association of DHA status with some parameters of the maturation of the visual pathway was seen both in the present study and in the study of Malcolm et al (4).

We conclude that supplementation of the diet of pregnant mothers with DHA offers an effective tool to improve the DHA status of the offspring, and suggest that the effects of supplementation on

neurodevelopment should be carefully monitored in long-term follow-up studies.

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REFERENCES

1. Uauy R, Hoffman DR, Mena P, Llanos A, Birch EE: Term infant studies of DHA and ARA supplementation on neurodevelopment: results of randomized controlled trials. J Pediatr 143:S17-S25, 2003
2. Guesnet P, Pugo-Gunsam P, Maurage C, Pinault M, Giraudeau B, Alessandri JM, Durand G, Antoine JM, Couet C: Blood lipid concentrations of docosahexaenoic and arachidonic acids at birth determine their relative postnatal changes in term infants fed breast milk or formula. Am J Clin Nutr 70:292-298, 1999
3. Böhles H, Arndt S, Ohlenschlager U, Beeg T, Gebhardt B, Sewell AC: Maternal plasma homocysteine, placenta status and docosahexaenoic acid concentration in erythrocyte phospholipids of the newborn. Eur J Pediatr 158:243-246, 1999
4. Malcolm CA, McCulloch DL, Montgomery C, Shepherd A, Weaver LT: Maternal docosahexaenoic acid supplementation during pregnancy and visual evoked potential development in term infants: a double blind, prospective, randomised trial. Arch Dis Child Fetal Neonatal Ed 88:F383-F390, 2003

YOUNG RESEARCHERS' WORKSHOP

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Abstract: Research in Europe needs multidisciplinary approaches and young researchers should get the opportunity to become familiar with new perspectives and future research topics. The workshop focused on some particular topics of research:

- new nutrients in infant formulas
- biochemical mechanisms of metabolic programming
- influence of early feeding on childhood obesity.

Key words: Gangliosides, intestinal microflora, appetite, obesity, infant formula, breastfeeding, leptin, adiponectin, sleeping metabolic rate, fat mass, doubly labelled water, uncoupling protein, birth weight, placental lipid genes, microarray, cholesterol biosynthesis, metabolic imprinting.

1. INTRODUCTION

This workshop was organized as a forum for young researchers. Research in Europe needs multidisciplinary approaches and young researchers should get the opportunity to become familiar with new perspectives and future research topics. The aim of this session was to convene new talented young researchers in the field of nutrition in pregnancy and childhood and to discuss recent work and projects for the future. A platform for young researchers in medicine, pharmacy, nutrition or any other biomedical research to present their work and to open it for discussion was provided. This workshop gave a good opportunity to receive valuable feed-back information, to get in contact with other researchers and to become familiar with other approaches and future research topics. Therefore, six abstracts on experimental research and clinical studies were selected. The background of the research area

was presented, current research problems and areas of interest were highlighted and time was made available for discussion. The audience, especially young researchers who were encouraged to participate in the session, was motivated to actively participate and to share information with other worldwide scientists. They were interested or conducted research in the field of perinatal nutrition and functioned as a channel of influence in policy, teaching, and clinical work. The workshop focused on some particular topics of research:

- new nutrients in infant formulas
- biochemical mechanisms of metabolic programming
- influence of early feeding on childhood obesity

All papers can be found in abstract form within the poster section of this book..

2. ENRIQUE VÁZQUEZ

Enrique Vázquez from the Abbott Laboratories in Granada, Spain, gave a presentation on 'Dietary gangliosides: beneficial effects for the neonate and potential mechanism of action'. Questions that arose from his presentation were:

- Are gangliosides as large molecules resistant to gastric fluid?
  Answer: some of them could be digested in the stomach but the major part seems to pass intact to the intestine, showing positive effects on gut immunity and intestinal microflora.
- What was the concentrations of the gangliosides used in this work?
  Answer: the concentration of gangliosides in the diet of the mice was 47 mg/kg, which represents the mean amount of gangliosides in maternal milk adapted to the percentage of solids in the milk of the mice.

3. DAREK GRUSZFELD

The next speaker was Darek Gruszfeld from Warsaw, Poland. As one of the co-workers in the European Childhood Obesity Project, which follows up children in Poland, Belgium, Italy, Spain, and Germany with regard to overweight, a presentation on interesting preliminary results on the 'Appetite control in breastfed and milk formula fed infants' was given. Questions and remarks arising from his presentation were:

- Has appetite been measured directly?
  Answer: No, it was measured indirectly by leptin concentration.
- How can you correct for higher leptin concentrations due to increased fat mass in the formula fed infants?
  Answer: up to now, corrections for higher fat mass has not been performed as the study has not been unblinded yet.
- Is increased fat mass caused by higher protein content of the formula milk or due to increased appetite?
  Answer: Since direct measurements of appetite have not been measured, it is difficult to estimate the cause for higher fat mass before 'unblinding' of the study.
- Children at the age of 6 months already get complementary foods. Was this taken into account?
  Answer: yes, food frequency protocols are performed by the mother on 3 days a month monthly, thus also at the time point of blood sampling.

The findings will contribute to the hormonal understanding of metabolic imprinting by a protein-rich diet. So, the final results after 'unblinding' of the formula milk groups will be very promising.

4. HINKE HAISMA

The next speaker was Hinke Haisma from Groningen, The Netherlands. She cooperated with researchers from London, Cambridge and Brazil. Dr. Haisma reported on the 'Effect of complementary feeding with cows' milk on sleeping metabolic rate in breast-fed infants'. Questions she was asked were:

- Can you please explain the significant differences on sleeping metabolic rate and energy expenditure between cow's milk fed and breast fed infants?
  Dr. Haisma showed us with the help of a table which significant differences could be detected.
- Further remarks were made that the breast and cow's milk fed babies composed a very heterogeneous group. Furthermore, the social inequity in Pelotas, Brazil, was quite remarkable.

5. JENNIE LITTEN

Another speaker was Jennie Litten from Nottingham, UK, who presented the research mainly conducted by Allison Mostyn from Wye, UK on 'The influence of birth weight on adipose tissue, skeletal muscle and lung uncoupling protein 2 and 3 expression in neonatal pigs'. Questions arising from her presentation were:

- Could you please delineate the mechanism for the different UCP expression in lung, fat and muscle tissue?
  Answer: The different UCP expression could be due to different immunological or nutritional parameters in the lung, but specific immunological markers were not measured.

6. TATJANA RADAELLI

The presentations continued with Tatjana Radaelli from Milan, Italy, who co-operated with researchers from Cleveland, Ohio, USA. She presented her work on 'Excess fetal adiposity is associated with programming of placental lipid genes'. Questions arising from her presentation were:

- Did some of the mothers receive insulin due to gestational diabetes and how could insulin influence the different expression?
  Answer: three from the six placentas were from mothers receiving insulin during pregnancy. An effect of insulin cannot be excluded.

- The expression of genes related to glucose metabolism is far less accented than the expression of fatty acid metabolism genes. This could be explained by the constitutive glucose transfer over the placenta.

7. THÉA DEMMERS

Our last speaker was Théa Demmers from McGill University in Ste. Anne de Bellevue, Canada, and she and her colleagues collaborated with Cincinnati Children's Hospital Medical Center, OH, USA. She reported on the "Effect of early cholesterol intake on cholesterol biosynthesis and plasma lipids". Questions arising from her talk were:

- How far is cholesterol biosynthesis responsive of diet?
  Answer: cholesterol biosynthesis is responsive to diet, but the response differs in different stages of life, it is highly responsive to infant dietary cholesterol intake and is down-regulated by a higher than usual cholesterol intake in adulthood but this response in adulthood is varied across individuals.
- Was cholesterol measured in mothers?
  Answer: plasma cholesterol of the mothers has not been taken into account, but as it might be of interest it will be compared with the children's plasma cholesterol and the fractional synthesis of cholesterol in the future.
- Will effects later in life be measured?
  Answer: there is a plan to follow-up the children in order to detect clinical effects.

8. CONCLUSIONS

The speakers, as well as the presenters and all the participants of this session, were thanked for their contribution. The participants had the opportunity to learn new interesting issues relating to obesity, fetal programming and effects of new nutrients for new supplemented infant formulas. The presenters gave the impression that their research was at a very high standard. They could learn that presenting their own results

and opening them up for discussion is a very important means of communication to even improve their research. They were motivated to keep up the good work and to profit from any discussion at the workshop and at the conference.

In fact, three of the presenters were elected as giving the best abstracts of the Workshop (1st: Tatjana Radaelli, 2nd: Hinke Haisma, 3rd: Théa Demmers).

CONSUMER NEEDS REGARDING DIETETIC PRODUCTS FOR PREGNANT AND LACTATING WOMEN AND FOR BABY FOODS

Focus Group Meeting

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Abstract: This meeting discussed the regulation, marketing and communication needs of these products from a consumer point of view. It was agreed, that clear and consistent messages are needed from health professionals, public authorities, industry and also the media. There is also a need to better understand the context in which choices of products are made. Research into these topics is reviewed in this paper, but it is obvious that more research into consumer and health professional behaviour is needed. This will allow best practices for communication of the use of dietetic foods for the important consumer group of lactating mothers and young parents.

1. INTRODUCTION

A key to appropriate infant feeding policies is an understanding of the scientific evidence on which recommendations are based (Foote and Marriott, 2003). Much of the literature relating to infant nutrition has focused on milk feeding, and the effect of introducing solid foods has been relatively neglected (Fewtrell et al., 2003). Recent research has established that there are short to medium term health implications associated with the age of solid food introduction for both preterm and full term infants (Morgan et al., in press). There is extensive evidence that consumption of certain types of food within an infant's balanced diet may have a positive and even protective effect on health. It appears that

certain foods in the weaning diet may have specific health benefits both preterm and full term infants (Morgan et al., 2003).

2. PROMOTION OF HEALTHY INFANT FEEDING

Given the importance of early nutrition programming, a major challenge is to promote healthy infant feeding by parents. However, this cannot be accomplished unless both parents and health professionals understand basic infant nutrition and its implications for later health. Infants are completely dependent on their carers to make decisions about their food choices. Parents gain knowledge from a wide range of sources including health professionals, the media, friends, and relatives. However, the quality of this information may vary and may not necessarily be evidence-based. We do not know whether the emergence of evidence of the relationship between early diet and later health has influenced the formulation and delivery of current advice on infant feeding practices. Knowledge and understanding of this link may further help parents offer health-enhancing diets to their infants.

Health professionals, who advise parents directly may be informed by national and international recommendations, although these may not be entirely evidence based or fully reflect the link between early diet and later health outcomes. Most studies of health professionals' understanding of early nutritional programming have investigated knowledge of breast-feeding (e.g. Freed et al., 1995) rather than weaning and infant feeding (Williams and Pinnington, 2003). Hyde's (1994) work on community-based health professionals concluded that parents are not always given clear and consistent advice. A study in Northern Ireland (Bleakney and McErlain 1996) found that staff knowledge improved significantly following the introduction of feeding guidelines. Some health professionals, however, were confused about the recommended ages for introducing cow's milk, vitamin supplements, fluoride drops and other food items (Bleakney and McErlain 1996).

Recent research (Williams and Pinnington, 2003) with paediatric nurses found the majority to be unaware of the current national recommendations and thus concluded that more work was needed if nurses are to continue to advise parents on aspects of weaning. Many health professionals believe that the decision to wean should be based solely on the assessment of the nutritional needs of the baby (Werk and

Alpert, 1998). There is a need for a broader and more international evidence base with regard to health professionals' knowledge and understanding of infant feeding. This should encompass consideration of varied sources that they may access, including the media, industry and the World Wide Web.

3. INFLUENCE OF MEDIA

It is important to understand the potential influence of the media on perceptions of infant feeding. There is little systematic research on this topic. A recent study of the portrayal of infant feeding in the British media (Henderson et al., 2000) found bottle feeding to be shown more often than breast feeding and presented as less problematic. The type of people seen to bottle feed were portrayed differently than those who breast fed, "ordinary" families and middle class or celebrity women respectively. Henderson and colleagues (2000) found that the health risks associated with formula milk and the potential health benefits of breast feeding were rarely referred to. Such portrayals can both be seen as a reflection of society's views on the topic and have the potential to influence parental decisions about infant feeding.

4. FOOD CHOICE

There is evidence that while dietary habits are an obvious public health issue, at an individual level many people see few health benefits in changing eating patterns (e.g. Jeffery 1994; Fishbein 1997). Given evidence of a lack of understanding of even basic infant nutrition messages such as the importance of relatively high fat, low fibre diets with optimal energy intake (e.g. Morgan et al., 1995; Hobie et al., 2000), it is unlikely that many parents are aware of the emerging strong relationship between infant feeding practices and health outcomes.

The issue of food choice is complex and influenced by a wide range of social and psychological factors, especially for a mother providing solid food to an infant for the first time (Lanigan et al., 2001). The decision to wean is one that is made by the mother after taking a number of factors into account and future health outcomes are by no means the sole driver of this decision (e.g. Morgan et al., 1995; Anderson et al.,

2001). Data collected by Murphy and her colleagues (1998; 1999) clearly show how mothers have to balance their babies' needs against their other obligations and their own personal needs and priorities. Hunger related behavioural changes are one of the main rationale for commencing weaning such behaviours have been identified as, the infant needing more frequent feeds, the infant crying after a feed, and changes in sleeping patterns. Carers make the decision on what to offer a baby and what to withhold; the infant may contribute to this decision by expressing dissatisfaction or refusing food, however the ultimate decision is one made by the carer. Food is sometimes used to control or distract babies, particularly in public settings (Murphy et al., 1998; 1999). The foods consumed and way and which food is consumed (e.g. self-feeding, use of a spoon) are sometimes seen as a measure of the child's progress and/or intelligence, mothers are thus often eager to encourage their babies to move on to 'the next stage' of feeding (1998; 1999).

5. PROTECTION MOTIVATION THEORY

Issues surrounding the promotion of health-enhancing behaviour are of central concern to health psychologists. Protection motivation theory (Rogers, 1983) links the likelihood of preventive action to the magnitude of the perceived threat, understanding of the seriousness of the consequences of engaging in high risk behaviour, assessment of the likelihood of own susceptibility and internal and external cues to action. Individual reactions to these factors are affected by demographic, social and psychological variables including age, gender, social class, ethnicity, personality and experience.

In the case of early nutrition some parents may not perceive a threat to the long-term wellbeing of their infant from unhealthy weaning and feeding practices, and external information from health professionals or public health messages may not reach them, and even then impact on their beliefs.

6. BARRIERS AND HOW TO OVERCOME THEM

Many barriers to healthy behaviour exist, but a prerequisite is accurate knowledge and understanding of the causes and consequences of particular diseases and the role of lifelong nutrition in increasing susceptibility or protecting against health problems in the future. Without an appreciation of the threat to health posed by certain behaviours, parents have little incentive to adopt health promoting feeding regimens for their infants. The difficulties surrounding the promotion of increased folic acid intake to reduce the risk of giving birth to a baby with neural tube defects highlight the complexity of changing behaviour in women of childbearing age (Raats et al. 1998). There is extensive evidence that adults have difficulty in translating nutritional messages into appropriate food choices (e.g. Nestle et al., 1998). This is likely to also be the case where they are selecting food on behalf of infants. Emphasis must therefore be placed on the development of practical, achievable and realistic advice that clearly explains the possible long-term health implications of poor nutritional regimes.

In studies of consumer choice criteria with respect to functional foods, the best predictor for willingness to use functional food was the perceived reward (Urala and Lähteenmäki in press). It has also been shown that increasing the scientific strength of a health-related claim did not automatically increase the perceived health benefit (Urala et al. 2003). Trustful respondents perceived the claims as more advantageous than did sceptical consumers. There is need to understand better the consumer choice criteria of parents with respect to weaning and baby foods, in order to also tailor the messages delivering the health information.

REFERENCES

Anderson AA, Guthrie CA, Alder EM, Forsyth S, Howie PW, Williams FLR. Rattling the plate—reasons and rationales for early weaning Health Educ Res 2001; 16:471-479.

Bleakney GM, McErlain S. Infant feeding guidelines: an evaluation of their effect on health professionals' knowledge and attitudes. J Hum Nut Diet 1996; (9): 437–450.

Fewtrell MS, Lucas A, Morgan JB. Factors associated with weaning in full term and preterm infants. Arch Dis Child Fetal Neonatal Ed 2003; 88(4):F296-F301.

Fishbein M. Predicting, understanding and changing socially relevant behaviors: lessons learned. In: McGarty C, Haslam SA, editors. The message of social psychology, Oxford: Blackwell; 1997, p. 77–91.

Foote K, Marriott L. Weaning in infancy. Arch Dis Child Fetal Neonatal Ed 2003; 88:488-492.

Freed GL, Clark SJ, Lohr JA, Sorenson JR. Paediatrician involvement in breast feeding promotion: a national study of residents and practitioners. Pediatr 1995; 96: 490–494.

Hobbie C, Baker S, Bayer C. (2000). Parental understanding of basic infant nutrition: misinformed feeding choices. J Pediatr Health Care 2000; 14:26–31.

Hyde L. Knowledge of basic infant nutrition amongst community health professionals. Matern Child Nurs J 1994; 19:27–32.

Jeffrey RW. Risk behaviors and health: contrasting individual and population perspectives. Am Psychol 1989;44:1194–202.

Henderson L, Kitzinger J, Green J. Representing infant feeding: content analysis of British media portrayals of bottle feeding and breast feeding. BMJ 2000;321:1196-1198.

Lanigan JA, Bishop J, Kimber AC, Morgan J. Systematic review concerning the age of introduction of complementary foods to the healthy full-term infant. Eur J Clin Nutr 2001; 55(5):309-320.

Morgan J, Lucas A, Fewtrell MS. Does weaning influence growth and health up to 18 months? Arch Dis Child. In press

Morgan J, Taylor A, Fewtrell MS. Meat consumption during weaning is positively associated with psychomotor outcome in children under 22 months of age. Arch Dis Child 2003; 88:837

Morgan JB, Kimber AC, Redfern AM, Stordy BJ. Healthy eating for infants – mothers' attitudes. Acta Paediatr 1995; 84:512-515.

Murphy EA, Parker S, Phipps C. Competing agendas in infant feeding. Br Food J 1998; 100 (3):128–132.

Murphy EA, Parker S, Phipps C. Motherhood, morality, and infant feeding, in Williams, L. and Germov, J. (eds), The Social Appetite: An Introduction to the Sociology of Food and Nutrition, Melbourne: Oxford University Press, 1999. pp. 242–258.

Nestle M, Wing R, Birch L, DiSogra L, Drewnowski A, Middleton S, Sigman-Grant M, Sobal J, Winston M, Economos C. Behavioral and social influences on food choice. Nutr Rev 1998; 56(5):S50–S64.

Raats MM, Thorpe L, Hurren C, Elliott K. Changing Preconceptions. Volume 2. The HEA Folic Acid Campaign 1995-1998. Research Report. London: Health Education Authority; 1998. p. 94.

Rogers RW. Cognitive and physiological processes in fear appeals and attitude change: a revised theory of protection motivation. In: Cacioppo J, Petty R, editors. Social Psychophysiology, New York: Guilford; 1983. p. 153-76.

Urala N, Arvola, A and Lähteenmäki L. Strength of health-related claims and their perceived advantage. Int J Food Sci Technol 2003; 38:815.

Urala N, Lähteenmäki L. Attitudes behind consumers' willingness to buy functional foods. Food Qual Pref in press

Werk L, Alpert J. Solid feeding guidelines. Lancet 1998; 352:1569.

Williams A, Pinnington LL. Nurses' knowledge of current guidelines for infant feeding and weaning. Journal of Hum Nutr Dietet 2003; 16(2): 73-80.

FOCUS GROUP: BREAKFAST MEETING: SMES AND THEIR CO-OPERATION WITH ACADEMIA

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Abstract: Co-operation between SMEs and Academia can be a win-win situation when each partner understands the constraints of the other. SMEs are often leaders in innovation; therefore more ready to share interest in research. They are flexible and dynamic. They need a short feed-back to sustain their co-operation. Academia is often more long-term oriented and more question- than answer-oriented. A code of conduct can ease the relationship because it can anticipate the potential problems.

Key words: SMEs, Co-operation, Research.

1. INTRODUCTION

Co-operation between infant food manufacturers and academia is essential. They both have the same final objective: to provide young children with the best products to ensure an adequate nutrition and to optimise the health potential of children.

2. DIFFERENCES BETWEEN INDUSTRY AND ACADEMIA

There are however some differences between both partners: Academia aims to measure the nutritional needs of children, the physiological pathways and the understanding of the mechanisms, with a specific attention to possible diseases. Unusually they have an extra

aim- to generate a new piece of knowledge and therefore a good paper in a good journal. Discovery is a powerful driver in Academia, and Nature is so complex, that it appears there is no end to the quest of knowledge. Food manufacturers, on the other hand, are aiming at always producing the best product, with the highest standard of hygienic quality and with the strict specifications. Food safety, adequate storage and stability of the formula are some of the daily concerns of the food producers.

3. CONSTRAINTS FOR MANUFACTURERS

Some of the constraints of the Infant Food Manufacturers were clearly stated in the presentation of Andrée Bronner from Paris. She emphasised the importance of the aim: to create the best alternative to breast milk. In fact the exact composition of the human breast milk is not totally known yet, and may never be known. The development of a new formula is a very long and complex process. Selection of adequate raw materials, identification of sustainable source of raw ingredients, adaptation of the industrial process to ensure the required standard of hygiene, without damaging the nutritional qualities of ingredients, adequate shelf-life and cost-efficiency are challenges that every producer must overcome. In fact there is no one perfect solution but many appropriate compromises.

It is so difficult and time and money consuming to make a formula, that manufacturers are not willing to change the process, or the recipe of a formula every day. They need convincing evidence that a new ingredient or a new process is needed before changing their specifications.

The co-operation with Academia is necessary to provide sounded suggestions for changing formula and also to provide guidelines for the assessment of new formula or new effects.

4. PARTICULAR PROBLEMS/OPPORTUNITIES FOR SMES

Small and Medium Enterprises (SMEs) expect clear information from Academia on their recently acquired scientific knowledge and

recommendation for implementation of new knowledge. EPSGHAN is doing such work in a fashionable manner, and this workshop, organised with the help of EU, Academia and Manufacturers aimed at selecting among the recent information the relevant information that must be translate into products.

SMEs are most of the time more reactive than large manufacturers and they have more flexibility to change recipes and/or specifications. Co-operation with Academia will be easier from this point of view. Similarly it may be easier to work on a smaller scale of production to make a new product for clinical testing. It is one of the strengths of SMEs to be more open to innovation. They are also interested in smaller markets than those of larger companies, and they are willing to develop specific products.

SMEs may need, more than larger companies with specific nutritional expertise, a guidance about new knowledge in the nutrition field. For example the conclusion of this workshop must be crystal clear: what information represents progress in science? For all producers including SMEs it is an incentive to look for a potential new ingredient. Further, what kind of knowledge is a significant breakthrough that will be implemented in recommendations and must be considered as opportunities by SMEs?

5. EXAMPLE OF SUCCESSFUL CO-OPERATION

Co-operation between SME and Academia can be successful. Mats Strömqvist from Sweden illustrated this with an example: the bile salt stimulated lipase. The discovery of a naturally occurring enzyme present in breast milk, a specific lipase, was the starting point of a collaboration between a University and an SME to implement the production of that lipase in transgenic animals, necessary to provide enough enzyme for clinical studies which are mandatory to demonstrate the efficiency of this new ingredient.

First academia was important in defining the potential benefits of such an ingredient: it is a first estimation of the potential market for the product. Then one SME was essential in using transgenic animals as providers of ingredients, and another SME in developing a formula for pre-term infant and considering this ingredient as a trigger for innovation within its market.

6. INFORMATION AND EDUCATION

Information and education is another fruitful field for co-operation between Academia and food producers. It is a common interest to educate the consumer and to harmonise the information sent to consumers as William Heird from Houston reported. One excellent principle is to start from the questions asked by the consumers and to provide a balanced answer with practical applications.

In the area of infant nutrition a balanced panel of experts from industry and academia can provide adequate answers to these questions and the food industry has the capacity to convey the message in different formats adapted to the different audiences. They also have the capacity to send that message to a very large group of people, and to send it regularly to disseminate a fairly balanced set of answers to the most frequently asked questions. The kind of co-operation is also an excellent training for Industry to understand the complexity of the scientific message that must be provided, and for Academia to work on the simplification of the message that must be understood by the largest group of the population. To make Science Simple and Understandable is another daily challenge where co-operation provide mutual benefits.

Science without application will not provide enough money to support new research. Food production without innovation and improvement towards an impossible target, to reproduce breast milk on an industrial scale, will not provide enough money to support new growth, and will not allow food producers to achieve their goals: to create the best product.

7. A WIN-WIN SITUATION

Co-operation does not mean loss of independence nor integrity; in fact it is the opposite. In a win-win situation, both Academia and food producers plays its part. Hugo Heymans from Amsterdam presented a set of principles, codes or rules, that ease the co-operation between Academia and food producers. There are different set of existing codes that must be used to define the rules before starting any co-operation.

Scientific integrity is of mutual interest: a long term profitable business cannot be built on uncertainty or weak science, especially for infant food. Intellectual property is important to assess throughout the process and there may be different perspectives on the value of information: Scientific knowledge is as important as industrial know-how to make a profitable success. Understanding the rules of patenting, keeping the information secret as long as possible, and being the first to publish, are important considerations to protect the interest of both parties.

8. CONCLUSIONS

The input of the EU is key to ease the process of sharing common interest. It can facilitate the development of competitive knowledge within academia and the implementation of this knowledge into the food industry, specifically the SMEs which are instrumental in all successful innovations.

ETHICAL ISSUES IN PERINATAL NUTRITION RESEARCH

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Abstract: This paper examines three ethical areas arising from perinatal nutrition research: the first is concerned with properly informed consent in a context of interventionist research; the second with the role of research ethics committees and the third with the relationship between research and public policy

Key words: ethics; Perinatal; research

1. INTRODUCTION

In the last decades, developments in ultrasound technologies have taught us a great deal about perinatal development and growth. Although this progress has led to great improvements in diagnosis of fetal diseases, it has not been followed by improvements in therapeutic strategies during intrauterine life, so that time of delivery is often still the only route available to reduce perinatal morbidity and mortality (Pardi, 2003). Very recently, research has developed around perinatal nutrition, both during intrauterine life and in the immediate neonatal period. This research is focused on two aspects:

1) understanding how the fetus/neonate "eats" and grows
2) developing interventionist therapeutical strategies for improving the rate of growth.

A number of studies point to long term risks related to alterations of the growth rate in the perinatal period and to the short and long term consequences for the health of children born small for gestational age (Lucas, 1991). Moreover, these children seem at higher risk when the restriction of growth *in utero* is not followed by catch up growth during the neonatal period (Eriksson, 1999). However, it is difficult to distinguish between risks related to genetic potential and those related to

changes in supply of nutrients. Both clinicians and scientists should not forget that alterations in fetal nutrition and growth represent a complex disease that requires multidisciplinary care to maximize the benefits provided to the parents and their baby.

2. PROPERLY INFORMED CONSENT IN A CONTEXT OF INTERVENTIONIST RESEARCH

2.1 What is informed consent?

Properly informed patient consent is now considered to be an essential feature of modern bioethics. If medical paternalism in the past allowed doctors and medical scientists to conduct forms of therapy or research without 'burdening' patients with information about their function or possible consequences, today this is no longer considered acceptable from either an ethical or a legal perspective. Even if such information is indeed a 'burden', it is now generally accepted that all competent patients do have a right to be properly informed. Some bioethicists even take the view that all patients must be given full and accessible information about medical interventions upon them whether they wish to have this information or not. Some argue that if it is patients' right to have such information, then it is the duty of the doctor/medical scientist to provide and their duty, in turn, to receive it.

We do not take this view ourselves. But we do take the view that all patients and subjects of medical research should be offered clear and accessible information about its function, consequences and possible risks. This will enable them to make a properly informed choice about whether or not to participate and even to withdraw from research if they have subsequent misgivings. For us, properly informed consent involves the provision of sufficient and understandable information and space for a patient who has the capacity to make a stable choice about medical interventions or research on herself, to do so responsibly in a manner considerate to others (see Stirrat and Gill, 2005).

2.2 Who should give consent?

The specific context of interventionist perinatal nutrition research does raise some very serious ethical issues. Clearly babies are not competent to give such consent themselves. But should it be just the mother or both the mother and the father who gives this consent? There are likely to be very different answers given here within the various nationalities and cultures that comprise the European Union. Surrogate consent, when there may be unknown risks involved, is always problematic. Increasingly perinatal nutrition research today involves genetic screening. Surrogate consent is particularly contentious here. As yet genetic science is rather better at diagnosing genetic conditions than in identifying a specific therapy for them. As a result, the genetic screening of babies now may, in the absence of any effective therapy, considerably disadvantage them in the future (for example, in terms of their future insurance or employment).

2.3 Risks and benefits

With or without genetic screening, interventionist perinatal nutrition research may well have no actual benefit for the baby involved. If, in addition, there is a risk (however unlikely) of serious harm to this baby, then it would normally be considered to be unethical. In bioethics it is usual to insist that benefits for that patient should outweigh any possibility of risk or burden. There are rare exceptions that are sometimes made to this principle – for example when an infant gives bone marrow to a sibling – but these are indeed rare, involve a minimal burden and arise from very specific medical crises. It would be difficult to justify such an exception in the context of perinatal nutrition research.

Insistence upon properly informed consent does place a duty upon doctors and scientists to provide clear and accessible information in a format that patients can really understand. This is especially difficult in technical scientific areas. There also needs to be careful consideration given to possible coercion, either because too much trust is given by some patients in their doctors (especially in pediatrics), or because doctors/medical scientists are too compromised by sponsoring bodies (such as the manufacturers of formula milk). Transparency is crucial at every level in perinatal nutrition research if properly informed consent is

to be achieved. It is also important for doctors/medical scientists to have some system of information feedback to research participants once the results of that research have been established.

3 ROLE OF RESEARCH ETHICS COMMITTEES

The role of ethics committees is often viewed as troublesome by medical scientists and researchers. The area of perinatal nutrition research is no exception. It is particularly confusing when different local ethics committees reach opposite conclusions about the ethical acceptability of particular research proposals. It can also be troublesome when such ethics committees lack specialist competence in obstetrics and/or paediatrics. Within the European Union as a whole, there are well known and sharp differences between nations on particular issues. For example, in the United Kingdom embryonic stem cell research is allowed (up to, but not beyond, fourteen days), whereas in Germany and a number of other European countries it is regarded as unethical and remains illegal (albeit not always for the same reasons). There is unlikely to be total consensus on such issues and this can be vexatious to the research community.

However, although consensus may not be achieved on everything, greater cross-European and international research co-operation and dialogue may reduce difficulties somewhat. For example, there is now a much greater consensus on the issue of properly informed consent and the parameters that we have already discussed are not specific to any one nation. They are shared widely and increasingly across Western countries and are also developing within the South as well. We believe that ethics committees do have an important role to play in many countries and that they have raised awareness of ethical issues involved in modern medicine and science. However, we also believe that their competence still needs to be enhanced and enriched through international co-operation and dialogue.

One area that deeply concerns many ethics committees is the research use of surplus human blood and tissue in research. Some bioethicists have argued that it is unethical to use any blood or tissue (whether identifiable or not) for anything other than the specific research for

which it was collected (and for which patient consent was explicitly given). Others have argued that such an approach would be very detrimental to research. Historical samples, for example, have been extremely important in developing knowledge about HIV/AIDS. There is no immediate way of resolving this difference. However, a number of governments are currently exploring either the possibility of generic consent when samples are taken or even presumed consent especially for discarded and unidentifiable tissue (leaving the onus upon those who object to their discarded tissue being used in research to do so).

4 THE TENSION BETWEEN RESEARCH AND PUBLIC POLICY

4.1 The MMR example

It is well known in bioethics that public health raises some very specific tensions between individual rights and the public good. For example, the recent triple-vaccination (MMR) controversy in the United Kingdom raised a serious dilemma for some parents. Reading national newspapers it seemed at the time (even though this was later discounted by the scientific community) that there was a connection between triple-vaccination and autism. While these parents could be assured that the high level of MMR vaccination in the UK insured that their babies were unlikely to be infected by these diseases, then it may have seemed that it was in their interests not to have their own babies vaccinated (or to have single vaccines spread over a longer period of time). Yet if every parent adopted this attitude, vaccination levels and thus 'herd immunity' would soon drop and unvaccinated (or single-vaccinated) children would then be at risk. On this understanding, the common good (herd immunity) requires all parents to have their babies MMR vaccinated, but a more individual consideration in a context of high herd immunity might suggest (at least to those who were not scientifically informed) that MMR vaccination should be avoided.

4.2 Perinatal nutrition research

Perinatal nutrition research may raise similar concerns. If the connection, say between formula milk early feeding and later obesity really is as established as researchers such as Lucas claim (see paper in this book), then public policy might adopt some very restrictive policies. Instead of simply instructing health visitors to encourage young mothers to breast feed, they might forbid the sale of formula milk without a prescription, provide serious tax incentives for young mothers who do breast feed (and thus penalise young mothers who do not), or seek to stigmatise or even criminalize formula feeding for young babies. But, of course, such draconian measures would seriously conflict with the ethos of many liberal, democratic societies and endanger individual rights. In any case, some of the other papers here raise proper scientific questions about the reliability or statistical significance of this particular link within perinatal nutrition research. The force of von Kries' paper, for example, is that such a link *may* be established but that it is more significant for populations than for individuals (since adults who know that they are slightly more at risk of obesity than their breast-fed peers can, after all, choose to eat less than them).

This raises crucial questions about the level of scientific certainty that is required in perinatal nutrition research in its relation to public policy. While a scientific area is still developing, is it reasonable to require politicians to act on the basis of knowledge that is still disputed? And, in any case, how coercive should public health policy actually be (a dilemma that is already strongly debated in many European countries about public smoking)? An answer to these questions necessarily involves some tension between individual rights and autonomy, on the one hand, and public good on the other. In the context of research conducted in the Third World/Global South this tension becomes even more vexed. It is very tempting for multi-national companies to conduct research in the Third World/Global South, because it can be less expensive and may meet less resistance in the populations there. But this raises very sharp ethical dilemmas that are receiving increasing attention from bioethicists. Many are seriously concerned about the risk of individual exploitation in contexts where local research ethics committees may be as yet ill-developed. Ethical vigilance is, we believe, very much needed in this area.

5 CONCLUSIONS

In itself, perinatal medicine is taking care of individuals that are not independent and able to make choices, and this implies particular consideration. The rapidly developing methods for prenatal diagnosis, and the potential for perinatal nutritional therapy represent a new ethical challenge. This possibility of early diagnosis, of foreseeing the future of these individuals and therefore trying to change their future, needs ethical approach, a discussion amongst scientists, clinicians and ethicists about what is accepted by society.

REFERENCES

Stirrat GM and Robin Gill, 'Autonomy in Medical Ethics after O'Neill', *Journal of Medical Ethics,* forthcoming 2005.

Pardi G, I. Cetin, A.M. Marconi, A. Lanfranchi, P. Bozzetti, E. Ferrazzi, Buscaglia M, F.C. Battaglia, 'Diagnostic value of blood sampling in fetuses with growth retardation', *New England Journal of Medicine*, 328: 692-696, 1993.

Lucas A 'Programming by early nutrition in man', *Ciba Foundation Symposium* 1991;156:38-50

Eriksson JG, T. Forsen, J.Tuomilehto, P.D.Winter, C.Osmond, D.J.Barker, 'Catch-up growth in childhood and death from coronary heart disease: longitudinal study', *British Medical Journal,* 1999;318:427-31.

EARLY PROGRAMMING OF DIABETES RISK – AN INTRODUCTION

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1. INTRODUCTION

The importance of perinatal nutrition is well recognized with regard to later growth and the risk of type 2 diabetes in adult life. However, nutrition in very early life has also implications with regard to the risk of type 1 diabetes in childhood. This field was reviewed in the presentations in a session at the Workshop "Early Nutrition and Its Later Consequences: New Opportunities" in Paris, July 2-3, 2004. A review of the relation between early nutrition and later diabetes risk is presented in this supplement by Knip and Åkerblom.

2. HISTORY

The cow's milk and type 1 diabetes hypothesis has been debated for some two decades. Experiments in BB rats (1) and NOD mice (2) have clearly demonstrated a deleterious effect of dietary proteins, such as cow's milk proteins in the disease process. The classical report of Elliott and Martin (1) was followed by a report from the same group (3) of the effect of cow's milk proteins appearing during a relatively narrow and early phase in the postnatal (weaning) period. Many ecological and epidemiological studies, but not all, in man have shown that exposure to

cow's milk proteins during the first months of life may be particularly important. The strongest indirect evidence in man comes from infant feeding studies, an inverse correlation being found between the duration of breast feeding and the incidence of type 1 diabetes in childhood (reviewed in ref. 4).

3. POSSIBLE MECHANISMS

The mechanism of the possible beta-cell lesion by cow's milk protein remains to be clarified, and several proteins in cow's milk have been suggested to be the diabetogenic compound.

In recent years much interest has been directed to the importance of the induction of immune tolerance in the gut. Several studies indicate that patients with type 1 diabetes have an enhanced humoral and cellular immunity to a series of cow's milk proteins. The findings of altered immune responsiveness to dietary antigens in type 1 diabetes suggest that the regulation of mucosal immunity may be disturbed. Accordingly it is possible that none of these antigen-specific immune responses may be directly involved in the pathogenesis of the disease, but they are induced as a consequence of dysregulated oral tolerance (4). This regulatory defect of the gut immune system may actually have a fundamental role in the pathogenetic process leading to type 1 diabetes (5,6).

The possibility that bovine insulin could be the pathogenetic compound has gained attention in recent years, and is presented in more detail in the article by Vaarala. The possibility that lymphocytes sensitized to bovine insulin in early infancy will later mature toward autoreactive insulin-specific lymphocytes in some individuals and lead to the destruction of insulin producing beta-cells is a new hypothesis in the pathogenesis of type 1 diabetes (6).

Gluten is another dietary protein of great interest with regard to the pathogenesis of type 1 diabetes in relation to early feeding. In BB rats wheat gluten in the diet increased the incidence of diabetes (7), and a gluten-free diet prevented diabetes in NOD mice (8). Recently it was

reported that gluten feeding in early infancy may lead to the appearance of autoimmune markers of type 1 diabetes (9,10).

4. CONCLUSIONS

Many important challenges remain for investigators in the field of early nutrition and the risk of type 1 diabetes, e.g. the mechanisms of the beta-cell lesion by foreign proteins, and the timing of exposure (9).

REFERENCES

1. ELLIOTT RB, MARTIN JM. Dietary protein: a trigger of insulin-dependent diabetes in the BB rat ? Diabetologia 26:297-299, 1984.
2. ELLIOTT RB, REDDY SN, BIBBY NJ, KIDA K. Dietary prevention of diabetes in the non-obese diabetic mouse. Diabetologia 31:62-64, 1988.
3. DANEMAN D, FISHMAN L, CLARSON C, MARTIN JM. Dietary triggers of insulin-dependent diabetes in the BB rat. Diabetes Res 5:93-97, 1987.
4. ÅKERBLOM HK, KNIP M. Putative environmental factors in Type 1 diabetes. Diabetes Metab Rev 14:31-67, 1998.
5. HARRISON LC, HONEYMAN MC. Cow's milk and type 1 diabetes. The real debate is about mucosal immune function. Diabetes 48:1501-1507, 1999.
6. VAARALA O. Gut and the induction of immune tolerance in Type 1 diabetes. Diabetes Metab Res Rev 15:353-361, 1999.
7. HOORFAR J, SCOTT FW, CLOUTIER HE. Dietary plant materials and development of diabetes in the BB rat. J Nutr 121:908-916, 1991.
8, FUNDA DP, KAAS A, BOCK T, TLASKALOVA-HOGENOVA H, BUSCHARD K. Gluten-free diet prevents diabetes in NOD mice. Diabetes Metab Rev Res 15:323-327, 1999.
9. NORRIS JM, BARRIGA K, KLINGENSMITH G et al. Timing of initial cereal exposure in infancy and risk of islet autoimmunity. JAMA 290:1713-1720, 2003.
10. ZIEGLER A-G, SCHMID S, HUBER D, HUMMEL M, BONIFACIO E. Early infant feeding and risk of developing Type 1 diabetes-associated autoantibodies. JAMA 290:1721-1728, 2003.

EARLY NUTRITION AND LATER DIABETES RISK

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Abstract: Early feeding may modify the risk of both type 1 (T1D) and type 2 diabetes (T2D) later in life. The information generated so far is, however, controversial. When evaluating studies on the impact of early feeding on risk of later diabetes, the data have to be assessed critically and possible confounding factors have to be considered. The study design may induce biases and there are considerable differences in early feeding practices across various countries and cultures. Accordingly it may not be possible to generalise observations based on one population. Long breastfeeding, exclusive breastfeeding in particular, and supplementation with vitamin D in infancy have been reported to confer partial protection against beta-cell autoimmunity and T1D. In contrast, early exposure to cow's milk proteins and cereals and heavy weight in infancy have been implicated as risk factors for T1D. Long breastfeeding has also been observed to protect against T2D in aboriginal populations. Poor fetal nutrition resulting in low birth weight has been identified as a factor contributing to later insulin resistance and T2D. Recent data indicate that current overweight and obesity are stronger determinants of insulin resistance than birth weight among preschool children. High-nutrient diet and rapid growth in early infancy have been reported to adversely programme the principal components of the metabolic syndrome including insulin resistance and T2D. It is an important scientific and public-health objective to define protective and predisposing effects of early nutrition on the development of diabetes, since early feeding can potentially be modified to minimise the risk of later chronic diseases.

Key words: Beta-cell autoimmunity, breastfeeding, cereals, cow's milk, growth, insulin resistance, type 1 diabetes, type 2 diabetes

1. INTRODUCTION

Diabetes is characterised by increased blood glucose concentrations. These are regulated by a loop comprising two components, the insulin-secreting beta cells and the insulin-sensitive tissues, i.e. liver, muscle and adipose tissue, which respond to insulin. Loss of blood glucose control is due to beta-cell failure, resulting in insulin deficiency, insulin resistance of the target tissues or a combination of both. Type 1 diabetes (T1D) is perceived as a chronic immune-mediated disease with a subclinical prodrome characterised by selective loss of insulin-producing beta cells in the pancreatic islets in genetically susceptible persons (Knip 2002). The preclinical disease phase is asymptomatic and may last from a few months up to more than 10 years. Acute clinical onset, ketoacidosis and dependency of exogenous insulin are characteristic features, and they result from absolute insulin deficiency. Type 2 diabetes (T2D) is typically a metabolic disorder of obese middle-aged or old people with slow clinical onset and non-insulin dependence (Wilkin 2001). The patients suffer from relative insulin deficiency because of impaired insulin action combined with the inability of the beta cells to compensate for the reduced insulin sensitivity. T2D is a component of the metabolic syndrome characterised by obesity, insulin resistance, lipid abnormalities, hypertension and cardiovascular disease.

Both T1D and T2D are caused by the interaction of a series of genetic and environmental factors. Although data available indicate that there is a stronger genetic predisposition to T2D than to T1D, more is known about the genetic background of the latter with a major part of the disease susceptibility conferred by *HLA* genes on the short arm of chromosome 6. Life style characteristics, such as obesity and physical inactivity, are well established exogenous risk factors for T2D, while the environmental determinants of T1D have remained more speculative (Åkerblom et al. 2002). Fetal malnutrition has been implicated as a risk factor for T2D (Hales and Barker 1992). Recent observations indicate, however, that current overweight is a stronger determinant of insulin resistance than birth weight among young children (Wilkin et al. 2002).

Short breastfeeding, early exposure to cow's milk proteins and cereals, failing supplementation with vitamin D and heavy weight in infancy have been implicated as risk factors of T1D related to early feeding (Virtanen and Knip 2003). Short exclusive breastfeeding, high-

nutrient diet and early growth acceleration in infancy have been reported to confer increased risk for T2D (Pettitt et al. 1997, Singhal and Lucas 2004). These risk factors will be discussed below.

2. LIMITATIONS OF STUDIES ON EARLY FEEDING - LATER DISEASE RELATIONS

The role of early feeding in the development of later chronic diseases has remained controversial in many respects despite an extensive research activity in this field. These controversies may at least partly reflect differences in study design. Many studies have been retrospective and such studies might be associated with substantial recall biases. Intervention studies which potentially provide the strongest evidence for a possible relationship between early feeding practices and subsequent health are so far very few in number in this area, partly due to the long follow-up time needed when assessing the consequences of infant feeding on later health.

There are also considerable differences in early feeding practices between various cultures and countries. The frequency and duration of breastfeeding varies conspicuously, and similarly the use of hydrolysed formulas appears to be very frequent in some countries, while it is extremely rare in other countries. About 15% of the infants in Colorado, USA, are exposed to cereals as their first foreign proteins, whereas this is exceptional e.g. in Finland. Accordingly observations made in one country are not necessarily generalisable to other geographical regions.

Some of the controversies may be explained by differences in exposure and outcome measures. A minority of the studies dealing with breastfeeding have differentiated between exclusive and total breastfeeding, although the biological effects of breastfeeding must be related to whether the infant is given breast milk only or supplemental food in addition to breast milk. The end of exclusive breastfeeding leads in most cases to exposure to cow's milk, and accordingly it may be difficult to define whether a later adverse health effect is related to short exclusive breastfeeding or alternatively to early exposure to cow's milk. Hence it would be important to define the dietary exposure in detail both qualitatively and quantitatively when assessing later health consequences of infant feeding.

3. EARLY FEEDING AND RISK OF TYPE 1 DIABETES

3.1 Breastfeeding and exposure to cow's milk

A series of retrospective studies have implied that breastfeeding may protect from T1D, but there are also reports suggesting no protective effect and even some indicating a predisposing effect of breastfeeding (see Virtanen and Knip 2003). Gerstein conducted already in 1994 a meta-analysis of all acceptable retrospective studies available by that time to assess whether early feeding practices had any influence on the risk of T1D. That meta-analysis showed that breastfeeding for a shorter period than 3 months was associated with a 1.4-fold risk for T1D, whereas exposure to cow's milk before the age of 4 months conferred a 1.6-fold disease risk. Prospective studies on the association between early feeding and the appearance of beta-cell autoimmunity, i.e. diabetes-associated autoantibodies, have also provided partly conflicting results. Three prospective studies from the United States, Germany and Australia did not observe any effect of breastfeeding/early exposure to cow's milk on the appearance of beta-cell autoimmunity, while a Finnish birth cohort study reported that short exclusive breastfeeding and early exposure to cow's milk predisposed young children to progressive beta-cell autoimmunity in the form of positivity for all four autoantibody specificities analysed (see Virtanen and Knip 2003).

A pilot intervention study conducted mainly in Finland eliminating exposure to cow's milk proteins over the first 6-8 months of life indicated that the early dietary intervention resulted in a reduction of 40-60% in the cumulative frequency of all diabetes-associated autoantibodies except GAD antibodies (see Knip 2003). Based on the experience from the pilot study a randomised, controlled and double-blinded trial proper was initiated in 2002 to answer the question whether it is possible to reduce the frequency of diabetes-associated autoantibodies and/or clinical T1D by the age of 6 years and the cumulative incidence of T1D by the age of 10 years by weaning to a highly hydrolysed formula over the first 6-8 months of life. This Trial to Reduce IDDM in the Genetically at Risk (TRIGR) will recruit 2032 infants with at least one affected family member and *HLA*-conferred susceptibility to T1D by the end of April 2006, and accordingly the final

answer will be available at the earliest in year 2012 illustrating the need for long follow-up periods in prospective and intervention studies exploring the association between early feeding and later health.

3.2 Early exposure to cereals

Two recent prospective studies have indicated that early exposure to cereals may increase the risk of seroconversion to positivity for diabetes-associated autoantibodies (Norris et al. 2003, Ziegler et al. 2003). The American report suggested that both early (before the age of 4 months) and late exposure (at the age of 7 months or later) to cereals were associated with an increased risk of beta-cell autoimmunity, while the German study implied that an increased risk was related to exposure to cereals before the age of 3 months. In addition the American survey indicated that both gluten-containing and non-gluten-containing cereals conferred an increased risk for beta-cell autoimmunity. Neither of the studies reported any data on the amount of cereals the infants were exposed to at various ages. Early exposure to cereals are against the infant nutrition recommendations in all developed countries and occurs only rarely. Accordingly one may ask whether early exposure to cereals is a proxy of other baby care practices predisposing to T1D.

3.3 Vitamin D supplementation

The daily requirement of vitamin D can not be met in infancy without oral supplementation, particularly not in the winter time with decreased daylight. A European retrospective multicentre survey indicated that vitamin D supplementation during infancy was associated with a reduced risk of T1D (EURODIAB 1999). A Finnish birth cohort study showed that vitamin D supplementation in the infant period was related to a decreased risk of T1D, whereas suspicion of rickets by the age of 2 years increased the risk of later diabetes (Hyppönen et al. 2001). These observations suggest that vitamin D supplementation in infancy confers partial protection against T1D.

3.4. Growth in infancy

Increased weight gain in infancy has been associated with an enhanced risk of T1D in case-control studies (see Virtanen and Knip

2003). In a Finnish survey current weight in the highest quartile at the age of 3 and 6 months resulted in a 1.5-fold risk of T1D later in childhood (Hyppönen et al. 1999). Whether accelerated linear growth in infancy contributes to an increased risk of T1D has remained controversial.

4. EARLY FEEDING AND RISK OF TYPE 2 DIABETES

4.1 Breastfeeding

Pettitt et al. reported in 1997 that exclusive breastfeeding for the first 2 months of life was associated with a significantly reduced rate of T2D among Pima Indians. A more recent study among Native Canadians indicated that breastfeeding for more than 12 months reduced the risk of subsequent T2D before the age of 18 years to about one fifth of the average risk (Young et al. 2002). It remains open, whether the protective effect of breastfeeding on the development of T2D is also operative among Caucasians, since so far no such observations have been reported.

4.2 Fetal and infant growth

According to the fetal origins hypothesis intrauterine malnutrition predisposes the individual to various components of the metabolic syndrome including cardiovascular disease, hypertension, obesity, dyslipidaemia and T2D (Barker 2002). A series of retrospective studies have shown that the rate of the above mentioned components of the metabolic syndrome are inversely related to birth weight.

Over the last few years evidence has accumulated indicating that early infant weight gain and current weight are more strongly associated with insulin resistance in young children than birth weight. A survey in 5-year-old British children indicated that current weight was the strongest determinant of insulin resistance both in boys and girls, and neither birth weight nor postnatal weight gain were independent predictors of insulin resistance (Wilkin et al. 2002).

The growth acceleration hypothesis suggests that early infant growth, weight gain in particular, is the common denominator of the metabolic syndrome (Singhal and Lucas 2004). According to this hypothesis the association previously observed between birth weight and the components of the metabolic syndrome would be the consequence of the phenomenon that infants born small for gestational age experience the fastest weight gain in early infancy. Singhal et al. reported in 2003 that early feeding with a nutrient-enriched formula in preterm infants resulted in higher circulating concentrations of 32-33 split proinsulin, a marker of insulin resistance, in adolescence compared to feeding with banked breast milk or a standard formula. The 32-33 split proinsulin concentrations in the peripheral circulation of the adolescents were related to the weight change over the first 2 weeks of life with the highest levels in those with the fastest weight gain. Another analysis of the same cohort showed that there was an inverse correlation between flow-mediated endothelium-dependent dilation in the brachial artery and early postnatal weight gain suggesting that high weight gain over the first 2 weeks of life predisposes to impaired vascular endothelial function in adolescence (Singhal et al. 2004). Insulin resistance has been shown recently to be associated with endothelial dysfunction (Hsueh et al. 2004).

5. CONCLUSIONS AND FUTURE DIRECTIONS

There is definitely a need to expand our present knowledge of the consequences of early feeding on later health and disease. Accumulated evidence suggests that early exposure to complex dietary proteins may be a risk factor for T1D. One can speculate that early weight gain in infancy may induce beta-cell stress making these cells more susceptible to cellular damage and thereby increasing the risk of T1D. Regular vitamin D supplementation in infancy may not only protect the child from rickets but also from later T1D.

Preliminary data indicate that exclusive breastfeeding for at least 2 months and prolonged overall breastfeeding protects against T2D, at least among aboriginal populations. High-nutrient diet and accelerated growth, weight gain in particular, in early infancy may be a risk factor for the development of the metabolic syndrome and T2D.

These observations argue in favour of strong promotion of exclusive breastfeeding over the first few months of life in practically all infants. It has been repeatedly shown that weight gain is slower on breastfeeding than on formula feeding, and already that consequence may reduce the subsequent risk of both T1D and T2D. The adverse effects of early weight gain and the fact that the energy content of formulas is usually higher than that of breast milk raises the issue whether there is a need to reduce the energy content of infant formulas in the developed countries. This is a question that needs further studies, preferably in the form of intervention trials implying that we have to wait for the definite answer for some time.

REFERENCES

Åkerblom HK, Vaarala O, Hyöty H, Ilonen J, Knip M. Environmental factors in the etiology of type 1 diabetes. Am J Med Genet 2002;115:18-29.

Barker DJ. Fetal programming of coronary heart disease. Trends Endocrinol Metab 2002;13:364-368.Hales C, Barker DJP. Type 2 (non-insulin dependent) diabetes mellitus: the thrifty genotype hypothesis. Diabetologia 1992;35: 595-601.

Gerstein HM. Cow's milk exposure and Type 1 diabetes mellitus; a critical overview of the clinical literature. Diabetes Care 1994; 17:13-19.

Hales C, Barker DJP. Type 2 (non-insulin dependent) diabetes mellitus: the thrifty genotype hypothesis. Diabetologia 1992;35: 595-601.

Hsueh WA, Lyon CJ, Quinones MJ. Insulin resistance and the endothelium. Am J Med 2004;117:109-117.

Hyppönen E, Kenward MG, Virtanen SM, Piitulainen A, Virta-Autio P, Knip M, Åkerblom HK, the Childhood Diabetes in Finland Study Group. Infant feeding, early weight gain and risk of type 1 diabetes. Diabetes Care 1999;22:1961-1965.

Hyppönen E, Läärä E, Reunanen A, Järvelin MR, Virtanen SM. Intake of vitamin D and risk of type 1 diabetes: a birth cohort study. Lancet 2001;358:1500-1503.

Knip M. Natural course of preclinical type 1 diabetes. Horm Res 2002; 57, Suppl. 1: 6-11.

Knip M. Cow's milk and the new trials for prevention of type 1 diabetes. J Endocrinol Invest 2003;26:265-267.

Norris JM, Barriga K, Klingensmith G, Hoffman M, Eisenbarth GS, Erlich HA, Rewers M: Timing of initial cereal exposure in infancy and risk of islet autoimmunity. JAMA 2003;290:1713-1720.

Pettitt DJ, Forman MR, Hanson RL, Knowler WC, Bennett PH. Breastfeeding and incidence of non-insulin-dependent diabetes mellitus in Pima Indians. Lancet 1997;350:166-168.

Singhal A, Cole TJ, Fewtrell N, Deanfield J, Lucas A. Is slower early growth beneficial for long-term cardiovascular health? Circulation 2004;109:1108-1113.

Singhal A, Fewtrell M, Cole TJ, Lucas A. Low nutrient intake and early growth for later insulin resistance in adolescents born preterm. Lancet 2003;361:1089-1097.

Singhal A, Lucas A. Early origins of cardiovascular disease: is there a unifying hypothesis? Lancet 2004;363:1642-1645.

The EURODIAB Substudy 2 Study Group. Vitamin D supplement in early childhood and risk for Type I (insulin-dependent) diabetes mellitus. Diabetologia 1999;42:51-54.

Wilkin TJ. The accelerator hypothesis: weight gain as the missing link between Type I and Type II diabetes. Diabetologia 2001;44: 914-922.

Wilkin TJ, Metcalf, BS, Murphy NJ, Kirkby J, Jeffery AN, Voss LD. The relative contributions of birth weight, weight change, and current weight to insulin resistance in contemporary 5-year olds: The EarlyBird Study. Diabetes 2002;51:3468-3472.

Virtanen SM, Knip M. Nutritional risk predictors of β-cell autoimmunity and type 1 diabetes at a young age. Am J Clin Nutr 2003;78:1053-1067.

Young TK, Martens PJ, Taback SP, Sellers EAC, Dean HJ, Cheang M, Flett B. Type 2 diabetes mellitus in children: prenatal and early infancy risk factors among Native Canadians. Arch Pediatr Adolesc Med 2002;156:651-655.

Ziegler A-G, Schmid S, Huber D, Hummel M, Bonifacio E: Early infant feeding and risk of developing Type 1 diabetes-associated autoantibodies. JAMA 2003;290:1721-1728.

IS TYPE 1 DIABETES A DISEASE OF THE GUT IMMUNE SYSTEM TRIGGERED BY COW'S MILK INSULIN?

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Abstract: The role of the gut immune system in the development of autoimmune type 1 diabetes is evaluated in this review with special emphasis in the hypothesis suggesting that dietary cow's milk insulin could trigger beta-cell autoimmunity when the mechanisms of oral tolerance are disturbed.

Key words: type 1 diabetes; insulin; gut; beta-cell autoimmunity; alpha4beta7-integrin; cytokines; breast milk, microflora, enterovirus

1. INTRODUCTION

The importance of gut immune system in the development of autoimmune diabetes was suggested by animal studies demonstrating that dietary factors modify the development of autoimmune diabetes in the animal models (Biobreeding (BB)-rats and NOD (non-obese diabetic)-mice). In these animal models autoimmune diabetes develops spontaneously, and the infiltration of pancreatic islets by lymphocytes (=insulitis) precedes the destruction of insulin-producing beta-cells. Weaning to the diet of hydrolyzed proteins protects from autoimmune diabetes in NOD-mouse and BB-rat models (Elliot et al., 1999, Kolb et al., 1997). Most importantly this kind of protective diet induces functional changes in the islets infiltrating lymphocytes (Kolb et al., 1997). The cytokine profile in the insulitis from cytotoxic immune response with high IFN-gamma expression is changed to less harmful response characterized by IL-4 and TGF-beta expression. This indicates that an immunological link between diet and autoimmune diabetes exists.

More direct evidence suggesting that dietary antigens could cause autoimmune diabetes dietary antigens comes from a transgenic mouse model (Blanas et al., 1996). When ovalbumin was expressed in beta-cells of transgenic mice, oral administration of ovalbumin resulted in the generation of OVA-specific islet-cell infiltrating CD 8 T-cells and development of destructive insulitis.

2. ROLE OF GUT IMMUNE SYSTEM

Accumulating evidence suggests that gut immune system plays a pivotal role in the development of type 1 diabetes. In NOD-mice, islet infiltrating lymphocytes express α4β7-integrin, which is a homing receptor to the gut mucosa (Hänninen et al., 1996 and 1998). Administration of monoclonal antibodies blocking this receptor or its ligand prevents the development of autoimmune diabetes. Furthermore, mesenterial lymphocytes transfer diabetes from NOD-mice to NOD/scid-mice (Hänninen et al., 1998). These studies indicate that lymphocytes homing to pancreatic islets in the inflammation are also able to home to the gut and this population of lymphocytes may circulate between the gut and the pancreas.

In humans, some reports suggest that autoreactive T-cells may originate from the gut immune system. T-cells derived from pancreas of a patient with type 1 diabetes adhered to mucosal and pancreatic endothelium (Hänninen et al., 1993). Autoreactive T-cells from patients with type 1 diabetes, expressed gut-associated homing receptor α4β7-integrin (Paronen et al., 1996).

Our studies in the intestinal biopsies from children with type 1 diabetes indicate that enhanced intestinal immune activation is associated with type 1 diabetes, at least in children. Increased expression of HLA class II-antigen, α4β7-integrin, IL-4 and IL-1β were found in intestinal mucosa of children with type 1 diabetes without association with celiac disease or signs of celiac diseases; i.e. antibodies to transglutaminase or increased number of intraepithelial lymphocytes (Westerholm-Ormio et al., 2003). In a recent studies, we have also found increased expression of IL-18 mRNA in intestinal biopsy samples from children with type 1 diabetes (Vaarala et al., unpublished observation). Our results indicate that primary aberrancies of the intestinal immune system are associated

with type 1 diabetes. It is possible that the intestinal immune activation fundamentally contribute to the development of type 1 diabetes.

3. EFFECT OF COW'S MILK

Epidemiological studies suggest that exposure to cow's milk (CM) proteins during the first 2 to 4 months of age is associated with about an 1.5-2 fold risk of type 1 diabetes (Gerstein 1994). An association between early CM exposure and risk of type 1 diabetes has not been observed in all epidemiological studies performed. Thus, prospective studies are needed to confirm the possible link between CM exposure and type 1 diabetes. No association of CM exposure with emergence of beta-cell autoimmunity was observed in German and North-American birth-cohort studies (Ziegler et al., 2003 ; Norris et al., 2003). In a Finnish birth-cohort study, exposure to CM formula before 4 months of age implied about five fold risk for the development of multiple autoantibodies and especially autoantibodies against tyrosine phosphatase (IA-2) in children with HLA DQB1*0302 risk allele of type 1 diabetes (Kimpimäki et al., 2001). In some epidemiological studies not only early exposure to CM proteins but also a high dietary intake of CM proteins later in life implied a risk of type 1 diabetes (Verge et al., 1994 ; Virtanen et al., 2000).

4. WHAT ARE THE PUTATIVE DIABETOGENIC FACTORS?

The putative diabetogenic factors in CM are not known, although several candidates have been identified. Bovine insulin in CM induces primary immunization to insulin in infants exposed to CM formulas (Vaarala et al., 1999; Paronen et al, 2000). According to our hypothesis, early immunization to insulin, during the time when gut maturation is incomplete, may result in aberrant immune response to insulin, which later could be activated against insulin-producing beta-cells (Vaarala 2002a). The failure of oral tolerance and intestinal immune activation associated with type 1 diabetes could favour the development of harmful immune response to insulin. It seems that the regulation of immune response to dietary insulin is aberrant in children who later develop beta-

cell autoimmunity. The levels of antibodies to dietary insulin increase during follow-up whereas the antibody levels decrease of remain stable in children without markers of type 1 diabetes related autoimmunity (Vaarala et al., 1999).

Furthermore, increased levels of antibodies binding to insulin are seen in the children with signs of enterovirus infection during the first 6 months of life (Vaarala et al., 2002b). Accordingly, enterovirus infections, which have been connected to the risk of type 1 diabetes, may lead to enhanced immunization to dietary insulin by increasing permeability of the gut or by changing the cytokine environment in the gut. Interestingly, we have found that the concentration of insulin in mother's breast milk modifies the humoral immune response to dietary insulin in the infants. The levels of antibodies to dietary insulin were lower in the infants who received breast milk with high insulin content when compared to the infants who received breast milk with low insulin content (Vaarala et al., unpublished observation).

The antigens and cytokines in breast milk may modify the development of immune response to food antigens. Furthermore, the composition of intestinal microflora may be important factor in regulation of immune response to dietary insulin.

5. CONCLUSIONS

Understanding the regulatory mechanisms of the intestinal immune response may reveal means for the prevention of type 1 diabetes and explain the changes in the incidence and etiological factors of type 1 diabetes in different populations.

REFERENCES

Blanas E, Carbone FR, Allison J, Miller JFAP, Heath WR, 1996, Induction of autoimmune diabetes by oral administration of autoantigen. *Science* **274**: 1707-1709.

Elliott RB, Reddy SN, Bibby NJ, Kida K, 1998, Dietary prevention of diabetes in the non-obese diabetic mouse. *Diabetologia* **31**: 62-4.

Gerstein HC, 1994, Cow's milk exposure and type I diabetes mellitus. A critical overview of the clinical literature. *Diabetes Care* **17**: 13-9.

Hanninen A, Salmi M, Simell O, Jalkanen S, 1996, Mucosa-associated (beta7-integrinhigh) lymphocytes accumulate early in the pancreas of NOD mice and show aberrant recirculation behavior. *Diabetes* **45**: 1173-1180.

Hanninen A, Jaakkola I, Jalkanen S, *1998*, Mucosal addressin is required for the development of diabetes in nonobese diabetic mice. *J Immunol* **160**: 6018-6025.

Hanninen A, Salmi M, Simell O, Jalkanen S, 1993,. Endothelial cell-binding properties of lymphocytes infiltrated into human diabetic pancreas. Implications for pathogenesis of IDDM. *Diabetes* **42**: 1556-1562.

Kimpimaki T, Erkkola M, Korhonen S, Kupila A, Virtanen SM, Ilonen J, Simell O, Knip M, 2001, Short-term exclusive breastfeeding predisposes young children with increased genetic risk of Type I diabetes to progressive beta-cell autoimmunity. *Diabetologia* **44**: 63-9.

Norris JM, Barriga K, Klingensmith G, Hoffman M, Eisenbarth GS, Erlich HA, Rewers M, 2003, Timing of initial cereal exposure in infancy and risk of islet autoimmunity. *JAMA* **290**:1713-1720.

Paronen J, Klemetti P, Kantele JM, Savilahti E, Perheentupa J, Akerblom HK, Vaarala O, 1997, Glutamate decarboxylase-reactive peripheral blood lymphocytes from patients with IDDM express gut-specific homing receptor alpha4beta7-integrin. *Diabetes* **46**: 583-8.

Paronen J, Knip M, Savilahti E, Virtanen SM, Ilonen J, Akerblom HK, Vaarala O, 2000, Effect of cow's milk exposure and maternal type 1 diabetes on cellular and humoral immunization to dietary insulin in infants at genetic risk for type 1 diabetes. Finnish Trial to Reduce IDDM in the Genetically at Risk Study Group. *Diabetes* **49**: 1657-65.

Scott FW, Cloutier HE, Kleemann R, Woerz-Pagenstert U, Rowsell P, Modler HW, Kolb H,1997, Potential mechanisms by which certain foods promote or inhibit the development of spontaneous diabetes in BB rats: dose, timing, early effect on islet area, and switch in infiltrate from Th1 to Th2 cells. *Diabetes* **46**: 589-98.

Vaarala O, Knip M, Paronen J, Hämäläinen A-M, Muona P, Väätäinen M, Ilonen J, Simell O, Åkerblom HK, 1999, Cow's milk formula feeding induces primary immunization to insulin in infants at genetic risk for type 1 diabetes. *Diabetes* **48**: 1389-1394.

Vaarala O, 2002a, Gut immune system and type 1 diabetes. *Ann N Y Acad Sci* **958**: 39-46

Vaarala O, Klemetti P, Juhela S, Simell O, Hyöty H, Ilonen J, 2002b,The effect of coincident enterovirus infection and cow's milk exposure on immunization to insulin in early infancy. *Diabetologia* **45**: 531-534.

Verge CF, Howard NJ, Irwig L, Simpson JM, Mackerras D, Silink M, 1994, Environmental factors in childhood IDDM. A population-based, case-control study. *Diabetes Care* **17**: 1381-9.

Westerholm-Ormio M, Vaarala O, Pihkanen P, Ilonen J, Savilahti E, 2003, Immunologic activity in the small intestinal mucosa of pediatric patients with type 1 diabetes. *Diabetes* **52**: 2287-2295.

Virtanen SM, Laara E, Hypponen E, Reijonen H, Rasanen L, Aro A, Knip M, Ilonen J, Akerblom HK, 2000, Cow's milk consumption, HLA-DQB1 genotype, and type 1 diabetes: a nested case-control study of siblings of children with diabetes. Childhood diabetes in Finland study group. *Diabetes* **49**: 912-7.

Ziegler AG, Schmid S, Huber D, Hummel M, Bonifacio E, 2003, Early infant feeding and risk of developing type 1 diabetes-associated autoantibodies. *JAMA* **290**:1721-1728.

GLUTEN-FREE DIET IN SUBJECTS AT RISK FOR TYPE 1 DIABETES: A TOOL FOR DELAYING PROGRESSION TO CLINICAL DISEASE?

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1. INTRODUCTION

We recently demonstrated that 6 months of gluten deprivation in first-degree relatives at high risk for type 1 diabetes did not influence the number and titres of autoantibodies, but had a beneficial effect on preservation of ß-cell function.

2. NEW STUDY

The study has now been extended to 22 first degree relatives positive for two or more antibodies among anti-GAD (GADA), anti-IA-2 (IA-2A) and anti-insulin (IAA) undergoing a total of 27 six-month periods of a gluten-free diet, followed by an equal number of six-month periods of normal gluten-containing diet. Five subjects developed clinical diabetes during follow-up. In the remainder, first phase insulin response (FPIR) to i.v. glucose tolerance test after the first 6 months of gluten deprivation increased in 19 of the 25 periods tested ($P = 0.021$ of 6 m vs baseline); 6-months after return to normal diet, FPIR decreased in 17 of 20 period tested ($P = 0.005$ of 12 vs 6 m). Patients were stratified as high responders (1st tertile), low responders (2nd tertile) and non responders (3rd tertile) according to FPIR after gluten deprivation and several immunological and metabolic markers have been evaluated for their possible predictive value of response to gluten deprivation.

3. RESULTS

Predictors of increased FPIR after gluten-free diet were low GADA titre, high IAA titre, high circulating CCL2 (MCP1), CCL17 and CCL11, and low circulating CXCL8 (IL-8). No other markers including 12 chemokines, 8 cytokines, IA-2A, CRP, adipocyte hormones and ghrelin were predictive of FPIR response to diet.

4. CONCLUSIONS

These findings confirm that 6 months of gluten deprivation have a beneficial effect on preservation of ß-cell function in subjects at risk for type 1 diabetes, with a more prominent response in a subgroup of individuals with a definite immune profile.

INSULIN LIKE GROWTH FACTOR REGULATION OF BODY MASS IN BREASTFED AND MILK FORMULA FED INFANTS

Data from the EU Childhood Obesity Programme

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Introduction: The data used for analysis was obtained from the European Union research project "Childhood obesity: programming by infant nutrition?". The trial takes place in five countries-it is a randomized double blind placebo controlled trial which remains still blinded. However some laboratory data are available for comparisons between all pooled formula fed infants and breast fed infants. Breast fed infants have a lower risk of obesity and receive less protein which lead to hypothesis of obesity programming by early protein intake- in this particular trial infants fed either low or high protein formula receive more protein than breast fed babies. Insulin-like growth factor axis seems to be stimulated in obese people and these hormonal effects could be also responsible for increasing risk of obesity with formula feeding. However, data on insulin-like growth factor axis in breast fed and formula fed infants are lacking.
The aim of our study was to investigate and compare the insulin-like growth factor 1 axis in formula and breast fed infants.

Methods: We used the preliminary laboratory results obtained from:

- 208 formula fed infants receiving either high protein (21 g/L or 15 % of energy) & low protein (12,7 g/l or 9,6 % of energy) formula pooled together (1:1)
- 113 breast fed infants (at least 3 m of breast-feeding).

Blood samples were taken at the age of 6 months for analysis of serum free IGF-I, total active IGF-I, IGF-BP-2 and IGF-BP3 (RIA tests- Diagnostic Systems Laboratories, INC., Webster, USA). The results were presented as mean±SD.

The two groups of infants studied were compared with the Mann-Whitney-U test. Differences were regarded to be statistically significant at $p<0.05$.

Results: Weight was increased in formula fed infants (7.81±0.89 kg vs. 7.60±0.89 kg, $p<0.05$) whereas length did not differ between the groups (67.5±2.4 vs. 67.7±2.4 cm). Body mass index was significantly higher in formula fed babies (11.6±1.2 vs. 11.1±1.1). In concordance with increased weight concentrations serum total active IGF-I and free IGF-I were significantly higher in formula fed infants (fig. 1,2). The inhibitory protein IGF-BP-2 decreased in formula fed infants, and the stimulatory protein IGF-BP-3 increased in formula fed infants (Fig. 3,4).

Discussion: The anthropometric results from our study confirm previous epidemiological observations of increasing weight and BMI in children fed formula in infancy (1,2). Already at age of 6 months formula fed infants have significantly higher weight and BMI in our trial. We can only speculate about possible protective role of lower protein intake in breast fed babies as we did not evaluate the effects of two different formulas.

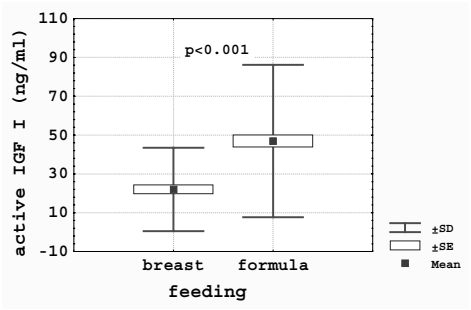

Figure 1. Total active IGF-1 serum concentration in breast fed and formula fed infants

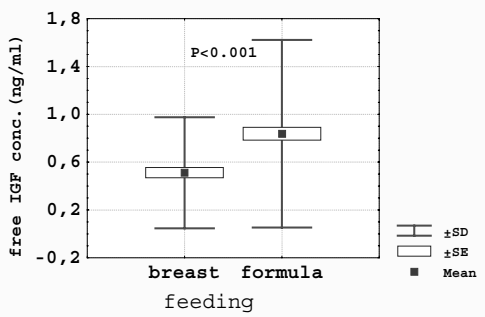

Figure 2. Free IGF-1 serum concentration in breast fed and formula fed infants

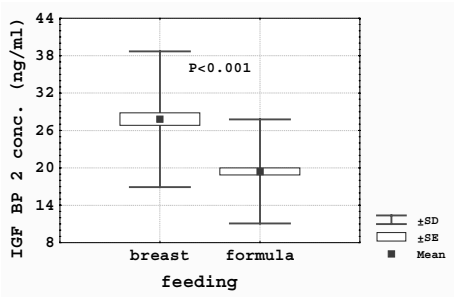

Figure 3. IGF-BP 2 serum concentration in breast fed and formula fed infants

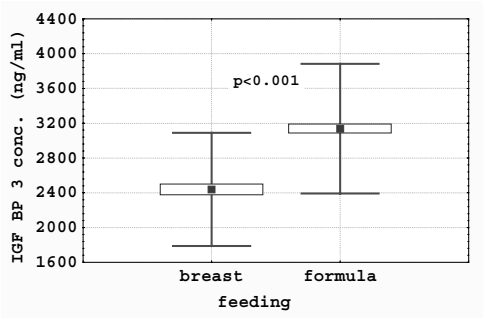

Figure 4. IGF-BP 3 serum concentration in breast fed and formula fed infants

Consistent results of insulin like growth factor axis stimulation point to the important role of this hormonal regulation of body weight. In fact, free IGF-1 increases in obesity that was documented in many studies (3,4,5). There were some conflicting results concerning total active IGF-1 serum levels: normal (3,4), low (5,6,7) or high (8,9,10) serum concentrations were described that may be explained by different populations studied with different types of adiposity. Recently studied pre- and post-menopausal women presented with various concentrations of total IGF-1 that was not clearly related to BMI (11). It may be expected that in pubertal, pre- and postmenopausal populations there are many different hormonal influences interfering with IGF-1 regulations. Our population of infants seems to be more homogenous and sex hormones do not interfere with body mass regulation at this age.

High concentration of free-IGF-1 may be also partially explained by lower levels of IGF-BP-2 , whose production is inhibited by insulin, usually increased with increasing weight. In other populations studied IGF-BP-2 decreased in obesity and the results are very consistent (3,4,5). Furthermore, IGFBP-3 has a complementary stimulatory activity as IGF-1 and this protein is increased in the infants studied. This finding can not be confirmed in all publications as obesity was shown to be associated with normal (4) or high (5) IGFBP-3 concentrations. Also IGFBP-3 serum levels did not show any clear association with BMI in pre- and postmenopausal women (11). On the other hand, a positive correlation between IGFBP-3 and free-IGF-I levels has been shown in obese children (5).
The results of our study seem to be very consistent to point out stimulatory effect of formula feeding on insulin-like growth factor axis. It can be speculated that increasing weight in formula fed infants is attributed to the stimulation of IGF axis. The role of high protein intake will be studied as soon as two groups of children fed high- and low-protein formulas are available for analysis.

Conclusions: The study presents preliminary results from the European Union research project "Childhood obesity programming by infant nutrition". Breast fed and formula fed infants at age of 6 months were compared in concern of insulin-like growth factor (IGF) axis: total and free IGF were significantly increased in formula fed infants, IGF-binding protein 2 was decreased and IGF-binding protein 3 was increased. The results point to stimulation of IGF axis by formula feeding that corresponds to higher body weight in these infants.

Key words: obesity, IGF-I, nutritional programming, insulin resistance

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References:

1. Von Kries R, Koletzko B, Sauerwald T et al. Breast feeding and obesity: cross sectional study. BMJ 1999, 319: 147-150
2. Toschke AM, Vignerova J, Lhotska L et al. Overweight and obesity in 6-14 year-old Czech children in 1991: Protective effect of breast-feeding. J Pediatr 2002, 141: 764-9
3. Frystyk J, Vestbo E, Skjaerbaek C, Mogensen CE, Orskov H. Free-insulin-like growth factors in human obesity. Metabolism 1995,44 (Suppl. 10): 37-44
4. Nam SY, Lee EJ, Kim KR et al. Effect of obesity on total and free-insulin-like growth factor (IGF)-1, and their relationship to IGF-binding protein (BP)-1, IGFBP-2, IGFBP-3, insulin, and growth hormone. Int J Obes 1997, 21:355-359
5. Argente J, Caballo N, Barrios V et al. Multiple endocrine abnormalities of the growth hormone and insulin-like growth factor axis in prepubertal children with exogenous obesity: effect of short and long-term weight reduction. J Clin Endocrinol Metab 1997, 82: 2076-2083.
6. Minuto F, Barreca A, Del Monte P, Fortini P et al. Spontaneous growth hormone and somatomedin-C/insulin-like growth factor-I secretion in obese subjects during puberty. J Endocrinol Invest 1988, 11: 489-495
7. Skaggs SR, Crist DM. Exogenous human growth hormone reduces body fat in obese women. Horm Res 1991, 35: 19-24.
8. Van Vliet G, Bosson D, Rummens E, Robyn C, Wolter R. Evidence against growth hormone-releasing factor deficiency in children with idiopathic obesity. Acta Endocrinol 1986, 279: 403-410
9. Loche S, Cappa M, Borrelli P et al. Reduced growth hormone response to growth-hormone-releasing hormone in children with simple obesity: evidence of somatomedin-C mediated inhibition. Clin Endocrinol 1987, 27: 145-153
10. Hochberg Z, Hertz P, Colin V et al. The distal axis of hrowth hormone (GH) in nutritional disorders: GH-binding protein, insulin-like growth factor-I (IGF-I), and IGF-I receptors in obesity and anorexia nervosa. Metabolism 1992, 41: 106-112.
11. Lukanova A, Lundin E, Zeleniuch-Jacquotte A et al. Body mass index, circulation levels of sex-steroid hormones, IGF-I and IGF-binding protein-3: a cross-sectional study in healthy women. European Journal of Endocrinology 2004, 150: 161-171

INVERSE ASSOCIATION BETWEEN TRANS ISOMERIC AND LONG-CHAIN POLYUNSATURATED FATTY ACIDS IN ERYTHROCYTE MEMBRANE LIPIDS IN PREGNANT WOMEN

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Methods:
Fatty acid composition of erythrocyte membrane phospatidylcholine lipids was investigated at the 20th week of gestation in pregnant women living in Germany (n = 23), Hungary (n = 34) and Spain (n = 44).

Results:
The sum of trans isomeric fatty acids was significantly (P < 0.01) higher in Spanish (0.71 [0.32], % wt/wt, median [IQR]) than in German (0.49 [0.24]) and Hungarian (0.56 [0.34]) mothers. In Spanish pregnant women, trans isomeric fatty acids were significantly inversely correlated to both arachidonic and docosahexaenoic acids (Table 1).

Conclusions:
This observation supports the concept that maternal trans fatty acid intake may be inversely associated to the long-chain polyunsaturated fatty acid status of the fetus.

Table 1. Linear correlation coefficients between fatty acids in erythrocyte membrane phosphatidylcholine lipids in Spanish expecting women (n = 44). * = P < 0.05, ** = P < 0.01

Fatty acid	C16:1t	C18:1t	Sum of trans isomeric
Linoleic	-0.08	+0.28	+0.15
Arachidonic	-0.47**	-0.33*	-0.48**

Fatty acid	C16:1t	C18:1t	Sum of trans isomeric
Alpha-linolenic	+0.08	-0.02	+0.05
Docosahexaenoic	-0.35*	-0.45**	-0.43**

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Comparison of essential fatty acid status among German, Hungarian and Spanish women at mid-gestation

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Methods:

Venous blood samples were obtained at the 20th week of gestation from women living in Germany, Hungary and Spain. Fatty acid composition of erythrocyte membrane phosphatidylethanolamine lipids was determined by capillary gas-liquid chromatography.

Results:

Arachidonic acid values were significantly higher in the Hungarian than in the German samples (Table 1). In contrast, values of docosahexaenoic acid were significantly lower in the Hungarian than in the German samples (Table 1).

Conclusions:

These data suggest that the controversial results in apparently similar studies on maternal long-chain polyunsaturated fatty acid supplementation in different populations may at least partly originate from the different fatty acid status at study entry.

Table 1. Fatty acid composition of erythrocyte membrane phosphatidyl-ethanolamine lipids in women at the 20th week of gestation. Data are % wt/wt, median (IQR), [a] = $P < 0.05$

Fatty acid	German (n =23)	Hungarian (n = 34)	Spanish (n = 44)
Linoleic	5.37 (1.35)	5.71 (1.87)	5.65 (1.30)
Arachidonic	18.49 (7.13)[a]	21.27 (3.39)[a]	18.55 (8.67)

Fatty acid	German (n =23)	Hungarian (n = 34)	Spanish (n = 44)
Alpha-linolenic	0.21 (0.16)	0.26 (0.23)	0.21 (0.36)
Docosahexaenoic	6.34 (1.83)[a]	4.93 (2.01)[a]	5.49 (1.76)

The study was supported by EU grant QLK1-CT-1999-00888

Trans isomeric fatty acids as confounding variables in studies on perinatal LC-PUFA supply

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Introduction In several *in vitro* studies and animal experiments, trans isomeric fatty acids were found to interfere with the availability of long-chain polyunsaturated fatty acids (LC-PUFAs). Here we attempt to systematically review data on this phenomenon in humans in the perinatal period.
Methods: A MEDLINE search was carried out by using the PubMed utility for the phrases (trans * fatty acid*) with (arachidonic acid or docosahexaenoic acid or essential fatty acid or LC*PUFA). The list of references in published articles and the expert opinions of colleagues were also utilized. Five publications reporting significant associations between trans fatty acids and LC-PUFA status or development in the perinatal period were identified.
Results: Significant inverse correlations were reported between trans isomeric fatty acids and LC-PUFAs in cord blood lipids both in healthy full-term infants (Ellias & Innis, 2001) and in full-term infants with an atopic trait (Decsi et al, 2001), as well as in cord vessel wall lipids in healthy full-term infants (Decsi et al, 2002), and in plasma lipids in very young preterm infants (Koletzko, 1992). Moreover, significant inverse correlation was found between infantile plasma trans fatty acids values and birth weight in preterm infants (Koletzko, 1992), and between maternal plasma trans fatty acid values and birth weight and gestational age in a group consisting of both preterm and full-term infants (Jendryczko et al, 1993).
Conclusions: It remains to be clarified whether the interference of trans fatty acids with LC-PUFAs might lead to any disturbance of perinatal development. It can be concluded even now, however, that trans isomeric fatty acids should be regarded as potential confounding variables in studies investigating the availability and role of LC-PUFAs in the perinatal period.

References:

Decsi T, Boehm G, Tjoonk HMR, Molnár S, Dijck-Brouwer DAJ, Hadders-Algra M, Martini I, Muskiet FAJ, Boersma RE: Trans isomeric octadecenoic acids are related inversely to arachidonic acid and DHA and positively to Mead acid in umbilical vessel wall lipids. Lipids 37:959-965, 2002

Decsi T, Burus I, Molnár S, Minda H, Veitl V: Inverse association between trans isomeric and long-chain polyunsaturated fatty acids in cord blood lipids of full-term infants. Am J Clin Nutr 74:364-368, 2001

Elias SL, Innis SM: Infant plasma trans, n-6, and n-3 fatty acids and conjugated linoleic acids are related to maternal plasma fatty acids, length of gestation, and birth weight and length. Am J Clin Nutr 73:807-814, 2001

Jendryczko A, Gruszczynski J, Tomala J, Szpyrka G: Nienasycone kwasy tluszczowe izomerii trans w osoczu krwi kobiet ciezarnych a masa urodzeniowa noworodka (Unsaturated fatty acids of trans isomers in plasma of pregnant women and newborn birth weight, in Polish with English summary). Gin Pol 64: 113-116, 1993

Koletzko B. *Trans* fatty acids may impair biosynthesis of long-chain polyunsaturates and growth in man. Acta Paediatr Scand 81:302-306, 1992

AN EIGHT YEARS PROSPECTIVE STUDY OF IRON DEFICIENCY ANAEMIA IN INFANCY

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Aim: To compare the development of 8-year-old children with and without iron deficiency anaemia (IDA) who had been tested for IDA at the age of 9 months.

Methods: 300 Portuguese infants were selected in São Marcos Hospital in 1994. Of those, 226 were recruited on the basis of their adequate weight for age and absence of preterm delivery. Blood count, iron, transferrin and ferritin (F) were tested in 201 infants. The definition of IDA is haemoglobin (Hb)<110g/l and F<12mg/ml and positive response to iron therapy. The Griffiths test (Gt) and the school performance reports were analysed at 8 y of age. GLM's associated with Gt scores were regarded as dependent variables and logistic regression (LR) with normal and non normal Gt, using Ivens' correction method. Gender, social class, parents' education, kindergarten attendance and IDA were regarded as independent variables.

Results: At age eight years, 33 out of the 39 children with IDA at the age of 9 months and 73 of the 78 controls were given the Gt. The mean (sd) of the general coefficient (GC) in IDA children and non IDA children was 94.1 (4.0) and 94.8 (3.2), p=0.280. The only scale with significant difference between IDA and non IDA children was the eye-hand scale and the mean (sd) obtained was 95.7 (7.0) and 98.5 (3.5), p=0.032. In GLM, kindergarten attendance was associated with an increased GC (F=4,350; p=0,040) and IDA was associated with a decreased eye-hand scale (F=7,353; p=0,008). In LR, with normal and non normal Gt, children with IDA had increased risk (OR: 9.4; p=0.007) of non normal eye-hand scale. Significant difference in school performance at 8 years of age was not found.

Conclusions: Children with infantile IDA had 9 times greater risk of non normal eye-hand scale at the age of 8y. The global development and the school performance do not seem to have been affected.

Keywords: IDA, infants, 8 y children, development, Griffiths test

NEW INSIGHTS IN THE POTENTIAL MECHANISM OF ACTION OF NUCLEOTIDES TO MODULATE IMMUNITY

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Introduction: Different immunological functions have been reported to be modulated by nucleotides, but most of these reports suggest that nucleotides exert the greatest impact on the immune system by modulating immunoglobulin production. This in turn may have a clinical relevance for the neonate preventing the incidence and duration of episodes of diarrhoea and infections. Our group has previously reported that dietary nucleotides modulate positively the production of IgA, but also of IgM. The main goal of this work is to describe a potential mechanism of action to explain how dietary nucleotides modulate immunoglobulin production.

Methods: The influence of dietary nucleotides on the expression of surface antigens characteristic of different intestinal lymphocyte subpopulations, on the expression of B-1 cell antigens by peritoneal cells, which constitutes the main reservoir of B-1 cells, and on the production of IL-2 and IFN-γ (Type 1 cytokines) and of IL-5 and IL-6 (Type 2 cytokines) by Peyer's patch lymphocytes, investigated through flow cytometry in mice at weaning.

Results: Dietary nucleotides significantly increased the percentage of Peyer's patch lymphocytes expressing CD22 (a B cell marker), and increased the percentage of lamina propria and peritoneal lymphocytes expressing CD5. CD5 is expressed by B-1 cells that are precursors of IgA-producing intestinal plasma cells and of IgM and IgA-producing plasma cells in peripheral blood. Moreover, dietary nucleotides modified the Peyer's patch lymphocyte production of Type 1 and Type 2 cytokines, that are involved in the differentiation of intestinal B cells to plasma cells that synthesize and secrete IgA. These results suggest that dietary nucleotides promote intestinal lymphocyte maturation, modulating the production of Type 1 (IL-2) and Type 2 (IL-6) cytokines that are involved in the differentiation of intestinal B cells to plasma cells synthesizing and secreting IgA. Moreover, nucleotides modulate positively the maturation and differentiation of B-1 cells.

Conclusion: All these data might explain how dietary nucleotides modulating the regional immunity can also modulate the systemic immunity and also substantiate the hypothesis that the mechanism by which nucleotides affect immune function is more likely to be a receptor-mediated event than simply being a raw material for nucleic acid replication.

Keywords: dietary nucleotides, B-1 cells, immunoglobulins, cytokines.

THRIVING OF MALNOURISHED BREASTFED INFANTS AFTER ADDITIONAL FORMULA MILK FEEDING

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Introduction: Breastfeeding is the ideal mode of nutrition for healthy infants, especially during the first months of life. Inadequate breastfeeding may result in critical infant failure to thrive. In such infants it is often necessary to implement nutritional intervention. We studied the efficiency of addition of formula milk to exclusively breastfed infants with failure to thrive, with formula offered after each breastfeeding. We aimed at catch up infant growth and preservation of breastfeeding as much as possible.

Methods: 33 full-term exclusively breast-fed malnourished infants, aged from 28 to 99 days, participated in the study. Insufficient weight gain was defined as ≤ 40% expected weight gain for age (600 g/month) and/or body weight ≤ 10. percentile for age (Eurogrowth, 2000). After each breastfeeding, formula milk was offered to the infants *at libitum,* and strategies to preserve breastfeeding were explained to the mother by a paediatrician. Human milk intakes during the first three and the last three days of the study were monitored with the test weighting method. Formula milk intake was registered during the whole study period. Contribution of breastfeeding to total milk intake (CBF, %) was calculated as human milk / (human milk + formula milk intake) x 100. Anthropometric data of the infants were analyzed using Euro Growth program.

Results: Mean (± SD) weight, length and head circumference gains during 31 days of nutritional intervention were 1282 ± 355 g, 4.0 ± 0.9 cm and 2.2 ± 0.9 cm. Absolute human milk intakes (mean ± SD) decreased from 471 ± 181 g/day at study start to 362 ± 282 g/day (study $days_{28-31}$) ($p < 0.05$). CBF (mean ± SD) decreased from 100 % at study start to 63 ± 19 % on study $days_{1-3}$ and 42 ± 35 % on study $days_{28-31}$ ($p < 0.001$). Total daily energy intake (human + formula milk) increased from 84 ± 27 kcal/kg at study start to 140 ± 27 kcal/kg during the catch-up growth (study $days_{1-3}$) and stabilized to 120 ± 24 kcal/kg (study $days_{28-31}$) ($p < 0.001$). In the subgroup of 9 infants with the highest contribution of breastfeeding to total milk intake (CBF > 75%) at the beginning of the study,

CBF at study ending (study days 29-31) only slightly decreased to 96% at seven infants, whereas it decreased to 37% at one infant.

Conclusions: Within one month of formula supplementation infants with failure to thrive successfully gained weight, while breastfeeding was maintained in most of the infants.

Acknowledgement: Supported by the Ministry of Education, Science and Sport, Republic of Slovenia (project L3-2091).
Key words: malnourished infants, breastfeeding, growth, formula feeding

ROLE OF MAMMARY GLAND LIPOPROTEIN LIPASE IN THE AVAILABILITY OF POLYUNSATURATED FATTY ACIDS FOR MILK SYNTHESIS

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Introduction: Mammary gland lipoprotein lipase (LPL) attached to the surface of capillary endothelium has been implicated in the clearance of circulating triacylglycerols (TAG) around parturition. By inhibiting the induction of mammary LPL expression around parturition, this study aimed to determine whether this enzyme plays a role in driving plasma polyunsaturated fatty acids (PUFA) to the mammary gland for milk synthesis.

Methods: From the onset of pregnancy, Sprague-Dawley rats were fed ad libitum a semi synthetic diet containing 10% of either olive oil or fish oil. From the 19th day of pregnancy half of the rats from each group were s.c. treated with 7 mg of progesterone/day whereas the other half received the medium (controls). All the animals were studied at day 23 of postfecundation.

Results: Mammary gland LPL activity was lower in rats receiving progesterone than in controls, whereas adipose tissue LPL activity did not differ between the two groups. Plasma TAG and FFA were higher in the progesterone rats than in controls. Whereas progesterone treatment enhanced the concentration of 16:0, 18:1, 18:2 and 22:6 in plasma, it decreased the proportion of 18:0, 18:2, 18:3 and 20:5 in mammary gland, although the degree of response varied between the animals fed fish oil diet as compared to the olive oil. In fact, whereas progesterone treatment enhanced the proportion of 20:4 n-6 in mammary gland of rats fed olive oil diet, it did not have any effect in rats fed fish oil diet.

Conclusions: Since decreased LPL activity in mammary gland is associated with enhanced levels of PUFA in plasma and a decreased proportion in the mammary gland, we propose that, around parturition, LPL plays a role in driving circulating PUFA for milk synthesis.

IS THE CRYING BEHAVIOUR IN INFANTS UP TO THE AGE OF 3 MONTHS INFLUENCED BY THE TYPE OF EARLY NUTRITION?

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Introduction: Colic is a frequent problem for parents and paediatricians. Objective of this study is to investigate whether the prevalence of colic is influenced by the type of early nutrition in children up to the age of 3 months. The study is embedded in the EU Childhood Obesity Programme, a double blind randomised clinical trial with two parallel study groups of formula-fed infants and a control group of breastfed children (www.childhood-obesity.org).

Method: After informed consent was obtained, parents and their healthy infants were invited to join the study. Criteria for inclusion were: breast-fed (BF)= $\geq$90 % of intake as human milk and formula-fed infants (FF)= 100 % formula fed before the end of the first month. Study formula was delivered to the parents. Recruitment was performed from October 2002 to October 2003 in Munich and Nürnberg, Germany. Information about socioeconomic factors, pregnancy and delivery, medical history of mother and child were collected and standardised anthropometric measures were performed. At the end of the 1st, 2nd and 3rd month self-administered questionnaires were completed by parents. These included food records, a questionnaire on infantile behaviour and in the 2nd and 3rd month additionally the Edinburgh postnatal depression scale. Diagnosis of colic was based on the Wessel criteria (crying and/or fussing for more than 3 hours a day, more than 3 days per week, and more than 3 weeks long).
After the 3rd month a questionnaire on reasons why the mother decided to breast-fed or formula-fed her child was included.

Results: In the 1st month 143 infants (66 BF, 77 FF) were enrolled, 125 (66BF, 59 FF) were followed in the 2nd month and 111 (63 BF, 48 FF) in the 3rd month , respectively. There was no significant difference in the prevalence of colic in BF- versus FF-infants. At the end of the 1st month was 12.1% in BF and 12.0% in FF, at the end of the 2nd month 4.7% and 12.5% and at the end of the 3rd month 0% and 4.3%, respectively. Total BF- and FF- infants did not differ in duration of crying per day.

Parents of BF-infants were slightly older, had a higher educational level and socioeconomic status and smoked less. The social status seems not to correlate with prevalence of colic in any of the first 3 months of life. We found no relation between postnatal depression and the type of milk fed (BF versus FF) nor with the prevalence of colic.
The main reasons why the mothers decided to breastfed her child were: breast milk is the 'best milk' for the child, it is the ´most natural infant nutrition', and it may prevent allergic diseases. Main reasons for FF were that the mother can better control the amount of milk given to the baby, that formula milk provides better satiety, and that the father can also feed the infant.

Conclusions: The results suggest that the prevalence of colic up to the age of 3 months is not influenced by the type of infant nutrition (BF vs. FF).

Key words: EU Childhood Obesity Programme - infant crying - infant nutrition prevalence colic – Edinburgh postnatal depression scale (EDPS)- reasons to breast-fed or formula-fed

DIETARY GANGLIOSIDES: BENEFICIAL EFFECTS FOR THE NEONATE AND POTENTIAL MECHANISM OF ACTION

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Introduction: The content and distribution of gangliosides in human milk differ from those found in milk from other mammalian species, and also selectively change during lactation. Therefore, milk gangliosides may have a role in newborn development during early infancy. This might be to contribute to brain and neurological development, but it has also been suggested that the intestine could be a target organ for dietary gangliosides. Our group has reported that ganglioside-supplemented infant formula modifies the intestinal ecology of preterm newborns, increasing the *Bifidobacteria* content and lowering that of *Escherichia coli*. Although the exact mechanism by which dietary gangliosides modify intestinal microflora is unknown, they might act as decoys of intestinal receptors for some strains of bacteria. In addition, the potential effect on intestinal immunity has been investigated in mice at weaning.
Methods: The luminal content of IgA was determined through ELISA and the number of intestinal IgA-secreting cells was analyzed by ELISPOT. In addition, to elucidate potential mechanisms of action, percentages of Th1 and Th2 lymphocyte subsets were quantified by ELISPOT and the effects of G_{D3} and G_{M3}, major gangliosides in colostrum and mature human milk respectively, on the proliferative state of resting intestinal lymphocytes was also evaluated through ^{3}H-Thymidine uptake.
Results: Dietary gangliosides increased the number of intestinal IgA-secreting cells and the luminal content of secretory IgA. In addition supplementation of the diet with gangliosides determined an earlier development in the number of cytokine-secreting cells, and a significantly higher number of Th1 and Th2 cytokine-secreting lymphocytes in lamina propria and Peyer´s patches. On the other hand, G_{D3} increased lymphocyte proliferation rates in all the intestinal lymphocyte populations, whereas G_{M3} stimulated the proliferation of intestinal lymphocytes excepting for those from Peyer's patches. All these results suggest that dietary gangliosides influence the maturation process of the intestinal immune system that takes place during weaning according to the following potential mechanism of action. Gangliosides modulate the cytokine network promoting the secretion of some cytokines involved in the stimulation of IgA

production and secretion. In addition, dietary gangliosides may have roles on the development of intestinal immunity by stimulating or inhibiting proliferative responses in intestinal lymphocytes.
Conclusion: In summary, dietary gangliosides may have an important role during early infancy modifying intestinal microflora and promoting the development of intestinal immunity in the neonate.
Keywords: dietary gangliosides, IgA, cytokines, intestinal immune system.

LEPTIN IN BREAST-FED AND FORMULA-FED INFANTS

Leptin and feeding in infants

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Introduction: Leptin is a hormone present in breast milk, but not in formula[1], which regulates food intake and energy metabolism; it could be one of the factors involved in preventing obesity.[2,4] Breast milk leptin provides a physiologic explanation for some of the advantages seen in normal growth, regulation of energy intake and immunological status in breast-fed (BF) compared with formula-fed (FF) infants.
Aim: To see whether leptin levels are different in BF or FF infants in the first months of life.
Methods: We studied 186 AGA healthy infants, in the first 22 m, without any disease of the gastrointestinal tract, admitted to our Department during the period between June 2000 and August 2003. Serum leptin concentration was determined at least 3 h post-feeding by RIA test (LEP-R44 Mediagnostic, Reutlingen, Germany). For each infant the parents filled up a form for the Ethical Committee. Statistical Analysis: Student t-test was performed. Statistical significance was set at $p<0.05$. Data have been normalized with natural logarithm.
Results: A significant difference ($p=0.044$) appeared in the first 4 m (n=82) between breast-fed (BF) (1.16 ± 0.99 ng/ml) and formula-fed (FF) (0.68 ± 1.11 ng/ml) infants. Between 4 and 8 m of age there were no differences in leptin levels between feeding groups (BF (n=21): 0.73 ± 1.03 ng/ml; FF (n=15): 0.55 ± 0.84 ng/ml). Similar results have been found in infants between 8 and 12 m (n=32; BF: 0.27 ± 1.01 ng/ml; FF: 0.71 ± 0.87 ng/ml).
Conclusions: Published data show that leptin levels are higher in breast-fed infants than in formula-fed ones. Breast-fed infants feed more frequently and take in less per feed compared with formula-fed infants.[3] The presence of leptin in breast milk may have a positive effect on satiety and regulation of energy intake. The long-term consequences of this difference between BF and FF infants and the role of leptin in promoting later obesity are unknown.

Key words: leptin, breast feeding, formula feeding, infancy

References

1. Savino F.et al. Leptin levels in breast-fed and formula-fed infants. Acta Paediatr 91:897-902; 2002.
2. Von Kries R. et al. Breast feeding and obesity: cross-sectional study. BMJ 1999; 319: 147-50

3. O'Connor D et al. Leptin is not present in infant formulas. J Endocrinol Invest 2003; 26: 490.
4. Locke R. Preventing obesity: the breast milk-leptin connection. Acta Paediatr 2002; 91: 891-896.

DIETARY FATTY ACIDS DURING PREGNANCY DETERMINES MATERNAL FATTY ACID PROFILE DURING LATE PREGNANCY AND THEIR AVAILABILITY TO THE FETUS EVEN DURING FASTING CONDITIONS

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Introduction: Anabolic changes taking place during early pregnancy are driven by an enhanced insulin sensitivity, which facilitates adipose tissue accumulation. whereas in late pregnancy an enhanced catabolic condition develops which may affect adipose tissue composition. By determining the fatty acid profile after a 24 h fast in pregnant rats fed with diets having different oil supplements, we wanted to determine whether an accelerated adipose tissue breakdown warrants the availability of polyunsaturated fatty acids (PUFA) to the fetus.

Methods: From the first day of pregnancy, Sprague-Dawley rats were fed *ad libitum* a semi synthetic diet containing 10% of either palm oil, sunflower oil, olive oil or fish oil. Half of the rats of each group were kept fed, whereas the other half were fasted from day 19 of pregnancy. All rats were studied at day 20.

Results: Fasting enhances plasma free fatty acids, glycerol, ketone bodies and triacylglycerols in all groups, the effect being smaller in rats fed the olive oil diet than in the other groups. Plasma levels of 16:0,18:0,18:1n-9,18:2 n-6,18:3n-3,20:4n-6 and 22:6 n-3 appeared higher in fasted than in fed rats under the different diets except in those on olive oil diet. Plasma levels of 20:4 n-6 appeared lower in rats fed the fish oil diet than in the other groups. The proportion of fatty acids in fetal liver follows a similar trend, with higher n-3 fatty acids and lower 20:4 n-6 in those from mothers fed fish oil diet, and maternal fasting enhanced the proportion of both 18:2 n-6 and 18:3 n-3 in fetal liver in all groups, with the smallest increase in those fed olive oil diet.

Conclusions: Dietary fatty acids during pregnancy determines maternal fatty acid profile during late pregnancy and their availability to the fetus even during fasting conditions.

EFFECT OF OIL-SUPPLEMENTED DIETS ON LIVER EXPRESSION OF PPAR ALPHA-RELATED GENES IN PREGNANT RATS

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Introduction: During late pregnancy, hypertriglyceridemia develops. The hepatic expression of genes related to lipid metabolism is modulated through the activation of the peroxisome proliferator-activated alpha receptor (PPAR alpha). Since fatty acids have been demonstrated to activate PPAR, we studied the effects of diets with different fatty acids compositions in pregnant rats on maternal triglyceridemia and the expression of PPAR related genes.

Methods: Pregnant rats were fed a diet containing 10% of either palm-, sunflower-, olive- or fish-oil throughout pregnancy, and killed on day 20. Pregnant rats feeding standard pellet were also studied in parallel. Liver and blood were collected for later determinations.

Results: The expression of carnitine palmitoyltransferase (CPT-I), a PPAR alpha target gene, was higher in those rats fed the different oil-supplemented diets as compared to those on pellets. These changes for CPT-I expression were related to those of plasma insulin levels. On the contrary, the fatty acid synthase expression was lower in all rats fed the oil-supplemented diets than those on pellets. Rats fed either sunflower- or fish-oil diets showed higher apolipoprotein B expression than those fed palm- or olive-oil diets. However, the expression of stearoyl-CoA desaturase seems to respond inversely to those different oil diets. Finally, the expression of the acute-phase protein, alpha2-macroglobulin, was reduced by all the oil-supplemented diets. These inter-group differences appeared similar to those found for maternal triglyceridemia.

Conclusions: Changes in gene expression caused by the oil-supplemented diets indicate an enhanced fatty acid catabolism and decreased lipogenesis and acute-phase response,. This would explain the significant reduction on triglyceridemia produced by the oil diets in pregnant rats.

EFFECT OF A NEW INFANT FORMULA ENRICHED WITH PREBIOTICS, PROBIOTICS, NUCLEOTIDES AND LC-PUFA ON RECOVERY AFTER INFECTION

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Introduction: During early life, infections are one of the main causes of disease. The aim of our study was to evaluate the effect of a new infant formula enriched with prebiotics, probiotics, nucleotides and LC-PUFAs with a low lactose content in infant recovery after different kinds of infections (respiratory, digestive...).

Methods: A multicentre, randomised, prospective out-patients clinical trial was conducted. 68 infants aged 12 months and younger, with a non-severe infection were distributed in 2 groups: control group and study (RE) group. Infants younger than 4 months were fed with an infant formula and infants from 5 to 12 months with a follow-on formula. Control group infants were fed standard infant and follow-on formula. RE group infants were fed with infant and follow-on formula enriched with prebiotics (galacto-oligosaccharides, nucleotides and LC-PUFA and with a low level of lactose. Follow-on formula used in the feeding of infants of RE group also contained probiotics (*Lactobacillus rhamnosus* and *Bifidobacterium infantis*). Clinical symptoms and duration if the infection was recorded at 0, 5, 10 and 15d to know the evolution of infants recovery.

Results: Appetite was recovered by day 6 in infants of RE group and by day 11 in Control group($p<0,001$)Weight recovery took place by the 5th day in RE group and by day 12 in the Control group($p<0,0001$)... Group RE showed a statistically significant reduction in the duration of digestive symptoms like vomiting and diarrhoea ($p=0,04$) in comparison with Control group. The mean duration of diarrhoea in Control group was 9 d and 4,8d in RE group. Taken into account all the variables, the global recovery of infants was quicker in group RE than in Control group (7,5 days for group RE and 11,7 days for Control group, $p=0,0003$).

Conclusions: The new range of infant formula enriched with prebiotics (galacto-oligosaccharides), probiotics , nucleotides and LC-PUFAs with a low

level of lactose has been shown to be useful. It can achieve a complete and fast recovery for infants suffering digestive or respiratory infections.

Keywords: Infant formula, prebiotics, probiotics, nucleotides, LC-PUFAs.

DOES HABITUAL PROTEIN INTAKE IN EARLY CHILDHOOD INFLUENCE AGE AND BODY MASS INDEX AT ADIPOSITY REBOUND?

Results of the DONALD Study

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Introduction: Both age and body mass index (BMI) at adiposity rebound (AR), the point of a child's minimum BMI before its gradual increase during childhood, have been shown to be associated with the risk for later obesity. High protein intake has been proposed to influence timing of the adiposity rebound (AR) and to increase the risk for overweight.

Methods: We examined the relations between habitual protein intake (assessed with annual 3-d weighed food records) in early childhood and age and BMI at AR in 131 children participating in the Dortmund Nutritional and Anthropometric Longitudinally Designed Study (DONALD Study). Height and weight were measured annually and AR was determined by visual inspection of individual BMI curves. Mean age and internal BMI-SDS at AR were compared between sex-specific tertiles of overall protein intake.

Results: Age at AR was not significantly different between the sex-specific tertiles of habitual energy-adjusted protein intake (% of energy) in girls and boys. There were no differences between tertiles of overall protein intake expressed as g/kg body weight in age and BMI at AR in both boys and girls. Adjustment for the potential confounders mother's BMI, internal BMI-SDS at birth, duration of pregnancy and breastfeeding did not change these results.

Conclusion: A high habitual protein intake averaged from at least three 3-day weighed dietary records between 0.5 and 2 years was not found to have consistent influence on timing of AR or BMI at AR.

DIETARY COMPLIANCE IN DIABETES PREVENTION PROJECT IN FINLAND

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Introduction:
Diabetes Prevention Project is an international, randomised, double-blinded trial. The hypothesis to be tested is whether hydrolysed infant formula compared to cow's milk based formula decreases the risk of developing type 1 diabetes in children with HLA conferred genetic disease susceptibility.

Methods:
During the first 6-8 months of life the infant will be given Study Formula if additional milk is needed. According to the protocol the infant should use Study Formula daily for at least 2 months before the age of 8 months. During the intervention period cow's milk, beef and foods containing these have to be avoided. Dietary compliance is monitored with dietary interviews every month of intervention. For the present study we used the data collected in Finland by the end of November 2003 (altogether 149 children at different stages of the intervention).

Results:
At 2 weeks of age 96% of the infants were breastfed and 39% were exclusively breastfed, at the 3-month interview 83% and 29%, and at the 6-month interview 60% and 2%, respectively. At 9 months of age 48% of the children were breastfed. During the first 2 weeks of life 33% of the infants received Study Formula daily, between 2 and 3 months of age 38%, and between 5 and 6 months of age 62%. Among the first 76 children to reach the end of the intervention 60 (79%) had received Study Formula for at least 2 months, 10 (13%) less than 2 months and 6 (8%) not at all. Of the children 22% had used food not recommended during the intervention (on average 1.2 deviations/child). The most common deviations were use of hydrolysed formula other than the Study Formula (9%), milk-based formula (8%) and lactic acid bacteria that may contain trace amounts of milk protein (5%).

Conclusions
The dietary compliance among the studied children was good, 79% had received Study Formula according to the study protocol.

Keywords: hydrolysed infant formula, cow's milk based infant formula, randomised clinical trial, dietary compliance

CHANGES OF PLASMA FATTY ACID PROFILE AND ANTIOXIDANT VITAMINS DURING NORMAL PREGNANCY

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Introduction: Hypertriglyceridemia associated with enhanced oxidative stress are characteristic features of normal pregnancy. These changes are counteracted by enhanced levels of vitamin E and other antioxidant vitamins, but they also depend on the susceptibility to oxidation due to variations in the fatty acid profile.

Methods: Healthy women were recruited at the first prenatal visit in the outpatient clinic of Hospital San Paolo in Milan. Their age was 31.4 ± 0.6 y, average BMI was 21.6 kg/m^2, and they underwent uncomplicated pregnancies. Blood samples were collected in EDTA-Na_2.

Results: Maternal plasma cholesterol, triglycerides and free fatty acids increased from the 1st trimester of gestation to delivery, suggesting an enhanced lipolytic activity. Plasma alpha- and gamma -tocopherols, and carotenoids also progressively increased with gestation, and values in cord plasma were lower than in mothers. Retinol levels declined with gestation and values in cord plasma were lower than in the mothers. The percentage of n-9 fatty acids remained stable during gestation, but slightly declined in cord plasma. Linoleic acid declined with gestation and further decreased in cord plasma, but arachadonic acid declined at the third trimester and at delivery and increased in cord plasma. The proportion of total n-3 fatty acids remained stable. The proportion of these n-3 fatty acids were similar in cord plasma and maternal plasma at delivery.

Conclusions: During gestation, the proportion of fatty acids reaching the fetus depends on both their concentration in the maternal circulation and specific placental transfer mechanisms. However, enhanced lipolytic activity and circulating levels of antioxidant vitamins at delivery may actively contribute to the availability of both fatty acids and these vitamins to the fetus in preparation for extra-uterine life.

OPTIMAL DESIGN FOR THE RECRUITMENT OF PARTICIPANTS AS A FACTOR FOR THE EFFECTIVE IMPLEMENTATION OF A CLINICAL TRIAL

Experiences of the TRIGR trial

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Introduction: In May 2002 the multinational TRIGR-Study (Trial to Reduce IDDM in the Genetically at Risk) was started in 14 countries all over the world². The probands of this study are newborns with high genetic risk to develop type 1diabetes. This very large primary prevention study will determine whether delayed exposure to intact food proteins will reduce the chances of developing type 1 diabetes later in life. The trial will also be able to analyze whether exclusive breastfeeding can reduce the risk to children of developing type 1 diabetes. Approximately one year after the official launch the recruitment rates varied from 0% to 90% (median 12%) of the rate predicted before the initiation of the study.

Aim: The objective of the present study was to analyse the characteristics of the centre and the information which were associated with a better recruitment rate. Methods. After one year time of recruitment 48 centres participating countries received a semi-standardised questionnaire (22 items) regarding the local structure of the centre, their information strategies and detailed procedures of recruitment. A representative sample 58% (n=28) responded.

Results: The majority of centres (82%, n=23) were familiar with the organisation of trials. Although 52% (n=12) took part in more than 2 trials per year, only 44% (n=10) had experience with newborns as probands. A fourth (n=7) collaborated with other specialised institutions to raise the awareness of the trial. Approaching professional caregivers through a targeted professional journal was used only in half of the centres. The internet (39%) was more frequently utilized than TV (21%). Almost all centres (96%) used meetings for public relation (PR) work. A majority of the centres (57%) had a recruitment rate of less than 50 probands per year. Only 9 centres were able to adhere to the planned schedule for the start of the recruitment. The centre with the best

recruitment rate had started with their PR efforts well before the initiation of recruitment and used primarily targeted information in conventional lay press.

Conclusions: The recruitment rate is influenced by factors related to the structure of the study centre, timing of the PR efforts with respect to the study initiation, using various media for raising awareness about the trial and identifying proper target groups. Using strategies that focus on the targeted patient groups, especially supplying information through the patient related conventional lay press are particularly successful. This approach achieved the best recruitment rate for a large scale multicentre primary prevention trial in newborns at increased diabetes risk.

References

1 Steiner, M., Pelster, D. (2002): Defizite und Auswege. Die Situation der Klinischen Forschung in Deutschland im internationalen Vergleich. DZKM, 11/12, S. 22

2 Akerblom, H.K.,Vaarala, O., Hyöty,H., Ilonen, J., Knip, M. (2002): Evironmental factors in the Etiology of Type 1 Diabetes. Am. Journ. of Med.Genetics (Semin.Med. Genet.) 115: 18-29

THE EFFECT OF PONDERAL INDEX ON PLASMA CONCENTRATION OF INSULIN-LIKE GROWTH FACTOR-1 (IGF-1) IN

NEONATAL PIGS.

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Introduction. Recent evidence has suggested that body shape, rather than birth weight alone, may be a more important diagnostic indicator of future growth and development[1]. It is well documented that IGF-1 plays an important role in the regulation of growth and energy metabolism. This study examined whether plasma concentrations of IGF-1 were influenced by body shape in neonatal pigs.

Methods. 171 newborn piglets were entered into the study and reared by their mother. Piglets were weaned at 28 days of age and were fed *ad libitum* (Growlean OP meal, 14 MJ kg-1, BOCM, UK). Individual body-weight and crown-to-rump length were measured on day 3 of life to enable Ponderal Index (PI: kg/m3) to be calculated. Fat-free mass (FFM) was estimated on day 3 of life via a TOBEC (Total Electrical Body Conductivity) analysing system using the following equation:√(TOBEC reading *CRL). A blood sample was taken from each pig on day 3 and 24 hours prior to slaughter (5-6 months) for subsequent determination of plasma IGF-1 concentration (ELISA kit: DSL-10-5600). A normal probability test was performed on the PI data to describe the distribution of the pigs (LOW, <10th, n=12: NORMAL, 11-89th, n=128: HIGH, >90th, n=31). Differences in plasma IGF-1 concentration were assessed using General Linear Model ANOVA with body weight as a covariate; values are presented as mean ±SEM and similar superscripts denote significant differences (a $P<0.001$; b $P<0.001$; c, d or e $P<0.05$). PI ($P<0.001$) and fat-free mass ($P<0.001$) were different between all sub-populations (Table 1).

Results. Plasma IGF-1 was elevated in the HIGH group ($P<0.01$) on day 3 of life but had returned to normal values by 6 m of age. In contrast, IGF-1 approximately doubled ($P<0.05$) over the first 6 months of life in the LOW group.

Table 1: PI, FFM and plasma IGF-1 concentration in the different sub-populations

	LOW (<10th) (n=5)	**NORMAL (11-89th) (n=96)**	**HIGH (>90th) (n=15)**
PI (kg/m^3)	11.5±0.5^{a}	15.4±0.1^{a}	21.8±0.3^{a}
FFM: day 3 (arb units)	333±11^{a}	247±3^{a}	176±3^{a}
IGF-1: day 3 (µg/ml)	66±35^{be}	81±8^{c}	166±25^{bcd}
IGF-1:5-6 months (µg/ml)	124±19^{e}	93±4	97±13^{d}

Conclusions. Body shape at birth has a pronounced influence on the changes in plasma IGF-1 from day 3 to 5-6 months of age. Piglets that are short-for weight (i.e. HIGH PI) in early life have elevated concentrations of IGF-1, which may in part be due to their greater body fat mass. The increasing plasma IGF-1 in LOW PI piglets may be related to catch up growth and enhanced promotion of fat deposition, similar to that seen in human babies that are long and thin at birth.

Keywords. Pigs; shape, growth, IGF1
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[1]Litten J.C. *et al.* 2002. Early Hum Dev. 66:1, 57-58.

EFFECTS OF PRENATAL EXPOSURE TO LOW AND HIGH DIETARY PROTEIN LEVELS ON MATERNAL AND FETAL AMINO ACID METABOLISM IN RATS.

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Introduction: There is increasing evidence that nutritional programming during fetal development might be involved in the world wide increasing obesity epidemic. In the rat model it has been shown that nutritional programming by low maternal protein intake during gestation results in intra-uterine growth restriction and subsequent development of metabolic disturbances in adult life such as hypertension and insulin resistance. There is some epidemiological evidence that high protein intake during early development might also influence later adiposity, but so far there is little direct experimental evidence. We have shown that a high (40%) protein intake of rats during gestation resulted in a decreased birth weight, a fast catch up growth during suckling period, and increased adiposity and decreased energy expenditure at 8 weeks of age compared to the adequate (20%) protein (Daenzer et al., J Nutr. 132: 142-144, 2002).

Methods We investigated parameters of protein metabolism of pregnant dams and their fetuses during feeding of different protein diets. Rats were time-mated and fed isocaloric diets containing low (LP, 10% w/w), adequate (AP, 20%) or high protein (HP, 40%) concentrations. Plasma amino acid (aa) concentrations during gestation were measured by HPLC. Additionally we studied the incorporation of orally applied ^{13}C-labelled lysine (lys) and leucine (leu) into maternal plasma, fetal, and placental protein.

Results Placental and fetal weights were not different in LP, NP or HP on gestation day 14 and 19. Urea in maternal plasma was highest in HP at all time points, indicating an increased aa oxidation. Maternal plasma concentrations of branched chain aa were also highest in HP. Interestingly, taurine concentrations were increased during gestation in LP and AP, but not in HP. Incorporation of ^{13}C-lys and ^{13}C-leu into maternal plasma protein was decreased with increasing protein intake (LP>NP>HP). Incorporation of ^{13}C-leu and ^{13}C-lys into both placental and fetal protein was decreased in HP compared to AP or LP.

Conclusions: These results are indicative of higher oxidation of dietary aa after HP feeding with the consequence of lower aa availability for fetal or placental protein synthesis. We conclude that feeding HP diets during pregnancy might have consequences for fetal development, especially during time of rapid tissue growth at gestation days 19-22.

COW'S MILK INTRODUCTION IN SPANISH INFANTS

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Background/aims: Cow's milk introduction in infant feeding is not recommended before 12 months of life. Although all paediatric services give parents information in this way, a significant percentage of parents introduce cow's milk in their infant's feeding too soon. The aim of this study was to evaluate the age of cow's milk introduction in Spanish infants and to compare it with Spanish guidelines.

Methods: A total of 926 mothers with infants from 1 to 4 years old took part in this survey. The questionnaire had a retrospective design and was divided in three major parts: Demographic variables, breastfeeding practices and age of introduction of different food during weaning period and semi-quantitative dietary data of their younger infant. The mean age of mothers in the study was 33,7 (SD4,5) years and the mean age of infants 2,4 (0,9)years. The study comprised 508 male and 419 female infants. Statistic analysis: ANOVA and correlation test were done using the computer program SPSS 11.0. A p-value of less than 0,05 was adopted as criteria of significance and 95% confidence intervals were used.

Results: The mean age of cow's milk introduction was 17,0 (5,6) months. 34,5% of infants began to take cow's milk at 12 months of life or before. 65% of infants introduce growth milks, cow's milks specially adapted to toddlers, enriched with vitamins and minerals and with a low protein content and vegetal fat, before standard cow's milk. 58,3% of infants began to take growth milks at 12 months of life or before. Age of cow's milk introduction was not related with maternal level of studies, number of children or Spanish geographical region, but it was found a relationship between maternal age and age of cow's milk introduction ($p < 0,05$). There were correlations between age of cow's milk introduction and breastfeeding duration ($p< 0,01$), age of follow-on formulae ($p<0,0001$), gluten ($p<0,01$) and weaning ($p<0,05$) introduction.

Conclusions: 1) 65,5 % of Spanish infants introduce in their complementary feeding cow milk after 12 months of life, following Spanish paediatric recommendations. 2) Parents must be informed about the similarities between growth milks and cow's milk and introduce both of them after 12 months of life, 3) Early use of cow's milk is associated with precocious introduction of wean

Key words: Cow milk introduction, weaning, complementary feeding, infant feeding.

LONGER TERM EFFECTS OF EARLY CHOLESTEROL INTAKE ON CHOLESTEROL BIOSYNTHESIS AND PLASMA LIPIDS: A RANDOMIZED CLINICAL TRIAL

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Introduction:
Although endogenous cholesterol (Ch) fractional synthesis rate (FSR) is inversely related to infant dietary Ch at 4 months of age, whether this effect is lasting and demonstrative of metabolic imprinting remains to be established.

Objective: To determine whether level of dietary Ch in infancy induced changes in FSR and plasma lipids that persisted at 18 months.

Methods: A prospective, clinical trial in 47 infants who received, from their first week of life until 18 months, human milk (HM) until weaned (n=15), or were randomized to receive modified cow-milk formula (MCF) with added Ch (n=15), or cow-milk formula (CF, n=17), for 12 months. Ch contents of HM, MCF, and CF were 120, 80, and 40 mg/L, respectively. FSR was measured at 4 and 18 months.

Results: At 4 months, FSR in the HM group (0.034 (0.005) pools/day) was lower ($p<0.02$) than in CF (0.052 (0.005) pools/day). There was no difference between HM and MCF (0.047 (0.005) pools/day), nor between MCF and CF. At 18 months, there were no differences in FSR between groups.

Conclusion: While Ch intake prior to weaning affects FSR and plasma lipids at 4 months, these differences do not persist after weaning to unrestricted diet at 18 months. This provides further evidence that there is no imprinting of FSR in infancy by differing dietary levels of Ch.

Key Words: cholesterol, breast-fed, formula-fed, fractional synthesis rate, deuterium

PATTERNS OF GROWTH AND ENERGY UTILIZATION OF THE DIET AFTER A PERIOD OF DIETARY RESTRICTION DURING THE WEANING PERIOD

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Introduction: In rats, weaning may be a critical window for nutrition programming further body growth. There is a dietary change from rat milk containing a high proportion of fat to a diet low in fat and rich in proteins, carbohydrates and fibre.

Aim: to test if deficient diets during the weaning period in rats, from the 21rst post natal day (P21) to P28, would affect the pattern of growth during a period of free access to food from P29 to P70 as compared to breast fed animals.

Methods: Forty male *Wistar* rats were randomly allocated to one of four groups of ten animals each: Group 1(G1): *ad libitum* breast feeding in litters of six pups per dam; G2: standardized laboratory chow *ad libitum*; G3: food restriction receiving half of the food ingested by a pair fed animal in G2; G4: no lipid diet. From P29 to P69, the standardized laboratory chow was fed *ad libitum* to all animals. The energy utilization rate (g/cal) from P29-P69 was calculated. The gain in weight from P29 to each four days after up to P70 was fitted to the Verhulst's function. The third derivative of this function (a constant expressing the rate of variation in growth acceleration ($g.d^{-2}$)) was estimated. The results were tested by a one factor ANOVA or Kruskall-Wallis test ($p \leq 0.05$) and when significant, the S.N.K method for multiple paired comparisons was employed.

Results: The median of the energy utilization of G3 was higher than that of all the other groups (G1:235 g/Cal, G2:250 g/Cal, G3:285 g/Cal, G4:261 g/Cal). The medians of the rate of variation in growth acceleration (G1:-0.0013, G2:-0.00089, G3:-0.00086, G4:-0.00089) of the groups G2, G3, and G4 were higher than G1 and were not significantly different one from the other.

Conclusions: Different diet restrictions had diverse effects on energy utilization and on the rate of variation in growth acceleration.

Key words: Nutritional programming; growth; young rats; dietary restrictions; weaning.

INFANT FORMULA FEEDING PATTERN AND WEANING INTRODUCTION IN SPANISH INFANTS

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Background/aims: Breast milk is recognised as the best option for feeding infants during first months of life. WHO recommends a duration of exclusive breastfeeding of six months and a total duration of breastfeeding of two years. The aim of this study was to evaluate the mean duration of breastfeeding in Spain, the feeding pattern with infant formulae and the mean age on weaning introduction.

Methods: A total of 926 mothers with infants from 1 to 4 years took part in this survey. The questionnaire had a retrospective design and was divided in three major parts: Demographic variables, breastfeeding practices and age of introduction of different food during weaning period and semi-quantitative dietary data of their younger infant. The mean age of mothers in the study was 33,7 ± 4,5 years and the mean age of infants 2,4 ± 0,9 years. The study comprised 508 male and 419 female infants. ANOVA and correlation test were done using the computer program SPSS 11.0. A p-value of less than 0,05 was adopted as criteria of significance and 95% confidence intervals were used.

Results: The mean duration of exclusive breastfeeding and total breastfeeding were 2,5 ± 2,1 and 3,8 ± 4,0, respectively, Only in 6,3 % of infants exclusive breastfeeding was maintained until 6 months of life and 32,2% of infants began to take infant formulae from the first day of life. Infant formulae were introduced at 2,4 ± 2,2 months and follow-on formulae at 6,7 ± 2,8 months. Weaning was introduced at 4,4 ± 1,3 months of life. At 4 months, 61,7 % of infants have introduced complementary feeding. The first food to be introduced were cereals (42,6%), fruits (22,4%) or cereals and fruits at the same time (26,2%).

Exclusive and total breastfeeding were not related to any demographic variable. Exclusive and total breastfeeding, and infant formulae and follow-on formulae introduction were related with weaning introduction.

Conclusions: 1) WHO recommendations about exclusive breastfeeding are only followed in a low percentage of Spanish infants. 2) Weaning introduction should be delayed. 3) It would be useful to inform mothers about the importance of extending exclusive and total breastfeeding and delaying weaning introduction.
Key words: Infant feeding, complementary feeding, infant formulae, breastfeeding.

VISUAL EVOKED POTENTIALS IN INFANTS AFTER DIETARY SUPPLY OF DOCOSAHEXAENOIC ACID AND 5-METHYLTETRAHYDROFOLATE DURING PREGNANCY

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Introduction: Docosahexaenoic acid (DHA) plays an important role in the visual development of infants and is preferentially transferred across the placenta. Supplementation with folic acid in pregnancy might enhance placental DHA transfer.

Methods: Women recruited in Spain (S) and Germany (G) were randomized to 4 supplementation groups: 0,5 g DHA and 0,4 mg 5-methyl-tetrahydrofolate (5-MTHF)/d, 0,5 g DHA/d, 0,4 mg 5-MTHF/d or placebo from week 20 of gestation until birth. Infants not fully breastfed were supplied with formulae $\pm$DHA according to the nutritional code assigned. Cortical visual evoked potentials (VEPs) were obtained at the age of 8 weeks. 249 pregnant women (152 S, 97 G) were recruited and in 109 children (77 S, 32 G) cortical VEPs suitable for evaluation were obtained.

Results: No significant differences in latencies, amplitudes and calculated minimal angle of resolution were detected between the 4 supplementation groups (S and G both separately and combined). The analysis of combined groups with DHA and without DHA showed a significant difference in the minimal angle of resolution between the DHA vs. non-DHA groups (1.4'$\pm$7.7' vs. 3.0'$\pm$4.9', $p<0.05$).

Conclusions: The maternal supply of 500 mg DHA per day from week 20 of gestation significantly enhanced infant visual resolution of 8 weeks after births.

Key words: cortical visual evoked potentials, visual development, docosahexaenoic acid, 5-methyltetrahydrofolate
Disclosure: The project has been supported by the Commission of the European Union within the 5th framework program (research grant nutrition an healthy lifestyle (NUHEAL), grant no. CLK1-CT-1999-00888).

ELECTRONIC DATA CAPTURE AND USE OF INTERNET TECHNOLOGIES IN A DOUBLE-BLIND RANDOMISED INTERVENTION TRIAL

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Introduction: The EU Childhood Obesity Programme, (http://www.childhood-obesity.org) is a multi-centre double-blind randomised clinical trial which is performed in 5 European countries (Germany, Belgium, Italy, Poland and Spain). In order to test the hypothesis that a high protein intake in early life is related to early markers of childhood obesity, a total of 1000 formula fed and 250 breast fed infants are being closely followed up for 2 years. The formula fed infants are either randomised to a high protein formula group or a low protein formula group. Managing a multi-centre clinical trial amongst other things requires efficient data collection, review and integration.

Methods: In comparison with traditional Data Capture (DC) where data is transcribed from paper records into a database, Electronic Data Capture (EDC) enables data to be recorded instantly on-site, using pre-configured software instead of the traditional paper form. EDC, therefore, eliminates the need for data transcription. Edit checks and validation can be applied immediately, eliminating errors that routinely create queries several months later in paper-based processes. In the EU Childhood Obesity Programme, EDC is used for remote data entry (RDE) which means that information entered on-site is transferred via the internet to a central database thus unifying the data of five clinical study centres.

Results: EDC for RDE was successfully implemented into the data management of the EU Childhood Obesity Programme. Extensive know-how is a prerequisite in the set up of an efficient and user-friendly RDE system. Performance is a limiting resource. In the EU Childhood Obesity Programme it became obvious that the total response time must not exceed 2 seconds, otherwise it is not possible to enter data directly into the EDC system in front of the study subject. Two updates have been installed to improve the functionality and user-friendliness of the software since its first release in January 2003. Although the study is still ongoing, it can be foreseen that researchers will benefit from the main advantage of EDC: rapid data availability. The study parameters (small number of study centres, large quantity of recordable data and numerous visits with repeated or similar design) argue for an EDC system rather than an

exclusively paper-based study since its use may substantially reduce project costs.

Conclusion: Considering the pros and cons of electronic data capture for remote data entry, in our study the advantages clearly outweigh the disadvantages. This methodology can therefore be recommended for the use in multi-centre clinical trials.
Keywords: Electronic data capture, remote data entry, randomized clinical trial

BREASTFEEDING AND BABY FRIENDLY HOSPITAL INITIATIVE IN SLOVENIA

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Introduction: Slovenia has a population of 2 million. Birth rate has been sharply declining from 30,000 of newborn infants in 1979 to 17,490 in 2002. A large proportion of mothers have traditionally been breastfeeding. We aimed to achieve the first national coverage data for the in hospital breastfeeding rates over ten years period and to evaluate the influence of the Baby-Friendly Hospital Initiative (BFHI) on initiation rate of breastfeeding.

Methods: The perinatal data of mothers and infants, hospital of birth and breastfeeding were collected by the National Perinatal Information System in all maternity hospitals (n=14) in Slovenia. Data were transferred to the computer National database at the Institute of Public Health of Slovenia. The present study includes data for all infants born in Slovenia from January 1st 1993 to December 31st 2002 (n = 184,560 live born infants; >99% were delivered at the maternity hospitals). Breastfeeding practices early postpartum (days 3-6 after delivery) were classified as exclusive breastfeeding (only human milk), partial breastfeeding (human milk or a combination of human milk and formula) and not breastfeeding at all. Ever-breastfeeding rate (%) was calculated as: exclusive breastfeeding rate (%) + breastfeeding rate (%). The trends in breastfeeding practices during observed time period were analyzed in comparison to the dynamics of BFHI.

Results: Average a) exclusive breastfeeding, b) breastfeeding and c) no breastfeeding rates (min, year - max, year) were: a) 90.2% (87.0%, 1999 - 92.7%, 1994), b) 6.6% (3.7%, 1994 – 10.3%, 1999) and c) 3.2% (2.3%, 2001 – 3.8%, 1993). Relatively high ever-breastfeeding rate has been increasing from 95.9% in 1995 to 97.7% in 2001. The main increase occurred in the group of partially breast-fed babies (from 3.9% in 1993 to 8.9% in 2002). BFHI in Slovenia started in 1998. By the year 2001 10 of 14 maternity hospitals were awarded BFH status. In 2002 84% of infants in Slovenia were born in BFH. This is by far the highest rate among 11 new EU Member States (range in other countries: 2–30% infants born in BFH; WHO publication EUR/03/5045442).

Conclusions: The prevalence of initiation of breastfeeding in Slovenia has been high (90% exclusive breastfeeding, 7% partial breastfeeding) during the observed ten-year period. Ever-breastfeeding rate has been increasing during the past six years, from 96.0%

in 1995 to 97.7% in 2001. The majority of infants, 85%, are born in BFH. The achievement of BFHI is that a large number of women changed from not breastfeeding to at least partial breast-feeding.

Key words: breastfeeding, Baby-Friendly Hospital, Slovenia.

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NUTRITIONAL STATUS IN YOUNG ADULTS WITH SCREEN-DETECTED SILENT/SUB-CLINICAL COELIAC DISEASE

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Introduction: We wanted to elucidate whether screen-detected clinically silent coeliac (CD) patients have nutritional deficiencies, thus further indicating a need of active case finding of the disease.

Methods: A cohort of 3654 schoolchildren was screened for endomysial (EMA) and/or tissue transglutaminase (tTG) antibodies, and 26 subjects (aged 16-25) proved to have CD and 9 had suspected CD. Control group was formed of 29 sero-negative healthy subjects from the same cohort. Nutritional status was assessed by serum tests and anthropometric measures.

Results: CD patients had lower whole blood folic acid (median 91 vs. 109 nmol/l, P=0.01) and pre-albumin (median 0.21 vs. 0.28 g/l, P=<0.001), and higher transferrin receptor (median 1.3 vs. 1.1, P=0.008) and transferrin receptor ferritin -index (median 1.2 vs. 0.7, P=0.006) than the controls. Females with CD were shorter (162 vs. 167 cm, P=0.018) than controls, but no difference was found in males (178 vs. 177 cm, NS). Body mass index was also similar in the groups (females 22 vs. 22, males 25 vs. 24). In CD patients, no association between villous-crypt measures in bulbus or mid-duodenum and nutritional parameters were found, but titres of tTG associated negatively with whole blood folic acid (r=-0.5, P=0.016) and positively with TfR-F Index (r=0.4, P=0.05).

Conclusion: Abnormalities in nutritional status, especially in folic acid and iron status are common in adolescents with silent/subclinical CD, and call for early diagnosis and dietary treatment of the disease in order to prevent progress of nutritional deficiencies.

Key words: *coeliac disease, silent, sub-clinical, mucosa, atrophy, nutritional status.*

LIPOPROTEIN LIPASE (LPL) MRNA EXPRESSION IN PLACENTAS FROM NORMAL AND IUGR (INTRAUTERINE GROWTH RESTRICTED) PREGNANCIES BY REAL-TIME PCR.

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Introduction: Adequate placental transfer is necessary for normal intrauterine growth and development of the fetus. Intrauterine growth restriction (IUGR) is a condition associated with reduced nutritional placental supply. Recently, IUGR has been associated with changes in polyunsaturated fatty acid fetal-maternal relationships, mostly for the long chain polyunsaturated fatty acids, suggesting a role for these nutrients in the pathogenesis of IUGR. Lipoprotein lipase (LPL) plays a crucial role in lipoprotein and energy metabolism: it participates in the uptake of cholesterol-rich remnant particles, by acting as a ligand for VLDL receptor, LDL receptor, LDL-receptor related protein (LRP), and it is involved in the hydrolysis of triglycerides (TG) present in chylomicrons and very low-density lipoprotein (VLDL) to generate fatty acids, a source of energy for peripheral tissues. This suggests that the level of LPL expression in a given tissue is the rate limiting process for the uptake of triglyceride-derived fatty acids. LPL is expressed in human placenta, where it participates in the uptake of triglyceride-derived fatty acids.

Methods: We compared the expression levels of LPL, at the mRNA level, in placentas from 11 AGA (appropriate for gestational age) and 30 IUGR pregnancies at the time of Caesarean section. Relative Real-Time quantification of RNA extracted from placental villi was performed by the Ct method, using beta-actin as house-keeping, normaliser gene.

Results: No significant relationship was observed between LPL expression levels and gestational age in either AGA or IUGR pregnancies. Average values of LPL mRNA were higher in IUGR (104.44 ± 77.68 a.u.) than in AGA (21.14 ± 10.09 a.u.) placentas, although not significantly. Moreover, a greater variability was observed in IUGR, with a sub-group of cases (40%) showing LPL mRNA expression over 60 a.u., a value never exceeded in normal cases.

Conclusions: The increased levels of lipoprotein lipase expression observed in this sub-group of IUGR pregnancies could represent a compensatory mechanism to increase placental fatty acid availability.
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Keywords: Intrauterine growth restriction – lipoprotein lipase – fatty acids – placental lipid metabolism – real-time PCR.

MATERNAL FASTING EFFECT ON NEONATAL HEALTH

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Introduction: Muslims fast from sunrise to sunset during 9th lunar month (Ramadan). It is optional for pregnant women to fast, but there are many Muslim women who do choose to fast during Ramadan.
Methods and materials: This historical cohort study was conducted among healthy women without any significant illnesses or drug consumption during their pregnancies that gave birth at two hospitals in Tehran. The fasted group were those with history of at least 10 days of Ramadan fasting and non-fasted group were those who did not have any Ramadan fasting.
Results: A total number of 539 healthy pregnant women enrolled in this study. The fasted group numbered 284 and non-fasted group255. Fasted group had history of 10-60(mean: 24 days of fasting. None of the mothers in either group had a history of smoking during pregnancy.

Table1. Maternal weight, height and BMI of two groups of mothers

Variable	Maternal Fasting State	Mean	Standard Deviation	T-test(sig 2-tailed)
Wt(before pregnancy	Fasted	65.81	12.18	>0.001
	Non-fasted	31.56	11.16	
Wt(at time of delivery)	Fasted	77.81	12.24	.707
	Non-fasted	74.15	11.26	
Ht	Fasted	159.20	6.55	>0.001
	Non-fasted	159.41	5.15	
BMI	Fasted	25.93	4.39	>0.001
	Non-fasted	24.19	3.94	

Table2.Premature birth in two groups of mothers

Variable	Neonatal Maturity		Total
	Term(%)	Preterm(%)	
Fasted	276(97.2)	8(2.8)	284

Non-fasted	245(96.1)	10(3.9)	255
Total	521(96.7)	18(3.3)	539

Table 3.Neonatal birth weight and height of two groups of mothers

Variable	Maternal Fasting State	Mean	Standard Deviation	T-test(sig 2-tailed)
Birth wt(g)	Fasted	3265	444	.009
	Non-fasted	3165	440	
Birth Ht(cm)	Fasted	49	1.9	1
	Non-fasted	49	1.8	

Table 4.Regression coefficient of two groups of mothers

Variable		R coefficient(β)	SE(β)	T-value	p-value
Maternal Fasting State	Non-fasted	0.00	-	-	-
	Fasted	71.7	43.4	1.65	0.1
Maternal BMI	<19.8	0.00	-	-	-
	19.8-25.9	110.6	75.2	1.47	0.14
	26-28.9	164.3	83.9	1.96	0.05
	>29	203.9	88.7	2.3	0.02
Constant		2959.8	146.1	20.3	0.0001

Conclusion: Neonates of fasted group of mothers were 100 grams heavier than those of non-fasted group, which was significant (p=0.009), but maturity and birth height did not differ significantly (P values were respectively 0.4 and 1). To avoid effect of confounding variables such as maternal BMI on neonatal birth weight we used multiple linear regression model which showed neonates of fasted group were 71 grams heavier which was not significant.

THE QUALITY OF SCHOOL CHILDREN'S NUTRITION IN SERBIA

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Introduction: Adequate nutrition through a healthy diet are the most important determinants of children's health, development, growth and nutritional status. Inadequate nutrient intakes present potential risk factors for chronic non-communicable diseases and malnutrition.

Aim: To determine the nutritional quality of the diets of schoolchildren.

Methods: A representative sample of 966 children, 15 years of age, in eighth grade of elementary schools from ten centers in Serbia were chosen for examination, as part of the PASCS Study. Food consumption was registered through a 7-day food records questionnaire, and the software "NUTQ" was used for calculating energy and nutrient intake.

Results: Mean energy intake of the children was 2803.7 kcal, and proteins were represented with 15%, fats with 40% and carbohydrates with 45% of the energy. Saturated fatty acids contributed with 12% of the energy, monounsaturated with 11%, and 7% came from polyunsaturated fatty acids. The different food groups contribution of the daily energy were: milk and products 10%, meat and products 17%, fat and oils 10%, cereals 31%, sugar 10%, vegetables 3%, fruits 8%, and finally fish only with about 1%. Proportion of fruits and vegetables in the diet were below the recommendations (10-15% and 15-25% respectively). Daily intake of dietary fibers was about 16 g/day (5.6 g/1000 kcal) and dietary cholesterol 220 mg/day (79 g/1000kcal). The nutritional quality of the diet raises concerns since 64% of the children had a daily fat intake above 30% of energy, including 57% with an intake of saturated fat above 10% of energy intake and 63% with a P/S ratio <0.4, 56% had a sugar intake above 10%of energy, 19% with a cholesterol intake above 300 mg, and all the children had a salt intake above 6 g/day and insufficient dietary fiber, and a considerable number with low intakes of many minerals and vitamins.

Conclusions: The diet of schoolchildren in the assessed area was characterized by relatively high content of total fat, high levels of saturated fat and sugar, high in salt, but low in vegetables, fruits, dietary fibers and some minerals and vitamins. The low nutritional quality and high nutritional risk profile of the diet point to a threat to health of the future adult population, through the high risk of developing chronic diseases. Primary prevention programs and health promotion strategies are therefore urgently needed both among children and the adult population, with a parallel strategy for addressing high-risk population groups. It needs to improving the nutritional quality of school meals, and use that as a means to include nutrition into the curricula at primary as well as secondary schools.

Key words: nutrition; schoolchildren; nutrient risk factors

References

WHO Technical Report Series 916, Diet, nutrition and the prevention of chronic diseases, Report of the Joint WHO/FAO Expert Consultation, Geneva Switzerland,World Health Organisation, WHO, 2003.

Pavlovic M., Majkic S.N., Bolits Z., Bjeloglav D., Kadvan A., Nutrition as the Potential Nutritive Risk Factor of Atherosclerosis , Jugoslov. Med. Biohem., 2001, 20:107-115

Williams C.L., Hayman L.L., Daniels S.R., Robinson T.N., Steinberger J., AHA Scientific Statement, Cardiovascular health in Childhood, A Statement for Health Professionals From the Committee on Atherosclerosis, Hypertension, and Obesity in the young (AHOY) of the Council on Cardiovascular Disease in the Young, American Heart Association, Circulation, 2002; 106: 143-160

TENDENCY TOWARDS OBESITY IN SYDNEY SCHOOL CHILDREN

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Introduction: The aim of this study was to investigate children's tendency towards obesity as measured by anthropometric measures {Body Mass Index(BMI),Total Body Fat (TBF) and Waist Girth (Waist)}in relation to blood pressure(BP), dietary, exercise, familial. and socio-demographic background factors in a cohort of 1,230 Sydney primary school children first measured in 1994 and again in 1997.

Results: We found in this community sample the mean measures of overweight and obesity and the prevalence of childhood overweight and obesity as pre-defined categories significantly increased in three years. In addition, obesity was more prevalent in children from schools in low socio-economic status, this was explained by children of Non English Speaking Background(excluding Asians). Children of NESB were at two fold significant risk of being obese (BMI > 22).
Overweight or obese children had higher systolic and diastolic BP and pulse rate than non-obese children: Obese (BMI > 22) children were at four fold risk of having a systolic BP > 117 which increased to a six fold risk three years later (BMI > 25). This was also reflected in obesity measures of high waist and TBF.
Obesity was also related to activity levels more strongly than to dietary factors: Obese children (by BMI, TBF or waist measures) were three times as likely to not exercise compared to their non obese peers.
Contrary to findings from other studies neither low birth weight or high BP of parents were related to childhood obesity, however BMI of either the mother or the father of the child was the strongest predictor of childhood obesity . Children who had an obese mother (BMI>30) had a two fold risk of obesity at 8 years old which increased to four fold at 11. If the father was obese (BMI > 30) the two fold risk at age 8 increased to five fold at age 11.

Conclusion: Health Promotion efforts should be made to increase children's exercise especially those of either non-English speaking background (excluding

Asians) or who have an obese parent. Blood Pressure should be monitored in overweight children as they may be at risk of heart disease and stroke in their adult life.

MONITORING AND SUPERVISING A DIETARY INTERVENTION TRIAL USING MODERN DATA PROCESSING SYSTEM

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Introduction: The recruitment for TRIGR started May 2002 and will continue until spring, 2006. Present status of TRIGR: 71 participating centers from 15 countries on 3 continents, by June 29, 2004 2398 families have been registered and 2028 infants have been randomized, 868 infants continue in the intervention after HLA screening from cord blood.
Methods: The Data Management Unit (DMU) established an electronic web-based data capture system for TRIGR. Study data is collected world wide using on-line web-based forms. The Oracle (internet) database is open continuously and the data can be entered round the clock. The data entry forms are linked to on-line logical controls and checks to reduce input errors. The TRIGR staff members have their own User Id and Password and the system is monitoring and keeping track of data entry and editing constantly. The laboratory data are captured to the DMU database using the FTP technique (File Transfer Protocol) via Internet. The tracking system for interviews, visits and blood samples is also available. Study Centers are asked to use it to report whether the scheduled contacts with the participating families have occurred. The tracking system has been designed to facilitate the monitoring of the Study Centers' compliance efforts. The Sametime videoconferencing program offers TRIGR Study members an inexpensive and effective alternative to e-mail, telephone calls and in person meetings on real time basis. To be able to design such a system, to get it ready on time, you need to have experienced staff with special knowledge and previous experience of similar trials. The management of an international multicentre intervention trial would be extremely complicated and challenging without modern technique.
Conclusions: The current system has proven its efficiency in monitoring, recruitment and compliance in study centres within TRIGR.

ANALYSIS OF DROP-OUTS IN A LONGITUDINAL STUDY

The Spanish Sample of the" EU Childhood Obesity Programme:Early Programming by Infant Nutrition (QLK1-2001-00389") (EPOC).

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Objective: to describe the drop-outs which occurred during the first year of recruitment by the Spanish group of the EPOC, analyzing the timepoints and factors that contributed to them.

Material & methods: This is a prospective randomized multicentre study, that compares different feeding patterns (2 isocaloric infant formulae with different protein amounts and a breastfed control group). We will analyze formula-fed babies (FF) > 9 months, and breastfed babies (BF) > 6 months.

Results: We have 314 FF, 80 of them dropped out (25.5%), and 127 BF, 59 of them dropped out (46,5%). In both groups, the largest number of drop-outs occurred during the first month of life: 34 FF (42.5%), and 37 BF (62.7%). In the following months, the number of drop-outs was much lower. Most of the FF drop-outs were due to "parents' no intention to continue" (33 babies, 41.3%). The second most frequent reason was the abandonment of the study formula due to digestive disorders (23 cases, 28.8%). There are no significant differences between the two types of study formula in the distribution of drop-outs. In the BF group, there were 29 drop-outs due to breastfeeding cessation, and 29 BF dropped out due to "parents' no intention to continue" (49% in both cases).

Conclusions: According to our results, it would be very useful to intensify the adherence strategies during the first two months to minimize the drop-outs. The large number of drop-outs due to breastfeeding cessation along the first month of life, suggests that it would have been a better strategy to recruit for the project when the baby was one month old.

Key words: infant nutrition, obesity, longitudinal study, randomized controlled trial, drop-outs

RECRUITMENT STRATEGIES OF THE SPANISH GROUP IN THE "EU CHILDHOOD OBESITY: PROGRAMMING BY INFANT NUTRITION"

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Aim: To describe the strategy used on the recruitment of 320 Spanish formula fed (FF) babies enrolled in the "EU CHILDHOOD OBESITY: PROGRAMMING BY INFANT NUTRITION"(EPOC) (QLK1-2001-00389) and the factors that made the recruitment successful.
Methods: Multicentre, prospective and randomized study, that compares different feeding patterns (2 infant formulae with different protein load and a breastfed (BF) control group) during the first year of life. Two main strategies were defined to optimise the recruitment:
1.Contact with the maximum number of new-borns in hospital: two full-time dieticians recruited 7 days a week checking all new-borns daily. Families, whose babies met the inclusion criteria, were invited to participate in the project during the 48h after delivery. BF babies who changed into FF within the first weeks of life were also phoned and included in the FF group.
2:To reach the highest percentage of acceptance: meetings with the hospital staff and an informative campaign addressed to the target population were organized before starting the recruitment.
Results: During one year, 3192 babies were born in the study hospitals, 2502 babies were checked (78,4%), 1720 of them met the inclusion criteria (68,7%), and 490 of these were FF since birth. From these ones, we offered the project to 422 families (86,1%), and 252 accepted (59,7%). Additionally, were recruited 73 babies that had abandoned breastfeeding. This comes to a total of 325 FF babies.
Conclusions: The success of our recruitment was due to the high number of families contacted. The successful percentage of acceptance was achieved due to the wide dissemination of the project. Another important factor was the personal effort expended by the recruitment team.
Keywords: infant nutrition, obesity, longitudinal study, randomized controlled trial, recruitment strategies.

DIET AND NUTRITIONAL RISK FACTORS IN SCHOOLCHILDREN

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Introduction: Inadequate diet and nutrient intakes present potential risk factors for malnutrition and chronic non-communicable diseases .
Aim: The objective of this study has been to determine the quality of family nutrition and nutritional status of schoolchildren.
Methods: A representative sample of 167 girls and 197 boys aged 15 from eight grade of elementary school were seen during systematic examination. Evaluation of nutritional status was done on the basis of BMI(kg/m2) NHANES I and biochemical parameters by software "CHILD". A 7 day food records of food consumption by questionnaire were used by the aid of software "NUTQ" for evaluation of energy and nutrient intake.
Results: Normal nutritional status (BMI P15-85) was noted at 65.5% of boys and 69.5% of girls, underweight (BMI<P5) at 4.0-7.0% of children, moderate underweight (BMI P5-15) at 7.8-17.0%, while overweight (BMI P85-95) was noted at 8.3-12.6% and obesity (BMI>P95) at 3.6-5.2% of boys and girls. Mean energy intake in family nutrition was 2528 kcal where the proteins were represented with 15%, fats with 40% and carbohydrates with 45%. Analysing the percentage supply of different food groups in daily energy, milk and products contributed with 11.3%, meat and products with 18%, fat and oils with 9%, cereals and grains with 32%, sugar and sweet 9%, vegetables 4%, fruit 7% and fish only with 1%.Nutritive risk factors of family nutrition exist in 65% of families in the form of increased intake of fats over 30% of energy value(EV), than sugar over 10% EV in 60%,saturated fatty acids over 10%EV in 57%, ratio P/Z<0.4 in 66%, cholesterol intake over 300mg/day in 15%,while in all families there were determined consumption of salt over 6g/day and insufficiency of dietary fibre, with insufficient intake of most vitamins and minerals. Increased values of total cholesterol can be seen in 6-8%, increased values of LDL-cholesterol in 4-7%, and hypertriglyceridemia in 16% of boys and girls. Majority risk factors are in connection with eating and lifestyle patterns already adopted in childhood and youth with tracking phenomenon which is the most important fact for primary prevention.

Conclusions: Registered inadequate nutritional status and nutritive risk factors points out the necessity of preventive measure like continually monitoring, early detection and treatment of high risk children with population nutrition and health promotion strategy.
Keywords: diet; schoolchildren; cardiovascular risk factors

References

1.Pavlović M., Kadvan A., Vukotić M, Nutritive risk factors in schoolchildrens diet, 17 th International Conference of Nutrition, Vienna, Austria, Avgust 27.-31. 2001., Ann Nutr Metab 2001; 45 (suppl 1): 564

2.Pavlovic M.,Vukotic M., Majkic N.S.,Simeunovic S.,Bolits Z., Nutritional status and lipid parameters in children from Yugoslav study of atherosclerosis precursors in schoolchildren (PASCS), Abstracts of the 12th Workshop European childhood obesity group, Insulin resistance in obese children, Prague, Czech Republic, May 23-25,2002:22

INFLUENCE OF TWO FORMS OF CASEINOPHOSPHOPEPTIDE ON IRON BIOAVAILABILITY

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Introduction: Iron deficiency is the most common nutritional problem around the world, both in developed and in developing countries. The main cause of this deficiency is its low bioavailability (5-10 %). Previous studies showed that phosphopeptides derived from casein hydrolysis of whole casein improves Fe absorption [1], but phosphopeptides (CPP) released by enzyme digestion of different caseins give conflicting results [2, 3]; that could impose restrictions to their widespread application in functional foods for man. CPP can be found in several different forms of structure but only two of them are important (βCPP(1-25), αs_1CPP (59-79)).The objective of this study was to evaluate the absorption of iron complexed to those two main forms of purified CPP (Fe-βCPP(1-25), Fe-αs_1CPP (59-79)) using the human intestinal Caco-2 cells model.

Methods: CPPs were first purified by Fast Protein Liquid chromatography on an anion exchange column, characterized by electrospray mass spectrometry (ESI-MS) coupled with HPLC (625 LC system) and complexed with Fe (Fe-βCPP(1-25), Fe-αs_1CPP (59-79)). Three iron transport solutions were prepared by mixing Fe complexes (Fe-βCPP(1-25), Fe-αs_1CPP (59-79)) and ferrous sulphate (control) with special Dulbecco's Modified Eagle Medium without iron (100 µmoles/l Fe). Bioavailability was assessed by applying the samples to human intestinal Caco-2 cells grown on filters for 21 days (n = 6) and incubating at 37 °C for 2 hours. Iron absorption was measured by Atomic absorption spectrometer (Perkin Elmer, 1100B).

Results: Uptake and net absorption of Fe-αs_1CPP (59-79)) complex were significantly lower ($p<0.0001$) than ferrous sulphate and Fe-βCPP(1-25) Net absorption of Fe-βCPP(1-25) complex was higher than ferrous sulphate (Table).

Table: Influence of two main forms of caseinophosphopeptides on iron bioavailability

	Total uptake (%Fe)	Net absorption (%Fe)
Ferrous sulphate (control)	7.78 ± 1.14	2.71 ± 0.07
Fe-βCPP(1-25)	6.69 ± 0.25	3.505 ± 0.25[a]
Fe-αs_1CPP (59-79)	1.87 ± 0.28**[a]	1.53 ± 0.22**[a]

Mean ± SD; ANOVA, p<0,01; **: different from Fe-βCPP(1-25) (p < 0,0001); [a]: different from ferrous sulphate group (p < 0,0001)

Conclusion: This study give for the first time experimental evidence to explain the conflicting results on the conflicting effects of caseins and their peptides on iron absorption [2,3] about cow milk proteins: βCPP(1-25) and αs_1CPP (59-79)) have opposite influences on iron bioavailability; αs_1CPP (59-79) inhibits iron absorption as opposed to βCPP(1-25) that creates its absorption improvement. The enhancing effect of β casein, which the main casein of breast milk could explain part of its enhancing effect on iron absorption.

References:[1] Hurell and al., (1989). Iron absorption in humans as influenced by bovine milk proteins; Am. J. Clin. Nutr., 49: 546-552 ;[2] Bouhallab et al., (1999). Sensitivity of b-caseinophosphopeptide-iron complex to digestive enzymes in ligated segment of rat duodenum ; J. Nutr. Biochem., 10: 723-727; [3] Yeung and al., (2002). Effects of iron source on iron availability from casein and casein phophopeptides; J. Food. Sci., 12: 292-299

Keywords: Iron uptake; Caseinophosphopeptides; Caco-2 cells

MODEL OF CHILDHOOD OBESITY PRIMARY PREVENTION PROGRAMME

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Introduction: The high frequency of obesity and overweight in our country highlights the necessity for immediate preventive measures.
Aim: The aim of this work has been to present the model of childhood obesity primary prevention programme in North Backa Region. Primary prevention program "My Heart, Healthy Heart" with parallel conducting the strategy of high risk and population nutrition and health promotion strategy by the aid of several pieces of software.
Methods: Monitoring of body weight and height recorded during a mass screening of 29.237 boys and 27.753 girls aged 1-19 years in health centres in the period 1995 - 2003. Evidence and statistical evaluation of data have been processed by software "CHILD" determining the nutritional status according to reference values of the BMI NHANES I and body weight in regard to age to NCHS/WHO. The individual level involves continuous longitudinal growth and nutritional status monitoring, identification of children with nutritive risk factors or with positive family history for selective screening of biochemical parameters and healthy lifestyle and nutrition counselling
Results: Analyzing the nutritional status of children age 6-18 overweight (BMI P85-95) was registered in 9.3%-11.1% girls and boys, and obesity (BMI>P95) in 5.9-6.2% of them. Body weight by age >P95 was established in 13.9-14.4% of children age 1-6. The population level starting from second year of life, by harmonizing life style and nutritional habits with recommendations. The main characteristics of the population strategy are: changing nutrition and lifestyle in whole population over 2 year, following the quality of family nutrition with recommendations for changing nutrition, following the quality of social nutrition with planning and corrections of menus, education about healthy nutrition within cooks, pedagogues, children, parents and by all means the whole society with mass-media help, as well as organising the celebration of World Food Day in October ,"Festival about healthy nutrition and physical activity" for preschool and schoolchildren in order to promote healthy nutrition and lifestyle.
Conclusions: Permanent nutrition promotion with monitoring of the nutritional status in children has been established in order to early discovery obesity and nutritive risk factors on individual an/or population level to childhood obesity prevention and realize optimal nutritional status of children and adolescents.
Key words: obesity; childhood; primary prevention

Mirjana Pavlovic, Agnes Kadvan, "Strategy and Model of prevention from Atherosclerosis", Acta Biol.Med.Exp.(2002), 27(2):37-39
Mirjana Pavlovic, Primordial Prevention: Primary Prevention of Risk factors is ishemic heart disease in childhood, in: Ostojic M. et al. (eds.) Prevention of ishemic heart disease, national committee for preparing guidelines for clinical practice in Serbia, 2002, National Guidelines for clinical practice, Belgrade; p 98-114.

PROBLEMS RELATED TO RECRUITMENT OF PARTICIPANTS FOR THE TRIGR PROJECT

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Introduction: The main objective of the TRIGR project (Trial to Reduce IDDM in the Genetically at Risk) is to answer the following question: can the risk of developing type 1 diabetes mellitus (T1D) in children with increased genetic predisposition to this disease be decreased by weaning to a highly hydrolysed formula? The potential candidates for TRIGR are: pregnant women affected by T1D, men with T1D who are to become fathers or families with an affected child who are going to have another child. Therefore process of identifying and recruiting participants for TRIGR is challenging. It includes three subsequent steps: recruitment, registration and randomisation. This complex procedure requires excellent cooperation between at least three different specialities: obstetrics, adult endocrinology and paediatric endocrinology. In our centre we work together in such a team.
Results: We started recruiting families in May 2003. Our aim was to recruit 35 each year. So far we have recruited 41 families. 11 families (26%) have refused to take part in the study. 30 participants have been registered and randomised. 22 of them (73%) were not eligible according to their genotype, and do not continue in the study; 8 (27%) had positive genetic results and are actively taking part in the study. Among those: 1 has all three family members affected, 1 has two diabetic parents, 1 has an affected sibling and 5 an affected mother. The longest follow-up period so far is 12 months.
Conclusions: Our results indicate that: good cooperation between doctors is crucial for successful recruitment; the majority of families invited to the TRIGR have agreed to participate; recruitment rate among affected by T1D mothers and children is good; we still lack a good strategy to attract men with T1D; in our centre only 27% of infants had the genetic predisposition to T1D, while the expected frequency was about 45%; in both families were more than one member was affected by T1D, genetic predisposition was confirmed.
Key words: TRIGR, diabetes mellitus type 1, prevention, genetic predisposition.

VITAMIN D STATUS AT BIRTH IN BRUSSELS- PRELIMINARY RESULTS

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Introduction : The 25(OH) vitamin D (25OHD) concentration measured in cord blood reflects the vitamin D status at birth and is correlated to the bone mineral content. The aims of the study were to evaluate the rate of neonatal vitamin D deficiency and to identify its risks factors in our city.

Methods : All infants born in our institution from April 2003 to March 2004 were prospectively included in the study. Cord serum 25OHD was measured using a competitive chemoluminescent immunoassay method (LIAISON-DIASORIN). We calculated the prevalence of moderate and severe vitamin D deficiency, defined as a cord serum 25OHD concentration ranging from 5 to 9.9 ng/mL and less than 5 ng/mL, respectively. Using contingency tables, we attempt to relate the rate of neonatal vitamin D deficiency to the following variables: maternal variables (ethnic origin, age, parity, interbirth interval), pregnancy variables (number of foetus, evolution, term, clothing and sun exposure habits, alimentary vitamin D intake, vitamin supplementation) and neonatal variables (gender, weight, height, birth weight percentile).

Results : Out of the 753 infants born during the study period, 716 were enrolled in the study. Serum cord 25OHD was less than 5 ng/mL in 119 (16.6%) and ranged from 5 to 9.9 ng/mL in another 207 (28.9%). The rate of vitamin D deficiency was significantly higher in infants born in winter and spring than in those born in summer and autumn ($p<0.05$) and in infants from mother originating from Maghreb and Near-East than in those originating from Europe and Africa ($p<0.05$). Vitamin D deficiency was also associated to covered clothing habits ($p<0.05$), to absence of any sunbathing ($p<0.05$) and to low (< 400 UI/day) vitamin D supplementation ($p<0.001$) of the mother during the pregnancy.

The prevalence of neonatal vitamin D deficiency was not related to maternal age, parity, number of foetus, interbirth interval, any pregnancy complication, term, birthweight and alimentary vitamin D intake during pregnancy.

Conclusion : Neonatal vitamin D deficiency is common in our institution. Skin exposure to sun (clothing habits, amount of sunbathing and season of birth) and low vitamin D supplementation seems to be its major determinants.
Key words : Vitamin D deficiency, newborn.

OBESITY AMONG YOUNG ADOLESCENT KUWAITIS

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Introduction: Kuwaitis suffer from one of the highest prevalences of obesity in the world, as has been previously documented. The purpose of this study was to assess the prevalence of overweight and obesity among young Kuwaiti adolescents aged 10-14 years.

Methods: Weights and heights of 14,659 (7205 males and 7454 females) adolescents, representing 17% of the target population, were measured, from which the body mass index (BMI) was calculated. Overweight and obesity were defined as BMI >85th and >95th centiles, respectively, of the NCHS reference data.

Results: The overall prevalence of overweight and obesity among males were 30.0 and 14.7%, respectively ($p<0.001$). The overall prevalence of overweight and obesity among females were 31.8 and 13.1%, respectively ($p<0.001$ and <0.01). The overall prevalence of overweight was lower in males than females but obesity was higher in males than females.

Conclusion: When compared to the NCHS reference population, the BMI of Kuwaiti adolescents exceeded that of the Americans in each centile category $\geq$50th centile. Health education programmes should be instituted to control this syndrome in order to prevent future risk of obesity-related diseases.

Key words: adolescents, Kuwaiti, overweight, obesity, comparison, Americans

DYNAMIC CHANGES IN ADIPOSITY FROM FETAL TO POST-NATAL LIFE ARE INVOLVED IN THE ADULT METABOLIC SYNDROME ASSOCIATED WITH REDUCED FETAL GROWTH

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Introduction: The association between a low birth weight and the metabolic syndrome (dyslipidemia, hypertension and cardio-vascular diseases, insulin-resistance and type-2 diabetes) or with one of its components has been substantially documented in various populations. The metabolic syndrome was originally described in man to cluster with insulin-resistance. It has been postulated that insulin-resistance also plays a central and primary role in the metabolic complications associated with reduced fetal growth, but this has never been clearly demonstrated and the natural history of the metabolic syndrome in subjects born with a low birth weight remains poorly documented. Therefore, the question arises as to when and how the metabolic syndrome emerges in this particular clinical situation. Our previous data have shown that insulin-resistance is detectable as early as 20y. However, one third of young adults born small for gestational age (SGA) seem to be affected. In these individuals insulin-resistance could not be explained by family history of metabolic diseases or obesity. Therefore the reason for this variable predisposition for insulin-resistance might be sought either in specific aspects of SGA, or among post-natal events directly linked to SGA.
Objectives: The aim of the present study was therefore to investigate the origins and mechanisms of this association. The study population is based on a regional cohort of young adults, precisely selected on birth data from a population-based registry located in the city of Haguenau (eastern France). This cohort includes 735 subjects born small for gestational age (SGA: birth weight < 10th percentile of the local distribution corrected for gestational age and gender) and 886 subjects born appropriate for gestational age (25th-75th percentile) in whom clinical and metabolic parameters of the MS were measured at a mean age of 22 years. Analyses of the metabolic syndrome components were performed after

adjustment for BMI, age, gender, smoking, family history of diabetes and oral contraception at the time of the study.

Results: Mean values of all components of the metabolic syndrome significantly differed between the two groups. In SGA subjects, the upper tertile of fasting insulinemia (reflecting the more insulin-resistant subjects) was associated with the highest values of systolic (p=0.001) and diastolic (p=0.02) blood pressure, triglyceridemia (p=0.005) and glycemia at fasting (p=0.0001) and during OGTT (p=0.0001), emphasizing therefore the key role of insulin-resistance in the development of the metabolic syndrome associated with reduced fetal growth.

However, whether the risk for IR is already determined at birth or influenced by the post-natal growth is not clarified. To address this question, we studied in the 735 subjects born SGA the effect of characteristics at birth and catch-up growth on the insulin-to-glucose ratio (I/G) taken as a surrogate marker for insulin-resistance. Neither gestational age (32-42 wks, p=0.62) nor birthweight (1130-3080 g, p=0.26) had a significant effect on the I/G, but BMI at birth was inversely related to the adult I/G. Catch-up height had no effect on the adult I/G (p=0.35). In contrast, catch-up in BMI was significantly associated with an increased I/G even when adjusted for adult BMI (p=0.004). As expected, catch-up in BMI was inversely related to BMI at birth, but subjects who experienced the larger BMI catch-up were not obese as young adults.

Conclusion: We have shown that all components of the insulin-resistant syndrome are clustered with insulin-resistance in subjects born SGA as early as 22 years. Additionally, our results demonstrate that thinness at birth, but not birth weight, influences the pots-natal growth and risk for the metabolic syndrome associated with SGA, suggesting therefore that fetal dynamic changes in adiposity are involved in the long-term metabolic consequences of reduced fetal growth. Thus a better knowledge of fetal growth patterns leading to SGA seems critical in order to unravel the mechanisms responsible for the post-natal outcome.

EXCESS FETAL ADIPOSITY IS ASSOCIATED WITH PROGRAMMING OF PLACENTAL LIPID GENES

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Introduction. Increased adiposity in fetuses of women with abnormal glucose tolerance has been associated with an increased risk to develop obesity and type-2 diabetes in later life. Maternal anthropometric and metabolic parameters can explain only part of the fetal fat mass variance, suggesting that other factors may be involved. As the primary interface between maternal and fetal circulation, the placenta is a major determinant of nutrient flux from mother to fetus. However the role of placental genes in controlling nutrient availability has not been widely studied. Knowledge is even more limited in understanding diabetes-induced modifications of placental functions. To gain insight into the molecular mechanisms that alter placental metabolic pathways, we have constructed the gene expression profile of placenta from women with normal glucose tolerance and with gestational diabetes (GDM).

Methods. Global gene screening was performed by oligonucleotide microarray analysis (Human U133 Affymetrix) of placenta obtained at term from lean controls, obese controls and women with gestational diabetes (GDM). Anthropometric data were obtained in the neonates within 48 hours from the delicery; neonatal body composition was estimated by total body electrical conductivity (TOBEC) and macrosomia was defined as % body fat > 14.

Results. A four-step selection criteria was applied to identify significantly modified genes in relation to fetal macrosomia. 136 genes encoding proteins for glucose and lipid metabolism were expressed in normal placenta. 38.2 % of these genes were significantly regulated in placenta of macrosomic neonates from GDM patients and only 5.1 % were modified in placenta of macrosomic neonates from obese women. In GDM, about half of the regulated genes were related to lipid metabolism, primarily encoding rate-limiting enzymes for fatty acid transport, triglycerides and cholesterol synthesis. Genes for leptin and leptin receptor were also up-regulated while lipoprotein lipase and NPY receptor were the only genes down-regulated in the lipid cluster. Despite the diabetic milieu, no genes were modified in relation to glucose transport but, the expression of glycogenin 2, a self initiator of glycogen synthesis was increased.

Conclusion. Using microarray technology we have identified major modifications of gene profiles in placentas of macrosomic fetuses of GDM mothers. These genes do not control glucose but primarily lipid metabolism pointing to a placental programming of lipid genes. We propose that the increase in placental lipid biosynthetic pathways have functional significance for fetal fat accretion as a step towards enhanced placental transfer of FFA.

Key words: fetal body composition; gestational diabetes; lipid metabolism, gene chips.

APPETITE CONTROL IN BREASTFED AND FORMULA FED INFANTS

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Introduction: Dysregulation of appetite control is suggested as a potential mechanism responsible for the higher risk of obesity in formula fed infants. Leptin seems to play a major role in appetite control and thus it may influence growth and weight gain. Adiponectin has some protective activity against weight gain and hyperglycemia.

Aim: To compare hormonal indicators of appetite control in breastfed and formula fed infants.

Methods: Preliminary laboratory findings from the EU Childhood Obesity Programme were analyzed to compare indicators of hormonal appetite control between the infants that were breastfed (N=87) and all pooled together infants that received formula feeding (N=158) at the age of 6 months. Infants from 4 countries participated in the study: Germany, Spain, Poland and Belgium. Breastfeeding lasted at least 3 months before blood sampling. We determined serum concentrations of leptin and leptin receptor as well as adiponectin.

Results: Leptin concentration increased in formula fed infants when compared to breastfed infants (10.3±6.7 ng/ml vs. 7,4±4,4 ng/ml; P<0,001) but leptin receptor concentration decreased (41,3±10,3 ng/ml vs. 47,1±11,8 ng/ml; P<0,001). Concentrations of adiponectin did not differ between groups (21,0±6,3 ng/ml in formula-fed vs. 21,6±5,7 ng/ml in breastfed infants).

Conclusions: Appetite control in infants seems to be influenced by formula feeding in comparison with breastfeeding as expressed by higher concentrations of leptin. To determine whether increased leptin concentration in formula fed

infants is only a secondary finding to higher body mass or direct consequence of feeding type needs further investigation.

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Key words: appetite; leptin; leptin receptor; adiponectin; childhood obesity

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4. DANONE INSTITUTE OVERALL ACTIVITIES

- Support to research:, Danone Institutes have, to date, funded more than 600 research studies, which accounted for more than 10 million EUR.
- Prizes and awards: more than 40 prizes and awards have been attributed to outstanding professional initiatives for 500 000 EUR.
- Symposia, workshops and educational meetings: Since 1991, 137 events involving top-level scientists have reached more than 24.000 health professionals.

• Publications related to health & nutrition: 72 publications and 6 newsletters present professionals with overviews of recent developments, promote consensus and/or explore controversy of relevant issues.
• Education programs for the general public. More than 70 programs towards the general public such as nutrition lectures, distribution of folders and brochures, etc. have been organised.

For more information, visit www.danoneinstitute.org.