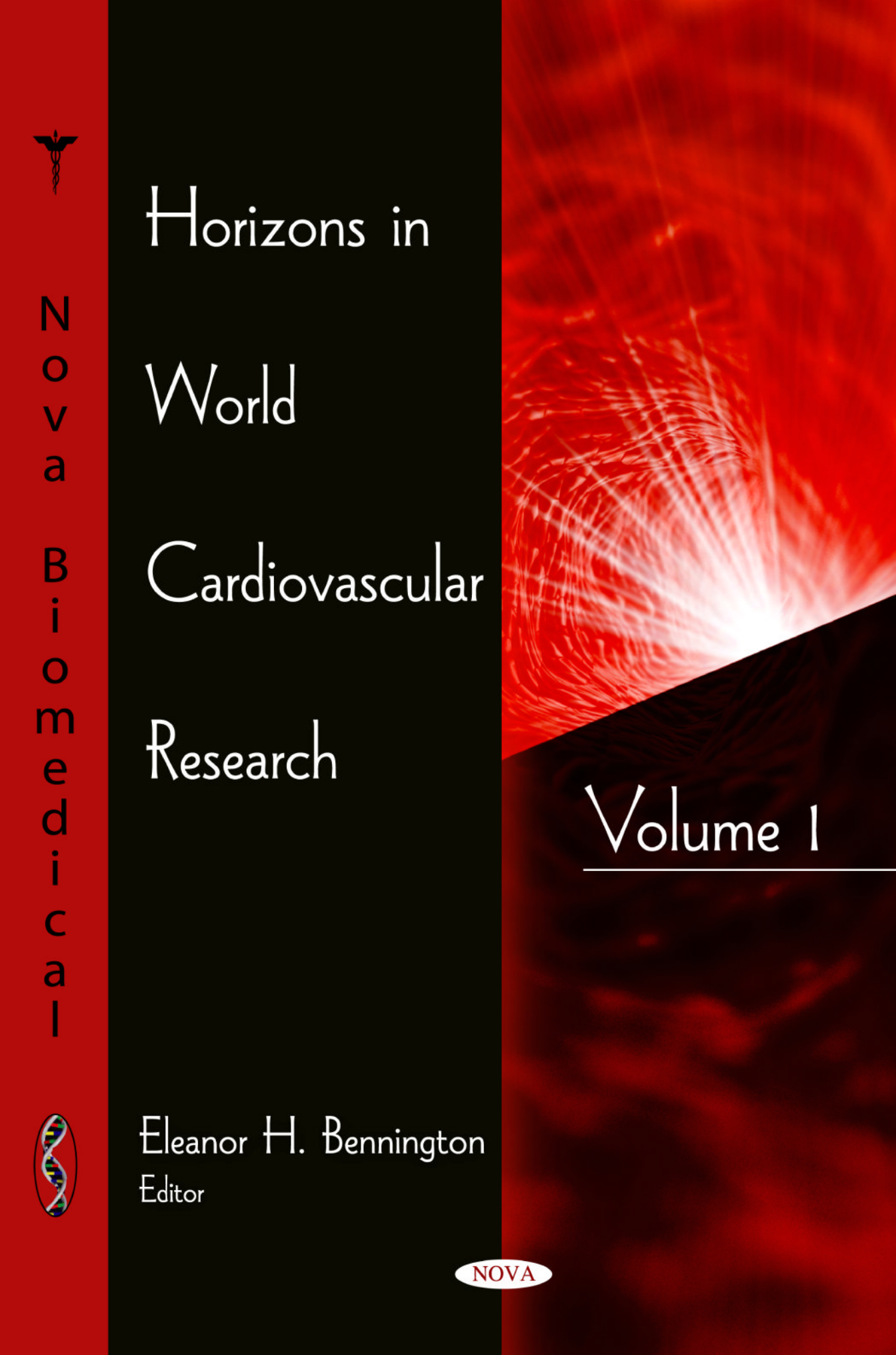

Nova Biomedical
Horizons in World Cardiovascular Research
Volume 1
Eleanor H. Bennington
Editor
NOVA

HORIZONS IN WORLD CARDIOVASCULAR RESEARCH SERIES

HORIZONS IN WORLD CARDIOVASCULAR RESEARCH. VOLUME 1.

HORIZONS IN WORLD CARDIOVASCULAR RESEARCH SERIES

Horizons in World Cardiovascular Research. Volume 1
Eleanor H. Bennington (Editor)
2010. ISBN: 978-1-60876-447-1

HORIZONS IN WORLD CARDIOVASCULAR RESEARCH SERIES

HORIZONS IN WORLD CARDIOVASCULAR RESEARCH. VOLUME 1.

ELEANOR H. BENNINGTON
EDITOR

Nova Biomedical Books
New York

Library of Congress Cataloging-in-Publication Data

Horizons in world cardiovascular research / editor, Eleanor H. Bennington.
p. ; cm.
Includes bibliographical references.
ISBN: 978-1-61470-052-4 (eBook)
1. Cardiovascular system--Diseases. I. Bennington, Eleanor H.
[DNLM: 1. Cardiovascular Diseases. WG 120 H8113 2009]

RC667.H67 2009
616.1--dc22

2009032000

Published by Nova Science Publishers, Inc. † New York

Contents

Preface

Cardiovascular disease refers to the class of diseases that involve the heart or blood vessels (arteries and veins). While the term technically refers to any disease that affects the cardiovascular system, it is usually used to refer to those related to atherosclerosis (arterial disease). Chronic heart failure (CHF) has emerged as a major worldwide epidemic. Recently, a fundamental shift in the underlying etiology of CHF is becoming evident, in which the most common cause is no longer hypertension or valvular disease, but rather long-term survival after acute myocardial infarction. Chronic artery disease (CAD) is the cause of CHF in the majority of patients, and CHF is the only mode of CAD presentation associated with increasing incidence of mortality. This new and important book gathers the latest research in cardiovascular disease with a focus on such topics as: diabetic cardiomyopathy, the promise of biological pacemakers, stem cells and repairs of the heart, cardiac autonomic function and sports activity, "tako-tsubo cardiomyopathy" and others.

Chapter I - There is increasing evidence that diabetes causes both anatomical and functional pathological changes in the myocardium. Diabetic cardiomyopathy (DC) is a myocardial disease caused by diabetes mellitus, which is unrelated to vascular pathology or systemic arterial hypertension and can occur in asymptomatic patients with diabetes alone. The coexistence of hypertension, diabetic cardiomyopathy, and myocardial ischemia increases with aging, especially among obese subjects. However relatively independent, each of these diseases seem to interact with the other in order to contribute to the biochemical, anatomical, and functional alterations in myocardial cells. The most important mechanisms involved in DC are (1) insulin resistance with consequent hyperinsulinemia and endothelial proliferation; (2) small-vessel disease (microangiopathy, impaired coronary flow reserve, and endothelial dysfunction); (3) metabolic disturbances like increased free fatty acid levels, carnitine deficiency, and changes in calcium homeostasis; (4) myocardial fibrosis (increases in angiotensin II activity, IGF-I, and inflammatory cytokines levels), and cardiac autonomic neuropathy (denervation and alterations in myocardial catecholamine levels). Abnormalities in both systolic and diastolic cardiac function have been demonstrated in diabetic subjects. Several lines of evidence indicate that left ventricular diastolic dysfunction represents the earliest preclinical manifestation of diabetic cardiomyopathy, preceding systolic dysfunction. Documentation of diastolic dysfunction should result in the initiation of therapy in order to prevent progression to Heart Failure (HF). Treatment of DC-HF is essentially not different

from treating HF caused by myocardiopathies of other etiologies, and it must follow the guidelines according to ventricular function. However, some particularities related to its diabetic etiology should be considered. Degree of glycemic control correlates well with the severity of microvasculature damage, which can be delayed or even prevented by keeping near normal serum glucose levels. Achievement of ideal glycemic control levels, preferably by reducing insulin resistance, is the essential step not only in treating diabetes itself, but also in preventing and managing DC. Besides dietetic therapy, increasing levels of endurance exercise should be encouraged in order to improve peripheral insulin resistance. There is large evidence that the use of ACE inhibitors, which might reverse left ventricular hypertrophy and myocardial fibrosis, is also able to prevent myocardial remodeling, improve endothelial function, and even contribute to lower insulin resistance. It is also known that ß-blockers and thiazolidinediones shift myocardium metabolism from the use of free fatty acids (FFAs) to that of glucose, which would be beneficial in DC. In addition, the thiazolidinediones have also been shown to decrease myocardial FFA levels in animals, besides improving ventricular function. In the near future, agents that decrease lipotoxicity or prevent/reverse glycosylation and cross-linking of collagen are promising.

Chapter II - Cardiomyopathy is a generic term for any heart disease in which the heart muscle is involved and functions abnormally. Recent developments and ongoing research in cardiology have led to descriptions of previously less recognized and/or incompletely characterized cardiomyopathies. These entities are being increasingly noticed in adult patient populations. Primary care providers, hospitalists, emergency medicine physicians and cardiovascular specialists need to be aware of the clinical features of these illnesses and the best strategies for diagnosis and management. In this chapter, we discuss the etiologies and diagnostic methods for identifying Tako-tsubo cardiomyopathy and ways to manage this entity. This cardiomyopathy is caused by intense emotional or physical stress leading to rapid, severe but reversible cardiac dysfunction. It mimics myocardial infarction with changes in the electrocardiogram and echocardiogram, but without obstructive coronary artery disease. This pattern of left ventricular dysfunction was first described in Japan and has been referred to as "tako-tsubo cardiomyopathy," named after a fishing pot with a narrow neck and wide base that is used to trap octopus. This syndrome is also known as "apical ballooning syndrome", "ampulla cardiomyopathy", "stress cardiomyopathy", or "broken-heart syndrome".

Chapter III - The autonomic nervous system (ANS) plays an important role in the pathophysiology of arrhythmogenesis due to increased sympathetic activity and reduced vagal tone. Traditional time and frequency heart rate variability (HRV) parameters have gained importance in recent years as techniques employed to explore the ANS. Available data support conclusions that decreased HRV is a strong predictor of increased cardiac mortality. On the other hand, inteventions that tend to increase HRV, such as regular sports activity, may have a cardio-protective effect. However, when analysing the effects of different sports disciplines and the effects of strenuous exercise on the cardiac autonomic function determined by HRV indices results remain conflicting due to a variable behavior of the used HRV time domain and spectral parameters.

Chapter IV - Cardiomyopathies are included in an heterogeneous group of diseases, characterized by different signs and symptoms, natural history, clinical outcome, and

different pattern of inheritance. The genetics of cardiomyopathies has born in 1989 with a single gene theory (one gene=one disese), but the complexity and wide heterogeneity of the disease has moved toward a different direction (one gene=many diseases, or genocopies). Elucidation of the molecular basis of cardiomyopathies has led to a categorization of the phenotypes according to their genetic etiology. The American Hearth Association and the European Society of Cardiology have recently proposed a different scheme of classification based on a distinction between primary (genetic, mixed, non genetic types) and secondary cardiomyopathies, or between the familial and non familial types, respectively. The possibility of a different approach of intervention (i.e. enzyme replacement therapy in metabolic cardiomyopathies) underlies the need to make an early and precise etiologic diagnosis.

Chapter V - Cardiovascular disease is the leading cause of morbidity and mortality in the United Kingdom (UK) and although the UK mortality rate has steadily declined since the early 1970's, the rate of premature death has fallen less than other European countries. Following a cardiac event, it is common for patients to experience debilitating physiological and psychological impairment. A reduced functional capacity and depression are frequent, which is associated with a worse outcome as well as directly impacting on the failure to return to work. Comprehensive cardiac rehabilitation is a multidisciplinary service that provides the majority of cardiac patients with long-term exercise prescription, education, cardiovascular risk factor modification, counselling and medical evaluation to facilitate recovery and improve overall functional capacity following a cardiac event. The provision of cardiac rehabilitation services has grown significantly and demonstrated improved patient health, increased exercise capacity, reduced overall mortality and reduced hospitalisation costs. However, this growth has not been matched by service quality with many programmes unable to adhere to national guidelines due to inadequate resources and the related inability to provide appropriate staff training. Deficiencies in cardiac rehabilitation provision are generally due to inadequate investment, professional barriers, and the relatively low level of priority directed to the service in many cardiology departments. It appears that efficient comprehensive cardiac rehabilitation for patients is a postcode lottery, with substantial variation in the management, organisation, and practice throughout the UK.

Chapter VI - Although there has been significant recent progress in the management of heart failure its associated mortality remains high. A large proportion of these patients die suddenly, termed sudden cardiac death (SCD), mostly from potentially reversible malignant cardiac arrhythmias. Despite the availability of a highly effective treatment in the form of an implantable cardioverter defibrillator (ICD), SCD in the heart failure population is still a significant problem. One important reason for this is the difficulty in identifying which patients are at highest risk of SCD and would benefit from an ICD. A number of tests are currently available to risk stratify heart failure patients at risk of SCD. However, used alone or in combination these are not sufficiently accurate and there is significant need for better risk stratification tools.

Multiple studies have demonstrated that serum biomarkers can accurately predict adverse outcomes in patients with heart failure of both ischaemic and non-ischaemic aetiology. A range of biomarkers predict both the occurrence of SCD in patients without ICDs and the occurrence of malignant arrhythmias in patients with devices, and in these studies individual

biomarkers are at least as accurate as the current best markers of SCD risk. The pathophysiology of SCD is a complex process with a range of electrophysiological and molecular alterations contributing to arrhythmogenesis in the failing heart. By providing an assessment of these various processes, serum biomarkers may improve prediction of SCD in heart failure and help guide ICD use. Furthermore, it is likely that optimal SCD risk stratification will require the combination of multiple tests that reflect these diverse upstream processes. As such the greatest potential benefit of biomarkers may be in measuring multiple complementary markers that assess distinct aspects of arrhythmic risk.

Chapter VII - The congenital long QT syndrome (CLQTS) is a genetic channelopathy that affects sodium and calcium kinetics, resulting in prolonged ventricular repolarization. This channelopathy is associated with increased propensity to syncope, malignant ventricular arrhythmias and sudden arrhythmic death in children with normal cardiac structure. Recently, the published data from the International LQTS Registry have established risk factors for sudden cardiac death and aborted cardiac arrest in children. β-blockers are the first-line drug therapy for congenital long-QT syndrome in children. Several β-blockers (propranolol, atenolol, nadolol, metoprolol,..) were used in CLQTS with a significant reduction of cardiac events in patients with LQT1 and LQT2 mutations, but no evident reduction in those with LQT3 mutations. Infrequently, additional Drugs (mexiletine and flecainide) were used in children with CLQTS. The implantable cardioverter defibrillator and left cervicothoracic sympathetic denervation are other therapeutic options in children who remain symptomatic despite β-blocker therapy. Genetic factors may be used to improve risk stratification in genotyped patients and to predict the response to β-blockers.

Chapter VIII - In modern day cardiology practice the insertion of electrical pacemaker devices is routine, with an estimated 434 devices being inserted per million people in the United States each year. Although the development of modern pacing devices revolutionised cardiology towards the end of the 20th century, electrical devices remain a palliation, rather than a cure, to an underlying disorder of cardiac rhythm. Thus in recent years the idea of a "biological" pacemaker, whereby artificial electrical components are replaced by cellular and genetic elements capable of producing intrinsic electrical activity, has taken several steps towards becoming a realistic therapeutic goal.

What advantages would such a development have over an already well-established method of treatment? Biological systems offer the promise of being more sensitive to the body's autonomic nervous system, thus providing a more natural control of physiological heart rate compared to current rate sensing pacemakers. Implantation of biological systems into the correct anatomical location would also allow electrical conduction to mimic the heart's intrinsic conduction system, such as the bundle of His, as closely as possible. Thirdly, many of downfalls of electrical pacemaker insertion, such as infection, battery replacement, and the induction of cardiac failure, could be reduced significantly, if not eliminated. For paediatric patients in particular, who face a lifetime of device changes, a biological pacemaker could prove to be a very effective cure.

Chapter IX - Different stem cell populations have been intensively studied in the last decade as a potential source of new cardiomyocytes to ameliorate the injured myocardium, compensate for the loss of ventricular mass and contractility and eventually restore cardiac

function. An array of cell types has been explored in this respect, including skeletal muscle, bone marrow derived stem cells, embryonic stem cells (ESC) and more recently cardiac progenitor cells. The best-studied cell types are mouse and human ESC cells, which have undisputedly been demonstrated to differentiate into cardiomyocyte and vascular lineages and have been of great help to understand the differentiation process of pluripotent cells. However, due to their immunogenicity, risk of tumor development and the ethical challenge arising from their embryonic origin, they do not provide a suitable cell source for a regenerative therapy approach.

Embryonic stem cells can differentiate into true cardiomyocytes, making them in principle an unlimited source of transplantable cells for cardiac repair, although immunological and ethical constraints exist. Somatic stem cells are an attractive option to explore for transplantation as they are autologous, but their differentiation potential is more restricted than embryonic stem cells. Currently, the major sources of somatic cells used for basic research and in clinical trials originate from the bone marrow. The differentiation capacity of different populations of bone marrow-derived stem cells into cardiomyocytes has been studied intensively. Only mesenchymal stem cells seem to form cardiomyocytes, and only a small percentage of this population will do so in vitro or in vivo. A newly identified cell population isolated from cardiac tissue, called cardiac progenitor cells, holds great potential for cardiac regeneration.

New approaches for cardiac repair have been enabled by the discovery that the heart contains its own reservoir of stem cells. These cells are positive for various stem/progenitor cell markers, are self-renewing, and exhibit multilineage differentiation potential. Recently has been developed a method for ex vivo expansion of cardiac-derived stem cells from human myocardial biopsies with a view to subsequent autologous transplantation for myocardial regeneration.

Chapter X - Cerebral hypoxia-ischaemia (HI) results in a multi-faceted complex cascade of events causing cell death and neurological dawmage to the central nervous system. Furthermore, cerebral ischaemia results in cardiovascular complications that can further confound the prognostic outcome of patients. This chapter addresses the cardiovascular changes that occur subsequent to an ischaemic insult, regulation of the insular cortex, changes in the autonomic nervous system and the role of various circulating cytokines (both pro-inflammatory and anti-inflammatory) and chemokines. In addition, markers of oxidative stress and cardiac enzyme release following an ischaemic insult are also discussed. Given that lack of treatment options available, the use of beta-blockers and pre- and post-conditioning paradigms as possible treatment option to prevent the occurrence of secondary cardiac abnormalities in addition to CNS injuries have also been addressed.

Chapter XI - The non-protein amino acid homocysteine (Hcy), a metabolite of the essential amino acid methionine, is implicated in the pathology of human cardiovascular and neurodegenerative diseases. In addition to its elimination by the remethylation and transsulfuration pathways, Hcy is also metabolized to the thioester Hcy-thiolactone in an error-editing reaction in protein biosynthesis when Hcy is mistakenly selected in place of methionine by methionyl-tRNA synthetase. In humans, the accumulation of Hcy-thiolactone can be detrimental because of its intrinsic ability to modify proteins by forming *N*-Hcy-protein adducts, in which a carboxyl group of Hcy is *N*-linked to ε-amino group of a protein

lysine residue. *N*-linked Hcy occurs in each protein examined and constitutes a significant pool of Hcy in human blood. *N*-Hcy proteins are recognized as neo-self antigens and induce an auto-immune response. As a result, IgG and IgM anti-*N*-Hcy-protein auto-antibodies, are produced in humans. Serum levels of anti-*N*-Hcy-protein IgG auto-antibodies are positively correlated with plasma total Hcy, but not with plasma cysteine or methionine levels, which is consistent with the etiology of these auto-antibodies. In a group of male patients with stroke, the levels of anti-*N*-Hcy-protein IgG auto-antibodies and total Hcy are significantly higher than in a group of healthy subjects. In a group of male patients with angiographically documented coronary artery disease, seropositivity for anti-*N*-Hcy-protein IgG auto-antibodies occurs 5-times more frequently than in controls and is an independent predictor of coronary artery disease. These findings show that an auto-immune response against *N*-Hcy-proteins is a general feature of atherosclerosis and provide support for a hypothesis that *N*-Hcy-protein is a neo-self antigen, which contributes to immune activation, an important modulator of atherogenesis. Plasma Hcy lowering by folic acid administration leads to significant decreases in anti-*N*-Hcy-protein IgG auto-antibody levels in control subjects, but not in coronary artery disease patients. The results of these Hcy-lowering treatments suggest that, while primary Hcy-lowering intervention is beneficial, secondary Hcy-lowering intervention in coronary artery disease patients may be ineffective in reducing the advanced damage caused by Hcy, and may explain at least in part the failure of vitamin therapy to lower cardiovascular events in recent Hcy-lowering trials. Chronic activation of immune responses towards *N*-Hcy-protein associated with hyperhomocysteinemia over many years would lead to vascular disease.

In: Horizons in World Cardiovascular Research. Volume 1 ISBN: 978-1-60876-447-1
Editor: Eleanor H. Bennington, pp. 1-31

Chapter I

Diabetic Cardiomyopathy, Insulin Resistance and Microangiopathy: Considerations on Treatment and Rehabilitation

Rômulo R. Lobo[1], Jarbas S. Roriz-Filho[2], Idiane Rosset[3] and Matheus Roriz-Cruz[4*]

[1]Division of Geriatrics. Department of Internal Medicine. University of São Paulo-RP, Brazil

[2]Division of Geriatrics. Department of Internal Medicine. University of São Paulo-RP, Brazil

[3]Division of Gerontological Nursing. Faculty of Nursing. Brazilian Federal University of Rio Grande do Sul State, Brazil

[4]Division of Geriatric Medicine. Department of Internal Medicine. Brazilian Federal University of Rio Grande do Sul State, Brazil

Abstract

There is increasing evidence that diabetes causes both anatomical and functional pathological changes in the myocardium. Diabetic cardiomyopathy (DC) is a myocardial disease caused by diabetes mellitus, which is unrelated to vascular pathology or systemic arterial hypertension and can occur in asymptomatic patients with diabetes alone. The coexistence of hypertension, diabetic cardiomyopathy, and myocardial ischemia increases with aging, especially among obese subjects. However relatively independent, each of these diseases seem to interact with the other in order to contribute to the biochemical, anatomical, and functional alterations in myocardial cells. The most important mechanisms involved in DC are (1) insulin resistance with consequent hyperinsulinemia and endothelial proliferation; (2) small-vessel disease

* Corresponding author: Matheus Roriz-Cruz: matheusroriz@hotmail.com

(microangiopathy, impaired coronary flow reserve, and endothelial dysfunction); (3) metabolic disturbances like increased free fatty acid levels, carnitine deficiency, and changes in calcium homeostasis; (4) myocardial fibrosis (increases in angiotensin II activity, IGF-I, and inflammatory cytokines levels), and cardiac autonomic neuropathy (denervation and alterations in myocardial catecholamine levels). Abnormalities in both systolic and diastolic cardiac function have been demonstrated in diabetic subjects. Several lines of evidence indicate that left ventricular diastolic dysfunction represents the earliest preclinical manifestation of diabetic cardiomyopathy, preceding systolic dysfunction. Documentation of diastolic dysfunction should result in the initiation of therapy in order to prevent progression to Heart Failure (HF). Treatment of DC-HF is essentially not different from treating HF caused by myocardiopathies of other etiologies, and it must follow the guidelines according to ventricular function. However, some particularities related to its diabetic etiology should be considered. Degree of glycemic control correlates well with the severity of microvasculature damage, which can be delayed or even prevented by keeping near normal serum glucose levels. Achievement of ideal glycemic control levels, preferably by reducing insulin resistance, is the essential step not only in treating diabetes itself, but also in preventing and managing DC. Besides dietetic therapy, increasing levels of endurance exercise should be encouraged in order to improve peripheral insulin resistance. There is large evidence that the use of ACE inhibitors, which might reverse left ventricular hypertrophy and myocardial fibrosis, is also able to prevent myocardial remodeling, improve endothelial function, and even contribute to lower insulin resistance. It is also known that ß-blockers and thiazolidinediones shift myocardium metabolism from the use of free fatty acids (FFAs) to that of glucose, which would be beneficial in DC. In addition, the thiazolidinediones have also been shown to decrease myocardial FFA levels in animals, besides improving ventricular function. In the near future, agents that decrease lipotoxicity or prevent/reverse glycosylation and cross-linking of collagen are promising.

1. Introduction and Epidemiology

Diabetes mellitus is the world's fastest-growing disease with high morbidity and mortality rates, predominantly as a result of heart disease. The growing incidence, particularly of the type 2 diabetes, is alarming, especially considering the increased levels of insulin resistance and diabetes in young adults and children [1,2]. The prevalence of diabetes is growing rapidly in both the developing and the developed countries. Annually, 7 million people are newly diagnosed with diabetes mellitus in the world and more than 3.8 million deaths take place for complications associated with the disease [3,4]. Data collected by the World Health Organization (WHO) showed the prevalence of DM to be 2.8% in 2000, equivalent to 171 million persons. It projected, still, for 2030 the prevalence of 4.4 % in the worldwide population, meaning that around 366 million persons would be attacked by the disease [5]. This dramatic increase will be almost entirely due to new cases of type II diabetes [6].

The criteria for DM were sharpened, with conditions such as impaired fasting glucose (IFG) and impaired glucose tolerance (IGT) being classified as intermediate stages between the ends of the spectrum; that is, normal glucose homeostasis and diabetes. This classification is based on fasting glucose levels and glucose overload levels[7] (Table 1). Precise statistical

data are lacking regarding the prevalence of IFG and IGT. Epidemiological data derived from Third National Health and Nutrition Examination Survey (NHANES III) and Diabetes Epidemiology: Collaborative analysis of diagnostic criteria in Europe (DECODE) studies, however, estimate the prevalence of these new diagnostic categories to be between 8 and 12% of the adult population [7,8].

Cardiovascular disease (CVD), including coronary heart disease (CHD), cerebrovascular disease, and peripheral vascular disease are the major causes of morbidity and the most common causes of death in people with diabetes [9]. Two-thirds of people with diabetes die of heart disease or stroke [10]. Diabetes predisposes patients to ventricular dysfunction and the development of concomitant coronary artery disease, endothelial dysfunction, hypertension, ventricular hypertrophy, coronary microvascular disease, autonomic neuropathies and metabolic abnormalities [10,11].

Heart failure (HF) is a common and serious comorbidity of diabetes. The Framingham study demonstrated the increased incidence of congestive HF in diabetic males (2.4:1) and females (5:1) independent of age, hypertension, obesity, coronary artery disease and hyperlipidaemia [12,13]. Several studies have shown that a 1% increase in HbA1c level increases the risk of developing HF by 8 to 15%, and that plasma glucose levels are associated with the risk of developing HF [7,14]. Besides, patients with diabetes account for >33% of all patients requiring hospitalization for HF [15]. Diabetic cardiomyopathy in the absence of CAD or hypertension is a common clinical feature of diabetes and is characterized mainly by diastolic dysfunction of the left ventricle and later on by a decrease of myocardial contractility [7,16].

Table 1. Definitions of normal and disturbed glucose metabolism according to World Health Organization (WHO)

Fasting plasma glucose (mmol/l)	2 h postload plasma glucose (mmol/l) (75 g glucose)		
	< 7.8	7.8–11.1	≥ 11.1
< 6.1	Normal	IGT	Diabetes
6.1–7.0	IFG	IFG + IGT	Diabetes
≥ 7.0	Diabetes	Diabetes	Diabetes

IFG, impaired fasting glucose; IGT, impaired glucose tolerance.

1.1. Definitions

The condition “diabetic cardiomyopathy” was originally described in 1972 on the basis of observations in four diabetic patients who presented with heart failure without evidence of hypertension, coronary artery disease, valvular or congenital heart disease [17].

Diabetic cardiomyopathy is a clinical condition, diagnosed when ventricular dysfunction develops in patients with diabetes in the absence of coronary atherosclerosis and hypertension [18]. It refers to a disease process which affects the myocardium in diabetic

patients causing a wide range of structural abnormalities eventually leading to left ventricular hypertrophy and diastolic and systolic dysfunction or a combination of these [12].

A significant number of diabetic patients exhibit diabetic cardiomyopathy. Accumulating data from experimental, pathological, epidemiological, and clinical studies have shown that diabetes mellitus results in cardiac functional and structural changes, independent of hypertension, coronary artery disease, or any other known cardiac disease, which support the existence of diabetic cardiomyopathy [18,19]. However, the frequency with which this occurs is not well defined. Although the existence of diabetic cardiomyopathy has been debated, substantial data now demonstrate that diabetes impairs ventricular function independently of other risk factors [20,21]. This specific form of cardiomyopathy has been associated with both type 1 and type 2 diabetes, but there is some evidence that it is uncommon in patients with type 1 diabetes in the era of intensive insulin therapy [22]. It is characterized by both systolic and diastolic dysfunction [4,23], presenting clinically with impaired diastolic function developing first [2,24].

Evidence now indicates that this cardiomyopathy is also seen in patients and animals predisposed to diabetes but presenting only with metabolic complications associated with insulin resistance [25]. Studies have suggested that increased risk of cardiovascular disease is not restricted to type II or type I diabetes mellitus, but extends to pre-diabetic stages such as impaired fasting glucose, impaired glucose tolerance, metabolic syndrome, and obesity. Insulin resistance impaired fasting glucose, impaired glucose tolerance, and diabetes mellitus would form a continuous sequence of risk for cardiovascular disease [7].

During diabetes, changes in cardiac metabolism occur early and precede the development of cardiomyopathy. Even though altered metabolism is inadequate to produce cardiac functional changes at this early time, it is likely that early metabolic damage is occurring at the cellular or subcellular levels. Overtime, these cumulative defects could be contributing to diabetic cardiomyopathy [26].

2. Pathophysiology

The three characteristic metabolic disturbances evident in diabetic states are hyperlipidemia (usually in the form of increased triglycerides and free fatty acids [FFAs]), early hyperinsulinemia followed by pancreatic-cell failure, which leads eventually to hyperglycemia [18]. The increase of triglycerides, insulin and glucose induces alterations in the activation of cellular transcription in the myocardium, altering the use of substrates, myocardium growth, and leading to endothelial dysfunction and increases stiffness [27]. Alterations in body mass (obesity) and adipocytokines (leptin, adiponectin) have also been implicated in the cardiovascular pathophysiology observed in diabetes. As such, the effects of increased FFAs, altered insulin action, and hyperglycemia can be considered triggers to the cardiac phenotype in diabetes [18].

2.1. Alterations in Substrate Supply and Utilization

Metabolic changes in diabetes are directly triggered by hyperglycemia. Increasing evidence suggests that altered substrate supply and utilization by cardiac myocytes could be the primary injury in the pathogenesis of this specific heart muscle disease [19,28]. A significant reduction in myocardial glucose supply and utilization has been observed in isolated diabetic cardiomyocytes [19] and diabetic patients [19,29]. A major restriction to glucose utilization in the diabetic heart is the slow rate of glucose transport across the sarcolemmal membrane into the myocardium, probably due to the cellular depletion of glucose transporters (GLUTs) 1 and 4, which can be corrected by insulin therapy [19,30]. A second mechanism of reduced glucose oxidation is via the inhibitory effect of fatty acid oxidation on pyruvate dehydrogenase complex due to high circulating FFAs. This has the net effect of reducing ATP availability and may be more important in type II diabetes, in which FFAs levels tend to be higher. The potential importance of this mechanism is exemplified by the observation that diabetic animals with minimal hypertriglyceridemia are resistant to the development of cardiomyopathy [31,32]. Both of these pathological mechanisms are potentially reversible in a short time frame, and the dynamics of each mechanism is compatible with the observation that cardiac dysfunction may be improved with improved metabolic control [19].

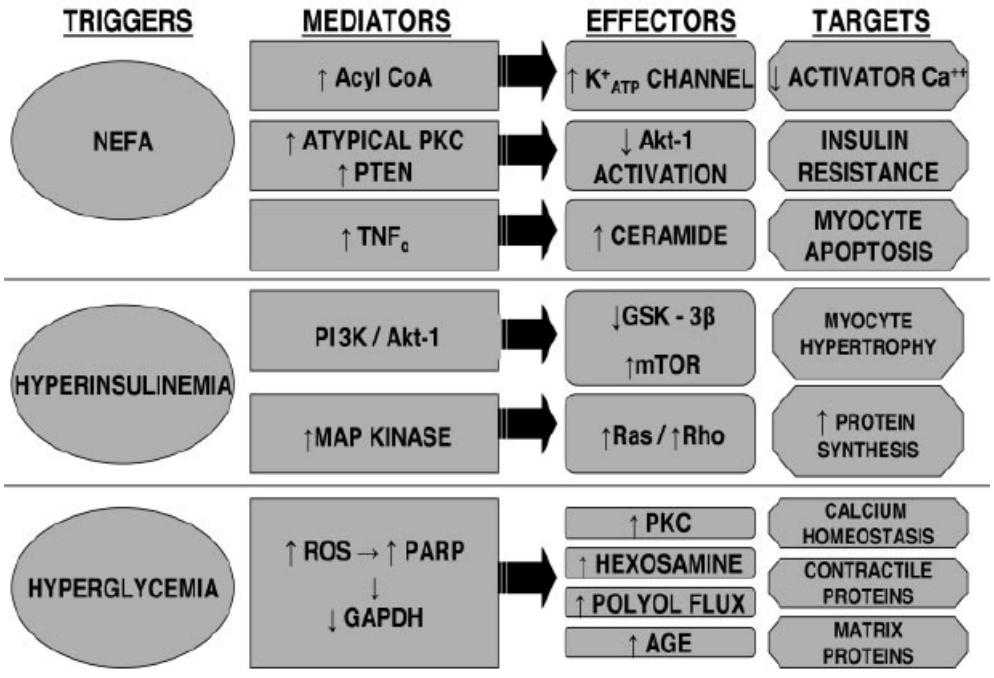


Figure 1. DIABETES LEADS TO CARDIOMYOPATHY. GAPDH: glyceraldehyde phosphate dehydrogenase; GSK-3B: glycogen synthases kinase-3B; MAP: mitogen-activated protein; PARP: poly (ADP ribose) polymerase; PKC: protein kinase C; PI3K: phosphatidylinositol 3-kinase; PTEN: phosphatase and tensin homolog; ROS: reactive oxygen species; TNF: tumor necrosis factor. (Adapted from reference 18.)

FFA metabolism: Elevated FFA levels are believed to be one of the major contributing factors in the pathogenesis of diabetes. FFAs enhance peripheral insulin resistance and trigger cell death. Disturbances of FFA metabolism may be an important contributor to abnormal myocardial function in diabetes. These changes are characterized by elevation of circulating FFAs caused by enhanced adipose tissue lipolysis, as well as high tissue FFAs caused by hydrolysis of augmented myocardial triglyceride stores. Moreover, in addition to the FFA-induced inhibition of glucose oxidation, high circulating and cellular FFA levels may result in abnormally high oxygen requirements during FFA metabolism and the intracellular accumulation of potentially toxic intermediates of FFAs, all of which lead to impaired myocardial performance and severe morphological changes [19]. FFAs play a central role in altering cellular insulin signaling through several mechanisms leading to insulin resistance and compensatory hyperinsulinemia [18,33,34]. Abnormalities in FFA metabolism have been demonstrated in idiopathic dilated cardiomyopathy in which the rate of FFA uptake by myocardium is inversely proportional to the severity of the myocardial dysfunction [35]. It is possible that similar defects contribute to the development of diabetic cardiomyopathy. The FFA-induced impairment of glucose oxidation may be a major factor in the development of diabetic cardiomyopathy, and would explain why cardiac function tends to improve upon metabolic improvement. Furthermore, the availability of carnitine, an essential substance for myocardial FFA metabolism, is usually reduced in diabetes [19]. Conversely, normalizing cardiac metabolism in diabetic animals reverses the development of cardiomyopathy [36,37].

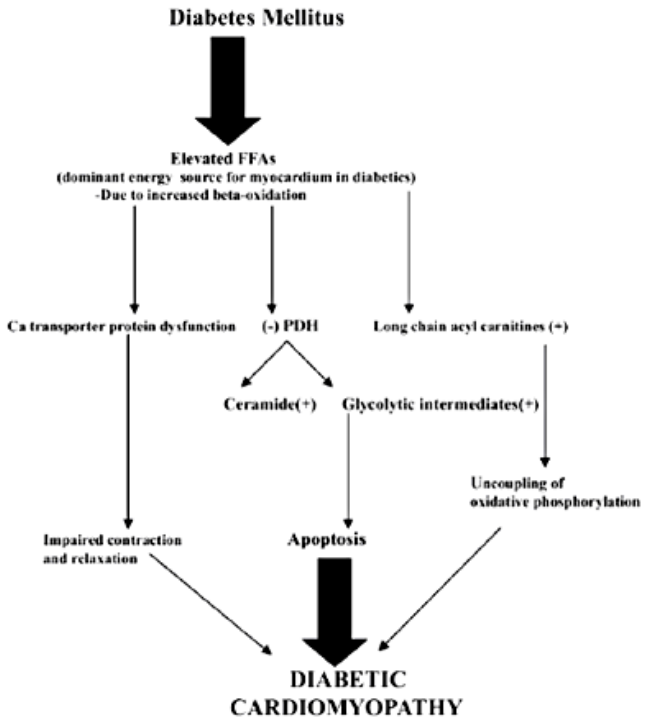


Figure 2. The role of altered myocardial metabolism in the development of diabetic cardiomyopathy. FFA = free fatty acid; PDH = pyruvate dehydrogenase. (Adapted from reference 74.)

Lipotoxicity: Lipotoxicity is the process by which excess fatty acids and associated triglyceride accumulation in parenchymal cardiac myocytes cause cellular dysfunction and death, and eventual myocardial dysfunction. An imbalance between fatty acid uptake and use leads to the inappropriate accumulation of free fatty acids and neutral lipids within cardiomyocytes. Long-chain nonesterified fatty acids and their products, such as ceramides and diacylglycerols, cause the majority of the toxic effects [38,39]. A number of studies have suggested that excessive fatty acid overload induces lipotoxicity and contributes to the initiation and development of cardiomyopathy [26,37,40]. With the use of transgenic mice, studies have shown that elevation of fatty acid uptake or utilization induces lipotoxicity in the absence of any systemic metabolic disturbance [26].

The mechanisms that mediate cardiac lipotoxicity are still not completely understood. One potential target is over production of reactive oxygen species (ROS) [41]. High rate of fatty acid oxidation increases mitochondrial action potential, leading to augmented ROS generation. Another potential mechanism for lipotoxicity is accumulation of lipids, when fatty acid uptake supersedes its oxidation. Regarding accumulation of triglycerides, the role of this neutral lipid in inducing contractile dysfunction is still unknown, although a strong association between triglycerides storage and lipotoxicity has been established in both animal models and human studies [42,43]. Taken together, there is strong evidence for lipotoxic mechanisms in rodents showing that lipid accumulation in the heart leads to heart failure [44]. Data indicate that the cardiac accumulation of triglycerides is related to FFA exposure, generalized ectopic fat excess, and peripheral vascular resistance and that these changes precede left ventricle overload and hypertrophy [44,45].

2.2. Insulin Resistance

Cellular insulin resistance may presage frank diabetes by a decade or more and requires compensatory increases in plasma insulin levels to maintain glucose homeostasis in the face of impaired cellular insulin action, principally in skeletal muscle and liver [46]. Systemic hyperinsulinemia may accentuate cellular insulin action in insulin responsive tissues, such as the myocardium, that do not manifest cellular insulin resistance. In this regard, the mitogenic actions of insulin on myocardium during chronic systemic hyperinsulinemia bear directly the commonly observed finding of cardiac hypertrophy in diabetic cardiomyopathy [18].

Myocardial changes seen in insulin-resistant individuals could be caused by the impaired ATP synthesis noted in these patients, despite reduced oxygen delivery or increased workload. Insulin resistance results in decreased myocardial glucose uptake and oxidation, increased fatty acid oxidation, and altered myocyte gene expression [38].The slow rate of glucose transport across the sarcolemma into the myocardium restricts the glucose usage in the hearts of patients with insulin resistance [38,47]. Excessive myocardial fatty acid uptake could enhance insulin resistance, promote cell dysfunction and trigger myocyte apoptosis, resulting in myocardial dysfunction [48].

The normal adaptive response by an injured/failing heart involves a complex series of enzymatic shifts and up-/downregulation of transcription factors, ultimately resulting in increased glucose metabolism and decreased FFA metabolism to maximize efficiency

[25,49,50]. In contrast, FFA metabolism is decreased, with decreased expression of the peroxisome proliferator-activated receptor (PPAR)-/retinoid X receptor complex and 2 enzymes critical to FFA metabolism, carnitine palmitoyl transferase-1 and medium-chain acyl-coenzyme A dehydrogenase [50,51,52]. These adaptive responses of the heart are inhibited in the setting of insulin resistance (Fig. 3). Although the initial myocardial metabolic switch in heart failure is down-regulation of FFA metabolism, the opposite occurs (up-regulation of FFA metabolism) in the setting of insulin resistance [53,54]. This increased reliance on FFA metabolism leads to increased oxygen consumption, decreased cardiac efficiency, and the potential for lipotoxicity [55,56]. Insulin resistance at its most fundamental level inhibits uptake and metabolism of glucose. It is likely this effect—preventing the heart from using its adaptive energy response to an insult—which contributes to heart failure and the vicious cycle of neurohormonal activation, serving to potentiate the myocardial dysfunction and further increasing energy requirements [25,51,57,58].

Importantly, cardiac dysfunction precedes the development of systemic hyperglycemia, implying that the altered cellular metabolism rather than systemic hyperglycemia is responsible for the cardiac dysfunction [59]. Treatment of insulin resistance in these models (with troglitazone, metformin, or exercise) prevents myocardial dysfunction, but therapy aimed at hyperglycemia itself without treating insulin resistance (sulfonylureas) showed no effect [60,61]. The prognostic impact of insulin resistance is independent of other variables, including peak oxygen consumption (VO2max) and left ventricular ejection fraction (LVEF), implying that insulin resistance is pathogenic rather than simply a marker for worsened heart failure [25,62].

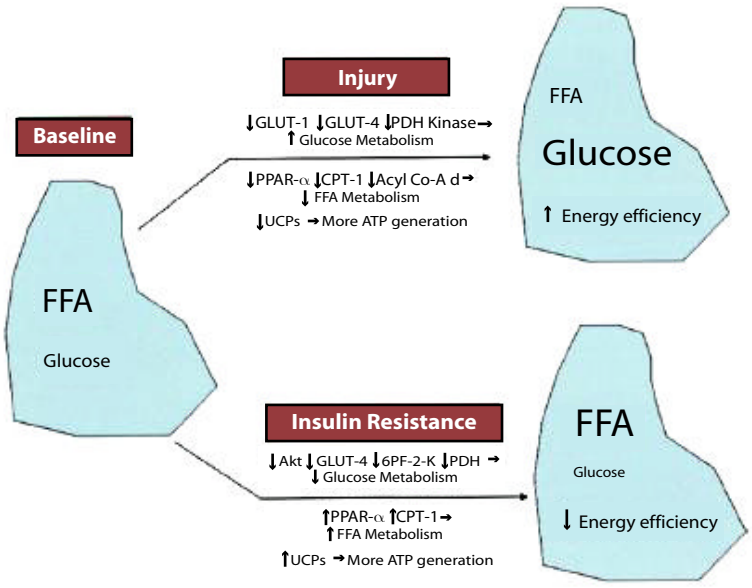


Figure 3. MYOCARDIAL ENERGY METABOLISM IN RESPONSE TO INJURY AND INSULIN RESISTANCE. FFA: free fatty acids; Acyl Co-A d: medium-chain acyl-coenzyme A dehydrogenase; CPT: carnitine palmitoyl transferase; GLUT: glucose transporter; PDH: pyruvate dehydrogenase; PPAR: peroxisome proliferator-activated receptor; UCP: uncoupling protein; 6PF-2K: 6-phosphofructo-2-kinase. (Adapted from reference 25.)

2.3. Hyperglycemia

The mechanism whereby hyperglycemia mediates tissue injury through the generation of reactive oxygen species has been elucidated largely through the work of the Brownlee and colleagues. Hyperglycemia leads to increased glucose oxidation and mitochondrial generation of superoxide [18]. Taken together, these data provide mechanistic evidence linking hyperglycemia to altered expression and function of both the ryanodine receptor (RyR) and sarco(endo)plasmic reticulum Ca2-ATPase (SERCA2) that may contribute to decreased systolic and diastolic function. Hyperglycemia-induced oxidative stress also activates poly(ADP-ribose) polymerase-1 (PARP) [63]. The activation of the PARP regulates several cellular reactions like repair of DNA, gene expression and cellular overlife. The effects of PARP include increase in the formation of advanced glycosilation end products (AGE´s), through the diversion of the route of degradation of the glucose. Meantime, the excessive activation of the PARP can begin several cellular processes and cause cellular damage. In addition, hyperglycemia contributes to altered cardiac structure through posttranslational modification of the extracellular matrix [18]. The response to hypoglycemic therapy further confirms the correlation of myocardial functional and structural changes with glycemic control. Taken together, hyperglycemia, through multiple pathways, causes cardiac cellular and functional changes, possibly contributing to the development of cardiomyopathy [26].

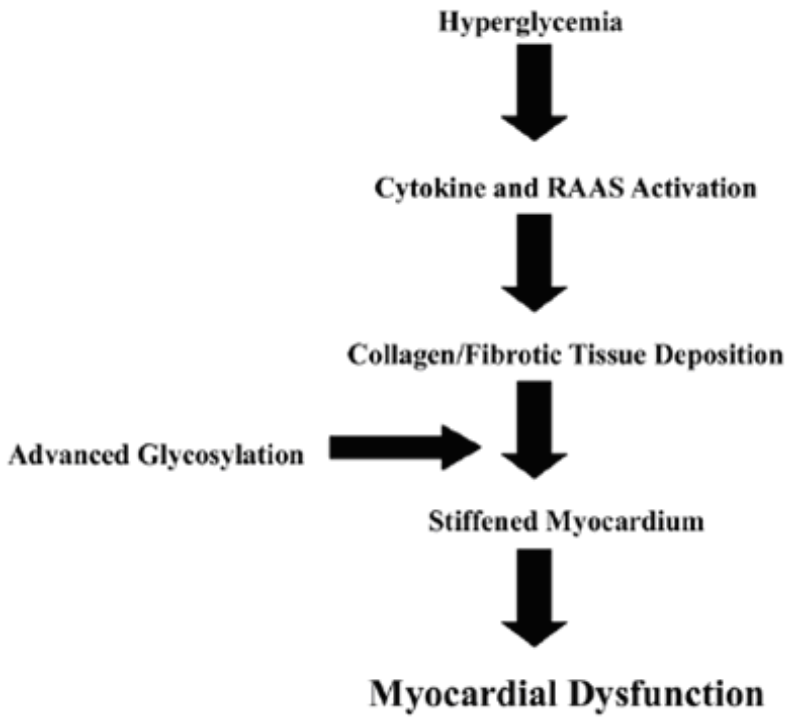


Figure 4. EFFECTS OF HYPERGLICEMIA ON THE DIABETIC CARDIOMYOPATHY. RAAS: renin-angiotensin-aldosterone system. (Adapted from reference 74.)

2.4. Abnormalities in the Regulation of Calcium Homeostasis

There are changes in the level of the cardiomyocyte, which are not solely attributable to impaired coronary blood flow or interstitial fibrosis, including altered functional activity of ion channels and pumps and changes in gene expression of regulatory and modulatory proteins of Excitation-Contraction (E-C) coupling. The cellular defects associated with E-C coupling manifest as prolonged action potentials, slowed cytosolic Ca2+ fluxes and slowed myocyte shortening and lengthening [2,64,65].

Oxidative stress caused by toxic molecules may play a critical role in subcellular remodeling and abnormalities of calcium handling that lead to subsequent diabetic cardiomyopathy. Alterations in regulatory proteins and contractile proteins, sarcoplasmic (endoplasmic) reticulum Ca2+-ATPase (SERCA2) and Na-Ca2 exchanger function may be important contributors to abnormal myocardial carbohydrate and lipid metabolism in diabetes. These changes likely result from accumulation of toxic molecules such as long-chain acylcarnitines, free radicals, and abnormal membrane lipid content[19]. Changes in gene expression that affect E-C coupling and cellular metabolism contribute to myocardial dysfunction in diabetic cardiomyopathy. At the cellular level, defective E-C coupling has been implicated as one of the root causes of the contractile dysfunction associated with diabetic cardiomyopathy. One of the most consistent and early changes seen in the hearts of individuals with diabetes is the prolongation of the ventricular action potential [4].

Cardiomyopathy in streptozotocin-induced type 1 diabetes is characterized by a decrease in the expression of SERCA2 [4,66], a change that is seen in most animal models of heart failure. In animal models of type 2 diabetes or insulin resistance, SERCA2 activity is also compromised, but a decrease in the expression of the protein is not always apparent [67]. The alteration in SERCA2 activity is most probably dependent on the severity and duration of diabetes. Impaired SERCA function has been consistently found to coincide with myocyte insulin resistance in animal and in vitro models of type 1 and 2 [diabetes68,69]. Furthermore, instigating insulin treatment in diabetic rats restored SERCA2a levels to normal, increased intracellular Ca2+ transient currents, and improved myocardial function following ischemia–reperfusion [70,71].

Besides, advanced glycosylation end products form irreversible cross-links within or between many proteins, such as SERCA2a, causing their inactivation and subsequently leading to abnormal cardiac relaxation and contractility [72,73].

2.5. Microvascular Disease

Diabetes is recognized by characteristic changes in microvascular architecture. These changes include abnormal capillary permeability, microaneurysm formation, subendothelial matrix deposition, and fibrosis surrounding arterioles. Coronary blood flow reserve in diabetic patients is reduced even in the absence of obstructive coronary artery disease and left ventricular hypertrophy [74]. Hyperglycemia also can lead to an enhanced synthesis of vasoconstrictor prostanoids by the endothelium and activation of protein kinase C. This vasoconstriction can promote myocardial hypertrophy, endothelial dysfunction, and

ventricular hypertrophy [74]. Protein kinase C, an intracellular signaling molecule, is activated in diabetes and can lead to endothelial dysfunction by reducing the bioavailability of nitric oxide while increasing oxygen-derived free radical production. It also can enhance leukocyte adhesion, increase albumin permeability, and impair fibrinolysis [74,75]. Therefore, activation of this enzyme contributes significantly to the development of microvascular complications, as seen in diabetic neuropathy and nephropathy.

There can be correlation between diabetic cardiomyopathy and microangiopathy, due to the similarities between diabetes and idiopatic miocardiopathy in what concerns the coronary disease [76]. About 72% of normotense diabetic patients present in around 72 % of the diabetic patients without arterial high blood pressure were watched obvious disease of small pots, whereas in non-diabetics this finding was only 12 % [77]. Besides, abnormalities of the reserve of coronary flow have been solidly demonstrated in diabetic patients without epicardic coronary arterial disease. Perivascular and interstitial fibrosis and miocardic hypertrophy were also frequent finds in diabetics [78].

The capacity of the vascular bed to meet metabolic demands may be impaired by abnormal epicardial vessel tone and microvascular dysfunction [76,79]. Diabetics have impaired endothelium-dependent relaxation [80], a defect that may be related to inactivation of nitric oxide by advanced glycosylation end products and increased generation of free radicals [81]. The abnormal vasodilator response in diabetes extends to the coronary microcirculation [82]. Besides, microcirculatory dysfunction in diabetics may be due in part to downregulation of the expression of vascular endothelial growth factor (VEGF).

Even in patients with no known coronary artery disease, microvascular dysfunction and decreased coronary flow reserve can be present. Such findings have been demonstrated particularly in the insulin-resistant/diabetic cardiomyopathy population [83,84]. In the absence of resting flow abnormalities, this is less likely to be a cause of resting left ventricular dysfunction but could contribute to left ventricular dysfunction with stress or exercise. In addition, a mismatch between coronary blood flow and myocardial glucose uptake has been demonstrated [85].

3. Clinical Picture and Symptoms

There are 2 important components in the clinical diagnosis of diabetic cardiomyopathy: the detection of myocardial abnormalities and the exclusion of other contributory causes of cardiomyopathy. An important challenge in the clinical diagnosis of diabetic cardiomyopathy has been the lack of any pathognomonic histological changes or imaging characteristics associated with the diagnosis [74].

The definitive diagnosis of diabetic cardiomyopathy is difficult to be established, principally because the signs, symptoms and finds of diagnostic examinations are unspecific. The diagnosis of diabetic cardiomyopathy currently rests on noninvasive imaging techniques that can demonstrate myocardial dysfunction across the spectra of clinical presentation [74]. Besides, the clinical picture and laboratorial what took the suspicion of diabetic cardiomyopathy can be resulting of pathologies very prevalent between the diabetic patients, like arterial high blood pressure, coronary disease and obesity. The clinical demonstration of

the diabetic cardiomyopathy is usually characterized for dyspnea due to the pulmonary congestion resulting from the diastolic dysfunction of the left ventricle. Subsequently, with the advancement of the disease, compromising of the systolic performance can occur, aggravating the severity of heart failure. The signs and symptoms of right heart failure, as well as the clinical form of dilated cardiomyopathy with global heart failure, are not common in the diabetic cardiomyopathy [86]. It is important to emphasize that with our current knowledge, there is still no consensus in the precise imaging definition of diabetic cardiomyopathy, but evidence of hypertrophy or diastolic dysfunction is likely crucial to support a diagnosis of diabetic cardiomyopathy, but is not specific to it [74].

Diastolic function parameters in diabetic patients are analogous to those in animal studies. Left ventricular ejection time is often reduced, and the length of the pre-ejection period and the ratio of pre-ejection period to left ventricular ejection time are often increased. Diastolic inflow patterns are frequently abnormal, reflecting underlying abnormalities in relaxation and/or reduced myocardial compliance. Left ventricular diastolic dysfunction appears to be quite common in well-controlled type II diabetic patients without clinically detectable heart disease [19].

Studies that have examined both systolic and diastolic dysfunction in both type I and type II diabetes suggest that the latter is more susceptible to preclinical changes. The lack of an association between diabetes and LV diastolic dysfunction in young diabetic subjects (35 yr) may relate to the prevalence of type I diabetes [19,87]. Nonetheless, another comparison of both type I and type II adult diabetic patients also showed no significant difference in mean rate-corrected pre-ejection period, left ventricular ejection time, electromechanical systole, and pre-ejection period/left ventricular ejection time ratio compared with those of age- and sex-matched normal subjects [88]. The mechanism of protection of type I diabetic patients may relate to protective effects of insulin therapy and lack of insulin resistance. Indeed, animal data suggest correction of abnormal function with insulin therapy, with indices of cardiac performance significantly greater in insulin-treated rats when compared with control rats [19].

A number of studies in both animals and humans have shown structural changes in parallel with the functional changes of diabetic heart disease, in the absence of hypertension, coronary artery disease, or intraventricular conduction defects [89,90,91]. These results indicate LV fibrosis in the early stages of type II diabetes. In another study using modern stereological techniques to quantify changes in the morphology accompanying streptozotocin-induced diabetes, the results showed that the time to peak tension and relaxation of papillary muscles was prolonged, the heart weight to body weight ratio was increased, and the volume of extracellular components was increased 3-fold in diabetic rats. At the same time, this study also demonstrated that the volume, surface density, and total surface area of capillaries as well as volume fraction of myocyte mitochondria were reduced, and oxygen diffusion distance to myocyte mitochondria was increased in the diabetic animals [92].

Similar structural alterations have been described in diabetic hearts without significant epicardial coronary disease in humans. The most prominent histopathological finding in diabetic patients is fibrosis, which may be perivascular, interstitial, or both. As the disease progresses, there is increased myocyte loss and replacement fibrosis. Thus, the increased

myocardial tissue reflectivity in diabetics may represent an early marker of diabetic cardiomyopathy [19].

4. Treatment

In the treatment of the diabetic cardiomyopathy is of basic importance the control of the DM in accordance with the directives in force, the control of the physical weight, the healthy food and the physical regular activity, besides the rigorous control of associate diseases, principally arterial high blood pressure, coronary disease and cholesterol.

Many of the established therapies in heart failure are also known to improve insulin resistance, even in non heart failure populations. Standard lifestyle recommendations (exercise, smoking cessation, weight loss) are all associated with improvements in insulin sensitivity [93,94]. Exercise improves both outcomes and insulin sensitivity in the non-ischemic heart failure population [95]. Angiotensin-converting enzyme inhibitors, angiotensin receptor blockers, and statins all exert favorable effects on glucose metabolism [96,97]. Although beta-adrenergic blocking medications usually worsen insulin resistance, carvedilol has a neutral-to-slight insulin sensitizing effect [98]. Whether this difference contributes to the reported improvements in outcomes for patients treated with carvedilol, compared with metoprolol, remains unclear [99].

Glycemic control: Poor glycemic control has been associated with an increased risk of cardiovascular mortality, with an increase of 11% for every 1% rise in HbA1c levels [100], and other study has shown a link between HbA1c and heart failure [101]. Thus it has been assumed that improving glycemic control should have a beneficial effect on cardiovascular morbidity and mortality. Evidence suggests that good glycemic control is beneficial, at least in the early stages of myocardial dysfunction [102,103]. Evidence also suggests that diabetic cardiomyopathy does not develop in patients with tightly controlled type 1 diabetes, supporting an important role for hyperglycemia in the pathogenesis of diabetic cardiomyopathy [104]. Hyperglycemia is responsible for microvascular complications in diabetes, and because microvascular alterations are thought to contribute significantly to the pathogenesis of diabetic cardiomyopathy, good glycemic control is perhaps the most important component in the overall management of diabetic cardiomyopathy [74].

β-Blockers: Chronic stimulation of the sympathetic nervous system leads to increased heart rate and altered gene expression, resulting in cardiac remodeling in both heart failure and diabetes [105,106]. Traditionally, there has been a reluctance to use β-blockers in patients with diabetes for fear of adverse effects on insulin resistance and an unawareness of hypoglycemia. However, with the recent advances in the understanding of heart failure and the realization of the importance of the sympathetic nervous system in the release of vasoactive substances, they have become an essential treatment for heart failure. Thus β-blockers have been shown to prevent and even reverse cardiac remodeling, resulting in improved LV function and a reduction in mortality [107]. In summary, β-blockers should be given to all diabetic patients with any evidence of HF, unless specifically contra-indicated.

This will result in a relative risk reduction in mortality; however, the effect is not as pronounced as the introduction of β-blockers in non-diabetic patients, but both groups derive significant prognostic benefit [12].

Neurohormonal antagonism: ACE inhibitors form the cornerstone for treatment of heart failure. The captopril multi-centre study demonstrated a significant improvement in exercise capacity and symptoms of heart failure without an effect on mortality [108]. The CONSENSUS study group was the first to show a significant reduction in mortality with enalapril in patients with severe heart failure [109]. The SOLV-D investigators confirmed these findings and also showed that enalapril was able to prevent onset of new heart failure [110,111].

The important role of the renin-angiotensin-aldosterone system in the pathogenesis of complications in diabetic patients is well described. Evidence supports the use of angiotensin-converting enzyme inhibitors in preventing myocardial fibrosis, cardiac hypertrophy, and myocardial mechanical dysfunction associated with diabetic cardiomyopathy [112]. Angiotensin-converting enzyme inhibition and angiotensin- 1 receptor blockade also have been shown to prevent coronary perivascular fibrosis and collagen deposition [113].

ARBs (angiotensin II type 1 receptor blockers) have been proposed to have additive effects on haemodynamic measurements, neurohumoral activity and left ventricular remodeling when added to ACE inhibitors in patients with chronic heart failure.

Ca2+ channel antagonists: An early animal study demonstrated an improvement in diabetic cardiomyopathy with verapamil [114]; however, trials of verapamil, diltiazem and nifedipine have shown a detrimental effect in heart failure [115]. Amlodipine and felodipine were investigated in the PRAISE and Val-HeFT III trials respectively, and no significant benefit was observed over conventional treatment [116,117].

Statins: The safety and efficacy of statin therapy in patients with moderate to severe heart failure has been demonstrated [118]. There is a significant reduction in mortality in patients with a non-ischemic heart failure, adding further support to the additional effects of statins beyond their reduction in cholesterol and prevention of progression of CAD [118]. There is a need for a large randomized, blinded, placebo-controlled trial to evaluate further the benefits in patients with heart failure.

Modulators of free fatty acid metabolism, such as trimetazidine, have proven useful in the management of angina, but their efficacy on diabetic cardiomyopathy is unknown [74].

Thiazolidinediones (TZDs): TZDs are a class of compounds for treating patients with Type II diabetes mellitus, which act by increasing insulin sensitivity in skeletal muscle and adipose tissue through binding and activation of PPAR-δ, a nuclear receptor that has a regulatory role in differentiation of cells. Additionally they also act on PPAR-α and increase serum HDL (high-density lipoprotein)-cholesterol, decrease serum triacylglycerols (triglycerides) and increase LDL cholesterol levels marginally (pioglitazone to a lesser extent) [119]. The TZDs, apart from insulin-sensitizing fat and skeletal muscle, increase the

expression and function of glucose transporters in the heart, leading to improved glucose metabolism, and reduce FFA utilization by the myocardium [120]. Unfortunately, their clinical utility in the HF population is limited, owing to their promotion of fluid retention/edema, an effect mediated via activation of amiloride-sensitive sodium channels in the collecting duct [130]. Recent controversy has also arisen over a possible association between rosiglitazone (1 of 2 TZDs approved in the U.S.) and increased rates of myocardial infarction [131].

Currently, the most promising potential medical therapies can be divided into 2 broad categories—metabolic modulators and diabetic medications [25].

Metabolic modulators: The agents in this group increase myocardial efficiency by increasing glucose metabolism and decreasing FFA metabolism. Interestingly, 3 of the agents are used as antianginals; it is by increasing energy efficiency that these agents are believed to produce their antianginal effect. One of the most promising potential treatment agents is trimetazidine. This medication—currently available in Europe but not in the U.S.—works by inhibiting the final enzyme in beta-oxidation of FFA. Trimetazidine administration results in improved myocardial ATP/phosphocreatine levels, a marker for myocardial energy stores [25,121].

A second agent that works by inhibiting FFA metabolism is perhexiline. Like trimetazidine, perhexiline is also used as an antianginal agent in other countries but is not approved in the U.S. Unfortunately, clinical use of this agent might be limited, owing to risks of hepatotoxicity and peripheral neuropathy [25,122].

Ranolazine is a third antianginal agent with potential as a metabolic modulator and is approved in the U.S. Unfortunately, it might not be an ideal choice for 2 reasons: 1. Although ranolazine does cause a switch from FFA to glucose, the degree of this effect is relatively limited at physiologic levels. Its main mechanism of action involves lowering intracellular calcium levels via inhibition of a slow-inactivating sodium current. 2. Ranolazine is associated with QT prolongation, although increased rates of ventricular arrhythmias have not been observed [123,124].

L-carnitine is an essential cofactor of fatty acid metabolism, shuttling the end-products of peroxisomal fatty acid oxidation into the mitochondria and modulating the intramitochondrial acyl-coenzyme A/coenzyme A ratio. Although its main role is enhancement of FFA metabolism, experimental evidence also supports an enhancement of glucose metabolism. Several human and animal studies support a modest benefit in left ventricular energetics and function with L-carnitine administration [125,126].

Diabetic medications: If insulin resistance—the fundamental feature of most cases of type II diabetes mellitus— plays a principal role in the pathogenesis of dilated cardiomyopathy in many patients, then agents used to treat patients with diabetes mellitus might also be useful for the insulin-resistant cardiomyopathy population [25].

Metformin, the only biguanide approved in the U.S., prevents worsened glucose metabolism in a non-HF, insulin-resistant population and can improve calcium handling in myocytes [127,128]. However, its use in heart failure patients is limited by the possible potential for lactic acidosis, and a myocardial imaging study showed no improvement in

myocardial glucose uptake with metformin administration. The same study did show increased myocardial glucose uptake with the administration of a TZD [129]. These agents work by activating PPAR-, a transcription factor that promotes insulin sensitivity and decreases circulating FFAs. Interestingly, TZDs seem to affect the myocardium, despite the near-complete lack of PPAR-receptors in the myocardium, indicating that the effects on the myocardium are due to decreased circulating FFA [25,26].

Insulin or insulin-secretagogues represent a potential class of antidiabetic agents that could be used to treat an insulin-resistant cardiomyopathy population. A beneficial impact of these agents could theoretically be gleaned by directly promoting glucose metabolism and decreasing circulating FFA. However, therapy with such agents has generally failed to inhibit insulin-resistant cardiomyopathy in animal models and is less attractive than the insulin-sensitizing agents, because it fails to address the underlying physiologic problem of insulin resistance and exposes the patient to the potential negative effects of hyperinsulinemia [25].

Recently, a new class of antidiabetic medications has been developed that acts on the glucagon-like peptide (GLP)-1 pathway. Glucagon-like peptide-1 is 1 of 2 main "incretins" in the body—hormones that promote post-prandial insulin secretion and improved insulin sensitivity [132]. Unfortunately, GLP-1 is impractical as a pharmacological therapy, because it is rapidly degraded in vivo by dipeptidyl peptidase (DPP)-IV, resulting in a 1- to 2-min half-life. Another option, exenatide, shares 53% homology with GLP-1 and works as a partial agonist of the GLP-1 receptor [132]. An alternative to administering a GLP-1 agonist is administering a DPP-IV antagonist. The first agent in this class, sitagliptin, was approved in the U.S. in October 2006, and several others are in development.

5. Cardiac Rehabilitation

Cardiac rehabilitation program is defined as a long-term program involving medical evaluation, prescribed exercise, cardiac risk factor modification, education and counseling. These programs are designed to limit the physiological and psychological effect of cardiac illness, reduce the risk of sudden death or recurrent ischemia, control cardiac symptoms, stabilize or reverse the atherosclerotic process, and enhance the psychosocial and vocational status of selected patients. Cardiac rehabilitation programs are prescribed for patients who have had a myocardial infarction, have had coronary bypass surgery, or have chronic stable angina pectoris [134].

The European Society of Cardiology defines cardiac rehabilitation program as the sum of interventions required to ensure the best physical, psychological and social conditions so that patients with chronic or post acute cardiac disease may, by their own efforts, preserve or assume their proper place in society [135].

The words "chronic" and "preserve" were added to the previous definition of the World Health Organization (WHO) in order to stress the concept of the importance of rehabilitation in the long term care of patients with chronic disease, including those who had not had recent acute events.

Cardiovascular health is achieved through interventions to enhance vascular protection. The use of these interventions can only be effective when patients and health professionals

are able to know the risk of recurrent vascular events, the treatment targets, the therapies needed and lifestyle modifications, reaching thus success in goal [136].

The promise of cardiac rehabilitation is that interventions such as exercise and risk factor reduction can achieve reduced cardiac events and mortality, likelihood of hospitalizations and need for invasive procedure [137,138]. Rehabilitation has improved cardiac mortality in patients with cardiovascular disease and has proved that the addiction of exercise to the standard pharmacological interventions currently recommended and prescribed can still produce additional patient benefits, notably after acute coronary events [138]. Several trials have shown significant improvements in cardiovascular risk factors and cardiovascular outcomes and have also documented benefits of cardiac rehabilitation in populations over the age 70 years [136,139].

Table 1. Core elements of the Canadian guideline of cardiac rehabilitation [136]

Patient referral process
– Patient assessment – Risk stratification – Exercise stress testing – Risk factor assessment
Lifestyle and risk factor modification
Nutritional counseling
– Risk factor counseling and management – Lipids – Hypertension – Smoking cessation – Diabetes – Psychosocial issues – Weight management, particularly abdominal obesity – Psychosocial management – Physical activity counseling and exercise training
Patient education programs – Lifestyle adherence strategies – Medication adherence strategies
Outcomes assessment programs – Health outcomes – Educational outcomes – Behavioral outcomes – Service outcomes
Continuous quality improvement programs
Continuous professional development programs

Cardiovascular rehabilitation showed positive results in reducing inflammatory cytokines, plasma fibrinogen concentrations, platelet aggregation, glucose intolerance, serum LDL, serum triglycerides and systolic blood pressure [136,140,141]. Besides, beneficial effects of cardiac rehabilitation may also be attributed to the comprehensive, proactive chronic disease management with respect to improved cardiovascular disease risk factor

management, and improved patient adherence to lifestyle interventions and prescribed medications [142].

Thus, both exercise and comprehensive, target-driven risk factor reduction can have significant benefits on patient outcomes, and the combination of the two is fully developed and completely expressed within contemporary cardiac rehabilitation programs [136].

Cardiac rehabilitation is a disease intervention whose success is derived not only from what interventions or therapies are prescribed but equally fro the process of how those interventions or therapies are delivered to patients.

The Canadian guideline of cardiac rehabilitation [136] proposes the following core elements of these programs:

- **Patient referral process:** to make easy the identification and inclusion of the patients in the program of cardiac rehabilitation. The use of algorithms and the commitment of health professional is necessary.

- **Patient assessment:** this include the welcome of the patient, being carried out a focused history and physical examination with particular emphasis on cardiovascular symptoms, exercise limitations, psychosocial problems and evidence of significant valvular heart disease or heart failure. Besides, accurate risk stratification is necessary, with the inclusion of exercise stress testing if necessary.

- **Lifestyle and risk factor modification:** any patient debit to receive a personalized exercise prescription and lifestyle recommendations focusing specifically on those risk factors relevant for each patient.
- **Nutritional counseling:** health professional should recommend a healthy diet bases on characteristics of each patient. A specialized professional can be necessary.

- **Risk factor counseling and management:** eliminate modifiable risk factors like smoking and dietary errors are a challenge. Cardiac rehabilitation staff should have a basic working familiarity with the principles of the model of behavior change, social cognitive theory and motivational interviewing techniques.

Persons with predominant abdominal adiposity (waist circumference larger than 102 cm in men and 88cm in women) are much more likely to have the metabolic syndrome, and cardiac rehabilitation professionals are reminded to measure the wais girth o all patients [143].

Physical inactivity and sedentary lifestyle are the most important, and seemingly the most prevalent, fundamental causes of atherosclerotic vascular disease. Physical inactivity worsens and potentiates the adverse effects of other cardiovascular risk factors such as hypertension, diabetes and dyslipidemia [144].

Depression and anxiety often complicate cardiovascular events and cause distress in their own right. Besides, depression reduces participation in cardiac rehabilitation programs [145,146].

- **Patient education programs:** to help patients to understand cardiovascular disease and improve lifestyle modifications and adherence to the program.

There are three recognized phases of cardiac rehabilitation [133].

Inpatient rehabilitation (Phase 1): Inpatient rehabilitation is now mostly limited to early mobilization, so that self care is possible by discharge, and brief counseling to explain the nature of the illness or intervention, to increase the patient's awareness of his or her risk factors and to reassure the patient about future progress and follow-up.

Ambulatory outpatient rehabilitation (Phase 2): Most cardiac rehabilitation is based upon supervised ambulatory outpatient programs conducted during convalescence. Attendance begins soon after discharge from hospital, ideally within the first few days.

Formal outpatient cardiac rehabilitation programs vary widely in content. Almost all contain an element of group exercise which is conducted by allied health professionals. Therefore, an educational and supportive element is inevitably delivered together with the exercise. Psychological and social support may be given on an individual basis, as required, or may be provided to groups of patients and family members.

Maintenance (Phase 3): A lifetime, maintenance stage follows the ambulatory program in which physical fitness and risk factor control are supported in a minimally supervised or unsupervised setting. They may consist of regular recall and review by physician or nurse. Patients may receive additional medication, further education, social support, exercise classes and behavioral intervention, as required. Some patients may be enrolled in special groups for specific reasons (for example, diabetes, obesity, smoking, lipid disorder, hypertension, heart failure) if clinics are established for the management of these particular risk factors or conditions. In other programs, patients may be enrolled in an ongoing exercise class.

The World Health Organization Expert Committee report "Rehabilitation after Cardiovascular Diseases, with Special Emphasis on Developing Countries" of 1993 [147] made the following recommendations:

1. Cardiac rehabilitation should be an integral component of the long-term, comprehensive care of cardiac patients.
2. Cardiac rehabilitation programs or services should be available to all patients with cardiovascular disease, both children and adults.
3. Rehabilitation services should be provided by any trained health professional caring for cardiac patients, since no sophisticated equipment or facilities are required. Both patients and their families should participate.
4. Rehabilitation programs should be integrated into the existing health care system; this can be done at modest cost. The major requirement is for health professionals to be trained in prescribing appropriate exercise and providing health education and vocational guidance.
5 Responsibility for the implementation of cardiac rehabilitation should be given to a designated health professional at the local level, trained as a coordinator. This

	individual should, in turn, be responsible to an appropriate physician or to a department, hospital, or other health care facility, which may operate under the auspices of the government or a nongovernmental organization or other agency.
6	All plans for the implementation of rehabilitative programs should include provision for evaluating the efficacy of the programs

The conclusion from the guidelines and policy statements is that cardiac rehabilitation services should be available to all patients with cardiac and vascular disease. There is uniformity of opinion to support the view that cardiac rehabilitation should include exercise, education, social support, behavioral change, follow-up of patients and program evaluation. However, there are significant differences between regions regarding specific aspects of the content of these programs; that is "how much of what and for whom?" and in methods of delivering programs [133].

Exercise in cardiac rehabilitation: Widely accepted recommendations regarding exercise training have come from many authoritative sources based upon literature review and consensus [133,135,148]. It is also well recognized that physical performance spontaneously recovers through resumption of normal activities after a period of physical inactivity following acute myocardial infarction or other illnesses. However, trials have demonstrated that exercise training produces a significantly more rapid recovery of physical function. Although previously thought to have been hazardous, progressive resistive exercise training is now recommended, particularly for those who have become inactive and weakened by muscle wasting [133]. There are clinical trials demonstrating improvement in psychological functioning (anxiety, depression and other measures) from exercise training alone, compared with standard medical care [149,150]. The benefit is more apparent with multifactorial rehabilitation.

Exercise training is recommended to improve subsequent exercise habits. However, programs should be followed by long-term availability of support and facilities for maintenance of activity. Exercise training extending beyond convalescence, with a maintenance or follow-up (phase 3) program is recommended to reduce morbidity, recurrent events, hospital readmissions and mortality. The evidence for these claims for secondary prevention comes from some studies with long-term follow-up support and from meta-analyses [151,152]. As suggested earlier, it may be that some benefits arise not from the exercise training itself, but from the comprehensive nature of the interventions.

Low to moderate intensity exercise training is recommended for all cardiac rehabilitation programs. Exercise training at low to moderate intensity has effects similar to those of moderate to high intensity exercise training. From these trials, it appears that a higher level of supervised exercise training has a small, positive relationship to maximal physical working capacity at the completion of the exercise program, but no significant difference is achieved in the long term. Thus, while the process of reconditioning appears to be accelerated through high intensity exercise, it is not associated with any recognizable or demonstrable other benefit. It is therefore reasonable to conclude that low to moderate intensity exercise is, with the single exception of physical working capacity, as effective as high intensity exercise, provided that home activity (particularly walking) is encouraged and undertaken [133,153].

Until recently, it was recommended that high risk patients should not be enrolled in exercise training programs (based upon moderate to high intensity exercise). Several trials and observational studies have now shown, however, that low levels of exercise lead to improvement in physical functioning and quality of life. This applies to patients with impaired ventricular function, with controlled cardiac failure and with symptomatic or asymptomatic residual ischemia.

Exclusion factors are serious conditions requiring attention before exercise is commenced. These are [148]:

- Significant hypertension or hypotension
- Severe aortic stenosis
- Uncontrolled arrhythmias
- Uncontrolled congestive heart failure
- Uncontrolled diabetes or metabolic disturbance
- High grade atrioventricular block without a pacemaker
- Current pericarditis or myocarditis
- Recent pulmonary or other embolism
- Recent stroke or transient ischemic attack
- Recent major surgery
- Terminal illness or severe disabling concurrent illness
- Acute febrile or systemic illness
- Physical or psychological disability preventing participation

An additional reason for exclusion is physician or patient refusal

Clinical risk stratification based upon history, examination and resting electrocardiogram is usually sufficient. Technological investigation of patients should be limited to specific tests to answer specific clinical questions applicable to individuals [133]. When necessary, complementary investigation must be performed before the onset of an exercise program.

6. Conclusions

Diabetic cardiomyopathy became a concrete reality after investigations performed in the last three decades. Diverse pathophysiologic mechanisms have been proposed to explain this condition, but hyperglycemia is the main candidate to be the process initiator. Research in the field of metabolic and structural changes has reached the development of therapeutic options and new medication must be used in the future.

Despite the absence of clear instruments for the diagnosis, diabetic cardiomyopathy should not be ignored by the clinicians and the rigorous control of diabetes is probably the better tool against the disease at the moment.

References

[1] Zimmet, P; Alberti, MM; Shaw, J. Global and societal implications of the diabetes epidemic. *Nature,* 2001; 414, 782–7.

[2] Davidoff, AJ. Convergence of glucose and fatty acid-induced abnormal myocardial excitation-contraction coupling and insulin signaling. *Clinical and Experimental Pharmacology and Physiology*, 2006, 33, 152–158.

[3] International Diabetes Federation (online 2006) Diabetes Atlas, 3rd edition [http://www.eatlas.idf.org/ media].

[4] Lebeche, D; Davidoff, AJ; Hajjar, RJ. Interplay between impaired calcium regulation and insulin signaling abnormalities in diabetic cardiomyopathy. *Nat Clin Pract Cardiovasc Med*., 2008, Nov;5(11), 715-24.

[5] Wild, S; Roglic, G; Green, A; Sicree, R; King, H. Global prevalence of diabetics: estimates for the year 2000 and projections for 2030. *Diabetes Care,* 2004, 27, 1047-53.

[6] King, H; Aubert, RE; Herman, WH. Global burden of diabetes 1995–2025: prevalence, numerical estimates and projections. *Diabetes Care,* 1998, 21, 1414–1431.

[7] Tziakas, DN; Chalikias, GK; Kaski, JC. Epidemiology of the diabetic heart. *Coronary Artery Disease,* 2005, Vol 16 (Suppl 1).

[8] Unwin, N; Shaw, J; Zimmet, P; Alberti, KG. Impaired glucose tolerance and impaired fasting glycaemia: the current status on definition and intervention. *Diabetic,* 2002, 19, 708–723.

[9] Meigs, JB. Epidemiology of cardiovascular complications in type 2 diabetes mellitus. *Acta Diabetol,* 2003, 40, S358–S361.

[10] Sowers, JR. et al. Diabetes, hypertension, and cardiovascular disease: an update. *Hypertension,* 2001, 7, 1053–1059.

[11] Boudina, S; Abel, ED. Diabetic cardiomyopathy revisited. *Circulation,* 2007, 115, 3213–3223.

[12] Hayat, SA; Patel, B; Khattar, RS; Malik, RA. Diabetic cardiomyopathy: mechanisms, diagnosis and treatment. *Clinical Science,* 2004, 107, 539–557.

[13] Kannel, WB; Hjortland, M; Castelli, WP. Role of diabetes in congestive heart failure: The Framingham Study. *Am. J. Cardiol*., 1976, 34, 29–34.

[14] Capes, SE; Hunt, D; Malmberg, K; Gerstein, HC. Stress hyperglycaemia and increased risk of death after myocardial infarction in patients with and without diabetes: a systematic overview. *Lancet,* 2000, 355, 773–778.

[15] Reis, SE; Holubkov, R; Edmundowicz, D; McNamara, DM; Zell, KA; Detre, KM., et al. Treatment of patients admitted to the hospital with congestive heart failure: specialty related disparities in practice patterns and outcomes. *J Am Coll Cardiol,* 1997, 30, 733–738.

[16] Bell, D. Diabetic cardiomyopathy. *Diabetes Care,* 2003, 26, 2949–2951.

[17] Rubler, S; Dlugash, J; Yuceoglu, YZ; Kumral, T; Branwood, AW; & Grishman, A. New type of cardiomyopathy associated with diabetic glomerulosclerosis. *Am. J. Cardiol*., 1972, 30, 595–602.

[18] Poornima, IG; Parikh, P; Shannon, RP. Diabetic Cardiomyopathy - The Search for a Unifying Hypothesis. *Circulation Research*. 2006, 98, 596-605.

[19] Fang, ZY; Prins, JB; Marwick, TH. Diabetic Cardiomyopathy: Evidence, Mechanisms, and Therapeutic Implications. *Endocrine Reviews*, 2004, 25(4), 543–567.

[20] Galderisi, M; et al. Echocardiographic evidence for the existence of a distinct diabetic cardiomyopathy (the Framingham Heart Study). *Am J Cardiol*, 1991, 68, 85–89.

[21] Fox, CS; et al. Increasing cardiovascular disease burden due to diabetes mellitus: the Framingham Heart Study. *Circulation*, 2007, 115, 1544–1550.

[22] Konduracka, E, Gackowski, A, Rostoff, P, et al. Diabetes-specific cardiomyopathy in type 1 diabetes mellitus: no evidence for its occurrence in the era of intensive insulin therapy. *Eur Heart J*, 2007, 28, 2465.

[23] Severson, D. Diabetic cardiomyopathy: recent evidence from mouse models of type 1 and type 2 diabetes. *Can J Physiol Pharmacol*, 2004, 82, 813–823.

[24] Gargiulo, P; Jacobellis, G; Vaccari, V; Andreani, D. Diabetic cardiomyopathy: Pathophysiological and clinical aspects. *Diabetes Nutr. Metab*., 1998, 11, 336–46.

[25] Witteles, RM; Fowler, MB. Insulin-resistant cardiomyopathy clinical evidence, mechanisms, and treatment options. *J Am Coll Cardiol*, 2008, 51, 93–102.

[26] An, D; Rodrigues, B. Role of changes in cardiac metabolism in development of diabetic cardiomyopathy. *Am J Physiol Heart Circ Physiol*, 2006, 291, 1489-1506.

[27] Hayat, SA; Patel, B; Khattar, RS; Malik, RA. Diabetic cardiomyopathy: mechanisms, diagnosis and treatment. *Clin Sci.*, 2004, 107, 539-57.

[28] Rodrigues, B; Cam, MC; McNeill, JH. Metabolic disturbances in diabetic cardiomyopathy. *Mol Cell Biochem*, 1998, 180, 53–57.

[29] Ohtake, T; Yokoyama, I; Watanabe, T; Momose, T; Serezawa, T; Nishikawa, J; Sasaki, Y. Myocardial glucose metabolism in noninsulin-dependent diabetes mellitus patients evaluated by FDG-PET. *J Nucl Med.*, 1995, 36, 456–463.

[30] Garvey, WT; Hardin, D; Juhaszova, M; Dominguez, JH. Effects of diabetes on myocardial glucose transport system in rats: implications for diabetic cardiomyopathy. *Am J Physiol*, 1993, 264, H837–H844.

[31] Liedtke, AJ; DeMaison, L; Eggleston, AM; Cohen, LM; Nellis, SH. Changes in substrate metabolism and effects of excess fatty acids in reperfused myocardium. *Circ Res.*, 1988, 62, 535–542.

[32] Rodriques, B; Cam, MC; Kong, J; Goyal, RK; McNeill, JH. Strain differences in susceptibility to streptozotocin-induced diabetes: Effects on hypertriglyceridemia and cardiomyopathy. *Cardiovasc Res.*, 1997, 34, 199–205.

[33] Shulman, GI. Cellular mechanisms of insulin resistance. *J Clin Invest*., 2000, 106, 171–176.

[34] Birnbaum, MJ. Turning down insulin signaling. *J Clin Invest*., 2001, 108, 655– 659.

[35] Yazaki, Y; Isobe, M; Takahashi, W; Kitabayashi, H; Nishiyama, O; Sekiguchi, M; Takemura, T. Assessment of myocardial fatty acid abnormalities in patients with idiopathic dilated cardiomyopathy using I123 BMIPP SPECT: correlation with clinicopathological findings and clinical course. *Heart*, 1999, 81, 153–159.

[36] Belke, DD; Larsen, TS; Gibbs, EM; Severson, DL. Altered metabolis causes cardiac dysfunction in perfused hearts from diabetic (db/db mice. *Am J Physiol Endocrinol Metab*, 2000, 279, E1104–E1113.

[37] Zhou, YT; Grayburn, P; Karim, A; Shimabukuro, M; Higa, M; Baetens, D; Orci, L; Unger, RH. Lipotoxic heart disease in obese rats: implications for human obesity. *Proc Natl Acad Sci USA*, 2000, 97, 1784–1789.

[38] Wong, C; Marwick, TH. Obesity cardiomyopathy: pathogenesis and pathophysiology. *Nature Clinical Practice*, 2007, vol 4 no 8.

[39] Listenberger, LL; Schaffer, JE. Mechanisms of lipoapoptosis: implications for human heart disease. *Trends Cardiovasc Med.*, 2002, 12, 134–138.

[40] Pillutla, P; Hwang, YC; Augustus, A; Yokoyama, M; Yagyu, H; Johnston, TP; Kaneko, M; Ramasamy, R; Goldberg, IJ. Perfusion of hearts with triglyceride-rich particles reproduces the metabolic abnormalities in lipotoxic cardiomyopathy. *Am J Physiol Endocrinol Metab*, 288, E1229–E1235, 2005.

[41] Finck, BN; Lehman, JJ; Leone, TC; Welch, MJ; Bennett, MJ; Kovacs, A; Han, X; Gross, RW; Kozak, R; Lopaschuk, GD; Kelly, DP. The cardiac phenotype induced by PPARalpha overexpression mimics that caused by diabetes mellitus. *J Clin Invest*, 2002, 109, 121–130.

[42] Christoffersen, C; Bollano, E; Lindegaard, ML; Bartels, ED; Goetze, JP; Andersen, CB; Nielsen, LB. Cardiac lipid accumulation associated with diastolic dysfunction in obese mice. *Endocrinology*, 2003, 144, 3483–3490.

[43] Sharma, S; Adrogue, JV; Golfman, L; Uray, I; Lemm, J; Youker, K; Noon, GP; Frazier, OH; Taegtmeyer, H. Intramyocardial lipid accumulation in the failing human heart resembles the lipotoxic rat heart. *FASEB J*, 2004, 18, 1692–1700.

[44] Van Herpen, NA; Schrauwen-Hinderling, VB. Lipid accumulation in non-adipose tissue and lipotoxicity. *Physiology & Behavior*, 2008, 94, 231–241.

[45] Kankaanpaa, M; Lehto, HR; Parkka, JP; Komu, M; Viljanen, A; Ferrannini, E, et al. Myocardial triglyceride content and epicardial fat mass in human obesity: relationship to left ventricular function and serum free fatty acid levels. *J Clin Endocrinol Metab*, 2006, 91, 4689–95.

[46] Shulman, GI. Cellular mechanisms of insulin resistance. *J Clin Invest*., 2000, 106, 171–176.

[47] Katz, EB; et al. Cardiac and adipose tissue abnormalities but not diabetes in mice deficient in GLUT4. *Nature*, 1995, 377, 151–155.

[48] Chiu, HC; et al. A novel mouse model of lipotoxic cardiomyopathy. *J Clin Invest*, 2001, 107, 813–822.

[49] Shah, A; Shannon, RP. *Insulin resistance in dilated cardiomyopathy*. Rev Cardiovasc Med., 2003, 4 Suppl 6, S50 –7.

[50] Davila-Roman, VG; Vedala, G; Herrero, P; et al. Altered myocardial fatty acid and glucose metabolism in idiopathic dilated cardiomyopathy. *J Am Coll Cardiol*, 2002, 40, 271–7.

[51] Taegtmeyer, H. Switching metabolic genes to build a better heart. *Circulation*, 2002, 106, 2043–5.

[52] Osorio, JC; Stanley, WC; Linke, A; et al. Impaired myocardial fatty acid oxidation and reduced protein expression of retinoid X receptoralpha in pacing-induced heart failure. *Circulation*, 2002, 106, 606 –12.

[53] How, OJ; Aasum, E; Severson, DL; Chan, WY; Essop, MF; Larsen, TS. Increased myocardial oxygen consumption reduces cardiac efficiency in diabetic mice. *Diabetes*, 2006, 55, 466 –73.

[54] Tuunanen, H; Engblom, E; Naum, A; et al. Decreased myocardial free fatty acid uptake in patients with idiopathic dilated cardiomyopathy: evidence of relationship with insulin resistance and left ventricular dysfunction. *J Card Fail*, 2006, 12, 644 –52.

[55] An, D; Rodrigues, B. Role of changes in cardiac metabolism in development of diabetic cardiomyopathy. *Am J Physiol Heart Circ Physiol*, 2006, 291, H1489–506.

[56] Finck, BN; Han, X; Courtois, M; et al. A critical role for PPARalphamediated lipotoxicity in the pathogenesis of diabetic cardiomyopathy: modulation by dietary fat content. *Proc Natl Acad Sci USA*, 2003, 100, 1226 –31.

[57] Taegtmeyer, H; McNulty, P; Young, ME. Adaptation and maladaptation of the heart in diabetes: part I: general concepts. *Circulation*, 2002, 105, 1727–33.

[58] Young, ME; McNulty, P; Taegtmeyer, H. Adaptation and maladaptation of the heart in diabetes: part II: potential mechanisms. *Circulation*, 2002, 105, 1861–70.

[59] Buchanan, J; Mazumder, PK; Hu, P; et al. Reduced cardiac efficiency and altered substrate metabolism sprecedes the onset of hyperglycemia and contractile dysfunction in two mouse models of insulin resistance and obesity. *Endocrinology*, 2005, 146, 5341–9.

[60] Davidoff, AJ; Mason, MM; Davidson, MB; et al. Sucrose-induced cardiomyocyte dysfunction is both preventable and reversible with clinically relevant treatments. *Am J Physiol Endocrinol Metab*, 2004, 286, E718 –24.

[61] Dutta, K; Podolin, DA; Davidson, MB; Davidoff, AJ. Cardiomyocyte dysfunction in sucrose-fed rats is associated with insulin resistance. *Diabetes*, 2001, 50, 1186 –92.

[62] Suskin, N; McKelvie, RS; Burns, RJ; et al. Glucose and insulin abnormalities relate to functional capacity in patients with congestive heart failure. *Eur Heart J*, 2000, 21, 1368 –75.

[63] Du, X; Matsumura, T; Edelstein, D; Rossetti, L; Zsengeller, Z; Szabo, C; & Brownlee, M. Inhibition of GAPDH activity by poly(ADP-ribose) polymerase activates three major pathways of hyperglycemic damage in endothelial cells. *J Clin Invest*, 112, 1049–1057, 2003.

[64] Lagadic-Gossmann, DL; Buckler, KJ; Le Prigent, K; Feuvray, D. Altered Ca2+ handling in ventricular myocytes isolated from diabetic rats. *Am. J. Physiol. Heart Circ. Physiol*. 1996, 270, H1529–37.

[65] Ren, J; Davidoff, AJ. Diabetes rapidly induces contractile dysfunctions in isolated ventricular myocytes. *Am. J. Physiol. Heart Circ. Physiol*. 1997, 272, H148–58.

[66] Zhong, Y; et al. Altered SR protein expression associated with contractile dysfunction in diabetic rat hearts. *Am J Physiol Heart Circ Physiol*, 2001, 281, H1137–H1147.

[67] Wold, LE; et al. Impaired SERCA function contributes to cardiomyocyte dysfunction in insulin resistant rats. *J Mol Cell Cardiol*, 2005, 39, 297–307.

[68] Dutta, K; et al. Depressed PKA activity contributes to impaired SERCA function and is linked to the pathogenesis of glucose-induced cardiomyopathy. *J Mol Cell Cardiol*, 2002, 34, 985–996.

[69] Davidoff, AJ; et al. Diabetic cardiomyocyte dysfunction and myocyte insulin resistance: role of glucose-induced PKC activity. *Mol Cell Biochem*, 2004, 262, 155–163.

[70] Shimoni, Y; et al. Short-term diabetes alters K+ currents in rat ventricular myocytes. *Circ Res.*, 1994, 74, 620–628.

[71] Yu, J; et al. Insulin improves cardiomyocyte contractile function through enhancement of SERCA2a activity in simulated ischemia/reperfusion. *Acta Pharmacol*, 2006, Sin 27, 919–926.

[72] Bidasee, KR; et al. Diabetes increases formation of advanced glycation end products on sarco(endo)plasmic reticulum Ca2+-ATPase. *Diabetes*, 2004, 53, 463–473.

[73] Bidasee, KR; et al. Chronic diabetes increases advanced glycation end products on cardiac ryanodine receptors/calcium-release channels. *Diabetes*, 2003, 52, 1825–1836.

[74] Aneja, A; Tang, WH; Bansilal, S; Garcia, MJ; Farkouh, ME. Diabetic Cardiomyopathy: Insights into Pathogenesis, Diagnostic Challenges, and Therapeutic Options. *American Journal of Medicine*, September 2008, Vol 121, No 9.

[75] Tesfamariam, B; Brown, ML; Cohen, RA. Elevated glucose impairs endothelium-dependent relaxation by activating protein kinase C. *J Clin Invest*., 1991, 87, 1643-1648.

[76] Rossen, JD. Abnormal microvascular function in diabetes: relationship to diabetic cardiomyopathy. *Coron Artery Dis.*, 1996, 7, 133-8.

[77] Zoneraich, S; Silverman, G; Zoneraich, O. Primary myocardial disease, diabetes mellitus, and small vessel disease. *Am Heart J.*, 1980, 100, 754-5.

[78] Chaves, FR; Jorge, PAR. Miocardiopatia diabética. *Arq Bras Endocrinol Metab*, 1998, 42, 134-9.

[79] Galderisi, M. Diastolic dysfunction and diabetic cardiomyopathy: evaluation by Doppler echocardiography. *J Am Coll Cardiol*, 2006, 48, 1548.

[80] Johnstone, MT; Craeger, SJ; Scales, KM; et al. Impaired endothelium-dependent vasodilatation in patients with insulin-dependent diabetes mellitus. *Circulation*, 1993, 88, 2510.

[81] Bucala, R; Tracey, KJ; Cerami, A. Advanced glycosylation products quench nitric oxide and mediate defective endothelium-dependent vasodilatation in experimental diabetes. *J Clin Invest*, 1991, 87, 432.

[82] Sebbag, L; Forrat, R; Canet, E; et al. Effects of experimental non-insulin requiring diabetes on myocardial microcirculation during ischemia in dogs. *Eur J Clin Invest*, 1994, 24, 686.

[83] Pop-Busui, R; Kirkwood, I; Schmid, H; et al. Sympathetic dysfunction in type 1 diabetes: association with impaired myocardial blood flow reserve and diastolic dysfunction. *J Am Coll Cardiol*, 2004, 44, 2368–74.

[84] Quinones, MJ; Hernandez-Pampaloni, M; Schelbert, H; et al. Coronary vasomotor abnormalities in insulin-resistant individuals. *Ann Intern Med.*, 2004, 140, 700–8.

[85] Iozzo, P; Chareonthaitawee, P; Dutka, D; Betteridge, DJ; Ferrannini, E; Camici, PG. Independent association of type 2 diabetes and coronary artery disease with myocardial insulin resistance. *Diabetes*, 2002, 51, 3020–4.

[86] Schannwell, CM; Schneppenheim, M; Pering, S; Plehn, G; Strauer, BE. Left ventricular diastolic dysfunction as an early manifestation of diabetic cardiomyopathy. *Cardiology*, 2002, 98, 33-9.

[87] Mathew, P; John, L; Jose, J; Krishnaswami, S. Assessment of left ventricular diastolic function in young diabetics–a two dimensional echo Doppler study. *Indian Heart J.*, 1992, 44, 29–32.

[88] Posner, J; Ilya, R; Wanderman, K; Weitzman, S. Systolic time intervals in diabetes. *Diabetologia*, 1983, 24, 249–252.

[89] Naito, J; Koretsune, Y; Sakamoto, N; Shutta, R; Yoshida, J; Yasuoka, Y; Yoshida, S; Chin, W; Kusuoka, H; Inoue, M. Transmural heterogeneity of myocardial integrated backscatter in diabetic patients without overt cardiac disease. *Diabetes Res Clin Pract*, 2001, 52, 11–20.

[90] Hileeto, D; Cukiernik, M; Mukherjee, S; Evans T; Barbin Y; Downey D; Karmazyn M; Chakrabarti S. Contributions of endothelin-1 and sodium hydrogen exchanger-1 in the diabetic myocardium. *Diabetes Metab Res Rev.*, 2002, 18, 386–394.

[91] Howarth, FC; Qureshi, MA; White, E; Calaghan, SC. Cardiac microtubules are more resistant to chemical depolymerisation in streptozotocin-induced diabetes in the rat. *Pflugers Arch*, 2002, 444, 432–437.

[92] Warley, A; Powell, JM; Skepper, JN. Capillary surface area is reduced and tissue thickness from capillaries to myocytes is increased in the left ventricle of streptozotocin-diabetic rats. *Diabetologia*, 1995, 38, 413–421.

[93] Knowler, WC; Barrett-Connor, E; Fowler, SE; et al. Reduction in the incidence of type 2 diabetes with lifestyle intervention or metformin. *N Engl J Med.*, 2002, 346, 393– 403.

[94] Tuomilehto, J; Lindstrom, J; Eriksson, JG; et al. Prevention of type 2 diabetes mellitus by changes in lifestyle among subjects with impaired glucose tolerance. *N Engl J Med.*, 2001, 344, 1343–50.

[95] Kemppainen, J; Tsuchida, H; Stolen, K; et al. Insulin signalling and resistance in patients with chronic heart failure. *J Physiol*, 2003, 550, 305–15.

[96] Freeman, DJ; Norrie, J; Sattar, N; et al. Pravastatin and the development of diabetes mellitus: evidence for a protective treatment effect in the West of Scotland Coronary Prevention Study. *Circulation*, 2001, 103, 357– 62.

[97] Pfeffer, MA; Swedberg, K; Granger, CB; et al. Effects of candesartan on mortality and morbidity in patients with chronic heart failure: the CHARM-Overall programme. *Lancet*, 2003, 362, 759–66.

[98] Bakris, GL; Fonseca, V; Katholi, RE; et al. Metabolic effects of carvedilol vs metoprolol in patients with type 2 diabetes mellitus and hypertension: a randomized controlled trial. *JAMA*, 2004, 292, 2227–36.

[99] Poole-Wilson, PA; Swedberg, K; Cleland, JG; et al. Comparison of carvedilol and metoprolol on clinical outcomes in patients with chronic heart failure in the Carvedilol

Or Metoprolol European Trial (COMET): randomised controlled trial. *Lancet*, 2003, 362, 7–13.

[100] Adler, AI; Neil, HA; Manley, SE; Holman, RR; Turner, RC. Hyperglycemia and hyperinsulinemia at diagnosis of diabetes and their association with subsequent cardiovascular disease in the United Kingdom prospective diabetes study *Am. Heart J*, 1999, 138, S353–S359.

[101] Iribarren, C; Karter, AJ; Go, AS. et al. Glycaemic control and heart failure among adult patients with diabetes. *Circulation*, 2001, 103, 2668–2673.

[102] Von Bibra, H; Hansen, A; Dounis, V; et al. Augmented metabolic control improves myocardial diastolic function and perfusion in patients with non-insulin dependent diabetes. *Heart*, 2004, 90, 1483-1484.

[103] Von Bibra, H; Siegmund, T; Hansen, A; et al. Augmentation of myocardial function by improved glycemic control in patients with type 2 diabetes mellitus. *Dtsch Med Wochenschr*. 2007, 132, 729-734.

[104] Konduracka, E; Gackowski, A; Rostoff, P; et al. Diabetes-specific cardiomyopathy in type 1 diabetes mellitus: no evidence for its occurrence in the era of intensive insulin therapy. *Eur Heart J*. 2007, 28, 2465-2471.

[105] Mann, DL; Kent, RL; Parsons, B; et al. Adrenergic effects on the biology of the adult mammalian cardiocyte. *Circulation*, 2000, 85, 624–628.

[106] Festa, A; D'Agostino, Jr R; Hales, CN; Mykkanen, L; Haffner, SM. Heart rate in relation to insulin sensitivity and insulin secretion in non-diabetic subjects. *Diabetes Care*, 2000, 23, 624–628.

[107] Lowes, BD; Gill, EA; Abraham, WT. Effects of carvedilol on left ventricular function mass, chamber geometry, and mitral regurgitation in chronic heart failure. *Am. J. Cardiol*., 83, 1201–1205.

[108] Captopril Multicentre Research Group. A placebo controlled trial of captopril in refractory chronic congestive heart failure. *J. Am. Coll. Cardiol*, 1983, 2, 755–763.

[109] The CONSENSUS Trial study Group. Effects of enalapril on mortality in severe congestive heart failure: results of the Cooperative North Scandinavian Enalapril Survival Study (CONSENSUS*). N. Engl. J. Med.*, 1987, 316, 1429–1435.

[110] The SOLVD Investigators. Effects of enalapril on survival in patients with reduced left ventricular ejection fractions. *N Engl J Med.*, 1991, 325, 293–302.

[111] The SOLVD Investigators. Effects of enalapril on mortality and the development of heart failure in asymptomatic patients with reduced left ventricular ejection fractions. *N Engl J Med.*, 1992, 327, 669–677.

[112] Al-Shafei, AI; Wise, RG; Gresham, GA; et al. Non-invasive magnetic resonance imaging assessment of myocardial changes and the effects of angiotensin-converting enzyme inhibition in diabetic rats. *J Physiol*., 2002, 538(Pt 2), 541-553.

[113] Zaman, AK; Fujii, S; Goto, D; et al. Salutary effects of attenuation of angiotensin II on coronary perivascular fibrosis associated with insulin resistance and obesity. *J Mol Cell Cardiol*., 2004, 37, 525-535.

[114] Afzal, N; Ganguly, PK; Dhalla, KS; Pierce, GN; Singal, PK; Dhalla, NS. Beneficial effects of verapamil in diabetic cardiomyopathy. *Diabetes*, 1988, 37, 936–942.

[115] Parameshwar, J; Poole-Wilson, P. The role of calcium antagonists in the treatment of chronic heart failure. *Eur Heart J,* 1993, 14, 38–44.

[116] Packer, M; O'Connor, C; Ghali, J; et al. Effect of amlodipine on morbidity and mortality in severe chronic heart failure: the PRAISE trial. *N Engl J Med.,* 1996, 335, 1107–1114.

[117] Cohn, J; Ziesche, S; Loss, L; et al. Effect of the calcium channel antagonist felodipine as supplementary vasodilator therapy in patients with chronic heart failure treated with enalapril: V-HeFT III. *Circulation,* 1997, 96, 856–863.

[118] Horwich, TB; MacLellan, WR; Fonarow, GC. Statin therapy is associated with improved survival in ischaemic and non-ischaemic heart failure. *J Am Coll Cardiol,* 2004, 43, 642–648.

[119] Florkowski, CM. Management of co-existing diabetes mellitus and dyslipidemia: defining the role of thiazolidinediones. *Am. J. Cardiovasc. Drugs,* 2002, 2, 15–21.

[120] Young, LH. Insulin resistance and the effects of thiazolidinediones on cardiac metabolism. *Am J Med.,* 2003, 115, 75S–80S.

[121] Aussedat, J; Ray, A; Kay, L; Verdys, M; Harpey, C; Rossi, A. Improvement of long-term preservation of isolated arrested rat heart: beneficial effect of the antiischemic agent trimetazidine. *J Cardiovasc Pharmacol,* 1993, 21, 128 –35.

[122] Lee, L; Campbell, R; Scheuermann-Freestone, M; et al. Metabolic modulation with perhexiline in chronic heart failure: a randomized, controlled trial of short-term use of a novel treatment. *Circulation,* 2005, 112, 3280–8.

[123] McCormack, JG; Barr, RL; Wolff, AA; Lopaschuk, GD. Ranolazine stimulates glucose oxidation in normoxic, ischemic, and reperfused ischemic rat hearts. *Circulation,* 1996, 93, 135– 42.

[124] Morrow, DA; Scirica, BM; Karwatowska-Prokopczuk, E; et al. Effects of ranolazine on recurrent cardiovascular events in patients with non-ST-elevation acute coronary syndromes: the MERLIN-TIMI 36 randomized trial. *JAMA,* 2007, 297, 1775– 83.

[125] Broderick, TL; Quinney, HA; Barker, CC; Lopaschuk, GD. Beneficial effect of carnitine on mechanical recovery of rat hearts reperfused after a transient period of global ischemia is accompanied by a stimulation of glucose oxidation. *Circulation,* 1993, 87, 972– 81.

[126] Ferrari, R; Merli, E; Cicchitelli, G; Mele, D; Fucili, A; Ceconi, C. Therapeutic effects of L-carnitine and propionyl-L-carnitine on cardiovascular diseases: a review. *Ann N Y Acad Sci.,* 2004, 1033, 79–91.

[127] Ren, J; Dominguez, LJ; Sowers, JR; Davidoff, AJ. Metformin but not glyburide prevents high glucose-induced abnormalities in relaxation and intracellular Ca2 transients in adult rat ventricular myocytes. *Diabetes,* 1999, 48, 2059–65.

[128] Knowler, WC; Barrett-Connor, E; Fowler, SE; et al. Reduction in the incidence of type 2 diabetes with lifestyle intervention or metformin. *N Engl J Med.,* 2002, 346, 393– 403.

[129] Hallsten, K; Virtanen, KA; Lonnqvist, F; et al. Enhancement of insulin-stimulated myocardial glucose uptake in patients with type 2 diabetes treated with rosiglitazone. *Diabet Med.,* 2004, 21, 1280 –7.

[130] Nesto, RW; Bell, D; Bonow, RO; et al. Thiazolidinedione use, fluid retention, and congestive heart failure: a consensus statement from the American Heart Association and American Diabetes Association, 2003. *Circulation,* 2003, 108, 2941– 8.

[131] Nissen, SE; Wolski, K. Effect of rosiglitazone on the risk of myocardial infarction and death from cardiovascular causes. *N Engl J Med.,* 2007, 356, 2457–71.

[132] Salehi, M; D'Alessio, DA. New therapies for type 2 diabetes based on glucagon-like peptide 1. *Cleve Clin J Med.,* 2006, 73, 382–9.

[133] Best Practice Guidelines for Cardiac Rehabilitation and Secondary Prevention. Heart Research Centre. *Department of Human Services Victoria,* April, 1999

[134] Feigenbaum, E; Carter, E. Cardiac rehabilitation services. Health technology assessment report, 1987, no 6. Rockville, MD: US Department of Health and Human Services, Public Health Service, National Center for Health Services Research and Health Care Technology Assessment. DHHS publication No. PHS 88– 3427. Aug. 1988.

[135] Long-term comprehensive care of cardiac patients. Recommendations by the Working Group on Rehabilitation of the European Society of Cardiology. *Eur Heart J.,* 1992, 13 Suppl C, 1–45.

[136] Canadian Guidelines for Cardiac Rehabilitation and Cardiovascular Disease Prevention, 2nd edition, 2004: Executive summary. *Can J Cardiol* Vol 21 Suppl D, Oct 2005.

[137] Goldhammer, E; Tanchilevitch, A; Maor, I; Beniamini, Y; et al. Exercise training modulates cytokine activity in coronary heart disease patients. *Int J Cardiol.,* 2005, 100, 93-9.

[138] Taylor, RS; Brown, A; Ebrahim, S; et al. Exercise-based rehabilitation for patients with coronary heart disease: Systematic review and meta-analyses of randomized controlled trials. *Am J Med.,* 2004, 116, 682-92.

[139] Dolansky, MA; Moore, SM. Effects of cardiac rehabilitation on the recovery outcomes of older adults after coronary artery bypass surgery. *J Cardiopulm Rehabil,* 2004, 24; 236-44.

[140] Thompson, PD. Exercise and physical activity in the prevention and treatment of atherosclerotic cardiovascular disease. *Arterioscler Thromb Vasc Biol.,* 2000, 23, 1319-21.

[141] Thompson, PD; Franklin, BA. From case report to meta-analysis- additional evidence for the benefits of exercise training in cardiac patients. *Am J Med.,* 2004, 116, 714-6.

[142] Murchie, P; Campbell, NC; Ritchie, LD; Simpson, JA; Thain, J. Secondary prevention clinics for coronary heart disease: Four year follow up of a randomized controlled trial in primary care. *BMJ,* 2003, 326, 84.

[143] Poirier, P; Després, JP. Waist circumference, visceral obesity, and cardiovascular risk. *J Cardiopulm Rehabil,* 2003, 23, 161-9.

[144] Myers, J. Cardiology patient pages. Exercise and cardiovascular health. *Circulation* 2003; 107: e2-5.145. Frasure-Smith, N; Lesperance, F; Talajic, M; Bourassa, MG. Gender, depression and one-year prognosis after myocardial infarction. *Psychosom Med.,* 1999, 61, 26-37.

[145] Ziegelstein, RC; Bush, DE; Fauerbach, JA. Depression, adherence bahaviour, and coronary disease outcomes. *Arch Intern Med.,* 1997, 157, 1921-9.

[146] World Health Organization Expert Committee. Rehabilitation after cardiovascular diseases, with special emphasis on developing countries. Technical report series number 831. Geneva: World Health Organization; 1993.

[147] American College of Sports Medicine. *Guidelines for exercise testing and prescription*. 5th ed. Philadelphia: Lea & Febiger, 1995.

[148] Taylor, CB; Houston-Miller, N; Ahn, DK; Haskell, W; DeBusk, RF. The effects of exercise training programs on psychosocial improvement in uncomplicated postmyocardial infarction patients. *J Psychosom Res.,* 1986, 30, 581–7.

[149] Newton, M; Mutrie, N; McArthur, JD. The effects of exercise in a coronary rehabilitation programme. *Scott Med J.*, 1991, 36, 38–41.

[150] Oldridge, NB; Guyatt, GH; Fischer, ME; Rimm, AA. Cardiac rehabilitation after myocardial infarction: combined experience of randomised clinical trials. *JAMA,* 1988, 260, 945– 50.

[151] O'Connor, GT; Buring, JE; Yusuf, S; Goldhaber, SZ; Olmstead, EM; Paffenbarger, RS; Hennekens, CH. An overview of randomized trials of rehabilitation with exercise after myocardial infarction. *Circulation,* 1989, 80, 234–44.

[152] Oberman, A; Fletcher, GF; Lee, J; Nanda, N; Fletcher, BJ; Jensen, B; et al. Efficacy of highintensity exercise training on left ventricular ejection fraction in men with coronary artery disease (The Training Level Comparison Study). *Am J Cardiol,* 1995, 76, 643–7.

In: Horizons in World Cardiovascular Research. Volume 1 ISBN: 978-1-60876-447-1
Editor: Eleanor H. Bennington, pp. 33-50

Chapter II

Tako-Tsubo Cardiomyopathy

Radhakrishnan Ramaraj[1*], Vincent L. Sorrell[2] and M. Reza Movahed[3]

[1]Resident Physician, Department of Internal Medicine, University of Arizona College of Medicine, 1501 N Campbell Avenue, Tucson, Arizona 85724

[2]Professor of Clinical Cardiology, Radiology and Pediatrics, The Allan C. Hudson and Helen Lovaas Chair of Cardiac Imaging, Section of Cardiology, Sarver Heart Center,University of Arizona College of Medicine, Tucson, Arizona 85724

[3]Associate Professor of Medicine, Director of Coronary Care Unit, Section of Cardiology, Sarver Heart Center, University of Arizona College of Medicine, Tucson, Arizona 85724

Abstract

Cardiomyopathy is a generic term for any heart disease in which the heart muscle is involved and functions abnormally. Recent developments and ongoing research in cardiology have led to descriptions of previously less recognized and/or incompletely characterized cardiomyopathies. These entities are being increasingly noticed in adult patient populations. Primary care providers, hospitalists, emergency medicine physicians and cardiovascular specialists need to be aware of the clinical features of these illnesses and the best strategies for diagnosis and management. In this chapter, we discuss the etiologies and diagnostic methods for identifying Tako-tsubo cardiomyopathy and ways to manage this entity. This cardiomyopathy is caused by intense emotional or physical stress leading to rapid, severe but reversible cardiac dysfunction. It mimics myocardial infarction with changes in the electrocardiogram and echocardiogram, but without obstructive coronary artery disease. This pattern of left ventricular dysfunction was first described in Japan and has been referred to as "tako-tsubo cardiomyopathy," named after a fishing pot with a narrow neck and wide base that is used to trap octopus. This

* Corresponding author: Radhakrishnan Ramaraj: University of Arizona College of Medicine, 1501 N Campbell Ave., Tucson, AZ 85725, drkutty2@gmail.com

syndrome is also known as "apical ballooning syndrome", "ampulla cardiomyopathy", "stress cardiomyopathy", or "broken-heart syndrome".

Introduction

In recent years, a new cardiac syndrome with transient left ventricular dysfunction has been described which was first identified in Japanese patients. This new entity has been referred to as "tako-tsubo cardiomyopathy" or "apical ballooning", named after the particular shape of the end-systolic left ventricle on ventriculography. [1] To date, tako-tsubo cardiomyopathy (TTC) has also been reported in western populations. Emotional or physical stress usually precedes the presentation of this cardiomyopathy. The mechanistic explanation responsible for this acute but reversible contractile dysfunction is still not known. Multivessel epicardial coronary artery vasospasm, coronary microvascular dysfunction or spasm, impaired fatty acid metabolism, transient obstruction of the left ventricular outflow, and catecholamine-mediated myocardial dysfunction has each been proposed as potential mechanism. [2-5] The optimal management of patients presenting with this syndrome depends mainly on the hemodynamic condition of the patient and remains primarily symptomatic in nature. Nearly 2 decades following the first report of this entity, it has been increasingly recognized. [6] Despite increased awareness, the pathophysiology of the condition remains uncertain, and few reports have suggested a specific mechanism, beyond high catecholamine levels, as a trigger for the syndrome. New variants of this disease, involving a different part of the left ventricular wall, have recently been described in the literature. [7-14] Based on these observations, a new term, "stress cardiomyopathy," is now commonly used in the medical community to describe all varieties of this condition.

What is Stress Cardiomyopathy?

Stress cardiomyopathy is a cardiac syndrome characterized by acute onset of chest pain and completely reversible regional contractile dysfunction (Table 1). On left ventriculography, typical wall motion abnormalities, such as apical and mid-ventricular akinesia and a hypercontractile base, can be identified. (Figure 1) Usually coronary angiography reveals no identifiable epicardial coronary artery disease. (Figure 2) Recently, a few cases of transient ballooning involving the mid-ventricular left ventricle, sparing the apical and basal segments, have also been documented. [9] Stress cardiomyopathy mimics symptoms of acute myocardial infarction with acute chest pain, electrocardiographic (ECG) changes, and a transient increase in blood levels of cardiac biomarkers including troponins although less marked than with acute myocardial infarction. In 1991 Sato and Dote first described this transient contractile dysfunction, naming it tako-tsubo cardiomyopathy. [1] Other groups called the syndrome apical "ballooning", "broken heart", "scared to death", "ampulla-syndrome", or "acute stress cardiomyopathy". [3]

Table 1. Symptoms and Stress at the time of presentation

Characteristics	(%) (N = 185)
Symptom	
Chest Pain	65.9 (122)
Dyspnea	16.2 (30)
Syncope	4.9 (9)
Chest pain and dyspnea	3.2 (6)
Nausea	1.6 (3)
ECG changes	1.6 (3)
CVA	1.1 (2)
Palpitations	1.1 (2)
V-fib	0.5 (1)
Back Pain	0.5 (1)
Fatigue	0.5 (1)
Cardiac Arrest	0.5 (1)
Not Reported	1.1 (2)
Precipitating Stress	
Emotional	47.9 (80)
Physical	29.3 (49)
None	22.8 (38)

The characteristic clinical syndrome of stress cardiomyopathy is acute left ventricular dysfunction, following a sudden emotional or physical stress. Patients typically present with chest pain similar to that of acute myocardial infarction (central, heavy, squeezing and crushing). On occasion, the discomfort radiates to the arms causing anxiety (Table 1). The pain can also mimic acute pericarditis, pulmonary embolism, acute aortic dissection and costochondritis. Although the coronary arteries have no flow-limiting lesions, acute changes on the ECG suggesting ischemia, and raised levels of cardiac enzymes, reflecting acute myocardial injury, are usually present. Left ventricular dysfunction and wall-motion abnormalities are typically seen, affecting the apical and, frequently, the midventricular left ventricular myocardium, but sparing the basal myocardium. On left ventriculography, echocardiography or cardiac magnetic resonance imaging (MRI), these functional abnormalities typically resemble a flask with a short, narrow neck and wide, rounded body. The shape of the ventricle at end systole resembles the Japanese fisherman's octopus pot—the tako-tsubo—from which the syndrome derives its original name. The hypercontractile basal myocardium can generate left ventricular outflow tract obstruction in the presence of apical and midwall hypokinesis. The final element of the syndrome is that left ventricular function and apical wall motion return to normal within days or weeks of the acute insult, in a similar manner to traditional myocardial stunning. Right ventricular involvement appears to be less common but has been reported in the literature. [15,16] Apical distribution of the right ventricular wall akinesia suggests that for some unknown reason, apical ballooning syndrome affects the heart in a geometrical way involving mostly the apex and the mid left ventricular

walls and does not follow a single coronary territory. [8] Some studies have found decreased flow in the apical left ventricular wall compared with the base, which could partially explain this geometrical involvement. [16,17]

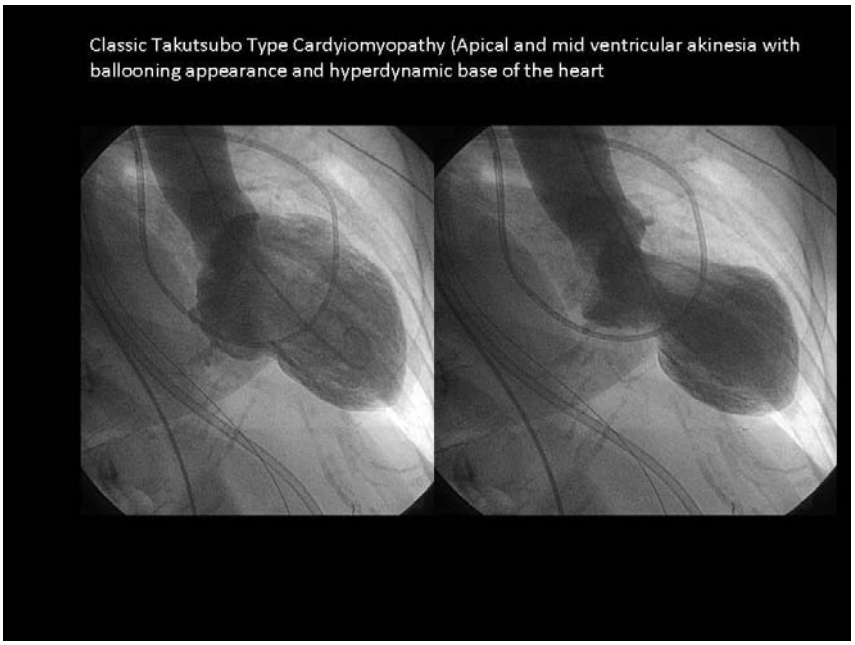


Figure 1. Classic Takutsubo Type Cardyiomyopathy (Apical and mid ventricular akinesia with ballooning appearance and hyperdynamic base of the heart

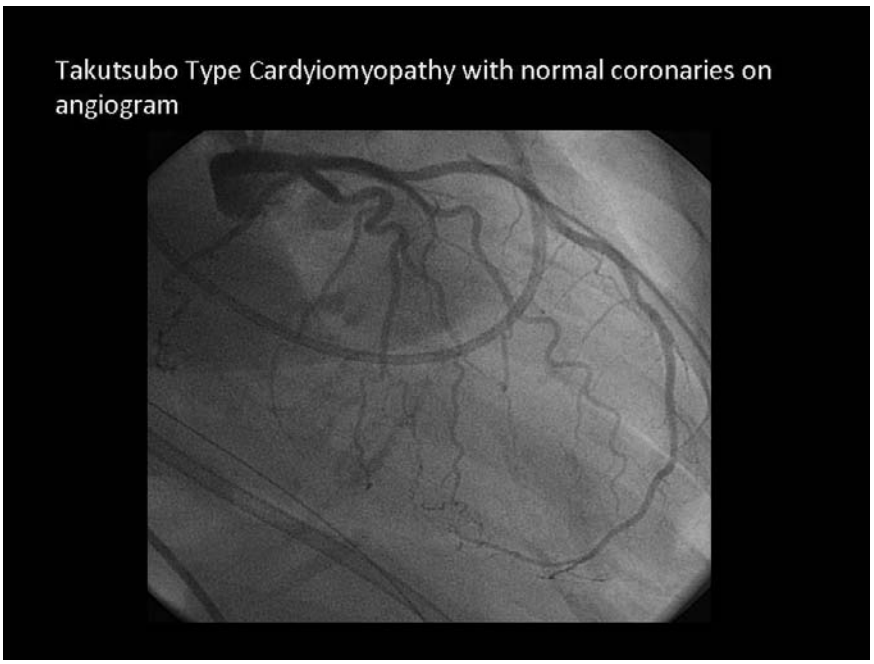


Figure 2. Takutsubo Type Cardyiomyopathy with normal coronaries on angiogram

Etiology

The cause of stress cardiomyopathy is unknown. However, all available evidence is consistent with the concept that this disease results from extreme emotional and/or physical stress. The disease shows a strong predominance for postmenopausal women. (Table 2) The seemingly increased susceptibility of women to stress-related left ventricular dysfunction and potential gender-related differences in response to catecholamines is not well understood.[5] However, sex hormones exert important influences on the sympathetic neurohumoral axis as well as on coronary vasoreactivity. The mechanisms underlying stress cardiomyopathy are unclear with exaggerated sympathetic stimulation probably being central to its causation. Thus, catecholamine excess has been implicated but not well documented.

Table 2. Patient characteristics

Characteristics	(%) (*N* = 185)
Mean age (years)	67.7
Female	93.5 (173)
Male	6.5 (12)
Race	
Asian	57.2 (83)
White	40 (58)
Other	2.8 (4)
Not reported	21.5 (40)

One hypothesis is that these patients experience myocardial ischemia as a result of epicardial coronary arterial spasm, secondary to increased sympathetic tone leading to vasoconstriction despite the absence of atherosclerotic coronary artery disease. [18] Another possible mechanism of direct myocardial injury is catecholamine-mediated myocardial stunning. Supporting this hypothesis is the well known fact that adrenoceptor density is higher in the cardiac apex compared with other areas of the myocardium. This might account for myocardial dysfunction and apical ballooning during catecholamine stress. [5] The elevated catecholamines can produce a concentration dependent decrease in myocyte viability, as demonstrated by a significant release of creatine kinase from the affected cells leading to decreased viability due to cyclic AMP-mediated calcium overload. [19] Abnormal coronary flow in the absence of obstructive coronary artery disease has recently been reported in patients with stress-related myocardial dysfunction. [17] Further correlations between this cardiomyopathy and specific genetic profiles are not known at this time. It has been hypothesized that stress cardiomyopathy is a form of myocardial stunning, but with a different cellular mechanisms than is seen during transient episodes of ischemia secondary to coronary stenosis.

Patients with stress cardiomyopathy usually have supra-physiological levels of plasma catecholamines and stress-related neuropeptides. Unlike polymorphonuclear inflammation seen with infarction, in stress cardiomyopathy there is contraction band necrosis, a unique form of myocyte injury characterized by hypercontracted sarcomeres, dense eosinophilic

transverse bands, and an interstitial mononuclear inflammatory response. Endomyocardial biopsy has demonstrated the presence of contraction band necrosis in patients with stress cardiomyopathy. [5] Contraction band necrosis is a type of cell death identified as early as 2 minutes after cell injury has occurred and can cause release of cardiac biomarkers. [20] Focal myocarditis and contraction band necrosis has been found in states of excess circulating catecholamine such as pheochromocytoma,[21] subarachnoid haemorrhage, [22,23] eclampsia [23] and fatal asthma. [24] Contraction band necrosis has also been documented in autopsies of patients with normal coronary vessels who suffered from coronary spasm due to various causes. [23,25] These findings all suggest that catecholamines may be a link between emotional stress and cardiac injury.

Epidemiology

Stress cardiomyopathy is still rarely diagnosed. Over the last few years, however, the number of published reports of patients presenting with this syndrome has steadily increased. Serial case studies coming from Japan reveal a prevalence of 1.2–2.0% among patients with acute coronary syndrome. [26] In a recent US study, stress cardiomyopathy was diagnosed in about 2.2% of patients admitted with suspected acute coronary syndrome. A German series reported an incidence of 0.1–2.3%; a study from France investigating this syndrome in a large urban population showed a prevalence of 0.9% and, the incidence of stress cardiomyopathy in an Italian investigation was 2%. [27-31] It is likely that prior to our current understanding, these patients were diagnosed as ‘acute myocardial infarction with normal coronaries’ secondary to coronary arterial spasm.

Classification

1. Stress cardiomyopathy can involve any segment of the left ventricular wall. There are now four different types, based on anatomic location, described in the literature. [32]
2. Classic type, which is the most commonly reported, is described as apical ballooning or Tako-tsubo type.
3. The second type is the reverse type; with hyperdynamic apex and akinesia of the base of the left ventricular wall (reverse Takotsubo or reverse apical ballooning type). This type is rarely described in the literature. [7,33-36]
4. The third type involves the mid left ventricular wall, sparing the base and the apex. It is called the “mid ventricular type.”[9,11,37]
5. The fourth type is localized wall motion abnormality affecting a segment of the left ventricular wall, usually the anterior wall. [10,12,14,34,38]

Theories behind Stress Cardiomyopathy

Several mechanisms have been proposed for stress cardiomyopathy

Epicardial Coronary Vasospasm

In all documented patients presenting with stress cardiomyopathy, relevant coronary artery obstruction has been excluded and coronary vasospasm is unlikely since the region of wall motion abnormalities does not correspond to the perfusion territory of a single coronary artery. Many studies have evaluated the presence of either spontaneous or provoked multivessel epicardial spasm during angiography. In a systematic review only a few patients experienced spontaneous multivessel epicardial spasm (1.4%). Using provocative tests such as infusion of ergonovine or acetylcholine, nearly 28% experienced multivessel spasm. However, results varied widely in different series. Taken together, epicardial vasospasm seems to be an unlikely mechanism as a cause of stress cardiomyopathy but may account for a few cases of elevated cardiac enzymes with normal coronary arteries. [39]

Microvascular Dysfunction

Investigators have reported that patients with stress cardiomyopathy have impaired coronary microcirculation since, using a Doppler guide wire, diminished coronary flow reserve (CFR) was observed. [40] These results were confirmed by other groups suggesting that microvascular dysfunction contributes substantially to the development of this syndrome. Recently, reduced coronary flow velocity in the absence of coronary artery stenosis was noted immediately after the onset of stress cardiomyopathy. Additionally, myocardial contrast echocardiographic studies revealed perfusion defects in the left ventricular apex which normalize after a follow up of 4 weeks, suggesting that microvascular dysfunction might have been responsible for the reversible contractile impairment. [4] However, it is unclear whether coronary microvascular dysfunction is the primary mechanism involved in the pathogenesis of the syndrome or whether it is simply an associated secondary phenomenon. Furthermore, the underlying cause of the potential microvascular dysfunction is unknown.

Catecholamine Induced Myocyte Injury

The most widely proposed hypothesis for stress cardiomyopathy relates to the role of stress. In the majority of cases, triggering conditions that preceded onset were said to involve exposure to endogenous (emotional) or exogenous stresses (trauma, surgical procedure, exacerbation of a pre-existing condition). This suggests that increased sympathetic activity plays a major role in the origin of this syndrome. One group of investigators described notably elevated norepinephrine concentrations in patients with stress cardiomyopathy. [41] This was confirmed by others who demonstrated significantly increased catecholamine

concentrations in comparison to patients with Killip class III myocardial infarction. Increased serum concentrations of catecholamines have been shown to generate direct myocyte injury. Oxidation of catecholamines results in the formation of highly toxic substances and free radicals causing intracellular calcium overload and myocardial cell damage. The typical histological signs of catecholamine toxicity, described as focal, mononuclear, inflammatory areas of fibrotic response and characteristic contraction bands, are also reported to be present in patients with stress cardiomyopthy. [5,42] Contraction bands have been reported in several clinical settings of extensive catecholamine production such as phaeochromocytoma or subarachnoid haemorrhage, showing that catecholamines may be an important link between emotional stress and cardiac injury. [5] The distinctive contractile pattern of stress cardiomyopathy may be explained by an enhanced responsiveness of apical myocardium to sympathetic stimulation. Alternatively, a base-to-apex perfusion gradient could result in regional differences in myocardial blood flow in the setting of catecholamine-mediated epicardial or microvascular vasoconstriction. [5] Interestingly, the wall motion abnormalities observed in stress cardiomyopathy are not the same as those found with subarachnoid haemorrhage or intracranial haemorrhage, in which only the basal segments of the left ventricle are affected. [43]

Obstruction of Left Ventricular Outflow Tract

Left ventricular outflow tract (LVOT) obstruction was observed in a report of three patients with tako-tsubo cardiomyopathy. [44] Other groups confirmed these abnormal findings, especially in women in the presence of abnormal myocardial functional architecture, such as localized mid-ventricular septal thickening. [3] It was hypothesized that in the presence of increased concentrations of catecholamines caused by emotional stress, this mid-ventricular septal thickening could lead to the development of severe transient left ventricular mid-cavity obstruction, resulting in subendocardial ischaemia unrelated to a specific coronary artery territory. However, it remains unclear whether the observed intraventricular gradient is a consequence rather than a cause of stress cardiomyopathy.

Diagnosis

The diagnosis of stress-induced stress cardiomyopathy is based on the following criteria:

(a) Transient akinesis or dyskinesis of the left ventricular wall (ballooning) seen on echocardiography (or any other imaging modality) accompanied by chest discomfort; commonly, but not universally, apically located;
(b) New electrocardiographic changes (either ST elevation or T wave inversion);
(c) No significant obstructive epicardial coronary artery disease;
(d) Absence of recent significant head trauma, phaeochromocytoma, myocarditis or hypertrophic cardiomyopathy.

Stress cardiomyopathy can present with the following changes on the ECG (Table 3)

- Diffuse symmetric T-wave inversion
- Pronounced prolongation of the QT interval
- Loss of R wave progression
- Prolonged PR interval
- Pathologic Q waves in leads V1, V2, V3 and aVL

Table 3. ECG findings & cardiac markers

Characteristics	(%) (N)
ST Elevation	87.5 (136)
T wave inversion	75 (104)
Q Waves	50 (22)
Positive cardiac markers	85.5 (117)

N = number of cases data was reported

Echocardiography findings in stress cardiomyoapthy are as follows:

- Transient, regional akinesis or dyskinesis, usually involving the entire LV (and RV) apex;
- A unique variant is “reverse stress cardiomyopthy” in which the apex is spared and only the basal portion of the LV myocardium is dysfunctional;
- Regardless of the location of regional dysfunction, the most important echo feature to distinguish this disease from an acute MI is a regional wall motion abnormality in multiple coronary artery territories rather than a single coronary artery zone, as well as involvement of the adjacent RV wall;
- Reduced left (and often right) ventricular ejection fraction and systolic dysfunction;
- Apical- ‘ballooning’ with abnormal wall motion of the mid and distal (and/or mid) left ventricle;
- Restoration of normal global and regional myocardial function with serial exams over time.

Electrocardiography

ST elevation (<2 mm) or T wave inversion in the anterior leads (V1–V6) have been the most commonly recorded findings mimicking acute MI. [5] In comparison to patients with anterior infarct, these ST elevations are less prominent. Electrocardiographic changes may be present for several hours followed by normalization and development of T wave inversion. Furthermore, in several cases transient prolongation of the QT interval was observed with a subsequent normalization within some weeks. [2] Even though QT interval prolongation is present in stress cardiomyopathy, rate adaptation of ventricular repolarization is not significantly altered in comparison to acute ST elevation myocardial infarction, suggesting a different effect of autonomic nervous activity on the ventricular myocardium. [45]

Laboratory Investigation

Blood values of myocardial creatine kinase (CK), CK-MB, and troponin are often only slightly elevated. There are also reports of increased concentrations of B-type natriuretic peptide (BNP) in stress cardiomyopathy patients. [46] Recently, serum concentrations of the N-terminal fragment of BNP (NT-proBNP) were shown to be a valuable marker for assessment of myocardial deterioration and recovery. [47] Moreover, low NT-proBNP values on admission were shown to be a reliable indicator of a favorable prognosis for patients presenting with stress cardiomyopathy.

Echocardiography

In the apical four-chamber view, typical akinesia of the left ventricular apex and/or the mid-portion of the left ventricle as well as a hypercontractile base are typically found. Interestingly, the wall motion abnormalities exceed the area assigned to one coronary vessel. In a few cases, LVOT obstruction with an end-systolic pressure gradient of up to 60 mm Hg was observed. [3] After normalization of myocardial function the pressure gradient disappeared. These findings of mid-cavity dynamic obstruction in the acute phase of stress cardiomyopathy correlate with localized mid-ventricular septal thickening when cardiac function returns to normal. Stress cardiomyopathy, can be reasonably suspected using careful evaluation of the initial echocardiographic examination in conjunction with ECG, laboratory, and clinical data. Wall motion analysis should reveal an apical ballooning appearance involving many coronary territories with mild elevation of cardiac enzyme levels or ECG changes. Furthermore, the additional presence of right ventricular apical akinesia during echocardiographic examination makes the diagnosis of this syndrome very likely. [8]

Coronary Angiography and Ventriculography

In all reported cases of stress cardiomyopathy, coronary angiography excluded relevant coronary artery obstruction in patients presenting with stress cardiomyopathy. Ventriculography usually displays typical apical ballooning and hypercontraction of the basal segments. In some cases, mid-ventricular ballooning sparing the basal and apical segments can be present. [9]

Cardiovascular Magnetic Resonance Imaging

Cardiovascular MRI provides morphologic and precise functional information of the left ventricle. More recently published data also documented regional wall motion abnormalities of the right ventricle in the acute phase of this syndrome.[48] Sporadically, focal signal increases in different left ventricular segments was detectable in the T2-weighted turbo-spin echo sequences, indicating myocardial edema. First-pass perfusion imaging did not show any

evidence of focal perfusion abnormalities, corresponding to a specific vascular territory. So far, in all cases, the observed endocardial delayed hyperenhancement was small in comparison to the extent of the wall motion abnormalities.[49,50] In view of the fact that in myocarditis areas of hyperenhancement originate from the epicardium, late MRI enhancement sequences can assist in the differential diagnosis of stress cardiomyopathy.

Myocardial Single Photon Emission Computed Tomography

Several reports describe thallium-201 (^{201}Tl) perfusion patterns with a perfusion defect in the apical LV region in the acute phase of stress cardiomyopathy despite normal coronary arteries. [51] These defects decrease with recovery. Investigators have reported a diminished accumulation of iodine-123 [123] metaiodobenzylguanidine (MIBG) in the hypokinetic region. [46] Investigators have also demonstrated impaired myocardial fatty acid metabolism rather than disturbed myocardial perfusion during the early phase.[52] In stress cardiomyopathy, technetium-99 m (^{99m}Tc)-tetrofosmin myocardial **single photon emission computed tomography (**SPECT) showed that myocardial perfusion in the apical region was impaired immediately after hospitalization with recovery after 3–5 days. [53]

Myocardial Biopsy

Several groups have investigated endomyocardial biopsies from both the right and left ventricle, revealing myocyte injury and a slight increase in connective tissue. From a systematic analysis it is known that stress cardiomyopathy is accompanied by severe cellular morphological alterations, with many vacuoles of different sizes contributing to cellular deterioration. The content of myocardial contractile material is reduced and detected in the border area of the cells. Contraction bands are sporadically present. Clusters of mitochondria with abnormalities in size and shape can be observed. The myocyte nuclei typically appear rounded or oval either in the middle or in the border area of the cells. Cell swelling associated with damage to the basal lamina or damaged mitochondria with flocculent densities are typical signs of oncotic cell death and are absent. Additionally, apoptotic and autophagic cell death can be excluded by electron microscopy and immunohistochemistry. Moreover, the interstitial space is widened and contains fibrotic material, including collagen fibrils, formations of cell debris, macrophages, and an increased number of fibroblasts. Most noteworthy, these alterations are transient and almost completely reversible after functional recovery. [42]

Management

The management of stress cardiomyopathy consists of supportive and symptomatic treatment. Initially patients are managed as if they had a myocardial infarction, including urgent coronary angiography with a view to performing a primary coronary intervention.

These patients should be treated with aspirin, low molecular weight heparin, and angiotensin-converting enzyme (ACE) inhibitors; ß-blockers and diuretics may also be administered. Beyond the standard care for congestive heart failure with diuretics and vasodilators, the treatment of stress cardiomyopathy largely remains unclear and involves only symptomatic management. With good initial medical support, patients with stress cardiomyopathy show good clinical and echocardiographic improvement in left ventricular function. [54] These patients also have an excellent short and long-term prognosis. Complications, such as cardiogenic shock, pulmonary edema or malignant arrhythmias, should be treated according to the usual management strategies (Table 4). However, the overall prognosis of patients presenting with this syndrome is favorable; the reported in-hospital mortality rates range from 0–8%. [55] Vasoactive agents should be used very carefully since they may further worsen the delicate situation. In cases of severe circulatory dysfunction, intra-aortic balloon counterpulsation should be considered. In a stable clinical setting, administration of anxiolytic agents is preferred. Data from an animal model of stress cardiomyopathy suggest that its development seems to be diminished after α- and ß-blockade.[56] Thus, ß-blockers should be given in the acute and chronic phases and may possibly help to prevent recurrences, which have been described as occurring in 2.7–8% of patients.[39] In order to prevent acute left ventricular thrombus formation, which has been observed in patients presenting with TTC, the administration of low molecular weight heparin is warranted. After restitution of contractile function, further anticoagulation with warfarin is not required. In the event of life threatening arrhythmias such as torsade de pointes tachycardia and ventricular fibrillation, the implantation of a cardioverter-defibrillator has to be considered.

Table 4. Complications and outcome

Complication	N(%)
Total patients with a complication	35 (18.9)
Shock	12 (6.5)
Thrombus	7 (3.8)
CHF	7 (3.8)
CVA	3 (1.6)
Ventricular tachycardia	3 (1.6)
Atrial fibrillation	2 (1.1)
LV rupture	1 (0.5)
Pneumothorax	1 (0.5)
Ventricular fibrillation	1 (0.5)
Ventricular septal defect	1 (0.5)
Death	6 (3.2)

Prognosis

Almost all patients with stress cardiomyopathy with left ventricular impairment demonstrate normal function within a few weeks. There is no data on frequency of patients

with residual long term left ventricular impairment.[54] Rarely, this syndrome can be complicated by left ventricular rupture, thus making stress cardiomyopathy a newly recognized cause of sudden death in up to 3% of patients.[13] However, the overall prognosis of patients presenting with this syndrome is favorable; the reported in-hospital mortality rates range from 0–8%. [55] In the majority of patients, left ventricular function returns to normal in 6±3 days. This syndrome may recur in up to 10% of patients, making it difficult to know how long to continue medical treatment.

Sex-Related Differences

In a study of transient left ventricular apical ballooning involving 185 cases, it was confirmed that most cases involved older women. [13] Many unanswered questions regarding stress cardiomyopathy remain. Among these the most puzzling one is the apparent increased incidence in females, who comprise over 90% of reported cases. Sex-related differences in the response of the adrenal medulla to sudden high-intensity sympathetic discharge and differing pharmacokinetics of epinephrine release could explain the increased rate in women. Of interest, basal plasma epinephrine levels are lower in women than in men.[6] This difference could reflect reduced synthesis, increased degradation or reduced basal release with more potential stores for sudden release. Estrogen has cardioprotective effects against acute injury through a variety of complex mechanisms.[57,58] Stress activates early gene expression in both the central nervous system and the ventricular myocardium in rodent models,[56] the myocardial changes in gene expression being mediated by activation of both α-adrenoceptors and β-adrenoceptors. Estrogen reduces these changes in gene expression, protecting against the apical ventricular dysfunction observed in this rodent model of stress cardiomyopathy (conscious immobilization).[56] Chronic (but not acute) exposure of the rat ventricular myocardium to estrogen reduces the catecholamine and ischemia/reperfusion enhanced expression of β_1 adrenoceptors. [59] Oophorectomy increases the expression of β_1 adrenoceptors, an effect that is reversed by estrogen supplementation. [60] Beyond the myocardium, greater vascular β_2 adrenoceptor-mediated sensitivity has been demonstrated in women than in men. [61] Estrogens could, therefore, influence the β_1 adrenoceptor:β_2 adrenoceptor signaling ratio in women in favor of the protective effects of β_2 adrenoceptors–G_i protein signaling following surges in catecholamine levels. This protection would occur at the mechanical cost of negative inotropism in regions with the highest density of β-adrenoceptors, namely the apical myocardium. Recently, Ueyama et al [56] suggested that estrogen supplementation partially prevents emotional stress-induced cardiovascular responses, both by an indirect action on the nervous system and by direct action on the heart. Thus, a reduction in estrogen levels following menopause might augment vascular reactivity to stress resulting in a high incidence of stress cardiomyopathy in post-menopausal women.

References

[1] Dote, K; Sato, H; Tateishi, H; Uchida, T; Ishihara, M. [Myocardial stunning due to simultaneous multivessel coronary spasms: a review of 5 cases]. *J Cardiol,* 1991, 21, 203-14.

[2] Tsuchihashi, K; Ueshima, K; Uchida, T; Oh-mura, N; Kimura, K; Owa, M; Yoshiyama, M; Miyazaki, S; Haze, K; Ogawa, H; Honda, T; Hase, M; Kai, R; Morii, I. Transient left ventricular apical ballooning without coronary artery stenosis: a novel heart syndrome mimicking acute myocardial infarction. Angina Pectoris-Myocardial Infarction Investigations in Japan. *J Am Coll Cardiol,* 2001, 38, 11-8.

[3] Merli, E; Sutcliffe, S; Gori, M; Sutherland, GG. Tako-Tsubo cardiomyopathy: new insights into the possible underlying pathophysiology. *Eur J Echocardiogr,* 2006, 7, 53-61.

[4] Ako, J; Takenaka, K; Uno, K; Nakamura, F; Shoji, T; Iijima, K; Ohike, Y; Kim, S; Watanabe, T; Yoshizumi, M; Ouchi, Y. Reversible left ventricular systolic dysfunction--reversibility of coronary microvascular abnormality. *Jpn Heart J,* 2001, 42, 355-63.

[5] Wittstein, IS; Thiemann, DR; Lima, JA; Baughman, KL; Schulman, SP; Gerstenblith; G; Wu, KC; Rade, JJ; Bivalacqua, TJ; Champion, HC. Neurohumoral features of myocardial stunning due to sudden emotional stress. *N Engl J Med.,* 2005, 352, 539-48.

[6] Lyon, AR; Rees, PS; Prasad, S; Poole-Wilson, PA; Harding, SE. Stress (Takotsubo) cardiomyopathy--a novel pathophysiological hypothesis to explain catecholamine-induced acute myocardial stunning. *Nat Clin Pract Cardiovasc Med.,* 2008, 5, 22-9.

[7] Bonnemeier, H; Schafer, U; Schunkert, H. Apical ballooning without apical ballooning. *Eur Heart J,* 2006, 27, 2246.

[8] Donohue, D; Ahsan, C; Sanaei-Ardekani, M; Movahed, MR. Early diagnosis of stress-induced apical ballooning syndrome based on classic echocardiographic findings and correlation with cardiac catheterization. *J Am Soc Echocardiogr,* 2005, 18, 1423.

[9] Hurst, RT; Askew, JW; Reuss, CS; Lee, RW; Sweeney, JP; Fortuin, FD; Oh, JK; Tajik, AJ. Transient midventricular ballooning syndrome: a new variant. *J Am Coll Cardiol,* 2006, 48, 579-83.

[10] Mazzarotto, P; Stecconi, P; Gemelli, F; Azzarito, M; Farnetti, F. [A case of ballooning syndrome with atypical anterior localization]. *Ital Heart J Suppl.,* 2005, 6, 730-4.

[11] Ohtsubo, M; Sakai, H; Takano, H; Kon, H; Okamoto, K; Yoshida, N; Fujita, M; [Atypical takotsubo cardiomyopathy with preservation of apical contraction: a case report including pathological findings]. *J Cardiol,* 2005, 46, 237-42.

[12] Suzuki, K; Osada, N; Akasi, YJ; Suzuki, N; Sakakibara, M; Miyake, F; Maki, F; Takahashi, Y. An atypical case of "Takotsubo cardiomyopathy" during alcohol withdrawal: abnormality in the transient left ventricular wall motion and a remarkable elevation in the ST segment. *Intern Med.,* 2004, 43, 300-5.

[13] Donohue, D; Movahed, MR. Clinical characteristics, demographics and prognosis of transient left ventricular apical ballooning syndrome. *Heart Fail Rev.,* 2005, 10, 311-6.

[14] Lamm, G; Auer, J; Eber, B. Atypical form of left ventricular ballooning after a violent attack. *Int J Cardiol,* 2007, 119, 395-7.

[15] Nyui, N; Yamanaka, O; Nakayama, R; Sawano, M; Kawai, S. 'Tako-Tsubo' transient ventricular dysfunction: a case report. *Jpn Circ J.* 2000, 64, 715-9.

[16] Nishikawa, S; Ito, K; Adachi, Y; Katoh, S; Azuma, A; Matsubara, H. Ampulla ('takotsubo') cardiomyopathy of both ventricles: evaluation of microcirculation disturbance using 99mTc-tetrofosmin myocardial single photon emission computed tomography and doppler guide wire. *Circ J,* 2004, 68, 1076-80.

[17] Bybee, KA; Prasad, A; Barsness, GW; Lerman, A; Jaffe, AS; Murphy, JG; Wright, RS; Rihal, CS. Clinical characteristics and thrombolysis in myocardial infarction frame counts in women with transient left ventricular apical ballooning syndrome. *Am J Cardiol,* 2004, 94, 343-6.

[18] Lacy, CR; Contrada, RJ; Robbins, ML; Tannenbaum, AK; Moreyra, AE; Chelton, S, Kostis, JB. Coronary vasoconstriction induced by mental stress (simulated public speaking). *Am J Cardiol,* 1995, 75, 503-5.

[19] Mann, DL; Kent, RL; Parsons, B. Cooper Gt. Adrenergic effects on the biology of the adult mammalian cardiocyte. *Circulation,* 1992, 85, 790-804.

[20] Hopster, DJ; Milroy, CM; Burns, J; Roberts, NB. Necropsy study of the association between sudden cardiac death, cardiac isoenzymes and contraction band necrosis. *J Clin Pathol,* 1996, 49,s 403-6.

[21] Wilkenfeld, C; Cohen, M; Lansman, SL; Courtney, M; Dische, MR; Pertsemlidis, D; Krakoff, LR. Heart transplantation for end-stage cardiomyopathy caused by an occult pheochromocytoma. *J Heart Lung Transplant,* 1992, 11, 363-6.

[22] Neil-Dwyer, G; Walter, P; Cruickshank, JM; Doshi, B; O'Gorman, P. Effect of propranolol and phentolamine on myocardial necrosis after subarachnoid haemorrhage. *Br Med J.,* 1978, 2, 990-2.

[23] Bauer, TW; Moore, GW; Hutchins, GM. Morphologic evidence for coronary artery spasm in eclampsia. *Circulation,* 1982, 65, 255-9.

[24] Drislane, FW; Samuels, MA; Kozakewich, H; Schoen, FJ; Strunk, RC. Myocardial contraction band lesions in patients with fatal asthma: possible neurocardiologic mechanisms. *Am Rev Respir Dis.,* 1987, 135, 498-501.

[25] Wu, DJ; Fujiwara, H; Matsuda, M; Ishida, M; Kawamura, A; Takemura, G; Kida, M; Uegaito, T; Fujiwara, T; Kawai, C. Clinicopathological study of myocardial infarction with normal or nearly normal extracardiac coronary arteries. Quantitative analysis of contraction band necrosis, coagulation necrosis, hemorrhage, and infarct size. *Heart Vessels,* 1990, 6, 55-62.

[26] Stollberger, C; Finsterer, J; Schneider, B. Tako-tsubo-like left ventricular dysfunction: clinical presentation, instrumental findings, additional cardiac and non-cardiac diseases and potential pathomechanisms. *Minerva Cardioangiol,* 2005, 53, 139-45.

[27] Pilliere, R; Mansencal, N; Digne, F; Lacombe, P; Joseph, T; Dubourg, O. Prevalence of tako-tsubo syndrome in a large urban agglomeration. *Am J Cardiol.,* 2006, 98, 662-5.

[28] Hertting, K; Krause, K; Harle, T; Boczor, S; Reimers, J; Kuck, KH. Transient left ventricular apical ballooning in a community hospital in Germany. *Int J Cardiol,* 2006, 112, 282-8.

[29] Wedekind, H; Moller, K; Scholz, KH. [Tako-tsubo cardiomyopathy. Incidence in patients with acute coronary syndrome]. *Herz,* 2006, 31, 339-46.

[30] Sato, M; Fujita, S; Saito, A; Ikeda, Y; Kitazawa, H; Takahashi, M; Ishiguro, J; Okabe, M; Nakamura, Y; Nagai, T; Watanabe, H; Kodama, M; Aizawa, Y. Increased incidence of transient left ventricular apical ballooning (so-called 'Takotsubo' cardiomyopathy) after the mid-Niigata Prefecture earthquake. *Circ J.*, 2006, 70, 947-53.

[31] Parodi, G; Del, Pace, S; Carrabba, N; Salvadori, C; Memisha, G; Simonetti, I,; Antoniucci, D; Gensini, GF. Incidence, clinical findings, and outcome of women with left ventricular apical ballooning syndrome. *Am J Cardiol.*, 2007, 99, 182-5.

[32] Movahed, MR; Mostafizi, K. Reverse or inverted left ventricular apical ballooning syndrome (reverse takotsubo cardiomyopathy) in a young woman in the setting of amphetamine use. *Echocardiography*, 2008, 25, 429-32.

[33] Bonnemeier, H; Ortak, J; Burgdorf, C; Bode, F; Schafer, U; Hartmann, F; Schunkert, H. "The artichoke heart": the inverse counterpart of left ventricular apical ballooning. *Resuscitation*, 2007, 72, 342-3.

[34] Haghi, D; Papavassiliu, T; Fluchter, S; Kaden, JJ; Porner, T; Borggrefe, M; Suselbeck, T. Variant form of the acute apical ballooning syndrome (takotsubo cardiomyopathy): observations on a novel entity. *Heart*, 2006, 92, 392-4.

[35] Ennezat, PV; Pesenti-Rossi, D; Aubert, JM; Rachenne, V; Bauchart, JJ; Auffray, JL; Logeart, D; Cohen-Solal, A; Asseman, P. Transient left ventricular basal dysfunction without coronary stenosis in acute cerebral disorders: a novel heart syndrome (inverted Takotsubo). *Echocardiography*, 2005, 22, 599-602.

[36] Simoes, MV; Marin-Neto, JA; Maciel, BC. Variable regional left ventricular dysfunction in takotsubo cardiomyopathy syndrome. *Echocardiography*, 2007, 24, 893, author reply 894.

[37] Tamura, A; Kawano, Y; Watanabe, T; Aso, T; Abe, Y; Yano, S; Kadota, J. A report of 2 cases of transient mid-ventricular ballooning. *Int J Cardiol*, 2007, 122, e10-2.

[38] Strunk, B; Shaw, RE; Bull, S; Adams, J; Baer, M; Gershengorn, K; Kao, A; Keeffe, B; Sklar, J; Sperling, D; Sperling, R; Wexman, M; Young, J. High incidence of focal left ventricular wall motion abnormalities and normal coronary arteries in patients with myocardial infarctions presenting to a community hospital. *J Invasive Cardiol*, 2006, 18, 376-81.

[39] Gianni, M; Dentali, F; Grandi, AM; Sumner, G; Hiralal, R; Lonn, E. Apical ballooning syndrome or takotsubo cardiomyopathy: a systematic review. *Eur Heart J*, 2006, 27, 1523-9.

[40] Sadamatsu, K; Tashiro, H; Maehira, N; Yamamoto, K. Coronary microvascular abnormality in the reversible systolic dysfunction observed after noncardiac disease. *Jpn Circ J*, 2000, 64, 789-92.

[41] Akashi, YJ; Nakazawa, K; Sakakibara, M; Miyake, F; Koike, H; Sasaka, K. The clinical features of takotsubo cardiomyopathy. *QJM*, 2003, 96, 563-73.

[42] Nef, HM; Mollmann, H; Kostin, S; Troidl, C; Voss, S; Weber, M; Dill, T; Rolf, A; Brandt, R; Hamm, CW. Elsasser A. Tako-Tsubo cardiomyopathy: intraindividual structural analysis in the acute phase and after functional recovery. *Eur Heart J*, 2007, 28, 2456-64.

[43] Zaroff, JG; Rordorf, GA; Ogilvy, CS; Picard, MH. Regional patterns of left ventricular systolic dysfunction after subarachnoid hemorrhage: evidence for neurally mediated cardiac injury. *J Am Soc Echocardiogr*, 2000, 13, 774-9.

[44] Villareal, RP; Achari, A; Wilansky, S; Wilson, JM. Anteroapical stunning and left ventricular outflow tract obstruction. *Mayo Clin Proc.*, 2001, 76, 79-83.

[45] Bonnemeier, H; Ortak, J; Bode, F; Kurowski, V; Reppel, M; Weitz, G; Barantke, M; Schunkert, H; Wiegand, UK. Modulation of ventricular repolarization in patients with transient left ventricular apical ballooning: a case control study. *J Cardiovasc Electrophysiol*, 2006, 17, 1340-7.

[46] Akashi, YJ; Nakazawa, K; Sakakibara, M; Miyake, F; Musha, H; Sasaka, K. 123I-MIBG myocardial scintigraphy in patients with "takotsubo" cardiomyopathy. *J Nucl Med*, 2004, 45, 1121-7.

[47] Nef, HM; Mollmann, H; Troidl, C; Weber, M; Hamm, C; Elsasser, A. Tako-Tsubo cardiomyopathy: NT-proBNP as a reliable parameter of a favourable prognosis? *Int J Cardiol*, 2008, 124, 237-8.

[48] Haghi, D; Athanasiadis, A; Papavassiliu, T; Suselbeck, T; Fluechter, S; Mahrholdt, H; Borggrefe, M; Sechtem, U. Right ventricular involvement in Takotsubo cardiomyopathy. *Eur Heart J.*, 2006, 27, 2433-9.

[49] Haghi, D; Fluechter, S; Suselbeck, T; Borggrefe, M; Papavassiliu, T. Delayed hyperenhancement in a case of Takotsubo cardiomyopathy. *J Cardiovasc Magn Reson*, 2005, 7, 845-7.

[50] Haghi, D; Fluechter, S; Suselbeck, T; Kaden, JJ; Borggrefe, M; Papavassiliu, T. Cardiovascular magnetic resonance findings in typical versus atypical forms of the acute apical ballooning syndrome (Takotsubo cardiomyopathy). *Int J Cardiol*, 2007, 120, 205-11.

[51] Ito, K; Sugihara, H; Katoh, S; Azuma, A; Nakagawa, M. Assessment of Takotsubo (ampulla) cardiomyopathy using 99mTc-tetrofosmin myocardial SPECT--comparison with acute coronary syndrome. *Ann Nucl Med.*, 2003, 17, 115-22.

[52] Kurisu, S; Inoue, I; Kawagoe, T; Ishihara, M; Shimatani, Y; Nishioka, K; Umemura, T; Nakamura, S; Yoshida, M; Sato, H. Myocardial perfusion and fatty acid metabolism in patients with tako-tsubo-like left ventricular dysfunction. *J Am Coll Cardiol*, 2003, 41, 743-8.

[53] Ito, K; Sugihara, H; Kawasaki, T; Yuba, T; Doue, T; Tanabe, T; Adachi, Y; Katoh, S; Azuma, A; Nakagawa, M. Assessment of ampulla (Takotsubo) cardiomyopathy with coronary angiography, two-dimensional echocardiography and 99mTc-tetrofosmin myocardial single photon emission computed tomography. *Ann Nucl Med.*, 2001, 15, 351-5.

[54] Ramaraj, R. Stress cardiomyopathy: aetiology and management. *Postgrad Med J*, 2007, 83, 543-6.

[55] Bybee, KA; Kara, T; Prasad, A; Lerman, A; Barsness, GW; Wright, RS; Rihal, CS. Systematic review: transient left ventricular apical ballooning: a syndrome that mimics ST-segment elevation myocardial infarction. *Ann Intern Med.*, 2004, 141, 858-65.

[56] Ueyama, T; Ishikura, F; Matsuda, A; Asanuma, T; Ueda, K; Ichinose, M; Kasamatsu, K; Hano, T; Akasaka, T; Tsuruo, Y; Morimoto, K; Beppu, S. Chronic estrogen

supplementation following ovariectomy improves the emotional stress-induced cardiovascular responses by indirect action on the nervous system and by direct action on the heart. *Circ J.*, 2007, 71, 565-73.

[57] Ling, S; Komesaroff, P; Sudhir, K. Cellular mechanisms underlying the cardiovascular actions of oestrogens. *Clin Sci (Lond)*, 2006, 111, 107-18.

[58] Patten, RD; Pourati, I; Aronovitz, MJ; Baur, J; Celestin, F; Chen, X; Michael, A; Haq, S; Nuedling, S; Grohe, C; Force, T; Mendelsohn, ME; Karas, RH. 17beta-estradiol reduces cardiomyocyte apoptosis in vivo and in vitro via activation of phospho-inositide-3 kinase/Akt signaling. *Circ Res.*, 2004, 95, 692-9.

[59] Kam, KW; Qi, JS; Chen, M; Wong, TM. Estrogen reduces cardiac injury and expression of beta1-adrenoceptor upon ischemic insult in the rat heart. *J Pharmacol Exp Ther*, 2004, 309, 8-15.

[60] Chu, SH; Goldspink, P; Kowalski, J; Beck, J; Schwertz, DW. Effect of estrogen on calcium-handling proteins, beta-adrenergic receptors, and function in rat heart. *Life Sci.*, 2006, 79, 1257-67.

[61] Kneale, BJ; Chowienczyk, PJ; Brett, SE; Coltart, DJ; Ritter, JM. Gender differences in sensitivity to adrenergic agonists of forearm resistance vasculature. *J Am Coll Cardiol*, 2000, 36, 1233-8.

[62] Van De Walle, SO; Gevaert, SA; Gheeraert, PJ; De Pauw, M; Gillebert, TC. Transient stress-induced cardiomyopathy with an "inverted takotsubo" contractile pattern. *Mayo Clin Proc.*, 2006, 81, 1499-502.

[63] Kuroko, Y; Yamazaki, T; Tokunaga, N; Akiyama, T; Kitagawa, H; Ishino, K; Sano, S; Mori, H. Cardiac epinephrine synthesis and ischemia-induced myocardial epinephrine release. *Cardiovasc Res.*, 2007, 74, 438-44.

[64] Sanchez-Recalde, A; Costero, O; Oliver, JM; Iborra, C; Ruiz, E; Sobrino, JA. Images in cardiovascular medicine. Pheochromocytoma-related cardiomyopathy: inverted Takotsubo contractile pattern. *Circulation*, 2006, 113, e738-9.

In: Horizons in World Cardiovascular Research. Volume 1 ISBN: 978-1-60876-447-1
Editor: Eleanor H. Bennington, pp. 51-65

Chapter III

Cardiac Autonomic Function and Sports Activity

Juan Sztajzel
Cardiology Service, University Hospital Geneva, Switzerland.

Abstract

The autonomic nervous system (ANS) plays an important role in the pathophysiology of arrhythmogenesis due to increased sympathetic activity and reduced vagal tone. Traditional time and frequency heart rate variability (HRV) parameters have gained importance in recent years as techniques employed to explore the ANS. Available data support conclusions that decreased HRV is a strong predictor of increased cardiac mortality. On the other hand, inteventions that tend to increase HRV, such as regular sports activity, may have a cardio-protective effect. However, when analysing the effects of different sports disciplines and the effects of strenuous exercise on the cardiac autonomic function determined by HRV indices results remain conflicting due to a variable behavior of the used HRV time domain and spectral parameters.

Introduction

In the course of the last two decades numerous studies in both animals and human beings have shown a significant relationship between the ANS and cardiovascular mortality. Perturbations of the ANS and its imbalance consisting of either increased sympathetic or reduced vagal activity may result in ventricular tachyarrhythmias and sudden cardiac death, which is presently one of the leading causes of cardiovascular mortality. [1] Conversely, increased vagal tone associated to decreased sympathetic activity may improve the cardio-vascular status. In this context, one of the most efficient means to reach this improvement is to perform regular physical activity.

This article reviews the structure and function of the ANS, its relationship with the normal and diseased heart, and the effects of regular sports activity and of strenuous exercise on the ANS using HRV to explore it.

Structure and Function of the Autonomic Nervous System

The ANS is composed of elements arising from the central and peripheral nervous systems and regulates the activities of structures not normally under voluntary control.[2, 3] It is predominantly an efferent system composed of the sympathetic and parasympathetic branches transmitting impulses from the central nervous system to the periphery (heart, arteries, veins, lungs, etc). While the sympathetic fibers are widely distributed throughout the body, the parasympathetic fibers supply more specific structures. These two divisions of the ANS are in many instances in functional opposition, but in other instances are synergistic. Their most important role is to preserve the body's ability to maintain internal stability or equilibrium and to balance opposing actions under the control of higher cerebral centers. [2, 3] In contrast to the parasympathetic system, the sympathetic system enables the body to respond to challenges to survival (fight or flight) or situations of hemodynamic collapse or respiratory failure. Sympathetic responses include an increase in heart rate (HR), blood pressure, and cardiac output; a diversion of blood flow from the skin and splanchnic vessels to those supplying skeletal muscle; bronchiolar dilation and a decline in metabolic activity.

The Autonomic Nervous System and the Normal Heart

The electrical and contractile activity of the myocardium is constantly dependent on the ANS. In physiological conditions, the sympathetic and parasympathetic branches have opposing actions on the different cardiac functions. The role of the ANS is to adapt the functions of the cardiac pump to the potential requirements of the body. [2, 3] The ANS regulates HR, myocardial contractility and vascular peripheral resistance, thereby controlling blood pressure, cardiac output and overall stability of the cardiovascular system. Neurohumoral regulation can produce important and rapid cardio-circulatory changes in a few seconds before other much slower mechanisms, such as those mediated by metabolic stimuli, circulating catecholamines or the renin-angiotensin system, exert any effect. [4]

Effects of Autonomic Nervous Regulation on the Heart

Although cardiac automaticity is intrinsic to different tissues with pacemaker properties, HR and cardiac rhythm are under the influence of the ANS. [5-7] Both divisions of the ANS tonically influence the cardiac sinus node. The sympathetic system enhances automaticity, whereas the parasympathetic system inhibits it.

Modulation of cardiac automaticity depends on intra-sinus displacements of rhythm control at the tissue level and on changes of ionic currents at the cellular level. Three types of ionic current are implicated in the regulation of slow diastolic depolarization of the pacemaker cells and are modulated by the ANS: the potassium current IKAch, the calcium current ICa and the hyperpolarization-activated "pacemaker" If current.

Interaction of Sympathetic and Parasympathetic Effects

Vagal and sympathetic activity constantly interact. However, under resting conditions in a normal subject, vagal tone predominates and variations in HR depend mainly on vagal modulation. Vagal tone has an inhibitory effect on sympathetic activity because of the presence of acetylcholine which attenuates adrenergic stimulation and diminishes release of norepinephrine. Sympathetic activation becomes significant at a low effort level or under any external influence. Administration of atropine results in immediate tachycardia while ß-adrenergic blockade only induces a weak decrease in HR at rest.

The Autonomic Nervous System and Heart Diseases

The ANS intervenes not only in various normal situations, but also in pathological situations, and thus may induce deleterious effects on the heart. Abnormalities of the ANS have been demonstrated in diverse conditions such as diabetic neuropathy, [8] coronary heart disease, particularly in the context of a myocardial infarction, [9] and in congestive heart disease. [10]

It is clearly established nowadays that a dysregulation in the autonomic nervous control of the cardiovascular system associating increased sympathetic and reduced parasympathetic tone plays an important role in coronary artery disease and in the genesis of.life-threatening ventricular arrhythmias. Increased adrenergic tone probably initially induces a dysfunction at the level of the vascular endothelium, which serves a dual role in the control of vascular tone by secreting both vasorelaxing (nitric oxide and prostacyclin) and vasoconstricting factors (endothelin-1). [11] Increased adrenergic tone may subsequently damage the endothelial bareer resulting in release of vasoactive substances, platelet aggregation and initiating thereby a mechanism for the formation of an atherosclerotic plaque and the risk of developping ischemic heart disease. [11]

The occurrence of ischemia and/or myocardial necrosis may induce a mechanical distortion of the afferent and efferent fibers of the ANS due to changes in the geometry related to necrotic and noncontracting segments of the heart. These geometrical ischemic changes result in denervation of myocardial regions, rendering the myocardium more sensitive to catecholamines. [12] Moreover, the resulting sympathetic overactivity may further increase platelet activation and coronary vasoconstriction, and be responsible for the development of ventricular arrhythmias. Available experimental and clinical data suggest that

sympathetic overdrive may favor the incidence of malignant cardiac arrythmias while the vagus has the opposite effect. [13,14]

Chronic left ventricular dysfunction induces a hyperadrenergic state and an elevated resting HR which is a detrimental compensatory mechanism for the heart to maintain cardiac output. This chronic activation of the sympathetic nervous system associated to reduced parasympathetic (vagal) tone can increase the risk of cardiovascular events.

The Autonomic Nervous System and Physical Activity

Physical activity is a complex mulitfactorial behaviour and is influenced by various enviromental and biologiocal factors. Physical training causes physiological changes in cardio-vascular adaptation mechanisms such as higher aerobic capacity, larger stroke volumes, heart hypertrophy and bradycardia. [15-18]

Bradycardia has been described as a charactersistic feature accompanying individuals who perform regular pyhsical activity. Reported data on this topic have considered that the main mechanism responsible for bradycardia probably depends on changes in the ANS which may be due to a chronic increase in vagal activity. Furthermore, this chronic increase in parasysmpathetic tone occuring with regular dynamic exercise has been associated with an increased cardio-protective effect, particularly with a decrease in the risk of potentially lethal arrhythmias during myocardial ischemia.

Although the cardio-vascular benefits of regular physical activity are widely recognized, the accompanying ANS responses and particularly the exact mechanism responsible for the slowing of the cardiac rhythm still remain unclear and need to be fully elucidated.

One way to answer this question is to consider the different available tools allowing to assess the effects of the two branches of the autonomic function on the cardiovacular response and to apply the different measures to various populations of well-trained individuals.

Measurements of the Autonomic Nervous System

There are presently various available methods available for assessing the status of the ANS:

1) Tests based on cardio-vascular reflexes

These test include the Valsalva manoeuver, [19] the test of HR response to standing [20], and the head-upright tilt (HUT) table test. [21]

2) Biochemical tests

The degree of neurohormonal activation can be assessed by increased plasma levels of norepinephrine, [22] or endothelins. [23] An elevated activity of these tests has been recognized as a valuable predictive indicator of mortality particularly in patients with impaired left ventricular function. However, these tests are not routinely performed in daily clinical practise.

3) Scintigraphic methods

Assessment of sympathetic regional neuron density with the iodine 123 metaiodobenzylguanidine (I123 MIBG) technique is an available biological method which may assess the degree of neurohormonal activation. [24] The I123 MIBG is a guanethidine analogue that shares the same uptake, storage and release pathway as norepinephrine. The I123 MIBG imaging of the heart has made it possible to evaluate the sympathetic activity and innervation of the left ventricle. Decreased or absent myocardial I123 MIBG uptake has been described in congestive heart failure, cardiomyopathies, and MI.

Myocardial infarction produces regional cardiac sympathetic and parasympathetic denervation in the infarcted area, thus creating increased sensitivity to circulating catecholamines with dispersion of refractoriness and conduction and subsequent risk of ventricular arrhythmias. [13, 14]

4) Non invasive technics based on the ECG

The most simple ones are measurements of HR response to exercise (reserve) and HR recovery after exercise. These markers have been shown to be independent predictors of mortality. [25] Thus, the higher the HR reached during the exercise test, the better the prognosis. Concerning the values of HR recovery after exercise which mainly reflect parasympathetic reactivation researchers have reported that the rate at which the HR recovers from exercise at 1 or 2 minutes powerfully predicts prognosis, the slower the HR recovers the higher the risk of death.

In recent years, other noninvasive techniques based on the ECG have been used as markers of autonomic modulation of the heart, including HRV, [26] baroreflex sensitivity (BRS), [27] QT interval, [28] and heart rate turbulence (HRT). [29]

We will focus mainly on the value of HRV as a noninvasive method to evaluate the sympatho-vagal balance in the field of sports activity.

Heart Rate Variability and Sports Activity

Heart rate variability expresses the total amount of variations of both instantaneous HR and RR intervals, acting as a "mirror" of the cardio-respiratory system. [26, 30-32] It reflects the influence of the ANS on the sinus node of the heart.

Heart rate variability as a clinical tool provides information about sympathetic and parasympathetic autonomic function in normal and pathological hearts. [31] It may be used in different clinical settings including diabetes, [33] arterial hypertension, [34] coronary artery

disease, [35-37] sudden cardiac death, [38, 39] and heart failure. [40] Furthermore, the effects of a variety of pharmacological interventions on HRV have been studied, such as antiarrhythmic drugs, [41] or anesthetics.[42] Finally, the effects of non pharmacological interventions on HRV have been analysed such as changes of position and sleep, [43] and more importantly for us the effects of physical effort, [44] and high level training [45]

It is important to point out that a large body evidence exists presently and which considers decreased global HRV as a strong predictor of increased all-cause, cardiac and/or arrhythmic mortality, particularly in patients at risk after myocardial infarction. Thus, interventions, such as physical exercise and training, that tend to improve HRV may reflect the beneficial effect they exert on the ANS.

Measurements of HRV

Measurements of HRV are generally performed on the basis of 24-hour Holter recordings or on shorter periods ranging from 0.5 to 5 minutes particularly in the field of dynamic electrocardiography. The parameters intervening in the analysis of HRV are time domain indices [46, 47] and frequency domain indices [46, 48] Time domain indices and frequency domain analysis constitute nowadays the standard clinically used parameters.

The 24-hour time domain indices are composed of statistical and geometric measures. Statistical measures, expressed in milliseconds (ms), comprise the standard deviation of all NN intervals (SDNN), the standard deviation of the averages of NN intervals in all 5-minute segments of the entire recording (SDANN), the root mean square of successive differences (RMSSD) and pNN50 (NN50 count divided by the total number of all normal RR intervals). Geometric measures include mainly the HRV triangular index (HRVi). SDNN and the HRVi are both estimates of overall HRV. Reduced SDNN and/or HRVi have been considered to reflect an increased sympathetic and a diminished vagal modulation of sinus node. [46] Conversely increased SDNN and/or HRVi reflect increased vagal tone. RMSSD and pNN50 reflect alterations in autonomic tone that are predominantly vagally mediated. [46]

Frequency domain or power spectral densitiy analysis describes the periodic oscillations of the HR signal decomposed at different frequencies and amplitudes and provides information on the amount of their relative intensity in the heart's sinus rhythm. [46-48] Power spectral analysis is generally preformed by a nonparametric method, the fast Fourier transformation (FFT), which is characterized by discrete peaks for the several frequency components.

Spectral components are evaluated in terms of frequency given in Hertz (Hz) and amplitude assessed by the area or power spectral density of each component (given in ms2). The following spectral bands are determined: the total power, the very low frequency band (VLF), the low frequency band (LF), the high frequency band (HF), and the LF/HF ratio. Spectral components are expressed in absolute values (ms2) and in natural logarithms (ln) of the power because of the skewness of the distributions. Furthermore, LF and HF powers may also be given in normalized units (nu). Normalisation is performed by substracting from the total power the VLF component, reducing thereby the effects of noise due to artifacts and minimizing the effects of the changes in total power on the LF and HF components.

$$\text{LF or HF norm (nu)} = \frac{\text{LF or HF (ms2)}}{\text{total power (ms2)-VLF (ms2)}} \times 100$$

The total power of RR interval variability is the total variance and corresponds to the sum of all spectral bands. The VLF component has been identified and proposed as a marker of sympathetic activity. The LF component is modulated by both the sympathetic and parasympathetic nervous systems and thus reflects a mixture of both autonomic inputs. The HF component is generally defined as a marker of vagal modulation. The LF/HF ratio provides a measure of the global sympatho-vagal balance. Table 1 summarizes the most frequently used parameters of the time and frequency domain.

Table 1. Standard HRV measurements

Variable	Unis	Description	Reference values[46]
SDNN	ms	standard deviation of all normal RR intervals	141±39
SDANN	ms	standard deviation of the averages of normal RR intervals in all 5-minute segments of the entire recording	127±35
RMSSD	ms	root mean square of successive differences	27±12
pNN50	%	NN50 count divided by the total number of all normal RR intervals	9±7
HRV index		HRV triangular index	
TP	ms2	variance of all NN intervals (FR: <0.4 Hz)	37±15
LF	ms2	low frequency power (FR: 0.04-0.15 Hz)	3466±1018
HF	ms2	high frequency power (FR : 0.15-0.4 Hz)	1170±416
LF/HF ratio		ratio of low-high frequency power	975±203
			1.5±2.0

TP = total power; ms = milliseconds; FR = frequency range.

Effects of Physical Training on HRV

Exercise training increases HRV, suggesting a beneficial influence on cardiac autonomic activity and playing thereby a cardio-protective role. [44, 49-54] Several researchers have reported an enhanced parasympathetic tone in endurance-trained males compared with sedentary subjects, possibly in association with an attenuation of sympathetic drive. [55-58] Several months of intensive physical training in older and in younger subjects resulted in a significant increase of HRV parameters. [44] In another study training resulted in increased parasympathetic tone, reflected by higher values of the HF component. [51]

However, data on HRV remain somewhat conflicting due to a variable behavior of HRV time domain and spectral parameters. [45, 54, 59-62] Some authors [45] have observed higher time, but lower frequency domain values, particularly a lower HF component, in a group of athletes. Other authors [54] found higher SDNN in highly trained male cyclists compared to male healthy, untrained controls. However, no significant differences were observed between the study groups for RMSSD and pNN50, reflecting vagal, as well as for

the LF and HF spectral bands. In a study comparing cyclists, weight lifters and controls no significant differences in any HRV parameters were found. [61] Another study [63] aimed to investigate whether endurance-trained female athletes demonstrate differences in cardiovascular autonomic control compared with sedentary controls. Analysis of the HRV showed a longer RR interval in the trained subject but only in the spontaneous breathing condition. Conversely, athletes exhibited higher normalized LF and lower normalized HF only during the controlled breathing condition, with a subsequently higher LF/HF ratio in the trained group in the same condition.

In a recently published study by our group [64] we evaluated temporal and spectral HRV parameters determined from 24-hour continuous ECG monitoring performed during all-day activity in 40 subjects, including 12 endurance athletes, 14 hockey players and 14 untrained male volunteers (control group). As seen in Table 2, compared with controls HR values were lower and all parasympathetic-related time domain indices, including RMSSD and pNN50, as well as the LF and HF spectral components were higher in both athletes groups. However, SDNN values, which determine global HRV, were significantly higher only in endurance athletes.

Table 2. Comparisons between time and frequency domain indices in endurance athletes, team players and control group

	Endurance athletes (n=12)	Team players (n=14)	Control group (n=14)
HR (bts/min)	58-9±0.4**	60.3±0.7**	69.6±1.9
SDNN (ms)	166.6±2.0†	133.8±2.3	127.2±2.9
RMSSD(ms)	53.0±1.8*	53.4±1.9*	31.0±0.8
pNN50 (%)	25.0±1.0*	23.3±1.4*	10.6±0.6
LF (ln ms2)	7.42±0.08*	7.36±0.06*	6.68±0.04
HF (ln ms2)	6.33±0.12*	6.37±0.11*	5.11±0.07
LF/HF ratio	1.18±0.001*	1.16±0.01*	1.31±0.01

All values are expressed as mean±SD.
*P<0.05 and **P<0.01 when comparing endurance athletes and team players to the control group.
†P<0.01 when comparing endurance athletes to team players and to the control group.

In our study compararing different athletic disciplines we found that both athletic endurance and team playing activity had a favorable day and nighttime effect on the vagal input of the ANS, mostly reflected by increased values of pNN50 and RMSSD, by a higher HF and by a lower LF/HF ratio. Moreover, endurance athletes had higher SDNN, which is a global index of HRV, suggesting thereby that this type of sports activity may have a more substantially favorable effect on the cardiac autonomic profile.

Thus, our data indicate that both endurance and team playing sports activities influence primarily the parasympathetic tone. Endurance disciplines, such as running and cycling have a particularly beneficial effect on the global cardiac autonomic activity.

Behaviour of HRV During Exercise

During the performance of an exercise test HR and blood pressure increase constantly which is presumed to be due to higher sympathetic tone associated to withdrawal of the parasympathetic input. When using spectral HRV parameters during an acute physical effort, it would be expected to assist to an increase of the LF and a decrease of the HF components with a concommitant increase of the LF/HF ratio, consistent with sympathetic hyperactivity and parasympathetic drive during recovery. Although there is evidence to suggest a shift towards sympathetic dominance, particularly at the peak of highly strenuous training regimens [65-67] the expected behaviour of HRV parameters has not been systematically observed. In some studies of HRV absolute LF and HF power decreased during exercise. However when quantifying HF and LF power in normalized units, paradoxical results have been found. Indeed, normalized HF power, a marker of vagal activity, instead of diminishing, it increased gradually during exercise, whereas normalized LF power, a marker of sympathetic activity, decreased during exercise. [55, 68, 69]

One possible explanation for this finding is a decline in the periodic nature of the spectral peaks during exercise testing and, thus, of their density.

We performed in a group of 15 high-level male athletes (unpublisehd data) two maximal graded stress tests on a cycle ergometer, the first one at 8.30 am and the second one at 4.30 pm, and one submaximal one-hour exercise test (endurance test) at 12.30 pm with a constant workload of 75 watts. The athletes were composed of 2 weight-lifters, 3 cyclists, 2 long-distance runners, 1 soccer and 7 hockey players. Mean age was 29±6 years. Each athlete was under a continuous 24-hour Holter recording over the whole test day. Spectral HRV parameters were taken from these 24-hour Holter recordings. Spectral values were given in absolute logarithmic (ln ms2) units. During the morning and the afternoon maximal stress tests when reaching a maximal HR the LF and the HF components as well as their ratio dramatically decreased (Table 3). During the post-effort period both components began to recover already after the third post-effort minute and entirely recovered one hour after the morning and the afternoon stress tests were completed.

Table 3. Morning and afternnon maximal graded stress tests

Variable	Basal	Peak	PE 3 min	PE 5 min	PE 10 min	PE 1 hour
HR (bts/min) 8.30am	72±2	176±4	107±3	96±4	93±3	72±2
4.30pm	72±3	176±3	104±4	100±5	93±2	65±4
LF (ln ms2) 8.30am	7.0±0.3	2.2±0.2	4.4±0.2	5.6±0.3	6.1±0.4	7.1±0.3
4.30pm	7.0±0.2	2.1±0.1	4.3±0.1	5.4±0.4	6.1±0.2	7.1±0.2
HF (ln ms2) 8.30am	6.4±0.5	2.4±0.3	4.6±0.3	4.9±0.1	5.8±0.3	6.4±0.1
4.30pm	6.4±0.4	2.3±0.3	4.6±0.2	4.8±0.2	5.8±0.4	6.5±0.2

PE = post-effort.

The behaviour of LF and HF components was somewhat different in the course of the endurance test performed at 12.30 pm with a constant workload of 75 watts (Table 4). Indeed, during this test a submaximal HR was achieved and this resulted only in a slight decrease of the LF component associated with a more marked decrease of the HF component. However, when compared with the findings during both maximal stress tests the changes were much less pronounced.

Table 4. Submaximal one-hour exercise test

Variable	Basal	Submaximal	Peak	PE 1 hour
HR (bts/min)	72±4	103±5	124±6	78±5
LF (ln ms2)	6.9±0.4	6.6±0.3	6.1±0.2	7.0±0.2
HF (ln ms2)	6.5±0.1	4.2±0.1	3.8±0.2	6.4±0.3

PE = post-effort.

In another study recently published by our group [70] we found that during an endurance mountain running all spectral components of HRV, particularly VLF and LF power, dramatically decreased during the ascent, and progressively normalized during descent and arrival. We concluded that the behaviour of our HRV data was due to an extreme activation of the sympathetic nervous system. The physiological response of the heart in this situation was a down-regulation of the ß-adrenergic receptors to protect the myocardial function with subsequent rise in parasympathetic tone, reflected by an increase of the high frequency (HF) power and a decrease of the LF/HF ratio.

Thus, when considering our data the decrease of LF and HF during strenuous exercise appears to correspond to a protective physiological response of the heart to the high level of circulating catecholamines. The rise in vagal tone after the effort expresses in a healthy population expresse an .integer autonomic function.

Conclusions

Regular sports activity increases HRV, mainly by enhancing the parasympathetic tone, suggesting thereby a beneficial influence on cardiac autonomic activity and playing thereby a cardio-protective role. The type of sports discipline performed may also play a role and have a variable effect on HRV parameters. Thus, endurance disciplines, such as running and cycling, may have a particularly beneficial effect on the global cardiac autonomic activity. During strenuous exercise spectral HRV parameters expressed in absolute values decrease, however when expressed in normalized units a paradoxical increase of the normalized HF power has been found.

References

[1] Zipes, DP; Wellens, HJJ. Sudden cardiac death. *Circulation*, 1998, 98, 2334-2351.

[2] Berne, RM; Levy, MN. Regulation of the Heartbeat. In: *Physiology.* 4th ed. St Louis: *Mosby*, 1998, 379-397.

[3] Loewy, AD. Central autonomic pathways. In: Loewy AD, Spyer, KM, eds. *Central Regulation of Autonomic Control.* New York, NY: Oxford University Press, 1990, 88-103.

[4] Brembilla-Perrot, B; Beurrier, D; Alsagheer, S; Suty-Selton, C; Terrier, De La Chaise, A; Thiel, B; Louis, P; Hadjaj, B. Variations de la fréquence cardiaque à l'arrêt de la stimulation ventriculaire: corrélations avec le tonus vagal. *Arch. Mal. Coeur.* 1994, 87, 1297-1302.

[5] Brown, HF; DiFrancesco, D; Noble, SJ. How does adrenaline accelerate the heart? *Nature.* 1979, 280, 235-236.

[6] Sakmann, B; Noma, A; Trautwein, W. Acetylcholine activation of single muscarinic K^+ channels in isolated pacemaker cells of the mammalian heart. *Nature.* 1983, 303, 250-253.

[7] Jalife, J; Michaels, DC. Neural control of sinoatrial pacemaker activity. In: Levy MN, Schwartz PJ, eds. *Vagal Control of the Heart: Experimental Basis and Clinical Implications.* Armonk, NY: *Futura*, 1994, 173-205.

[8] Hosking, DJ; Bennett, T; Hampton, JR. Diabetic autonomic neuropathy. *Diabetes.* 1978, 27, 1043-1055.

[9] Rothschild, M; Rothschild, A; Pfeifer, M. Temporary decrease in cardiac parasympathetic tone after acute myocardial infarction. *Am. J. Cardiol.* 1988, 18, 637-639.

[10] Packer, M. The neurohumoral hypothesis: a theory to explain the mechanism of disease progression in heart failure. *J. Am. Coll. Cardiol.* 1992, 20, 248-254.

[11] Sztajzel, J; Mach, F; Righetti, A. Role of the vascular endothelium in patients with angina pectoris or acute myocardial infarction and normal coronary arteries. *Postgrad. Med. J.*, 2000, 76, 16-21.

[12] Kjellgren, O; Gomes, JA. Heart rate variability and baroreflex sensitivity in myocardial infarction. *Am. Heart J.* 1993, 204-214.

[13] Corr, PB; Yamada, KA; Witkowski, FX. Mechanisms controlling cardiac autonomic function and their relation to arrhythmogenesis. In: Fozzard HA, Haber E, Jennings RB, Katz AN, Morgan HE, eds. *The Heart and Cardiovascular System.* New York, NY: Raven Press, 1989, 1343-1403.

[14] Schwartz, PJ; Priori, SG. Sympathetic nervous system and cardiac arrhythmias. In: Zipes, DP; Jalife, J, eds. *Cardiac Electrophysiology: From Cell to Bedside. Philadelphia*, PA: WB Saunders Co, 1990, 330-343.

[15] Ahmed, AK; Harness, JB; Mearns, AJ. Respiratory control of the heart. *Eur. J. Appl. Physiol.* 1982, 50, 95-104.

[16] Hammond, HK; Froehlicher, VF. Normal and abnormal heart rate response to exercise. *Prog. Cardiovasc. Dis.*, 1985, 27, 271-296.

[17] Maron, BJ. Structural features of the athlete heart as defined by echocardiography. *J. Am. Coll. Cardiol.* 1986, 7, 190-203.

[18] Smith, L; Hudson, DL; Graitzer, H; Raven, P. Exercise training bradycardia: the role of autonomic balance. *Med. Sci. Sports Exerc.* 1989, 21, 40-44.

[19] Korner, PI; Tonkin, AM; Uther, JB. Reflex and mechanical circulatory effects of graded Valsava maneuvre in normal man. *J. Appl. Physiol.* 1976, 40, 434-440.

[20] Ewing, DJ; Hume, L; Campbell, IW; Murray, A; Neilson, JMM; Clarke, BF. Autonomic mechanisms in the initial heart rate response to standing. *J. Appl. Physiol.* 1980, 49, 809-814

[21] Robotis, DA; Huang, DT; Faubert, JP. Head-up tilt-table testing: an overview. *Ann. Non Invas. Electrocardiol.* 1999, 4, 212-218

[22] Benedict, CR; Shelton, B; Johnstone, DE; Francis, G; Greenberg, B; Konstam, M; Probstfield, JL; Yusuf, S. Prognostic significance of plasma norepinephrine in patients with asymptomatic left ventricular dysfunction. The SOLVD investigators. *Circulation.* 1996, 94, 690-697.

[23] Pousset, F; Isnard, R; Lechat, P; Kalotka, H; Carayon, A; Maister, G; Escolano, S; Thomas, D; Komajda, M. Prognostic value of plasma endothelin-1 in patients with chronic heart failure. *Eur. Heart J.* 1997, 18, 254-258.

[24] Dae, MW, Botvinick, EH. Imaging of the heart using metaiodobenzylguanidine. *J. Thorac. Imaging.* 1990, 5, 31-36.

[25] Kannel, W. On checking the pulse. *Eur. Heart J.* 2000, 21, 91-98.

[26] Stein, PK; Bosner, MS; Kleiger, RE; Conger, BM. Heart rate variability: A measure of cardiac autonomic tone. *Am. Heart J.* 1994, 127, 1376-1381.

[27] Pagani, M; Lombardi, F; Guzzetti, S; Rimoldi, O; Furlan, R; Pizzinelli, P; Sandrone, G; Malfatto, G; Dell'Orto, S; Piccaluga, E; Turiel, M; Baselli, G; Cerutti, S; Malliani, A. Power spectral analysis of heart rate and arterial pressure variabilities as a marker of sympatho-vagal interaction in man and conscious dog. *Circ. Res*. 1986, 59, 178-103.

[28] Schwartz, PJ; Wolf, S. QT interval prolongation as predictor of sudden death in patients with myocardial infarction. *Circulation.* 1978, 57, 1074-1077.

[29] Schmidt, G; Malik Barthel, P; Schneider, R; Ulm, K; Rolnitzky, L; Camm, AJ; Bigger, JT; Schomig, A. Heart rate turbulence after ventricular premature beats as a predictor of mortality after acute myocardial infarction. *Lancet.* 1999, 353, 1390-1396.

[30] Le Heuzey, J. La variabilité sinusale: intérêt en rythmologie. *Arch. Mal. Coeur*. 1992, 85, 37-43.

[31] Van Ravenswaaij-Arts, CMA; Kollée, LAA; Hopman, JCW; Stoelinga, GBA; Van Geijn, HP. Heart rate variability. *Ann. Intern. Med.*, 1993, 118, 436-447.

[32] Malik, M. Clinical implications and use of heart rate variability. In: Malik M, Camm J, eds. Heart Rate Variability. Armonk, NY: *Futura.* 1995, 331-538.

[33] Malpas, SC; Maling, TJB. Heart rate variability and cardiac autonomic function in diabetes. *Diabetes.* 1990, 39, 1177-1181.

[34] Chakko, S; Mulingtapang, RF; Huikuri, HV; Kessler, KM; Materson, BJ; Myerburg, RJ. Alterations in heart rate variability and its circadian rhythm in hypertensive patients with left ventricular hypertrophy free of coronary artery disease. *Am. Heart J*. 1993, 126, 1364-1372.

[35] Kleiger, RE; Miller, P; Bigger, JT; Moss, AJ. And the Multicenter Post-infarction Research Group. Decreased heart rate variability and its association with increased mortality after acute myocardial infarction. *Am. J. Cardiol.* 1987, 59, 256-262.

[36] Bigger, JT; Kleiger, RE; Fleiss, JL; Rolnitzky, LM; Steinmann, RC; Miller, JP; Moss, AJ; And the Multicenter Post-Infarction Research Group. Components of heart rate variability measured during healing of acute myocardial infarction. *Am. J. Cardiol.* 1988, 61, 208-215.

[37] Bigger, JT; Fleiss, JL; Steinmann, RC; Rolnitzky, LM; Kleiger, RE; Rottmann, JN. Frequency domain measures of heart period variability and mortality after myocardial infarction. *Circulation.* 1992, 85, 164-171.

[38] Martin, GJ; Magid, NM; Myers, G; Barnett, PS; Schaad, JW; Weiss, JS; Lesch, M; Singer, DH. Heart rate variability and sudden death secondary to coronary artery disease during ambulatory electrocardiographic monitoring. *Am. J. Cardiol.* 1987, 60, 86-88.

[39] Dougherty, CM; Burr, RL. Comparison of heart rate variability in survivors and nonsurvivors of sudden cardiac arrest. *Am. J. Cardiol.* 1992, 70, 610-615.

[40] Saul, JP; Arai, Y; Berger, RD; Lilly, LS; Colucci, WS; Cohen, RJ. Assessment of autonomic regulation in chronic heart failure by heart rate spectral analysis. *Am. J. Cardiol.* 1988, 61, 1292-1299.

[41] Zuanetti, G; Latini, R; Neilson, JMM; Schwartz, PJ; Ewing, DJ. Heart rate variability in patients with ventricular arrhythmias: effect of antiarrhythmic drugs. *J. Am. Coll. Cardiol.* 1991, 17, 604-612.

[42] Latson, TW; McCarroll, SM; Mirhej, MA; Hyndman, VA; Whitten, CW; Lipton, JM. Effects of three anesthetic induction techniques on heart rate variability. *J. Clin. Anesth.* 1992, 4, 265-276.

[43] Ewing, DJ; Neilson, JMM; Shapiro, CM; Steward, JA; Reid, W. Twenty-four hour heart rate variability: effects of posture, sleep, and time of day in healthy controls and comparison with bedside tests of autonomic function in diabetic patients. *Br. Heart J.* 1991, 65, 239-244.

[44] Stein, PK; Ehsani, AA; Domitrovich, PP; Kleiger, RE; Rottman, JN. Effect of exercise training on heart rate variability in healthy older adults. *Am. Heart J.* 1999, 138, 567-576.

[45] Sacknoff, DM; Gleim, GW; Stachenfeld, N; Coplan, NL. Effect of athletic training on heart rate variability. *Am. Heart J.* 1994, 127, 1275-1278.

[46] Task Force of the European Society of Cardiology and the North American Society of Pacing and Electrophysiology. Heart rate variability. Standards of measurement, physiological interpretation, and clinical use. *Circulation.* 1996, 93, 1043-1065.

[47] Kleiger, RE; Stein, PK; Bosner, MS; Rottman, JN. Time domain measurements of heart rate variability. *Amb. Electrocardiol.* 1992, 10, 487-498.

[48] Malliani, A; Pagani, M; Lombardi, F; Cerutti, S. Cardiovascular neural regulation explored in the frequency domain. *Circulation.* 1991, 84, 428-92.

[49] Bernardi, L; Valle; F; Coco, M; Calciati, A; Sleight, P. Physical activity influences heart rate variability and very-low-frequency components in Holter electrocardiograms. *Cardiovasc. Res.* 1996, 2, 234-237.

[50] De Meersman, RE. Heart rate variability and aerobic fitnes. *Am. Heart J.* 1993, 125, 726-731.

[51] Goldsmith, RL; Bigger, JT; Steinmann, RC; Fleiss, JL . Comparison of 24-hour parasympathetic activity in endurance trained and untrained young men. *J. Am. Coll. Cardiol.* 1992, 20, 552-558.

[52] Levy, WC; Cerqueira, MD; Harp, GD; Johannessen, KA; Abrass, IB; Schwartz, RS; Stratton, JR. Effect of endurance exercise training on heart rate variability at rest in healthy young and older men. *Am. J. Cardiol.* 1998, 82, 1236-1241.

[53] Molgaard, H; Sorensen, KE; Bjerrejgaard, P. Circadian variation and influence of risk factors on heart rate variability in healthy subjects. *Am. J. Cardiol.* 1991, 68, 777-784.

[54] Pluim, BM; Swenne, CA; Zwinderman, AH; Maan, AC; Van Der Laarse, A; Doornbos, J; Van Der Wall, EE. Correlation of heart rate variability with cardiac functional and metabolic variables in cyclists with training induced left ventricular hypertrophy. *Heart.* 1999, 81, 612-617.

[55] Dixon, BM; Kamath, MV; McCartney, N; Fallen, EI. Neural regulation of heart rate variability in endurance athletes and sedentary controls. *Cardiovasc. Res.*, 1992, 26, 713-719.

[56] Bonaduce, D; Petretta, M; Cavallaro, V; Apicella, C; Ianniciello, A; Romano, M; Breglio, R; Marciano, F. Intensive training and cardiac autonomic control in high level athletes. *Med. Sci. Sports Exerc.*, 1998, 30, 691-696.

[57] O'Sullivan, S; Bell, C. The effects of exercise and training of human cardiovascularreflex control. *J. Auton. Ner. Syst.*, 2008, 81, 16-24.

[58] Tulppo, M; Hautala, A; Makikallio, T; Laukkanen, R; Nissila, S; Hughson, R; Huikuri, H. Effects of aerobic training on heart rate dynamics in sedentary subjects, *J. Appl. Physiol.* 2003; 95: 364-372.

[59] Puig, J; Freitas, MJ; Carvalho, NP., et al. Spectral analysis of heart rate variability in athletes. *J. Sports Med. Phys. Fitness.* 1993, 33, 44-48.

[60] Yataco, AR; Fleisher, LA; Katzel, LI. Heart rate variability and cardiovascular fitness in senior athletes. *Am. J. Cardiol.* 1997, 80, 1389-1391.

[61] Lazoglu, AH; Glace, B; Gleim, GW; Coplan, NL. Exercise and heart rate variability. *Am. Heart J.* 1996, 131, 825-827.

[62] Reiling, MJ; Seals, DR. Respiratory sinus arrhythmia and carotid baroreflex control of heart rate in endurance ahtletes and untrained controls. *Clin. Physiol.* 1988, 8, 511-519.

[63] Middleton, N; De Vito, G. Cardiovascular autonomic control in endurance-trained and sedentary young women. *Clin Physiol Func Imaging*, 2005, 25, 83-89.

[64] Sztajzel, J; Jung, M; Sievert, K; Bayes De Luna, A. Cardiac autonomic profile in different sports disciplines during all-day activity. *J. Sports Med. and Phys. Fitness.* 2008, 48, 495-501.

[65] Furlan, R; Piazza, S, Dell'Orto, S; Gentile, E; Cerutti, S; Pagani, M; Malliani, A. Early and late effects of exercise and athletic training. on neural mechanisms controlling heart rate. *Cardiovasc. Res.*, 1993, 27, 482-488.

[66] Pichot, V; Roche, F; Gaspoz, J; Enjolras, F; Antoniadis, A; Minini, P; Costes, F; Busso, T; Lacour, J; Barthelemy, J. Relation between heart rate variability and training load in middle-distance runners. *Med. Sci. Sports Exerc.*, 2001, 32, 1729-1736.

[67] Iellamo, F; Legramante, JM; Pigozzi, F; Spataro, A; Norbiato, G; Lucini, D; Pagani, M. Conversion from vagal to sympathetic predominance with strenuous training in high performance world class athletes. *Circulation.* 2002, 105, 2719-2724.

[68] Malpas, SC. Neural influences on cardiovascular variability: Possibilities and pitfalls. *Am. J. Physiol. Heart Circ. Physiol.* 2002, 282, H6-H20.

[69] Pichon, AP; De Bisschop, C; Roulaud, M; Denjean, A; Papelier, Y. Spectral analysis of heart rate varibility during exercise in trained subjects. *Med. Sci. Sports Exerc.*, 2004, 36, 1702-1708.

[70] Sztajzel J, M. Jung M, K. Sievert K and A. Bayes de Luna A. Effects of extreme endurance running on cardiac autonomic nervous modulation in healthy trained subjects. *Am. J. Cardiol.* 2006, 97, 276-278.

In: Horizons in World Cardiovascular Research. Volume 1 ISBN: 978-1-60876-447-1
Editor: Eleanor H. Bennington, pp. 67-83

Chapter IV

Diagnosis of Cardiomyopathies and Rare Diseases: From "Phenocopy" to "Genocopy" Era

Giuseppe Limongelli*[1]*, Giuseppe Pacileo, Paolo Calabro', Raffaella D'Alessandro, Alessandra Rea, Valeria Maddaloni, and Raffaele Calabro
Monaldi Hospital, Second University of Naples, Naples, Italy

Keywords: *cardiomyopathies, phenocopy, genocopy, diagnosis, management*

Abstract

Cardiomyopathies are included in an heterogeneous group of diseases, characterized by different signs and symptoms, natural history, clinical outcome, and different pattern of inheritance. The genetics of cardiomyopathies has born in 1989 with a single gene theory (one gene=one disese), but the complexity and wide heterogeneity of the disease has moved toward a different direction (one gene=many diseases, or genocopies). Elucidation of the molecular basis of cardiomyopathies has led to a categorization of the phenotypes according to their genetic etiology. The American Hearth Association and the European Society of Cardiology have recently proposed a different scheme of classification based on a distinction between primary (genetic, mixed, non genetic types) and secondary cardiomyopathies, or between the familial and non familial types, respectively. The possibility of a different

[1] Address for Correspondence: Giuseppe Limongelli, MD, PhD, EDBT, FESC, MAHA, Department of Cardiothoracic Sciences, Second University of Naples, Monaldi Hospital, Via L Bianchi, 80131, Naples, Italy, Email: limongelligiuseppe@libero.it,Work-phone:+390817062852, Mobile: +393381041147, FAX:+390817062683.

approach of intervention (i.e. enzyme replacement therapy in metabolic cardiomyopathies) underlies the need to make an early and precise etiologic diagnosis.

Introduction

Cardiomyopathies are diseases of heart muscle. They represent an important and heterogeneous group of diseases. The awareness of cardiomyopathies in both the public and medical communities historically has been impaired by persistent confusion surrounding definitions and nomenclature. However, many classifications offered in the literature and in textbooks are to some degree contradictory in presentation. For more than 30 years, the term “cardiomyopathies” has been used to describe disorders of the heart with particular morphological and physiological characteristics.

Old Classifications

In 1980, the World Health Organization (WHO) in defined cardiomyopathies as "heart muscle diseases of unknown cause," to distinguish cardiomyopathy (including: Hypertrophic cardiomyopathy, Dilated cardiomyopathy, and Restrictive cardiomyopathy) from cardiac dysfunction due to known diseases such as hypertension, ischemic heart disease, or valvular disease. Heart muscle disorders of known aetiology (eg, ischemic or hypertensive cardiomyopathy) were classified as secondary diseases [1].

In1995, the WHO/International Society and Federation of Cardiology (ISFC) Task Force on the Definition and Classification of the Cardiomyopathies expanded the classification to include all diseases affecting heart muscle and to take into consideration etiology as well as the dominant pathophysiology. Cardiomyopathies were defined as "diseases of the myocardium associated with cardiac dysfunction", and they were classified according to anatomy and physiology into the following four types: Hypertrophic cardiomyopathy, HCM; Dilated cardiomyopathy, DCM; Restrictive cardiomyopathy, RCM; Arrhythmogenic right ventricular cardiomyopathy, ARVC, and Unclassified cardiomyopathies. Cardiomyopathies that are associated with specific cardiac or systemic disorders generally fall into one of these categories. These include ischemic, valvular, hypertensive, inflammatory, toxic, mitochondrial, neuromuscular, metabolic, and inherited disorders (FIGURE 1) [2].

These disorders have been also indicated as “phenocopies”. The term “specific cardiomyopathy” or “phenocopies” was probably the first important step toward a new classification, reflecting the fact that the genetic basis of the cardiomyopathies was being elucidated. Indeed, over the last two decades, clinical and molecular insights helped to better understand aetiology and management of cardiomyopathies. Many disorders considered before as “idiopathic” or “primary” disorders have been associated to specific genetic or non genetic defects, clinical features and outcome.

The American Heart Association Classification

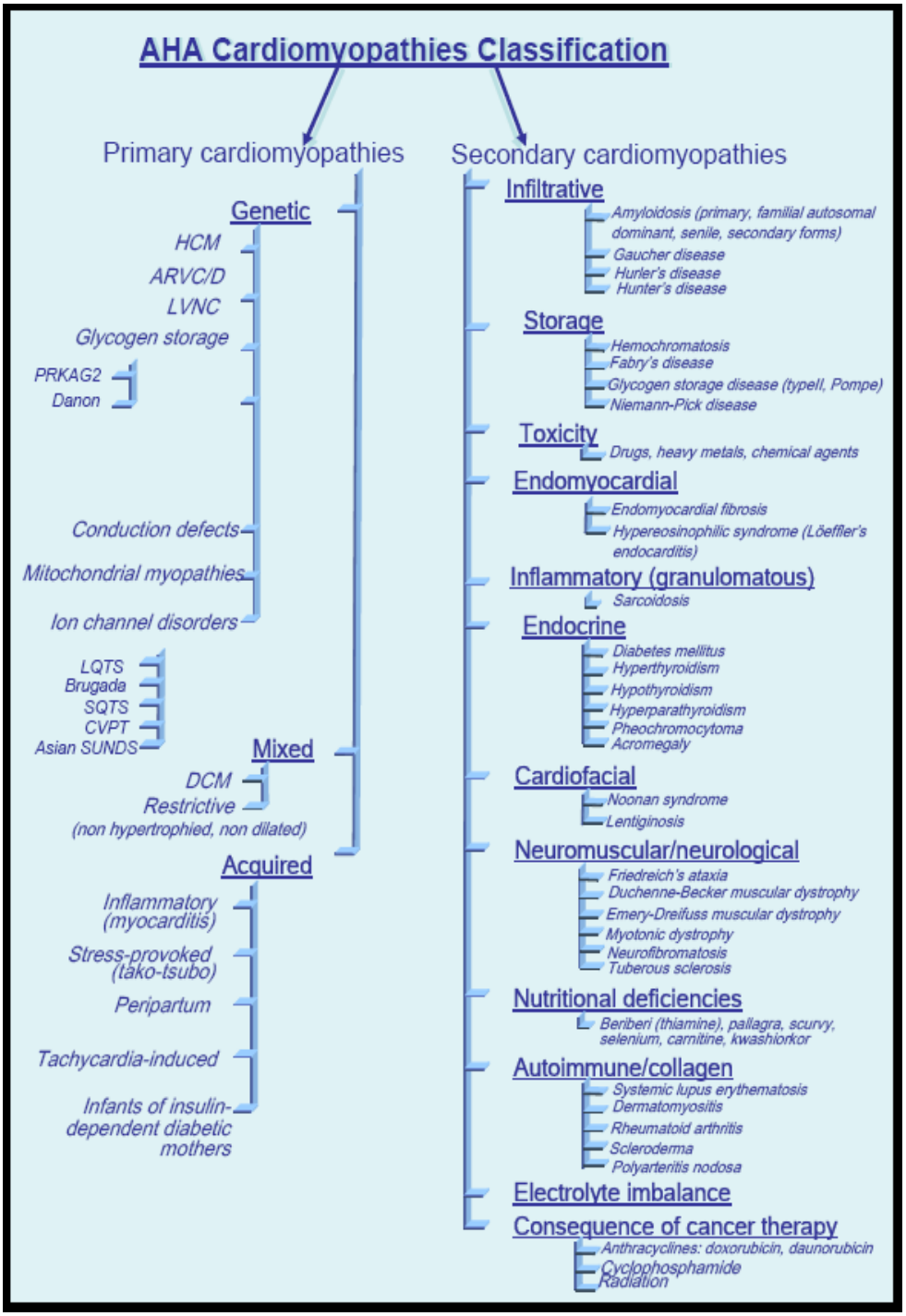


Figure 1. The American Heart Association classification of cardiomyopathies.

In 2006, an expert committee of the American Heart Association proposed the following definition of cardiomyopathies: "Cardiomyopathies are a heterogeneous group of diseases of the myocardium associated with mechanical and/or electrical dysfunction that usually (but not invariably) exhibit inappropriate ventricular hypertrophy or dilatation and are due to a variety of causes that frequently are genetic. Cardiomyopathies either are confined to the heart or are a part of generalized systemic disorders, often leading to cardiovascular death or progressive heart failure-related disability."

They also proposed a new scheme of classification, in which the term "primary" is used to describe diseases in which the heart is the sole or predominantly involved organ and "secondary" to describe diseases in which myocardial dysfunction is part of a systemic disorder. Primary cardiomyopathies have been sub-classified in genetic forms, mixed forms (genetic and non genetic), or acquired forms.

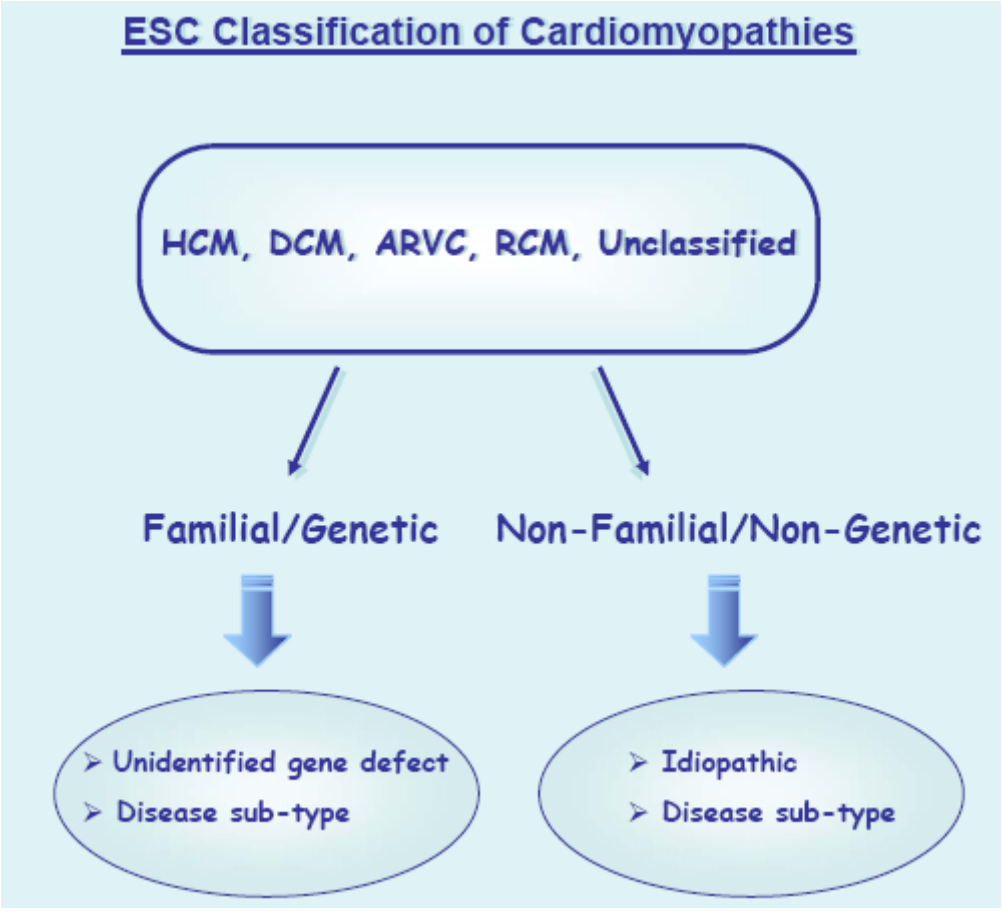


Figure 2. The European Society of Cardiology classification of cardiomyopathies.

The main departure of the proposed AHA Scientific Statement definition from previous classifications is the inclusion of the ion channelopathies as primary cardiomyopathies, despite the absence of gross structural abnormalities (FIGURE 2) [3].

The European Classification

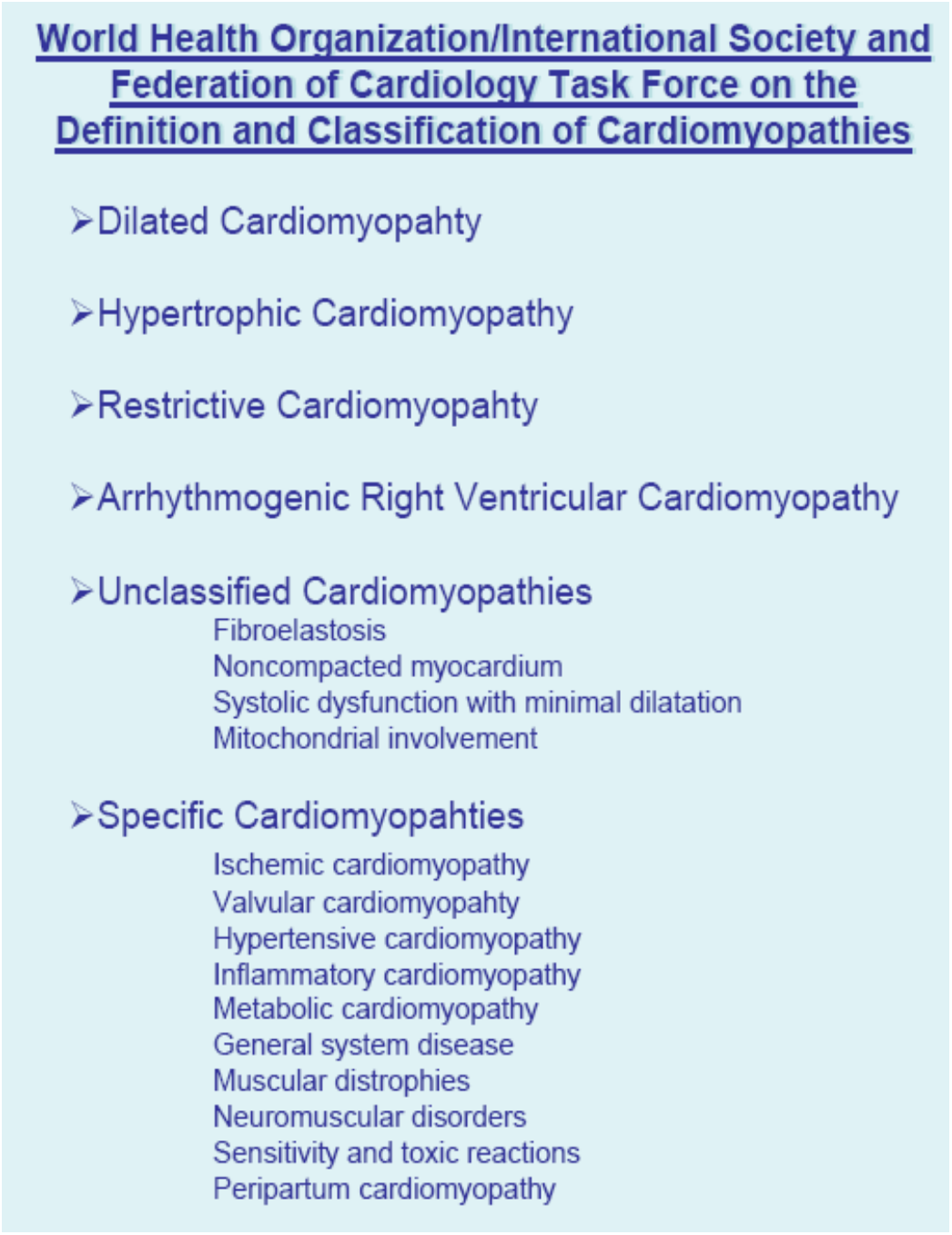


Figure 3. The World Health Organization classification of cardiomyopathies.

In 2007, the Working Group on Myocardial and Pericardial Diseases of the European Society of Cardiology proposed an update of the WHO/ISFC classification, defining cardiomyopathy as: "A myocardial disorder in which the heart muscle is structurally and

functionally abnormal in the absence of coronary artery disease, hypertension, valvular disease and congenital heart disease sufficient to explain the observed myocardial abnormality." Cardiomyopathies are grouped into specific morphological and functional phenotypes: each phenotype is then subclassified into familial/genetic and non-familial/non genetics forms. Like the 2006 AHA proposal, it focuses on the established morphological distinctions (hypertrophic cardiomyopathy, dilated cardiomyopathy, arrhythmogenic right ventricular cardiomyopathy, restrictive cardiomyopathy). Unlike the AHA classification, heart disease secondary to coronary heart diseases, valvular and congenital disorders are not included. Channelopathies are excluded as well (FIGURE 3) [4].

Genetics of Cardiomyopathy: A Long Way to Go

From "One Gene-One Disease"…

In 1989, Christine and Jon Seidman and co-workers reported the first association between an inherited gene defect and a primary cardiomyopathy [5]. In a subsequent study, they reported the first beta myosin missense mutation in a French Canadian family with hypertrophic cardiomyopathy, leading to the equation "one gene=one disease" [6].

…To "One Disease-Many Genes"…

Since then, substantial progress has been made in elucidating further (sarcomeric and non sarcomeric) gene defects in cardiomyopathies. To date, over 450 mutations in sarcomere protein genes (thick and thin filaments) have been identified in patients with HCM: β-myosin heavy chain (chromosome 14); cardiac troponin T (chromosome 1); cardiac troponin I (chromosome 19); troponin C (chromosome 3); α-tropomyosin (chromosome 15); cardiac myosin-binding protein C (chromosome 11); the essential and regulatory myosin light chains (chromosomes 3 and 12, respectively); cardiac actin (chromosome 15), titin (chromosome 2), and α-cardiac myosin heavy chain (chromosome 14). Some patients with sporadic disease, have similar genetic abnormalities as those with familial disease. De novo mutations in cardiac myosin binding protein-C, cardiac beta-myosin heavy chain, cardiac troponin T or alpha-tropomyosin genes have been found in isolated case reports of individuals with sporadic HCM [7].

HCM was then characterized as a disease of the sarcomere. As a consequence, DCM was indicated as a disease of the cytoskeleton and extracellular matrix, ARVC of the desmosome, and so on.

…To "One Disease-Many (Different)Genes"…

However, this "systematic" view became, again, in contrast with the rapidly growing genetic knowledge. Indeed, mutations in sarcomeric genes account for approximately 50-60%

of all cases of HCM, which is the most know form of cardiomyopathy both in the clinical and genetic setting, so far. The absence of sarcomeric gene mutations in the remaining HCM population seems to be related to a shortcomings in current mutation detection methods and strategies, or to be a result of disease-causing mutations in yet unidentified genes [7].

Rare causes of HCM have been associated with mutations in sarcomere-related protein genes (myosin light chain kinase, muscle LIM protein, LIM binding domain 3, telethonin, vinculin and metavinculin, caveolin, titin, α-actinin 2, myozenin 2, and junctophilin 2), or functional genes (phospholamban, RAF-1) [7].

Another possible explanation for the low proportion of cases thought to be caused by sarcomere gene mutations is that the population of hypertrophic cardiomyopathy without sarcomeric gene mutations may carry one of the several diseases that mimic the phenotypic expression of sarcomeric hypertrophic cardiomyopathy (the so called "phenocopies" of the disease), including metabolic-mitochondrial-neuromuscular diseases, inherited syndromes, and chromosomal abnormalities [7].

Mutations in the genes encoding the gamma-2 regulatory subunit of adenosine monophosphate (AMP)-activated protein kinase (PRKAG2) and lysosome-associated membrane protein 2 (LAMP2) have been associated with hypertrophic cardiomyopathy in association with Wolff-Parkinson-White (WPW) syndrome [7,8]. Similar to PRKAG2 and LAMP2, Fabry's disease, an X-linked lisosomial disorder, can express predominant cardiac features of left ventricular hypertrophy. Over the years, mutations in GLA-encoded alpha-galactosidase A have been found in patients with this multisystem disorder [7,9]. Friedreich ataxia, an autosomal-recessive disease involving sclerosis of the spinal cord, is often associated with cardiomyopathies, and its cardiovascular manifestations may precede the neurological symptoms by up to a decade in some cases [7,10]. Chromosome Abnormalities, including Down syndrome and trisomy 18, Autosomal Dominant Cardiofacial Disorders (Noonan syndrome, LEOPARD syndrome, Cardiofaciocutaneous syndrome Costello syndrome) or Phakomatoses (Neurofibromatosis, Tuberous sclerosis) have been associated with a variety of cardiac defects, including hypertrophic cardiomyopathy [7,11-14].

…To "One Gene-Many Diseases"

The major cardiomyopathies are genetically heterogeneous diseases for which the causative genes are partially overlapping. The evidence that a complex of genes (i.e. sarcomeric protein genes) may be responsible of a "spectrum" of different phenotypes, including HCM, DCM, RCM and recently LVNC, represent a further step in the knowledge and understanding of cardiomyopathies[15]. The identification of sarcomeric mutations in familial LVNC, and an alpha-actin mutation in HCM with LVNC and atrial septal defect together with the observation of late onset LVNC in a Duchenne patient, suggests that the aetiology of LVNC extends beyond an arrest in embryonic cardiac development (i.e. the possibility of late onset LVNC[16,17]. The current findings expand the genetic heterogeneity of LVNC, and the identification of sarcomeric defects in familial LVNC suggests that LVNC may be part of a cardiomyopathy spectrum including HCM, RCM, and DCM[16,17].

Whether this means that these are different diseases or rather different manifestations (phenotypes) of the same pathological mechanism is presently not clear.

… Or to "One Gene-Many (Different)Diseases"…

It is now evident that a large number of mutations in different genes, albeit largely within the same class, could cause the same phenotype. Moreover, mutations in one gene could cause multiple phenotypes, as best illustrated in the case of lamin A/C, whereby mutations can cause 13 different diseases, including DCM, conduction defects, Emery Dreifuss muscular dystrophy, familial partial lipodystrophy, premature aging, axonal neuropathy, and insulin resistance [18]. In addition, SCN5A (sodium channel) gene mutations may cause phenotypes that combine features of LQT3 (Long QT Syndrome 3), Brugada syndrome, conduction disease and dilated cardiomyopathy ("one gene-different diseases") [19].

One Disease or Many Diseases? From " Phenocopy" to " Genocopy"

Genomic medicine has entered clinical practice, and the recognition of the diagnostic utility of genetic testing for cardiomyopathies (particularly, hypertrophic cardiomyopathy) is growing. With expanding knowledge of the genetic background of these diseases, primary cardiomyopathies have recently been subclassified into genetic, mixed, and acquired cardiomyopathies (AHA 2006) or familial and non familial disease (ESC 2007), shifting the general view of cardiomyopathies from a "phenocopy" to a "genocopy" model.

However, although a number of cardiomyopathies susceptibility genes involving different pathways have been identified, the search for novel mutations in new genes continues. As a result of the increasing genetic heterogeneity of HCM, a classification based on functional genetics might seem very helpful, but in the light of the low yield of mutations in a large number of these genes as well as the commercial availability of just a small number of these genes, a phenotypic classification might be a more useful tool in looking at this disease from a clinical-practice vantage point. With the growing number of cardiomyopathy-associated genes discovered, strategic choices have to be made in clinical practice.

Diagnosis of Cardiomyopathy

The utility of an accurate diagnosis and distinction from a phenocopy state is well illustrated in certain circumstances, such as Fabry disease, which could be clinically indistinguishable from HCM caused by mutations in sarcomeric proteins [9]. Enzyme replacement therapy with alpha-galactosidase, the enzyme responsible for Fabry disease, has been shown to impart considerable clinical benefit in management of patients with Fabry disease, while the conventional treatment offered for true HCM would render no significant benefit in such patients [20].

The age of onset (infancy, childhood, adolescency, or adulthood), the pattern of inheritance (autosomic, X linked or matrilinear), symptoms/signs at onset, physical abnormalities (dysmorphic features, myopathy, mental retardation), ECG abnormalities (i.e. short PR in metabolic or mitochondrial disorders), the echocardiographic pattern (i.e. left ventricular non-compaction cardiomyopathy associated with specific neuromuscular disorders), and other biochemical or functional tests (i.e. premature lactic acidosis and flat oxigen pulse during metabolic stress test in mitochondrial disorders) may be relevant to discriminate between different causes of cardiomyopathies. Detailed clinical evaluation and mutation analysis are, therefore, important to provide an accurate diagnosis in order to enable genetic counselling, prognostic evaluation and appropriate clinical management [21].

Physical Examination

Physical abnormalities can be characteristics of specific disorders and lead to the final clinical diagnosis. Macroglossia, carpal tunnel syndrome, reticular lung infiltrates and Bence-Jones proteinuria may be hallmarks of plasma-cell-dyscrasia-related systemic amyloidosis. Metabolic disorders and syndromes are associated with characteristic physical abnormalities (dysmorphic features, myopathy, mental retardation) and symptoms at onset. Patients with inborn errors of metabolism involving impaired energy production or the accumulation of toxic metabolites often have signs and symptoms of multiple organ dysfunction. Dysmorphic features may characterize malformation syndromes as well as storage diseases, and therefore other minor and major malformations should also be sought. In patients with primary neuromuscular disorder, skeletal muscle weakness usually precedes the cardiomyopathy and dominates the clinical picture. Occasionally, however, skeletal myopathy is subtle, and the first symptom of disease may be cardiac failure. Encephalopathy is characteristically seen in the mitochondrial syndromes MELAS (Mitochondrial Encephalopathy, Lactic Acidosis, and Strokelike episodes), MERRF (Myoclonic Epilepsy, Ragged Red Fibers), Kearns-Sayre syndrome, and Leigh disease. Acute worsening can occur in these syndromes in association with intercurrent illness or metabolic stressors. In general, the neurological features of these syndromes (epilepsy, strokelike episodes, dementia, and ophthalmoplegia) predominate, and the cardiomyopathy typically occurs later in the clinical course [21,22].

Electrocardiogramm

Almost all (95%) patients with hypertrophic cardiomyopathy have an abnormal ECG. The most frequent ECG changes are left atrial enlargement, repolarization abnormalities, and pathologic Q waves, most commonly in the inferolateral leads. Voltage criteria for left ventricular hypertrophy alone are non-specific and are often seen in normal young adults. Giant negative T waves in the mid-precordial leads are characteristic of hypertrophy confined to the left ventricular apex. Some patients have a short PR interval, including metabolic/storage (Danon, PRKAG2, Fabry disease) or mitochondrial disorders. Patients with amyloidosis often show low voltages in the precordial leads[7-10, 21-23].

Non Invasive Imaging Technology

Standard echocardiography, new echocardiographic technologies, and Cardiac magnetic resonance (CMR) provide information on myocardial structure and have been suggested as a potential tool to discriminate between different phenocopy states.

Standard Echo

Left ventricular hypertrophy associated to congenital heart defects is frequently seen in malformation syndromes (such as pulmonary valve abnormalities in Noonan and LEOPARD syndrome). An abnormal texture of the interventricular septum ("granular sparkling" aspect), especially if associated with biatrial dilation, pericardial effusion and restrictive phenotype, may be diagnostic of amyloid. However, other infiltrative diseases (i.e. metabolic myopathies, Gaucher, Hunter's, and Hurler's diseases) or storage cardiomyopathies (haemochromatosis, Fabry's disease, glycogen storage, and Niemann-Pick disease) should be considered. In advanced haemochromatosis all cardiac chambers may be dilated. Mucopolysaccharidosis and Gaucher's disease may lead to aortic and mitral stenosis. In hypothyroidism, other than amyloidosis, a pericardial effusion can be present. Pieroni et al. showed in 83% of Fabry's cardiomyopathy patients (95% of FC patients with LVH) a binary appearance of endocardial border absent in all HCM, hypertensive, and healthy subjects (sensitivity 94%; specificity 100%), reflecting an endomyocardial glycosphingolipids compartmentalization, consisting of thickened glycolipid-rich endocardium, free glycosphingolipid subendocardial storage, and an inner severely affected myocardial layer with a clear subendocardial-midwall layer gradient of disease severity. On the other hand, Kounas et al. showed the binary sign in 8/28 patients with HCM (3 patients) and with Fabry's cardiomyopathy (5 patients). The sensitivity and specificity of the binary sign as a discriminator of AFD from HCM were 35% and 79%, respectively. The authors suggest that the binary endocardial appearance lacks sufficient sensitivity and specificity to be used as an echocardiographic screening tool. In neuromuscular disorders like glycogenosis, mitochondriopathy and myotonic dystrophy, myocardial thickening, hypertrabeculation/noncomnpaction and systolic dysfunction are found. The coexistence of left ventricular non-compaction and localised inferobasal left ventricular akinesia are almost pathognomonic of dystrophinopathies. Finally, a diagnosis of neuromuscular/metabolic/mitochondrial cardiomyopathy is favored in presence of concentric/asymmetric/apical, non-obstructive hypertrophic cardiomyopathy, with or without hypertrabeculation of the apex, especially when associated with an early onset impairment of LV systolic function. However, metabolic and mitochondrial cardiomyopathy might also be presented with dilated type, and hypertrophy may become dilated in the later stage [7,9,10-12, 24-26].

Recently, genotype–phenotype studies from the Majo Clinic Cardiomyopathy Group have discovered an important relationship between the morphology of the left ventricle, its underlying genetic substrate and the long-term outcome of this disease. They observed that the septal contour was the strongest predictor of the presence of a myofilament mutation,

regardless of age. Intriguing conclusions can be drawn from these observations. Whereas in initial morphological studies, sigmoidal HCM seemed to be associated with older age, the underlying genotype rather than age appears to be the predominant determinant of septal morphology. Furthermore, Z-disc HCM seems to have a predilection for sigmoidal contour status These observations may facilitate echo-guided genetic testing by enabling informed genetic counseling about the a-priori probability of a positive genetic test based upon the patient's expressed anatomical phenotype[27].

New Imaging Techonologies

Weidemann et al. have investigated in a prospective study whether regional non-ischaemic fibrosis in hypertrophic myocardium can also be detected by ultrasonic strain-rate imaging based on specific visual features of the myocardial deformation traces. This diagnostic study aimed to define left ventricular fibrotic segments in 30 patients with hypertrophic cardiomyopathy (n = 10), severe aortic valve stenosis (n = 10), Fabry disease cardiomyopathy (n = 10), and 10 healthy controls. In total, 42 segments showed late enhancement by magnetic resonance imaging. Using strain-rate imaging, all late enhancement positive segments displayed a characteristic pattern consisting of a first peak in early systole followed by a rapid fall in strain rate close to zero and a second peak during isovolumetric relaxation. This 'double peak sign' was never seen in segments of healthy controls. However, it was detected in 10 segments without late enhancement. These 'false-positive' segments belonged to Fabry patients who often develop a fast progressing fibrosis. In a follow-up magnetic resonance imaging study after 2 years, all these segments had developed late enhancement. Therefore, the 'double peak sign' in strain-rate imaging tracings seems to be a reliable tool to diagnose regional fibrosis[26].

CMR

Moon JC et al. have shown that late gadolinium enhancement cardiovascular magnetic resonance can visualize myocardial interstitial abnormalities. Late enhancement was demonstrated in nine patients with different specific cardiomyopathies, with a mean signal intensity of 390 +/- 220% compared with normal regions. The distribution pattern of late enhancement was unlike the subendocardial late enhancement related to coronary territories found in myocardial infarction. The affected areas included papillary muscles (sarcoid), the mid-myocardium (Anderson-Fabry disease, glycogen storage disease, myocarditis, Becker muscular dystrophy) and the global sub-endocardium (systemic sclerosis, Loeffler's endocarditis, amyloid, Churg-Strauss). Focal myocardial late gadolinium enhancement have been found in these specific cardiomyopathies, and the pattern is distinct from that seen in infarction. CMR hyperenhancement pattern is very characteristic for cardiac involvement of amyloidosis and can therefore be used to discriminate this disease from other forms of restrictive or hypertrophic cardiomyopathies. Although most profound in the subendocardial layer of myocardium, amyloid deposition occurs throughout the entire myocardium, causing

the entire myocardium to have a higher signal on delayed contrast enhancement images than normal myocardium [28].

Biochemical/Metabolic Tests

Biochemical analysis represents an step for the diagnosis of mitochondrial, metabolic and neuromuscular cardiomyopathies. The presence of hypoglycemia, primary metabolic acidosis with an increased anion gap, or hyperammonemia should alert the physician to the possibility of a metabolic disorder. The insulin-excess states of Beckwith-Wiedemann syndrome and the infant of a diabetic mother can produce hypoketotic hypoglycemia but are distinguished by low free fatty acid levels by characteristic clinical features. Disorders in fatty acid metabolism can be identified as defects of fatty acid ß-oxidation or of carnitine-dependent transport depending on quantitative carnitine levels in blood, urine, and tissue; acylcarnitine profile in blood; and urine organic acids (fatty acids, dicarboxylic, and hydroxydicarboxylic acids). In Fabry disease, electrolyte imbalances and proteinuria reflecting renal failure may be seen. Level of globotriaosylceramide (Gb3 or GL-3) a glycosphingolipid may be elevated. Enzymatic analysis performed by using plasma or leukocytes may show a deficiency of alpha-galactosidase A. However, levels of Gb3 and alpha-galactosidase A may be normal in female heterozygote Fabry patients. Therefore, genetic and/or molecular diagnosis is necessary to confirm Fabry disease if suspected based on clinical features of proteinuria and acroparesthesias that were invariably present in both men and women with Fabry mutation and cryptogenic stroke. Elevated serum creatine kinase levels can be associated with diagnosis of a neuromuscular disease. Although clinical signs and laboratory tests are useful for identifying and classifying diseases of the lower motor unit, in isolation, they rarely lead to a specific diagnosis. However, a markedly elevated serum creatine kinase level (10 to 100 times higher than normal) is invariably found early in the clinical course of Duchenne muscular dystrophy and almost always in its milder allelic form, Becker-type muscular dystrophy, whereas the serum creatine kinase level is usually lower in other muscular dystrophies and myopathies (1 to 10 times higher than normal). Because creatine kinase levels can vary markedly among different patients with the same disease and may fluctuate in a given patient over time, clinical judgment is necessary to interpret these values. A premature lactic acidosis, a very low VO_2 and a flat oxygen pulse may represent markers of metabolic/mitochondrial diseases. The diagnosis can be confirmed on measurement of blood and cerebrospinal fluid lactate and pyruvate levels, histological analysis of skeletal muscle, assay of respiratory chain enzymes, and/or mitochondrial DNA analysis[21, 29].

Genetic Testing

The clinical application of mutation analysis is technically possible, but has been hindered by logistics and high cost. Given the cost of mutation analysis, however, a strategic approach based on probabilities should be employed where possible. Careful phenotyping should identify the most common phenocopies of cardiomyopathies.

The major goals of genetic testing in patients with cardiomyopathies are:

- -to contribute to diagnosis;
- -to provide prognostic and therapeutic benefits;
- -most important, to detect relatives affected or at risk to develop the disease (carriers).

Once a mutation has been detected in a proband, the possibility of genetic testing should be suggested to first-degree relatives (who have a 50% probability of being gene-positive in autosomal disorders: 'cascade' screening). This type of screening enables close clinical management of mutation carriers, and identifies genetically normal family members, obviating the need for them to undergo clinical screening and repeat follow-up examinations. Appropriate genetic counselling, performed by a well trained physician (clinicians, geneticist) or genetic counsellor, should precede and follow genetic testing to help the patient and his/her family to comprehend the reasons to perform the test and the clinical significance and impact of a positive/negative diagnosis. A specially trained and experienced nurse may serve as coordinator of the investigations and as contact person for the family [3,4,7,15,22].

Organ-Specific and Skeletal Muscle Biopsy

Biopsy with Congo red staining and immunostaining is the procedure of choice for the diagnosis of amyloidosis. Stain the tissue with an alkaline solution of Congo red, and examine it under polarized light, where positive (green) birefringence is detectable in the presence of amyloidosis of any type. The nature of the fibril precursor can be established by immunohistochemical staining with antibodies specific for the major amyloid precursors (Amyloid A, immunoglobulin L chains of k or l type, antitransthyretin). In Amyloid A amyloidosis, only the Amyloid A is positive. The amyloid nature of the deposit can by confirmed by staining with an antiserum specific for serum amyloid P-component. In amyloidosis, the tissue with the highest yield, particularly in the presence of proteinuria or renal failure, is the kidney (technically adequate samples have a diagnostic yield close to 100%). If renal biopsy is deemed too risky for a specific patient or if amyloidosis without renal disease is suspected, 2 sites have been shown to be useful in obtaining tissue for histologic and immunochemical analysis. Subcutaneous fat aspiration is positive in approximately 60% of individuals with Amyloid A amyloidosis, except in the case of familial Mediterranean fever, when it rarely, if ever, is positive. Rectal biopsy is more useful than subcutaneous fat aspiration in Amyloid A amyloidosis. It has been found to produce positive results (assuming that submucosa is included in the biopsy specimen) in 80-85% of patients ultimately found to have tissue amyloid at a clinically relevant site. Samples from either the subcutaneous fat aspirate or the rectal biopsy can be stained as conventional tissue biopsies to determine the presence and nature of the amyloid precursor. Occasionally, patients have positive results on subcutaneous fat aspirates in the presence of a negative result on rectal biopsy, while others may have deposits in the rectal tissue and not in the aspirate. Use of both procedures may increase the yield to 90%. Abdominal subcutaneous fat biopsy results are not

very sensitive in Amyloid A caused by familial Mediterranean fever and in dialysis-related amyloidosis. The results are usually negative, probably because beta2-microglobulin does not accumulate in this tissue.

A skeletal muscle biopsy is often necessary, especially in infants, when the clinical and laboratory findings are nonspecific. If a muscular dystrophy is suspected, particularly in a boy, molecular analysis of the dystrophin gene and/or protein is indicated. Dystrophin, a cytoskeletal protein normally found in all muscle cell types, is thought to stabilize the plasma membrane of the muscle cell and may be important in the regulation of intracellular calcium. Approximately 65% of patients with Duchenne muscular dystrophy or Becker-type muscular dystrophy have deletions of the dystrophin gene that can be detected by PCR in blood lymphocytes. In the other 35% of patients, including manifesting female carriers for whom PCR results are difficult to interpret, a muscle biopsy is required to detect a reduced amount of the dystrophin protein or abnormalities of its size. The presence of dystrophic changes in a skeletal muscle biopsy specimen is also an indication for molecular analysis of dystrophin [22,30,31].

Endomyocardial Biopsy

Although the role of endomyocardial biopsy (EMB) in the diagnosis and treatment of adult and pediatric cardiovascular disease remains controversial, a recent joint AHA/ACC/ESC statement recommends endomyocardial biopsy (class I, evidence B) in patients with suspected myocarditis, including

1) new onset heart failure of less than two weeks duration associated with a normal sized or dilated left ventricle and haemodynamic compromise;
2) new onset heart failure of 2 weeks to 3 months duration associated with a dilated left ventricle and new ventricular arrhythmias, second- or third-degree heart block, or failure to respond to usual care within 1 to 2 weeks. In addition, endomyocardial biopsy is reasonable (IIA; evidence C):

- in the clinical setting of unexplained heart failure of >3 months' duration associated with a dilated left ventricle and new ventricular arrhythmias, Mobitz type II second- or third-degree AV heart block, or failure to respond to usual care within 1 to 2 weeks;
- in the setting of unexplained heart failure associated with suspected anthracycline cardiomyopathy;
- in the setting of heart failure associated with unexplained restrictive cardiomyopathy;
- in the setting of unexplained heart failure associated with a DCM of any duration that is associated with suspected allergic reaction in addition to eosinophilia.
- in the setting of suspected cardiac tumors, with the exception of typical myxomas whereas adenovirus is most commonly associated with histological [32].

Conclusions

In 1968, the World Health Organization defined cardiomyopathies as "diseases of different and often unknown etiology in which the dominant feature is cardiomegaly and heart failure". This statement was updated in 1980 and defined cardiomyopathies as "heart muscle diseases of unknown cause", thereby differentiating them from specific identified heart muscle diseases of known cause such as myocarditis. In 1995, a World Health Organization/International Society and Federation of Cardiology Task Force on cardiomyopathies classified the different cardiomyopathies by the dominant pathophysiology or by etiological/pathogenetic factors **(**Phenocopy era**).** Over the last two decades, the importance of gene defects in the etiology of cardiomyopathies has been recognized, and several new disease entities have been identified with the introduction of molecular biology into clinical medicine (Genocopy era**)**, rendering previous classifications and formal cardiomyopathies concepts obsolete, and leading to different reclassification of cardiomyopathies by the AHA and the Working Group of the ESC.

However, given the extreme heterogeneity of cardiomyopathies, there probably is no single classification or "model" that can be regarded as generally acceptable to all the interested parties from diverse disciplines (researchers, clinicians, epidemiologists, geneticists). Nevertheless, cardiologists and cardiomyopathy specialists need to become familiar with the basic principles of molecular biology and clinical genetics, in order to generally understand the basis of the disease, to provide a correct characterization of the clinical phenotype and to eventually guide the genotype, to understand and manage the implications of a positive genetic diagnosis for the proband and his/her family.

References

[1] Report of the WHO/ISFC task force on the definition and classification of cardiomyopathies. *Br. Heart. J.* 1980; 44:672–673.

[2] Richardson P, McKenna W, Bristow M, et al. Report of the 1995 World Health Organization/International Society and Federation of Cardiology Task Force on the definition and classification of cardiomyopathies. *Circulation* 1996; 93:841–842.

[3] Maron BJ, Towbin JA, Thiene G, et al. Contemporary definitions and classification of the cardiomyopathies: an American Heart Association Scientific Statement from the Council on Clinical Cardiology, Heart Failure and Transplantation Committee; Quality of Care and Outcomes Research and Functional Genomics and Translational Biology Interdisciplinary Working Groups; and Council on Epidemiology and Prevention. *Circulation* 2006; 113:1807– 1816

[4] Elliott P, Andersson B, Arbustini E, et al. Classification of the cardiomyopathies: a position statement from the European society of cardiology working group on myocardial and pericardial diseases. *Eur. Heart. J*. 2007; 29(2):270-6.

[5] Jarcho JA, McKenna W, Pare JA, et al. Mapping a gene for familial hypertrophic cardiomyopathy to chromosome 14q1. *N. Engl. J. Med.* 1989; 321: 1372–1378.

[6] Geisterfer-Lowrance AA, Kass S, Tanigawa G, et al. A molecular basis for familial hypertrophic cardiomyopathy: a beta cardiac myosin heavy chain gene missense mutation. *Cell* 1990; 62:999–1006.

[7] Keren A, Syrris P, McKenna WJ. Hypertrophic cardiomyopathy: the genetic determinants of clinical disease expression. *Nat. Clin. Pract. Card Med.* 2008;5:158-168.

[8] Arad M, Maron BJ, Gorham JM, et al. Glycogen storage diseases presenting as hypertrophic cardiomyopathy. *N. Engl. J. Med.* 2005; 352:362–372.

[9] Sachdev B, Takenaka T, Teraguchi H, et al. Prevalence of Anderson–Fabry disease in male patients with late onset hypertrophic cardiomyopathy.*Circulation* 2002; 105:1407–1411.

[10] Van Driest SL, Gakh O, Ommen SR, et al. Molecular and functional characterization of a human frataxin mutation found in hypertrophic cardiomyopathy. *Mol. Genet. Metab.* 2005; 85:280–285.

[11] Limongelli G, Hawkes L, Calabro R, et al. Mutation screening of the PTPN11 gene in hypertrophic cardiomyopathy. *Eur. J. Med. Genet.* 2006;49(5):426-30.

[12] Limongelli G, Pacileo G, Marino B, et al. Prevalence and clinical significance of cardiovascular abnormalities in patients with the LEOPARD syndrome. *Am. J. Cardiol.* 2007;100:736-41

[13] Limongelli G, Pacileo G, Digilio MC, et al. Severe, obstructive biventricular hypertrophy in a patient with Costello syndrome: Clinical impact and management. *Int. J. Cardiol.* 2008; 130: e108-e110.

[14] Limongelli G, Pacileo G, Melis D, et al. Trisomy 18 and hypertrophy cardiomyopathy in an 18-year-old woman. *Am. J. Med. Genet. A*. 2008;146(3):327-9

[15] Marian AJ. Phenotypic plasticity of sarcomeric protein mutations. *J. Am. Coll Cardiol.* 2007 Jun 26;49:2427-9.

[16] Klaassen S, Probst S, Oechslin E, et al. Mutations in Sarcomere Protein Genes in Left Ventricular Noncompaction. *Circulation* 2008;117;2893-2901.

[17] Hoedemaekers YM, Caliskan K, Majoor-Krakauer D et al. Cardiac b-myosin heavy chain defects in two families with non-compaction cardiomyopathy: linking non-compaction to hypertrophic, restrictive, and dilated cardiomyopathies. *Eur. Heart J.* 2007 Nov;28(22):2732-7.

[18] Capell BC, Collins FS. Human laminopathies: nuclei gone genetically awry. *Nat Rev. Genet.* 2006;7:940 –52.

[19] Remme CA, Wilde AA, Bezzina CR. Cardiac sodium channel overlap syndromes: different faces of SCN5A mutations. *Trends Cardiovasc. Med.* 2008 Apr;18(3):78-87.

[20] Wilcox WR, Banikazemi M, Guffon N, et al. Long-term safety and efficacy of enzyme replacement therapy for Fabry disease. *Am. J. Hum. Genet* 2004;75:65–74.

[21] Schwartz ML, Cox GF, Lin AE. Clinical Approach to Genetic Cardiomyopathy in Children. *Circulation.* 1996;94:2021-2038.

[22] Anan R, Nakagawa M, Miyata M, et al. Cardiac involvement in mitochondrial diseases: a study on 17 patients with documented mitochondrial DNA defects. *Circulation.* 1995;91:955-961

[23] Stern S. Electrocardiogram: Still the Cardiologist's Best Friend. *Circulation* 2006;113:753-756.

[24] Cueto-Garcia L, Reeder GS, Kyle RA, et al. Echocardiographic findings in systemic amyloidosis: spectrum of cardiac involvement and relation to survival. *J. Am. Coll. Cardiol.* 1985; 6:737–743.

[25] C Rapezzi, O Leone, E Biagini et al. Echocardiographic clues to diagnosis of dystrophin related dilated cardiomyopathy. *Heart.* 2007;93:10.

[26] Weidemann F, Strotmann JM. Use of tissue Doppler imaging to identify and manage systemic diseases. *Clin. Res. Cardiol.* 2007; 96:1–9 .

[27] Bosa JM, Ommen SR, Ackerman MJ. Genetics of hypertrophic cardiomyopathy: one, two, or more diseases? *Curr. Opin. Cardiol.* 22:193–199.

[28] Silva C, Moon JC, Elkington AG, et. al. Myocardial late gadolinium enhancement in specific cardiomyopathies by cardiovascular magnetic resonance: a preliminary experience. *J. Cardiovasc. Med.* (Hagerstown). 2007;8:1076-9.

[29] Di Lenarda A, Arbustini E. Diagnosis of dilated cardiomyopathy: how to improve clinical and etiological definition. *Ital. Heart J. Suppl*. 2002; 3:375-7.

[30] Obici L, Perfetti V, Palladini G, et al. Clinical aspects of systemic amyloid diseases. *Biochim. Biophys. Acta.* Nov 10 2005;1753(1):11-22.

[31] Finsterer J, Stöllbergerb C. The Heart in Human Dystrophinopathies. *Cardiology* 2003;99:1-19.

[32] Cooper LT, Baughman KL, Feldman AM, et al. The role of endomyocardial biopsy in the management of cardiovascular disease: a scientific statement from the American Heart Association, the American College of Cardiology, and the European Society of Cardiology. *Circulation*. 2007;116:2216-33.

In: Horizons in World Cardiovascular Research. Volume 1 ISBN: 978-1-60876-447-1
Editor: Eleanor H. Bennington, pp. 85-96

Chapter V

Inequalities in the Training and Implementation of Cardiac Rehabilitation in the United Kingdom

Jamie O'Driscoll[1*] and Rajan Sharma[2]
[1]Thames Valley University, London
[2]Ealing Hospital NHS Trust, London

Abstract

Cardiovascular disease is the leading cause of morbidity and mortality in the United Kingdom (UK) and although the UK mortality rate has steadily declined since the early 1970's, the rate of premature death has fallen less than other European countries. Following a cardiac event, it is common for patients to experience debilitating physiological and psychological impairment. A reduced functional capacity and depression are frequent, which is associated with a worse outcome as well as directly impacting on the failure to return to work. Comprehensive cardiac rehabilitation is a multidisciplinary service that provides the majority of cardiac patients with long-term exercise prescription, education, cardiovascular risk factor modification, counselling and medical evaluation to facilitate recovery and improve overall functional capacity following a cardiac event. The provision of cardiac rehabilitation services has grown significantly and demonstrated improved patient health, increased exercise capacity, reduced overall mortality and reduced hospitalisation costs. However, this growth has not been matched by service quality with many programmes unable to adhere to national guidelines due to inadequate resources and the related inability to provide appropriate staff training. Deficiencies in cardiac rehabilitation provision are generally due to inadequate investment, professional barriers, and the relatively low level of priority directed to the service in many cardiology departments. It appears that efficient

[*] Corresponding Author: Thames Valley University, Department of Sport, Health, and Exercise Sciences, Faculty of Health and Human Sciences, Wellington Street, Slough, SL1 1YG, E-mail Address: jamie.o'driscoll@tvu.ac.uk, Telephone Number: 01753 697706

comprehensive cardiac rehabilitation for patients is a postcode lottery, with substantial variation in the management, organisation, and practice throughout the UK.

Inequalities in the Training and Implementation of Cardiac Rehabilitation in the United Kingdom

Cardiovascular disease (CVD) is the leading cause of morbidity and premature mortality in the United Kingdom (UK) (Dalal and Evans 2003) and accounts for approximately 198,000 deaths each year (Allender 2008). The UK persists as one of the worst developed European countries for CVD mortality and as such the Government has set appropriate targets to reduce the epidemic (Allender 2008). However, despite Government recognition of the health impact, the associated health care costs and development of sophisticated interventions that ultimately aim to restore patient functional capacity, CVD is estimated to remain the highest ranked cause of death worldwide through to 2020 (Murray and Lopez 1997). With this in mind, the need and demand for health care services is increasing disproportionately compared to the available resources. This is primarily due to an ageing population, the development of new knowledge and technology, increasing patient expectations and greater professional expectations (Thompson and Stewart 2002).

Due to improved medical intervention and revascularisation, increasing numbers of patients are living with CVD and after the acute phase of care, the majority of patients require longer-term management and rehabilitation in order to restore their functional capacity and in many to return to work (Stokes 2000). Cardiac rehabilitation (CR) is a multidisciplinary service that provides the majority of cardiac patients with long-term exercise prescription, education, cardiovascular risk factor modification, counselling and medical evaluation (Balady et al. 2000) and is increasingly recognised as a core component of the continuum of care for patients with CVD (Balady et al. 2007; Leon et al. 2005; Wenger et al. 1995). As such the provision of CR services has grown considerably in recent years and is now recommended as useful and effective (Class 1) by the American Heart Association (AHA) and the American College of Cardiology in the treatment of patients with forms of CVD (Antman et al. 2004; Balady et al. 2007; Braunwald et al. 2002; Gibbons et al. 2003; Hunt et al. 2005).

In the UK, comprehensive CR is divided into four phases (Bethell et al. 2009) and programmes target improvements in both physiological and psychosocial aspects of recovery from cardiac injury or intervention to improve/restore patient functional capacity. Physiological parameters include improved exercise tolerance and adherence, cessation of smoking, and optimisation of coronary artery disease (CAD) risk factors, such as body weight, blood pressure and lipid profiles. Psychosocial aspects include the amelioration of negative emotional repercussions of cardiac trauma, such as stress, anxiety and depression and the appropriate return to occupation, which is considered beneficial for both the individual and society (Wenger 2008). The range of knowledge and skills necessary to manage and address the physiological and psychosocial elements is extensive and requires multidisciplinary guidance from cardiologists, clinical exercise scientists, nutritionists,

nurses, physiotherapists, psychologists, occupational therapists and social workers (Stokes 2000).

Traditionally, most patients recruited onto CR programmes were patients following a myocardial infarction (MI) or coronary artery bypass graft (CABG) surgery. However, in the era of evidence based health care, contemporary use of CR services include patients following percutaneous coronary intervention (PCI); heart or heart and lung transplantation recipients; patients with stable angina or stable chronic heart failure (CHF); those with peripheral arterial disease (PAD) with claudication; and patients following cardiac surgical procedures for heart valve repair or replacement (Wenger 2008). As a result CR has evolved into a much broader-based multidisciplinary service requiring a range of knowledge and skill mix to achieve the desired outcomes. However, while this is embraced, demands and expectations have been increased without adequate funding targeted at service provision. Additionally, the needs, expectations, and experiences of patients and health care professionals as well as the local resources, priorities, and performance management of CR services are different throughout the UK (Thompson 2002). Furthermore, the National Health Service (NHS) is continually facing financial challenges and CR services are not a priority, despite investment in cardiac technology and intervention. Indeed, the British Heart Foundation (BHF) detailed that the outlook for CR programmes are less secure than in 2006, despite support from the governments national director for heart disease and stroke (BHF 2007).

The patient uptake in CR services throughout the UK and Europe is inadequate (Beswick et al. 2005; Bethell et al. 2001; Kotseva et al. 2009; Kotseva 2004; Wood et al. 2008) and is underrepresented by ethnic minorities, women, older people, and patients living in socially deprived areas (Beswick et al. 2005; Jackson et al. 2005; McGee and Horgan 1992; Taylor et al. 2001; Tod et al. 2001). Commonly, individuals with the greatest functional impairment who are most likely to significantly benefit from CR services do not participate in programmes (Harlan et al. 1995). The culmination of these factors has been previously described as a collective failure of medical practice (Wood et al. 2008).

Cardiac rehabilitation is a cost effective (Bethell et al. 2009; BHF 2007; Levin et al. 1991; Oldridge et al. 1993; Papadakis et al. 2005) proven evidence based intervention, which significantly reduces hospitalisation costs and improves cardiovascular disease risk factors (Clark et al. 2005; Taylor et al. 2004; Thompson 2002; Zwisler et al. 2008). Programmes are now firmly established in the UK and involvement of a multidisciplinary team is paramount in the delivery of a broad range of CR interventions (Child 2004). However, CR suffered from a lack of national direction (Child 2004), until the British Association of Cardiac Rehabilitation (BACR) (Coats 1995) and the Department of Health (DoH) published the National Service Framework (NSF) for Coronary Heart Disease guidelines (DoH 2000b), which sets explicit standards for implementing secondary prevention measures and the provision of effective CR programmes (Dalal and Evans 2003).

Despite evidence of the effectiveness of CR and the introduction of the NSF guidelines, there is support to suggest wide variation in the provision, practice, organisation and management of CR services in the UK (Davidson 1995; O'Driscoll et al. 2007; Stokes 2000; Thompson et al. 1997) with failure to meet the national guidelines (Bethell 2000; BHF 2007; O'Driscoll et al. 2007; Thompson et al. 1996) and the fact that few physicians play an active

role and/or endorse CR programmes (Bethell et al. 2009; Jackson et al. 2005; Lewin et al. 1998). Involvement of a clinical lead, such as a consultant cardiologist or general practitioner (GP) with a specialist interest in cardiology as occurs in other European countries may improve facilitation and provision of CR services (Bethell et al. 2009). In addition, health outcome studies consistently demonstrate gaps in applying the clinical evidence of CR into practice, which contributes to sub-optimal patient outcome (Clark et al. 2005; Majumdar et al. 2004). Support for and active referral to CR programmes from the patients doctor is an effective way to encourage CR attendance (McGee and Horgan 1992). Furthermore, education on the benefits and application of CR may improve referral and uptake (Bittner et al. 1999).

The majority of CR programmes in the UK have been initiated, co-ordinated, and delivered by nurses (Stokes 2000; Thompson and Stewart 2002) with the earliest programmes developed during the 1970s. Between 1989 and 1999 there was a rapid growth in the number of CR programmes (six-fold increase) and now every hospital in the UK who treats acute cardiac problems are able to access CR services (Bethell et al. 2009) with the majority remaining hospital based. However, this field is relatively undeveloped as a speciality in terms of an established training or career pathway and the nurses involved in the majority of CR programmes have developed from different career backgrounds with varying degrees of experience or training in cardiac care (Stokes 2000). In taking on new roles and responsibilities, many of which evolved spontaneously and without any methodical design or forecast, there is a risk of health care professionals and in particular nurses focusing exclusively on particular aspects of medical intervention (individual knowledge strengths) rather than concentrating on the entirety of patient care (Thompson and Stewart 2002). As a result the provision of CR services throughout the UK is extremely diverse (O'Driscoll et al. 2007; Stokes 2000) and the configuration of the multidisciplinary health care team is variable (Davidson 1995; Thompson et al. 1997) with minimal input from disciplines other than nursing and physiotherapy (Stokes 2000). Indeed, in a random sample of 120 CR programmes the individual contact with patients was provided by nurses and physiotherapists, with other disciplines mainly involved during lectures or group discussions (Lewin et al. 1998). Furthermore, of the CR programmes within the UK, only 60% had a physiotherapist, 20% had a dietician, and 10% had a psychologist (Bethell et al. 2009). However, this may simply be a reflection of insignificant funding or inadequate planning and organisation.

The need for specialist health care professionals being associated with CR programmes is essential for optimal patient outcome. This is particularly evident in the lack of psychological support available for patients during rehabilitation. Following a cardiac event, depression is common and extremely debilitating. Indeed, patient perceptions of symptoms and their sense of control are significantly associated with quality of life (Lau-Walker et al. 2008) and a depressed mood is a predictor of returning to work following a cardiac event (Bhattacharyya et al. 2007). Therefore, the management of early depression may promote the resumption of employment and enhance the quality of life of cardiac patients (Bhattacharyya et al. 2007). Furthermore, continuous adjustment of goals/tasks during CR, such as increasing exercise intensity and improving self-confidence is positively related to increased cardiopulmonary fitness, reduced depression, weight loss and return to work (Burns and Evon 2007).

The nursing role in CR services did not significantly develop until the late 1980's and it's this paradigm shift that has changed nursing care to a holistic model. The diversity of CR programme personnel is directly influenced by the rapid, continual adjustments in medical health care, especially advancing technology and the emergence of how important multifaceted CR services are in patient rehabilitation from cardiac injury/disease or intervention. Therefore the level of education and qualifications attained by CR health care professionals may vary significantly (Bennett and Pescatello 1997). In addition, the change from disease orientated care, such as working on a coronary care ward, to health orientated care, such as the reinforcement of behaviour change, which is necessary for effective and comprehensive CR services, may be challenging for many nursing personnel (Stokes 2000).

Due to the changing roles and identities of health care professionals working within CR services, research suggests that the training and experience already acquired, may not completely equip them for such expanded roles in co-ordinating, delivering, and auditing care directed at health promotion and chronic disease management (Wiles 1997). The changing health care professionals' roles due to for example, the introduction of rehabilitation services may impact negatively on their own individual motivation and morale, which may significantly influence the patient's return to health (O'Driscoll et al. 2007). Indeed, nurses report a lack of preparedness for educative, managerial, and leadership roles, which may result in disengagement from and disinterest in their work and contribute to the development of an unhealthy working environment (Conway et al. 2006). Furthermore, job dissatisfaction appears common within the nursing profession (Solman et al. 2004) with up to 24% of nurses reporting decreased job satisfaction and commitment (McNeese-Smith and van Servellen 2000).

Adequate training is required to prepare and equip health care professionals for their individual and multidisciplinary role in educating and supporting patients. Indeed, the inadequate training and lack of professional accreditation available for CR service provision may be one of the major influences on patient recruitment, adherence, and outcome within the UK. In addition, inadequate staff training can result in blurred objectives as well as undefined roles, identities, and skills of health care professionals (O'Driscoll et al. 2007; Stokes 2000). In contrast to the United States of America (USA), programme accreditation has not been established and core competences for health care professionals working in CR programmes have not been formally identified within the UK (Stokes 2000). Despite the arguments surrounding health care professionals and their ability to provide best practice CR services, there is an open debate as to whether or not pre-registration nurse training competently prepares nurses for clinical practice as a whole, since there is no blueprint for nurse education or for the quality of nursing education (Bradshaw 1997). However, despite the USA having advanced and established infrastructure for their CR services, in a study analysing 108 CR programmes, only 40.7% of the staff reported that they met the minimum training/qualification recommendations and only 7% met the preferred recommendations (Bennett and Pescatello 1997).

Changes within the NHS have resulted in a subsequent drive for new and innovative nursing roles (DoH 2000a, c). This has resulted in a change in skill mix (Jenkins-Clarke et al. 1998), where nurses are increasingly being employed instead of doctors in some areas of work (Pearson 1998). These changing roles and identities across professional boundaries

within the NHS, creates a culture of uncertainty that has the potential to both inspire and threaten innovation in health care (Williams and Sibbald 1999). In recent years, Government pressure to improve the cost effectiveness of health care provision has focused attention on the possible benefits of moving care from expensive to cheaper providers, in particular from doctors to nurses. The subsequent boundary changes may create uncertainty in relation to professional identity in connection with aspects of the health care professionals work and/or role. This highlighted uncertainty is not limited to displacement of work between doctors and nurses, but also creates tension between different groups of health care professionals due to the potential overlap with other disciplines (Williams and Sibbald 1999), which may be more common in CR programmes due to its multidisciplinary nature, such as exercise prescription, nutritional advice, and psychological support etc. The tension and uncertainty between different groups of staff leads to a loss of professional networking and support, which can lead to demoralization and a sense of diminished autonomy (Hiscock 1996). This in turn, may lead to a breakdown in communication between colleagues and different health care professionals undermining each other as well as leaving both staff and patients feeling extremely vulnerable (O'Driscoll et al. 2007; Williams and Sibbald 1999). Indeed multidisciplinary learning is perceived as beneficial; however little evidence exists of this working in practice and potential barriers include structural and organisational difficulties and failure to agree common aims (Stokes 2000). The need to address how uncertainty and, therefore, changing roles and identities can inspire rather than threaten innovation in health care is critical.

Current educational preparation of nurses, whether at pre-or post registration levels, generally fails to prepare practitioners to play a more prominent role in rehabilitation programmes (Stokes 2000). Furthermore, with few opportunities available in the UK for a structured learning programme specifically developed for CR and with no system of evaluation or accreditation for those that do train it is not surprising to see such diversity throughout the UK in service provision. Providing specialist training for CR programme facilitators and empowering health care professionals with the ability and skills to transfer knowledge across professional boundaries and into different health care settings may improve the safety and quality of patient care and could be one answer to further improving CR services. Future development is necessary and will require greater emphasis on training and education in CR service provision.

Summary

Comprehensive CR is essential within the continuum of care for patients with cardiovascular disease for the restoration of functional capacity, regardless of age or gender. Contemporary CR is a proven evidence based intervention that reduces CVD risk factors and may significantly reduce the current CVD epidemic in the UK. The multifaceted composition of CR requires extensive knowledge and skills in order to deliver and achieve nationally recommended objectives. In the UK, current expertise and experience of health care professionals involved in CR provision is inadequate. Furthermore, there is substantial variation in the management, organisation, content, staffing and funding of CR programmes

with no formal training throughout the UK. This may directly impact on patients and create the prospect of obtaining efficient comprehensive rehabilitation from cardiac injury or intervention a postcode lottery.

Cardiac rehabilitation programme development appears to have occurred without structured planning with the general outcome of disorganized service provision. As such there is a general lack of aligned roles and identities amongst health care professionals and without adequate modifications to CR infrastructure there is a danger of increased levels of dissatisfaction and high attrition rates within the nursing profession.

To improve CR service provision, a review of the education process, professional development opportunities, and overall training is required. Continual education and training to develop health care professionals is paramount for CR service progression. This process is complex and will need to address role function and boundary crossover as well as be co-ordinated throughout the UK with specific qualifications, core competencies, and programme and individual accreditation processes in place. Continued evaluation and monitoring of this logical approach will be necessary to ensure that the health care professionals involved in CR provision are adequately trained to deliver nationally recognised care.

It is vital for greater investment and improved planning to permit professional development and enable current and future CR service providers the ability to align their specialist roles with the direction in which health care is moving.

References

Allender, S., Peto, V., Scarborough, P., Kaur, A. & Rayner, M. (2008). "Coronary Heart Disease Statistics." ed. Department of Public Health. 2007 Edition. University of Oxford: British Heart Foundation Health Promotion Research Group.

Antman, E. M., Anbe, D. T., Armstrong, P. W., Bates, E. R., Green, L. A., Hand, M., Hochman, J. S., Krumholz, H. M., Kushner, F. G., Lamas, G. A., Mullany, C. J., Ornato, J. P., Pearle, D. L., Sloan, M. A. & Smith, Jr., S. C. (2004). "ACC/AHA guidelines for the management of patients with ST-elevation myocardial infarction--executive summary. A report of the American College of Cardiology/American Heart Association Task Force on Practice Guidelines (Writing Committee to revise the 1999 guidelines for the management of patients with acute myocardial infarction)." *J Am Coll Cardiol, 44(3),* 671-719.

Balady, G. J., Ades, P. A., Comoss, P., Limacher, M., Pina, I. L., Southard, D., Williams M. A. & Bazzarre, T. (2000). "Core components of cardiac rehabilitation/secondary prevention programs: A statement for healthcare professionals from the American Heart Association and the American Association of Cardiovascular and Pulmonary Rehabilitation Writing Group." *Circulation, 102(9),* 1069-1073.

Balady, G. J., Williams, M. A., Ades, P. A., Bittner, V., Comoss, P., Foody, J. M., Franklin, B., Sanderson, B. & Southard. D. (2007). "Core components of cardiac rehabilitation/secondary prevention programs: 2007 update: a scientific statement from the American Heart Association Exercise, Cardiac Rehabilitation, and Prevention Committee, the Council on Clinical Cardiology; the Councils on Cardiovascular Nursing,

Epidemiology and Prevention, and Nutrition, Physical Activity, and Metabolism; and the American Association of Cardiovascular and Pulmonary Rehabilitation." *Circulation, 115(20),* 2675-2682.

Bennett, S. B. & Pescatello. L. S. (1997). "A regional comparison of cardiac rehabilitation personnel. Adherence to the 1995 American Association of Cardiovascular and Pulmonary Rehabilitation Guidelines by Staff Position." *J Cardiopulm Rehabil, 17(2),* 92-102.

Beswick, A. D., Rees, K., West, R. R., Taylor, F. C., Burke, M., Griebsch, I., Taylor, R. S., Victory, J., Brown J. & Ebrahim, S. (2005). "Improving uptake and adherence in cardiac rehabilitation: literature review." *J Adv Nurs., 49(5),* 538-555.

Bethell, H. J. (2000). "Cardiac rehabilitation: from Hellerstein to the millennium." *Int J Clin Pract., 54(2),* 92-97.

Bethell, H. J., Turner, S. C., Evans, J. A. & Rose, L. (2001). "Cardiac rehabilitation in the United Kingdom. How complete is the provision?" *J Cardiopulm Rehabil, 21(2),* 111-115.

Bethell, H., Lewin, R. & Dalal, H. (2009). "Cardiac rehabilitation in the United Kingdom." *Heart, 95(4),* 271-275.

Bhattacharyya, M. R., Perkins-Porras, L., Whitehead, D. L. & Steptoe, A. (2007). "Psychological and clinical predictors of return to work after acute coronary syndrome." *Eur Heart J., 28(2),* 160-165.

BHF. (2007). "The National Audit of Cardiac Rehabilitation: *Annual Statistical Report."* British Heart Foundation.

Bittner, V., Sanderson, B., Breland, J. & Green, D. (1999). "Referral patterns to a University-based cardiac rehabilitation program." *Am J Cardiol, 83(2),* 252-255, A255.

Bradshaw, A. (1997). "Defining 'competency' in nursing (Part I): A policy review." *J Clin Nurs, 6(5),* 347-354.

Braunwald, E., Antman, E. M., Beasley, J. W., Califf, R. M., Cheitlin, M. D., Hochman, J. S., Jones, R. H., Kereiakes, D., Kupersmith, J., Levin, T. N., Pepine, C. J., Schaeffer, J. W., Smith, E. E., 3rd, Steward, D. E., Theroux, P., Gibbons, R. J., Alpert, J. S., Faxon, D. P., Fuster, V., Gregoratos, G., Hiratzka, L. F., Jacobs, A. K. & Smith, S. C. Jr. (2002). "ACC/AHA 2002 guideline update for the management of patients with unstable angina and non-ST-segment elevation myocardial infarction--summary article: a report of the American College of Cardiology/American Heart Association task force on practice guidelines (Committee on the Management of Patients With Unstable Angina)." *J Am Coll Cardiol, 40(7),* 1366-1374.

Burns, J. W. & Evon, D. (2007). "Common and specific process factors in cardiac rehabilitation: independent and interactive effects of the working alliance and self-efficacy." *Health Psychol, 26(6),* 684-692.

Child, A. (2004). "Cardiac rehabilitation: goals, interventions and action plans." *Br J Nurs., 13(12),* 734-738.

Clark, A. M., Hartling, L., Vandermeer, B. & McAlister, F. A. (2005). "Meta-analysis: secondary prevention programs for patients with coronary artery disease." *Ann Intern Med., 143(9),* 659-672.

Coats, A., McGee, H., Stokes, H. & Thompson, D. (1995). British Association for Cardiac Rehabilitation (BACR) Guidelines for Cardiac Rehabilitation. Oxford: Wiley: Blackwell Science.

Conway, J., McMillan, M. A. & Solman, A. (2006). "Enhancing cardiac rehabilitation nursing through aligning practice to theory: implications for nursing education." *J Contin Educ Nurs., 37(5),* 233-238.

Dalal, H. M. & Evans, P. H. (2003). "Achieving national service framework standards for cardiac rehabilitation and secondary prevention." *BMJ, 326(7387),* 481-484.

Davidson, C., Reval, K., Chamberlain, D. A., Pentecost, B. & Parker, J. (1995). "A report of a working group of the British Cardiac Society: Cardiac rehabilitation services in the United Kingdom 1992 " *British Heart Journal, 73,* 210-212.

DoH. (2000a). "A Health Service for All Talents." ed. *Department of Health.* London.

DoH. (2000b). "National Service Framework for Coronary Heart Disease: *Modern Standards and Service Models."* London: Department of Health.

DoH. (2000c). "The NHS Plan." ed. Department of Health. London.

Gibbons, R. J., Abrams, J., Chatterjee, K., Daley, J., Deedwania, P. C., Douglas, J. S., Ferguson, T. B., Jr., Fihn, S. D., Fraker, T. D., Jr., Gardin, J. M., O'Rourke, R. A., Pasternak, R. C., Williams, S. V., Alpert, J. S., Antman, E. M., Hiratzka, L. F., Fuster, V., Faxon, D. P., Gregoratos, G., Jacobs, A. K. & Smith, S. C. Jr. (2003). "ACC/AHA 2002 guideline update for the management of patients with chronic stable angina--summary article: a report of the American College of Cardiology/American Heart Association Task Force on Practice Guidelines (Committee on the Management of Patients With Chronic Stable Angina)." *Circulation, 107(1),* 149-158.

Harlan, W. R., 3rd, Sandler, S. A., Lee, K. L., Lam, L. C. & Mark, D. B. (1995). "Importance of baseline functional and socioeconomic factors for participation in cardiac rehabilitation." *Am J Cardiol, 76(1),* 36-39.

Hiscock, J. & Pearson, M. (1996). "Professional costs and invisible value in the community nursing market." *Journal of Interprofessional Care, 10,* 23-31.

Hunt, S. A., Abraham, W. T., Chin, M. H., Feldman, A. M., Francis, G. S., Ganiats, T. G., Jessup, M., Konstam, M. A., Mancini, D. M., Michl, K., Oates, J. A., Rahko, P. S., Silver, M. A., Stevenson, L. W., Yancy, C. W., Antman, E. M., Smith, S. C., Jr., Adams, C. D., Anderson, J. L., Faxon, D. P., Fuster, V., Halperin, J. L., Hiratzka, L. F., Jacobs, A. K., Nishimura, R., Ornato, J. P., Page, R. L. & Riegel, B. (2005). "ACC/AHA 2005 Guideline Update for the Diagnosis and Management of Chronic Heart Failure in the Adult: a report of the American College of Cardiology/American Heart Association Task Force on Practice Guidelines (Writing Committee to Update the 2001 Guidelines for the Evaluation and Management of Heart Failure): developed in collaboration with the American College of Chest Physicians and the International Society for Heart and Lung Transplantation: endorsed by the Heart Rhythm Society." *Circulation, 112(12),* e154-235.

Jackson, L., Leclerc, J. Erskine, Y. & Linden, W. (2005). "Getting the most out of cardiac rehabilitation: a review of referral and adherence predictors." *Heart, 91(1),* 10-14.

Jenkins-Clarke, S., Carr-Hill, R. & Dixon, P. (1998). "Teams and seams: skill mix in primary care." *J Adv Nurs., 28(5),* 1120-1126.

Kotseva, K., Wood, D., De Backer, G., De Bacquer, D., Pyorala, K. & Keil, U. (2009). "Cardiovascular prevention guidelines in daily practice: a comparison of EUROASPIRE I, II, and III surveys in eight European countries." *Lancet, 373(9667),* 929-940.

Kotseva, K., Wooda, D. A., De Bacquer, D., Heidrich, J. & De Backer, G. (2004). "Cardiac rehabilitation for coronary patients:lifestyle, risk factor and therapeutic management. Results from the EUROASPIRE II survey." *European Heart Journal 6* (supplement J), J17-J26.

Lau-Walker, M., Cowie, M. R. & Roughton, M. (2008). "Coronary heart disease patients' perception of their symptoms and sense of control are associated with their quality of life three years following hospital discharge." *Journal of Clinical Nursing, 18,* 63-71.

Leon, A. S., Franklin, B. A., Costa, F., Balady, G. J., Berra, K. A., Stewart, K. J., Thompson, P. D., Williams, M. A. & Lauer, M. S. (2005). "Cardiac rehabilitation and secondary prevention of coronary heart disease: an American Heart Association scientific statement from the Council on Clinical Cardiology (Subcommittee on Exercise, Cardiac Rehabilitation, and Prevention) and the Council on Nutrition, Physical Activity, and Metabolism (Subcommittee on Physical Activity), in collaboration with the American association of Cardiovascular and Pulmonary Rehabilitation." *Circulation, 111(3),* 369-376.

Levin, L. A., Perk, J. & Hedback, B. (1991). "Cardiac rehabilitation--a cost analysis." *J Intern Med., 230(5),* 427-434.

Lewin, R. J., Ingleton, R. Newens, A. J. & Thompson, D. R. (1998). "Adherence to cardiac rehabilitation guidelines: a survey of rehabilitation programmes in the United Kingdom." *BMJ, 316(7141),* 1354-1355.

Majumdar, S. R., McAlister, F. A. & Furberg, C. D. (2004). "From knowledge to practice in chronic cardiovascular disease: a long and winding road." *J Am Coll Cardiol, 43(10),* 1738-1742.

McGee, H. M. & Horgan, J. H. (1992). "Cardiac rehabilitation programmes: are women less likely to attend?" *BMJ, 305(6848),* 283-284.

McNeese-Smith, D. K. & Van Servellen, G. (2000). "Age, developmental, and job stage influences on nurse outcomes." *Outcomes Manag Nurs Pract, 4(2),* 97-104.

Murray, C. J. & Lopez, A. D. (1997). "Alternative projections of mortality and disability by cause 1990-2020: Global Burden of Disease Study." *Lancet, 349(9064),* 1498-1504.

O'Driscoll, J. M., Shave, R. & Cushion, C. J. (2007). "A National Health Service Hospital's cardiac rehabilitation programme: a qualitative analysis of provision." *J Clin Nurs., 16(10),* 1908-1918.

Oldridge, N., Furlong, W., Feeny, D., Torrance, G., Guyatt, G., Crowe, J. & Jones, N. (1993). "Economic evaluation of cardiac rehabilitation soon after acute myocardial infarction." *Am J Cardiol, 72(2),* 154-161.

Papadakis, S., Oldridge, N. B., Coyle, D., Mayhew, A., Reid, R. D., Beaton, L., Dafoe, W. A. & Angus, D. (2005). "Economic evaluation of cardiac rehabilitation: a systematic review." *Eur J Cardiovasc Prev Rehabil, 12(6),* 513-520.

Pearson, P. (1998). "Equal but different." *Community Practitioner,* 71, 165-166.

Solman, A., Conway, J. & McMillan, M. (2004). "Stepping out: education for cardiac recovery." *Contemp Nurse, 17(1-2),* 159-166.

Stokes, H. C. (2000). "Education and training towards competency for cardiac rehabilitation nurses in the United Kingdom." *J Clin Nurs., 9(3),* 411-419.

Taylor, F. C., Victory, J. J. & Angelini, G. D. (2001). "Use of cardiac rehabilitation among patients following coronary artery bypass surgery." *Heart, 86(1),* 92-93.

Taylor, R. S., Brown, A., Ebrahim, S., Jolliffe, J., Noorani, H., Rees, K., Skidmore, B., Stone, J. A., Thompson, D. R. & Oldridge, N. (2004). "Exercise-based rehabilitation for patients with coronary heart disease: systematic review and meta-analysis of randomized controlled trials." *Am J Med., 116(10),* 682-692.

Thompson, D. R. (2002). "Improving cardiac rehabilitation: a view from the United Kingdom." *Eur J Cardiovasc Nurs., 1(2),* 95-99.

Thompson, D. R., Bowman, G. S., Kitson, A. L., De Bono, D. P. & Hopkins, A. (1996). "Cardiac rehabilitation in the United Kingdom: guidelines and audit standards. National Institute for Nursing, the British Cardiac Society and the Royal College of Physicians of London." *Heart, 75(1),* 89-93.

Thompson, D. R., Bowman, G. S., Kitson, A. L., De Bono, D. P. & Hopkins, A. (1997). "Cardiac rehabilitation services in England and Wales: a national survey." *Int J Cardiol, 59(3),* 299-304.

Thompson, D. R. & Stewart, S. (2002). "Nurse-directed services: how can they be made more effective?" *Eur J Cardiovasc Nurs., 1(1),* 7-10.

Tod, A. M., Wadsworth, E., Asif, S. & Gerrish, K. (2001). "Cardiac rehabilitation: the needs of South Asian cardiac patients." *Br J Nurs., 10(16),* 1028-1033.

Wenger, N. K. (2008). "Current status of cardiac rehabilitation." *J Am Coll Cardiol, 51(17),* 1619-1631.

Wenger, N. K., Froelicher, E. S., Smith, L. K., Ades, P. A., Berra, K., Blumenthal, J. A., Certo, C. M., Dattilo, A. M., Davis, D., DeBusk, R. F., et al. (1995). "Cardiac rehabilitation as secondary prevention. Agency for Health Care Policy and Research and National Heart, Lung, and Blood Institute." *Clin Pract Guidel Quick Ref Guide Clin., (17),* 1-23.

Wiles, R. (1997). "Empowering practice nurses in the follow-up of patients with established heart disease: lessons from patients' experiences. SHIP Collaborative Group. Southampton Heart Integrated care Project." *J Adv Nurs., 26(4),* 729-735.

Williams, A. & Sibbald, B. (1999). "Changing roles and identities in primary health care: exploring a culture of uncertainty." *J Adv Nurs., 29(3),* 737-745.

Wood, D. A., Kotseva, K., Connolly, S., Jennings, C., Mead, A., Jones, J., Holden, A., De Bacquer, D., Collier, T., De Backer, G. & Faergeman, O. (2008). "Nurse-coordinated multidisciplinary, family-based cardiovascular disease prevention programme (EUROACTION) for patients with coronary heart disease and asymptomatic individuals at high risk of cardiovascular disease: a paired, cluster-randomised controlled trial." *Lancet, 371(9629),* 1999-2012.

Zwisler, A. D., Soja, A. M., Rasmussen, S., Frederiksen, M., Abedini, S., Appel, J., Rasmussen, H., Gluud, C., Iversen, L., Sigurd, B., Madsen, M. & Fischer-Hansen, J. (2008). "Hospital-based comprehensive cardiac rehabilitation versus usual care among patients with congestive heart failure, ischemic heart disease, or high risk of ischemic

heart disease: 12-month results of a randomized clinical trial." *Am Heart J, 155(6),* 1106-1113.

In: Horizons in World Cardiovascular Research. Volume 1 ISBN: 978-1-60876-447-1
Editor: Eleanor H. Bennington, pp. 97-115

Chapter VI

Sudden Cardiac Death Risk Stratification in Heart Failure – The Potential Role of Biomarkers

P. A. Scott[1], J. M. Morgan[1] and P. A. Townsend[2*]
[1]Wessex Cardiothoracic Centre, Southampton University Hospitals NHS Trust, SO16 6YD, UK
[2] Human Genetics Division, University of Southampton, SO16 6YD,UK

Abstract

Although there has been significant recent progress in the management of heart failure its associated mortality remains high. A large proportion of these patients die suddenly, termed sudden cardiac death (SCD), mostly from potentially reversible malignant cardiac arrhythmias. Despite the availability of a highly effective treatment in the form of an implantable cardioverter defibrillator (ICD), SCD in the heart failure population is still a significant problem. One important reason for this is the difficulty in identifying which patients are at highest risk of SCD and would benefit from an ICD. A number of tests are currently available to risk stratify heart failure patients at risk of SCD. However, used alone or in combination these are not sufficiently accurate and there is significant need for better risk stratification tools.

Multiple studies have demonstrated that serum biomarkers can accurately predict adverse outcomes in patients with heart failure of both ischaemic and non-ischaemic aetiology. A range of biomarkers predict both the occurrence of SCD in patients without ICDs and the occurrence of malignant arrhythmias in patients with devices, and in these studies individual biomarkers are at least as accurate as the current best markers of SCD risk. The pathophysiology of SCD is a complex process with a range of electrophysiological and molecular alterations contributing to arrhythmogenesis in the failing heart. By providing an assessment of these various processes, serum biomarkers

* Corresponding author: Reader in Molecular Cell Biology and Head of Transcription Regulation Group Human Genetics Division, Duthie Building MP808, Southampton General Hospital, Tremona Road, Southampton, SO16 6YD, UK, 023 80 798692 (tel), 023 89 794264 (fax), p.a.townsend@soton.ac.uk

may improve prediction of SCD in heart failure and help guide ICD use. Furthermore, it is likely that optimal SCD risk stratification will require the combination of multiple tests that reflect these diverse upstream processes. As such the greatest potential benefit of biomarkers may be in measuring multiple complementary markers that assess distinct aspects of arrhythmic risk.

Introduction

There has been significant recent progress in the management of heart failure with advances in neurohormonal blockade and the advent of device therapy. In spite of this the mortality associated with heart failure remains high - 80% of men and 70% of women under the age 65 with heart failure will die within 8 years [1]. A large proportion of these patients die suddenly, termed sudden cardiac death (SCD), mostly from potentially reversible malignant arrhythmias. Despite the availability of a highly effective treatment, in the form of an implantable cardioverter defibrillator (ICD), SCD in the heart failure population is still a significant problem. One important reason for this is the difficulty in identifying which patients are at highest risk of SCD and would benefit from an ICD. In this chapter we review the importance and pathophysiology of SCD in heart failure, detail the currently available tools for SCD risk stratification, and consider the potential role of biomarkers.

The Impact of Sudden Cardiac Death in Heart Failure

Cardiac death soon after symptom onset - termed sudden cardiac death - is a major health problem. It is the commonest mode of death in the developed world and causes approximately 100,000 adult deaths per year in the United Kingdom and four times that in the United States [2-4]. In patients who die within an hour of the onset of symptoms or during sleep, more than 90% will be due to cardiac arrhythmias [5], and most of these events are likely to be caused by potentially reversible ventricular tachyarrhythmias [6].

SCD is a major cause of mortality in heart failure irrespective of its aetiology. Early data concerning the importance of SCD in heart failure came from epidemiological studies. Among 652 members of the Framingham Heart Study who developed congestive heart failure, 5-year survival rates after disease onset were 25% in men and 38% in women, and up to half of these deaths were sudden [7,8]. These findings still hold despite contemporary management. Mozaffarian et al assessed the mode of death in 10,538 ambulatory patients with New York Heart Association class II-IV heart failure enrolled in 6 randomised trials or registries [9]. Ischaemic heart disease accounted for 62% of cases. During 16,735 person-years of follow-up, 2014 deaths occurred, including 1014 sudden deaths and 684 pump-failure deaths. Though overall sudden death was the commonest mode of death, pump-failure was more frequent in advanced heart failure. Solomon et al studied 14,609 patients with asymptomatic left ventricular dysfunction or heart failure after myocardial infarction [10]. Over a median follow-up of 180

days there were 1067 cardiac arrests, 903 leading to death, which accounted for approximately a third of all deaths.

The Pathophysiology of Sudden Cardiac Death in Heart Failure

Most cases of SCD in heart failure result from a malignant ventricular arrhythmia, either ventricular fibrillation or ventricular tachycardia [11]. This is supported by data from patients dying suddenly while undergoing Holter recording. In 157 episodes of SCD in ambulatory patients undergoing monitoring, 84% were secondary to ventricular arrhythmias, most commonly ventricular fibrillation (62%), while bradycardias accounted for only 16% [12]. Though these were not exclusively patients with heart failure, it is probable that the mechanisms in heart failure are similar.

The underlying electrophysiological and molecular processes that lead to these malignant arrhythmias are incompletely understood. However there is likely to be a complex interplay between acquired abnormalities of cardiac structure and function, and genetic predisposition. The acquired changes include alterations in myocardial repolarisation, calcium homeostasis and neurohormonal signalling [13-15]. Two of the more important processes are action potential prolongation, due to changes in ion channel expression, and alterations in neurohormonal signalling.

Action Potential Prolongation and Ion Channel Expression

Prolongation of the action potential (AP) is a consistent finding in the ventricular myocardium of failing hearts irrespective of the cause [16]. The underlying physiological basis of the changes in AP duration is alteration in the functional expression of ion channel proteins, including potassium and sodium channels. The ventricular myocardium has a number of distinct classes of voltage-gated potassium ion channels. The most consistent finding in human and animal heart failure models is the downregulation of the I_{to} protein, but changes in the potassium channels I_{Kr} and I_{Ks} have also been noted [17-19]. Furthermore the importance of different potassium channels may vary depending on the aetiology of the heart failure [20]. Changes in sodium channels, which are important in the maintenance of the plateau phase of the action potential, have also been implicated [21].

The AP prolongation that occurs as a result of these changes in ion channel expression is inhomogeneous, leading to spatial and temporal heterogeneity in ventricular repolarisation [22]. It is this dispersion of repolarisation that may provide the substrate for the occurrence of malignant ventricular arrhythmias that lead to SCD [23]. These changes in repolarisation can be detected on the surface electrocardiogram (ECG), and form the basis of the risk stratification test Microvolt T-wave Alternans described below [24].

Altered Neurohormonal Signalling

Abnormal neurohormonal activation plays an integral role in the genesis of ventricular arrhythmias. Although the exact details of altered neurohormonal signalling are debated there is widespread acceptance of the importance of the autonomic nervous system and the renin-angiotensin-aldosterone (RAAS) system. Modulation of these neurohormonal systems have been shown to improve prognosis in patients with heart failure, including sudden death, and therapies that target them are now the mainstay of treatment for heart failure [25]. Further evidence of the importance of the sympathetic nervous system comes from the observation that there is a circadian variation in the frequency of SCD [26].

Myocardial infarction leads to sympathetic dennervation in the infarct zone [27]. This may be followed by neurilemma cell proliferation and axonal regeneration (nerve sprouting) leading to increased sympathetic nerve density or hyperinnervation in some areas of the myocardium [28]. In the normal human ventricle sympathetic activation causes a reduction in the action potential duration and a decrease in the dispersion of repolarisation [29]. In the failing heart the juxtaposition of dennervated and hyperinnervated myocardium may lead to spatial heterogeneity in ventricular repolarisation during sympathetic activation, predisposing to ventricular arrhythmogenesis [28]. Measuring these alterations in autonomic function has been demonstrated to be predictive of SCD, though such tests are not currently in widespread clinical use.

The RAAS system, through its two main effectors angiotensin II and aldosterone, has a range of effects on the myocardium that may predispose to malignant arrhythmias. These include induction of myocardial hypertrophy, increased collagen synthesis, promotion of inflammation and thrombosis, and modulation of active membrane properties [13].

Genetic Predisposition

Evidence for genetic predisposition to SCD comes from epidemiological data. Jouven et al assessed the occurrence of SCD in 7746 middle-aged men in the Paris Prospective Study. The risk of sudden death was increased by 80% in men who had a parental history of SCD, and nearly 9 times with a history in both parents [30].

It is well established that mutations in genes coding for cardiac ion channels underlie a range of heritable conditions that predispose to ventricular arrhythmias and SCD, including Long QT and Brugada syndrome [31]. It is also becoming clear that some gene polymorphisms, while not causing monogenic inherited arrhythmogenic syndromes, can increase susceptibility to proarrhythmic drugs by reducing “repolarisation reserve” [32]. It may be that specific polymorphisms in cardiac ion channel genes similarly predispose patients with heart failure to arrhythmias.

Implantable Cardioverter Defibrillators

Since their introduction in the 1980s ICDs have revolutionised the management of patients at high risk of SCD. Multiple large randomised controlled trials have demonstrated that ICDs reduce mortality from SCD in high risk patients [33]. They are currently given to two groups of patients: survivors of life-threatening arrhythmias (secondary prevention) and patients at high risk for developing a life-threatening arrhythmia (primary prevention). In both of these settings they are both highly efficacious and cost effective [11,34].

Despite considerable effort to improve the results of out-of-hospital cardiac arrest, survival remains relatively low. Annual survival rates to hospital discharge of out-of-hospital cardiac arrest secondary to ventricular fibrillation are between 24% and 33% [35]. The use of ICDs for primary prevention of SCD is therefore of paramount importance in reducing overall SCD rates. In this respect the key issue is risk stratifying patients for SCD to identify which groups are at highest risk. While selecting patients for a secondary prevention ICD is relatively straightforward, identifying patients for primary prevention device therapy is more difficult.

Traditional Risk Stratification Tools to Guide Primary Prevention ICD use

Risk stratification has been studied primarily in patients with congestive heart failure (CHF) or asymptomatic left ventricular dysfunction, as these groups are well known to be at increased risk of SCD. A large number of tests have been evaluated. These include tests of left ventricular function, autonomic function, ventricular repolarisation, and the presence or absence of spontaneous or inducible ventricular arrhythmias. The diverse nature of these tests reflects the complex underlying pathophysiology of ventricular arrhythmogenesis. The clinically relevant risk stratification tests are:

Left Ventricular Ejection Fraction (LVEF)

Depressed LVEF, as measured by echocardiography, contrast and radionuclide ventriculography, or magnetic resonance imaging, has long been recognised to be the most important determinant of all-cause mortality in patients with IHD [36,37]. More recently, a reduced LVEF has been demonstrated to be consistently the strongest predictor of SCD in both ischaemic and non-ischaemic cardiomyopathy.

In 14,609 post-MI patients enrolled in the VALIANT trial, depressed LVEF was the most powerful predictor of SCD [10]. In the first 30 days following MI each decrease in 5 percentage points in LVEF was associated with a 21 percent increase in the risk of sudden death or cardiac arrest with resuscitation. In a prospective study of 343 patients with idiopathic dilated cardiomyopathy, LVEF was the only significant predictor of arrhythmic events in multivariate analysis, with a relative risk of 2.3 per 10% decrease in ejection fraction [38].

As a result of this robust data a depressed LVEF has been the main entry criterion used in the randomised controlled clinical trials of primary prevention ICD therapy in heart failure [39,40].

Ambulatory Monitoring

A number of studies have suggested an association between the presence of non-sustained ventricular tachycardia (NSVT) on ambulatory monitoring and SCD in both ischaemic and non-ischaemic cardiomyopathy [41-43] However, although it is used as an important determinant in the latest UK NICE guidance on ICD use, more recent evidence has cast doubt on its predictive accuracy in the modern era [34,44].

Electrophysiological Studies (EPS)

Following the finding that post-MI patients with inducible ventricular arrhythmias had a significantly increased risk of SCD, EPS was for a long time considered the "gold standard" SCD risk stratification test in IHD patients [45-47]. However more recent studies have suggested that non-inducible patients are still at high risk of SCD, casting doubt on the prognostic value of EPS in IHD [48,49]. EPS has no significant prognostic role in non-ischaemic cardiomyopathy [50,51].

Microvolt T-wave Alternans (MTWA)

The electrocardiogram, or ECG, is a surface recording of the electrical activity of the heart. It records both ventricular depolarisation (the QRS complex) and repolarisation (the T-wave). Abnormalities in ventricular repolarisation, which are integral to arrhythmogenesis, are reflected in changes in the shape and size of the T-wave.

MTWA, which is a change in the size or shape of the T-wave on alternate beats, can be detected by complex computerised techniques. Multiple trials have demonstrated that MTWA testing is predictive of malignant arrhythmias. A meta-analysis of 19 studies, evaluating MTWA in 2608 patients over an average of 21 months follow-up, found a positive predictive value of 19.3% and negative predictive value of 97.2% [52]. There was no difference in predictive value between ischaemic and nonischaemic heart failure subgroups. However, patients with an indeterminate result were excluded from the analysis, and the high proportion of such patients (20-40%) is a significant limitation of MTWA. In addition there are currently a lack of prospective trials in which MTWA has been used to guide ICD use, and both of these issues will need addressing before MTWA is in routine clinical use [53].

Other Tests

In addition a number of other risk stratification tests have some predictive ability though they are not in widespread clinical use. These include tests of autonomic function, the signal-averaged ECG, and changes in the ECG QT segment [6].

Overall LVEF is consistently the strongest and most widely used predictor of SCD and the role of additional tests is currently unclear. Most contemporary guidelines suggest that heart failure patients with severely depressed LVEF (<30-35%) should be considered for an ICD without the need for additional testing, while patients with higher ejection fractions may benefit from further evaluation with additional risk stratification tests prior to ICD implantation [11,34].

The Limitations of Current Risk Stratification Systems

Despite their proven benefits and universal recommendation in national and international guidelines [11,34,54], uptake of ICDs has been variable, and the majority of patients who might benefit from a device for 'primary prevention' of SCD do not receive one [55-58]. The reasons for this under-use are likely multifactorial. Firstly, implanted ICDs are often unused. Four year follow-up in two large trials, MADIT-II and SCD-HeFT, which used contemporary risk stratification tools to direct device use, showed under 40% of patients with ICDs received appropriate anti-tachycardic therapy [39,40]. Secondly, serious device-associated complications such as inappropriate device therapy and infection, though uncommon in trials, are increasingly recognised in routine practice [55,59,60]. Thirdly, at an estimated cost of £20102 per device, ICDs are an expensive technology [61,62].

The development of more accurate risk stratification systems would enable better targeting of ICD use. This would ensure devices are used in patients most likely to benefit and avoided in those who are unlikely to benefit but may still have complications. There is therefore significant value in developing improved risk stratification systems using existing and/or novel markers of SCD.

Serum Biomarkers in Cardiac Disease

There has been a wealth of interest over the last decade in the use of biomarkers in cardiac disease. Many individual biomarkers have demonstrated associations with adverse cardiovascular outcomes, including C-reactive protein (CRP), interleukin-6, fibrinogen, d-dimer, albuminuruia, and plasminogen activator inhibitor type 1 [63-67]. Supported by systematic reviews confirming their value and consensus recommendations supporting their use, two specific serum markers, cardiac troponin (cTn) and brain natriuretic peptide (BNP), are now in widespread clinic use [68-71].

There is some evidence combining multiple cardiac biomarkers improves outcome prediction [72-74], though the magnitude of benefit is uncertain. For example, Wang et al

studied 10 biomarkers, including CRP and BNP, in 3209 people in the Framingham Heart Study over 7 years and reported high “multimarker" scores increased the risks of death (hazard ratio 4.08) and major cardiovascular events (hazard ratio 1.84) [72]. However, they also noted that adding multimarker scores to conventional risk factors delivered only small increases in risk classification.

Serum Biomarkers in Heart Failure

Evidence of the value of serum biomarkers to predict SCD in heart failure comes from two types of study. Firstly, studies that have evaluated the relationship of biomarker levels to overall mortality or sudden cardiac death in heart failure. Secondly, studies that have evaluated biomarkers in patients with ICDs, using malignant ventricular arrhythmias as surrogate markers of SCD.

Serum Biomarkers to Predict Overall Mortality in Heart Failure

Heart failure is a clinical syndrome associated with complex molecular, endocrine and inflammatory changes [75]. The prognostic value of numerous serum biomarkers that reflect these underlying pathophysiological processes have been evaluated. Markers of neurohormonal activation, myocyte injury, myocardial stretch, and inflammation have all shown to be predictive of adverse outcomes [76].

Table 1. Studies evaluating the association of serum biomarkers with sudden cardiac death in patients with heart failure or left ventricular dysfunction.

Study	Year	No. of patients	Aetiology of heart disease	Biomarkers	Results
Berger et al. [83]	2002	452	IHD, NICM	BNP, NT-BNP NT-ANP, big endothelin	All 4 biomarkers predictive of SCD in univariate analysis On multivariate analysis only BNP predictive
Tapanainen et al. [84]	2004	521	IHD	BNP, ANP, NT-ANP	All 3 biomarkers predictive of SCD in univariate analysis On multivariate analysis only ANP and BNP predictive

IHD, ischaemic heart disease; NICM, non-ischaemic cardiomyopathy; BNP, brain natriuretic peptide; NT-BNP, N-terminal brain natriuretic peptide; NT-ANP, N-terminal atrial natriuretic peptide; ANP, atrial natriuretic peptide.

Multiple studies have demonstrated that levels of serum inflammatory cytokines predict long-term heart failure mortality [77-79]. Rauchaus et al prospectively evaluated the

predictive value of inflammatory cytokine levels in 152 patients with heart failure (121 patients in NYHA class II-III) [78]. During a mean 34 months follow-up there were 62 deaths. In univariate analyses tumour necrosis factor-alpha (TNF-α) and soluble TNF-receptors 1 and 2 (sTNF-R1/sTNFR2) ($p<0.0001$), interleukin-6 ($p=0.005$), and soluble CD14 receptors ($p=0.0007$) were all predictive of death. In multivariate analysis the strongest predictor was sTNF-R2 ($p<0.001$), which proved better than depressed LVEF. Serum cardiac troponin (cTn) is also an independent predictor of adverse outcomes, including mortality, in both stable and decompensated heart failure [80-82].

The majority of these studies were small and evaluated the relationship of biomarkers to overall mortality rather than SCD. However the commonest mode of death in all but the most advanced heart failure is sudden death [9]. Therefore it is probable that these biomarkers predict SCD as well as overall mortality. This is supported by data from the VEST trial [79].

Deswal et al analysed circulating levels of two inflammatory cytokines (TNF and IL-6) and their cognate receptors in 1200 patients enrolled in a multicentre placebo-controlled trial of Vesnarinone, an inotropic drug, in advanced heart failure [79]. All patients were NYHA class III-IV and the aetiology of heart failure in the majority was IHD (58%). In the placebo group (384 patients) there were 65 deaths, 31 each due to SCD and pump failure. Data from these 384 patients demonstrated serum levels of tumor necrosis factor ($p=0.02$), IL-6 ($p=0.002$), sTNF-R1 ($p=0.0001$), and sTNF-R2 ($p=0.0001$) were all independent predictors of overall mortality in multivariate analysis. Although the predictive relationship of biomarkers to SCD was not specifically evaluated, levels of TNF and IL-6 were not significantly different between the SCD and pump failure groups.

Serum Biomarkers to Predict Sudden Cardiac Death in Heart Failure

The value of serum biomarkers to predict SCD in heart failure has been specifically evaluated in two prospective studies (Table 1) [83,84]. One enrolled patients with chronic heart failure of ischaemic and non-ischaemic aetiology [83], and the other post-MI patients [84]. Both demonstrated a significant association between a single serum biomarker measurement and subsequent SCD risk.

Berger et al examined the association of 4 serum biomarkers - BNP, N-terminal BNP (NT-BNP), N-terminal atrial natriuretic peptide (NT-ANP), and big endothelin - with SCD in 452 ambulatory patients with heart failure and LVEF <35% [83]. The aetiology of heart failure in the majority of these patients (65%) was non-ischaemic. During follow-up (592+/-387 days) there were 89 deaths of which 44 were sudden. Using univariate analyses the only significant predictors of sudden death were log BNP ($p=0.0006$), log N-ANP ($p=0.0028$), LVEF ($p=0.0054$), log N-BNP ($p=0.0057$), systolic blood pressure ($p=0.0138$), big endothelin ($p=0.0326$), and NYHA class ($p=0.0375$). However in multivariate analysis only log BNP ($p=0.0006$) was still significantly associated with SCD. The use of specific cardiac medication including beta-blockers, ACE-I and amiodarone, as well as the presence of IHD and diabetes, were not predictive of SCD.

Tapanainen et al prospectively evaluated the accuracy of plasma ANP, N-ANP, BNP and depressed LVEF in predicting SCD in 521 survivors of acute MI [84]. During a mean follow-up

of 43 +/-13 months there were 33 deaths of which 16 were due to SCD. In univariate analysis, BNP (relative risk 4.4, p=0.011), ANP (RR 4.1, p=0.014) and N-ANP (RR 3.4, p=0.018) had similar accuracy as LVEF (RR 4.9, p=0.013) in predicting SCD. In multivariate analysis, after adjusting for clinical variables, only elevated BNP (p = 0.02) and low LVEF (<40%) (p = 0.03) remained as significant predictors of SCD. It should be noted that there was a high use of contemporary post-MI medical therapy in the cohort, including 97% beta-blockade.

Serum Biomarkers to Predict ICD Discharges

Implantable cardioverter defibrillators are extremely effective in terminating episodes of ventricular fibrillation (VF) and ventricular tachycardia (VT) that may otherwise have led to SCD. Therefore evaluating the relationship of biomarkers to SCD in patients with ICDs is potentially difficult. In addition to this therapeutic role however, ICDs also accurately record the occurrence of these malignant arrhythmias and the treatment given by the device, termed anti-tachycardic therapy. Thus the incidence of potentially life-threatening arrhythmias, as determined by device interrogation, may be used as a surrogate marker of SCD in these patients.

Table 2. Studies evaluating the association of serum biomarkers with malignant ventricular arrhythmias in ICD recipients.

Study	Year	No. of patients	Aetiology of heart disease	Biomarkers	End-point	Results
Manios et al [87]	2005	35	IHD	NT-proBNP	Appropriate device therapy for VT/VF	NT-proBNP predictive
Verma et al. [86]	2006	345	IHD, NICM	BNP, CRP	Appropriate device therapy for VT/VF	BNP predictive CRP not predictive
Biasucci et al [91]	2006	65	IHD	CRP	Appropriate device therapy for VT/VF	CRP predictive
Klingenberg et al [90]	2006	50	IHD	NT-proBNP	Appropriate device therapy for VT/VF	NT-proBNP predictive
Christ et al [85]	2007	123	IHD, NICM	BNP	Appropriate device therapy for VT/VF, death or heart transplantation	BNP predictive
Yu et al [89]	2007	99	IHD	NT-proBNP	Appropriate device therapy for VT/VF	NT-proBNP predictive EPS not predictive
Blangy et al [88]	2007	121	IHD	PINP, PIIINP, TIMP1, BNP, CRP	Appropriate device therapy for VT/VF	All markers predictive
Konstantino et al [92]	2007	50	IHD, NICM	BNP, CRP, IL-6, TNF-α	Appropriate device therapy for VT/VF	No markers predictive

IHD, ischaemic heart disease; NICM, non-ischaemic cardiomyopathy; BNP, brain natriuretic peptide; CRP, C-reactive protein; NT-proBNP, N-terminal pro-brain natriuretic peptide; PINP procollagen type I aminoterminal peptide; PIIINP, procollagen type III aminoterminal peptide, TIMP1, membrane metalloproteinase I; IL-6, interleukin 6; TNF-α, tumour necrosis factor alpha; VT, ventricular tachycardia; VF, ventricular fibrillation.

The ability of a range of serum biomarkers to predict malignant arrhythmias in ICD recipients has been assessed in 8 studies, enrolled 890 patients (Table 2) [85-92]. These studies, 6 of which were prospective, enrolled patients with both ischaemic and non-ischaemic heart failure. All but one study found biomarkers were able to predict the occurrence of malignant ventricular arrhythmias. The only study with negative findings was small and examined only 50 patients over 6 months [92].

Six studies have investigated BNP (or N-terminal pro-BNP) and demonstrated it independently predicts malignant arrhythmias in patients with ICDs [85-90]. Three of the larger studies reported patients with BNP levels over the 50th centile had significantly more malignant arrhythmias (risk ratios between 2.2 and 3.8) [86,88,89]. Multivariate regression analyses in these studies examining traditional clinical and echocardiographic risk factors for SCD, found BNP most strongly predicted malignant arrhythmias and performed better than reduced LVEF.

Two studies investigated a broader range of serum biomarkers. Blangy et al prospectively evaluated markers of cardiac fibrosis [procollagen type I aminoterminal peptide (PINP), procollagen type III aminoterminal peptide (PIIINP), membrane metalloproteinase I (TIMP1)], myocardial pressure overload [brain natriuretic peptide (BNP)] and inflammation [high sensitivity (hs)-C-reactive protein] [88]. They observed 121 patients with IHD over 12 months. During this time 38 patients had appropriate device therapy for VT. In a multivariate analysis, LVEF <0.35 (OR = 2.19, P = 0.049), an increased serum BNP (OR = 3.75, P = 0.014), an increased hs-C-reactive protein (OR = 3.2, P = 0.006), an increased PINP (OR = 3.71, P = 0.009), and a decreased PIIINP (OR = 0.21, P = 0.003) were associated with a higher VT incidence. Biasucci et al studied 65 patients and confirmed the association with hsCRP [91].

One study has compared the predictive value of N-terminal pro-BNP (NT-pro-BNP) to the gold-standard of EPS [89]. Yu et al prospectively studied 99 patients with ICDs for prevention of SCD following MI. EPS and measurement of NT-pro-BNP were performed at study entry. During a mean follow-up of 556 (+/-122) days 23 patients received appropriate device therapy for VF/VT. On multivariate Cox regression analysis, only NT–pro-BNP level at or greater than median (497 ng/L) was a significant predictor for VT/VF occurrence (p=0.047). Neither univariate or multivariate analysis demonstrated any relationship between inducibility at EPS and the study end-points.

Serum Biomarkers to Guide ICD use?

Multiple studies have demonstrated that serum biomarkers can accurately predict adverse outcomes in patients with heart failure and asymptomatic left ventricular dysfunction of both ischaemic and non-ischaemic aetiology. A range of biomarkers predict both the occurrence of SCD in patients without ICDs and the occurrence of malignant arrhythmias in patients with devices (Table 3). In these studies individual biomarkers are at least as good as the current best marker of SCD risk, depressed LVEF. In the only trial to compare biomarkers to electrophysiological testing, serum NT-BNP was considerably more accurate than EPS in predicting malignant arrhythmias [89].

As predictive tests, biomarkers have significant advantages over current tools. Assessment of LVEF can be expensive, if performed by the gold-standard magnetic resonance imaging, and inaccurate, if performed using two-dimensional transthoracic echocardiography. EPS is expensive, invasive, associated with small but important risks to the patient, and often only available in larger cardiac centres. Ambulatory monitoring, to look for spontaneous ventricular arrhythmias, is not particularly reproducible [93]. In contrast, biomarker measurement is simple, relatively inexpensive, reproducible, and without direct patient risk.

Table 3. Biomarkers demonstrated to predict the occurrence of sudden cardiac death or ventricular arrhythmias in patients with heart failure.

Biomarker	Role of biomarker	No. of studies	Aetiology of heart failure in studies
Brain Natriuretic Peptide (BNP)	A natriuretic peptide largely released from the ventricles, in response to increases in intraventricular pressure and myocardial stretch	6	IHD, NICM
N-terminal pro Brain Natriuretic Peptide (NT-proBNP)	An N-terminal fragment that is co-secreted with BNP	3	IHD, NICM
Atrial Natriuretic Peptide (ANP)	A natriuretic peptide largely released from the atria in response to increases in intraatrial pressure and stretch	1	IHD
N-terminal Atrial Natriuretic Peptide (NT-ANP)	An N-terminal fragment that is co-secreted with ANP	2	IHD, NICM
C-reactive protein (CRP)	An acute phase reactant marker of systemic inflammation	3	IHD, NICM
Big endothelin	A precursor to endothelin, a vasoactive peptide involved in vascular homeostasis	1	IHD, NICM
Procollagen type I aminoterminal peptide	A marker of collagen turnover and myocardial fibrosis	1	IHD
Procollagen type III aminoterminal peptide	A marker of collagen turnover and myocardial fibrosis	1	IHD
Membrane metalloproteinase I	A marker of extracellular matrix remodelling	1	IHD

IHD, ischaemic heart disease; NICM, non-ischaemic cardiomyopathy.

The genesis of ventricular arrhythmias that lead to SCD is a complex process requiring the presence of both an abnormal myocardial substrate, needed to initiate and sustain an arrhythmia, and pro-arrhythmic triggers [11]. A range of electrophysiological and molecular alterations contribute to arrhythmogenesis in the failing heart, including changes in ion

channel expression and neurohormonal modulation, and serum biomarkers may provide an assessment of these various processes. It is likely that optimal SCD risk stratification will require the combination of multiple tests that reflect these diverse upstream processes. As such the greatest potential benefit of biomarkers may be in measuring multiple complementary markers that assess distinct aspects of arrhythmic risk, or in combining biomarkers with traditional risk stratification tools. Currently there have been no studies evaluating this.

Conclusion

Despite the availability of a number of well characterised tests, risk stratification of SCD in patients with heart failure is currently sub-optimal. The value of serum biomarkers in cardiovascular disease is well established. There is increasing data to suggest that individual serum biomarkers predict SCD at least as well as established risk stratification tools in heart failure patients. Biomarkers are available that provide an assessment of the diverse pathophysiological processes that are central to ventricular arrhythmogenesis, including myocardial stretch, inflammation, and neurohormonal activation. There is therefore significant need for further studies to evaluate the potential role of biomarkers, individually or in combination, in patient selection for ICDs.

References

[1] Rosamond, W; Flegal, K; Friday, G; et al; American Heart Association Statistics Committee and Stroke Statistics Subcommittee. Heart disease and stroke statistics--2007 update: a report from the American Heart Association Statistics Committee and Stroke Statistics Subcommittee. *Circulation,* 2007, 115, e69-171.

[2] Morgan, JM; Cowan, JC; Camm, AJ; McComb, JM. Sudden cardiac death: opportunities for prevention. *Heart,* 2006, 92, 721-3.

[3] Zipes, DP; Wellens, HJJ. Sudden cardiac death. *Circulation,* 1998, 98, 2334 – 51.

[4] Zheng, ZJ; Croft, JB; Giles, EH; et al. Sudden cardiac death in the United States, 1989-1998. *Circulation,* 2001, 104**,** 2158-63.

[5] Hinkle, LE; Jr, Thaler, HT. Clinical classification of cardiac deaths. *Circulation,* 1982, **65,** 456-64.

[6] Kusmirek, SL; Gold, MR. Sudden cardiac death: the role of risk stratification. *Am Heart J,* 2007, 153, (4 Suppl), 25-33.

[7] Ho, KKL; Anderson, KM; Kannel, WB; et al. Survival after the onset of congestive heart failure in Framingham Heart Study subjects. *Circulation,* 1993, 88**,** 107-115.

[8] Kannel, WB; Plehn, JF; Cupples, LA. Cardiac failure and sudden death in the Framingham Study. *Am Heart J,* 1988, 115, 869-75.

[9] Mozaffarian, D; Anker, SD; Anand, I; et al. Prediction of mode of death in heart failure: the Seattle Heart Failure Model. *Circulation,* 2007, 116, 392-8.

[10] Solomon, S; Zelenkofske, S; McMurray, JJV; et al. Sudden death inpatients with myocardial infarction and left ventricular dysfunction, heart failure, or both. *N Engl J Med.*, 2005, 352, 2581 - 8.

[11] ACC/AHA/ESC 2006 Guidelines for Management of Patients With Ventricular Arrhythmias and the Prevention of Sudden Cardiac Death: a report of the American College of Cardiology/American Heart Association Task Force and the European Society of Cardiology Committee for Practice Guidelines (writing committee to develop Guidelines for Management of Patients With Ventricular Arrhythmias and the Prevention of Sudden Cardiac Death): developed in collaboration with the European Heart Rhythm Association and the Heart Rhythm Society. *Circulation,* 2006, 114, 385-484.

[12] Bayes De Luna, A; Coumel, P; Leclercq, JF. Ambulatory sudden cardiac death: mechanisms of production of fatal arrhythmia on the basis of data from 157 cases. *Am Heart J*, 1989, 117, 151–9.

[13] Tomaselli, GF; Zipes, DP. What causes sudden death in heart failure? *Circ Res.*, 2004, 95, 754-63.

[14] Zipes, DP; Rubart, M. Neural modulation of cardiac arrhythmias and sudden cardiac death. *Heart Rhythm*. 2006, 3, 108-13.

[15] Rubart, M; Zipes, DP. Genes and cardiac repolarization: the challenge ahead. *Circulation*, 2005, 112, 1242-4.

[16] Tomaselli, GF; Marban, E. Electrophysiological remodeling in hypertrophy and heart failure. *Cardiovasc Res.*, 1999, 42, 270–283.

[17] Beuckelmann, DJ; Nabauer, M; Erdmann, E. Alterations of K currents in isolated human ventricular myocytes from patients with terminal heart failure. *Circ Res.*, 1993, 73, 379–385.

[18] Kaab, S; Nuss, HB; Chiamvimonvat, N; et al. Ionic mechanism of action potential prolongation in ventricular myocytes from dogs with pacing-induced heart failure. *Circ Res.*, 1996, 78, 262–273.

[19] Tsuji, Y; Opthof, T; Kamiya, K; et al. Pacing-induced heart failure causes a reduction of delayed rectifier potassium currents along with decreases in calcium and transient outward currents in rabbit ventricle. *Cardiovasc Res.*, 2000, 48, 300–309.

[20] Koumi, S; Backer, CL; Arentzen, CE. Characterization of inwardly rectifying K_ channel in human cardiac myocytes. Alterations in channel behavior in myocytes isolated from patients with idiopathic dilated cardiomyopathy. *Circulation*, 1995, 92, 164–174.

[21] Undrovinas, AI; Maltsev, VA; Kyle, JW; et al. Gating of the late Na channel in normal and failing human myocardium. *J Mol Cell Cardiol*, 2002, 34, 1477–1489.

[22] Akar, FG; Rosenbaum, DS. Transmural electrophysiological heterogeneities underlying arrhythmogenesis in heart failure. *Circ Res.*, 2003, 93, 638–645.

[23] Weiss, J; Garfinkel, A; Karagueuzian, HS; et al. Chaos and the transition to ventricular fibrillation: a new approach to antiarrhythmic drug evaluation. *Circulation,* 1999, 99, 2819 –26.

[24] Narayan, SM. T-wave alternans and the susceptibility to ventricular arrhythmias. *J Am Coll Cardiol*., 2006, 47, 269-81.

[25] ACC/AHA 2005 Guideline Update for the Diagnosis and Management of Chronic Heart Failure in the Adult: a report of the American College of Cardiology/American Heart Association Task Force on Practice Guidelines (Writing Committee to Update the 2001 Guidelines for the Evaluation and Management of Heart Failure): developed in collaboration with the American College of Chest Physicians and the International Society for Heart and Lung Transplantation: endorsed by the Heart Rhythm Society. *Circulation*, 2005, 112, e154-235.

[26] Muller, JE; Ludmer, PL; Willich, SN; et al. Circadian variation in the frequency of sudden cardiac death. *Circulation*, 1987, **75,** 131-138.

[27] Barber, MJ; Mueller, TM; Davies, BG; et al. Interruption of sympathetic and vagal-mediated afferent responses by transmural myocardial infarction. C*irculation*, 1985, 72, 623–631.

[28] Chen, LS; Zhou, S; Fishbein, MC; Chen, PS. New perspectives on the role of autonomic nervous system in the genesis of arrhythmias. *J Cardiovasc Electrophysiol*, 2007, 18, 123-7.

[29] Takei, M; Sasaki, Y; Yonezawa, T; et al. The autonomic control of the transmural dispersion of ventricular repolarization in anesthetized dogs. *J Cardiovasc Electrophysio*, 1999, 10, 981–989.

[30] Jouven, X; Desnos, M; Guerot, C; Ducimetière, P. Predicting sudden death in the population: the Paris Prospective Study I. *Circulation*, 1999, 99, 1978-83.

[31] Brugada, J; Brugada, R; Brugada, P. Channelopathies: a new category of diseases causing sudden death. *Herz*, 2007, 32, 185-91.

[32] Remme, CA; Bezzina, CR. Genetic modulation of cardiac repolarization reserve. *Heart Rhythm*, 2007, 4, 608-10.

[33] Ezekowitz, JA; Rowe, BH; Dryden, DM; et al. Systematic review: implantable cardioverter defibrillators for adults with left ventricular systolic dysfunction. *Ann Intern Med.*, 2007, 147, 251-62.

[34] TA95 Implantable cardioverter defibrillators for arrhythmias. http://guidance.nice.org.uk/TA95/guidance/pdf/English

[35] Cobb, LA; Weaver, WD; Fahrenbruch, CE; et al. Community-based interventions for sudden cardiac death. Impact, limitations, and changes. *Circulation*, 1992, 85, 98 –102.

[36] Multicenter Post Infarction Research Group. Risk Stratification and survival after myocardial infarction. *N Engl J Med.*, 1983, 309, 331-6.

[37] Nelson GR, Cohn PF, Gorlin R. Prognosis in medically-treated coronary artery disease: influence of ejection fraction compared to other parameters. *Circulation*, 1975, 52, 408-12.

[38] Grimm, W; Christ, M; Bach, J; et al. Noninvasive arrhythmiarisk stratification in idiopathic dilated cardiomyopathy: results of the Marburg cardiomyopathy study. *Circulation*, 2003, 108, 2883-91.

[39] Bardy, GH; Lee, KL; Mark, DB; et al. Amiodarone or an implantable cardioverter-defibrillator for congestive heart failure. *N Engl J Med.*, 2005, 352, 225-37.

[40] Moss, AJ; Zareba, W; Hall, WJ; et al. Prophylactic implantation of a defibrillator in patients with myocardial infarction and reduced ejection fraction. *N Engl J Med.*, 2002, 346, 877 - 83.

[41] Anderson, KP; DeCamilla, J; Moss, AJ. Clinical significance of ventricular tachycardia (3 beats or longer) detected during ambulatory monitoring after myocardial infarction. *Circulation*, 1978, **57,** 890-7.

[42] Bigger, Jr JT; Fleiss, JL; Kleiger, R; et al. The relationships among ventricular arrhythmias, left ventricular dysfunction, and mortality in the 2 years after myocardial infarction. *Circulation*, 1984, 69, 250-8.

[43] Grimm, W; Christ, M; Maisch, B. Long runs of non-sustained ventricular tachycardia on 24-hour ambulatory electrocardiogram predict major arrhythmic events in patients with idiopathic dilated cardiomyopathy. *PACE*, 2005, 28, S207-10.

[44] Ma¨kikallio, TH; Barthel, P; Schneider, R; et al. Prediction of sudden cardiac death after acute myocardial infarction: role of Holter monitoring in the modern treatment era. *Eur Heart J*, 2005, 26, 762-9.

[45] Bourke, JP; Richards, DA; Ross, DL; et al. Routine programmed electrical stimulation in survivors of acute myocardial infarction for prediction of spontaneous ventricular tachyarrhythmias during follow-up: results, optimal stimulation protocol and cost-effective screening. *J Am Coll Cardiol*, 1991, 18, 780-8.

[46] Iesaka, Y; Nogami, A; Aonuma, K; et al. Prognostic significance of sustained monomorphic ventricular tachycardia induced by programmed ventricular stimulation using up to triple extrastimuli in survivors of acute myocardial infarction. *Am J Cardiol*, 1990, 65, 1057- 63.

[47] Nogami, A; Aonuma, K; Takahashi, A; et al. Usefulness of early versus late programmed ventricular stimulation in acute myocardial infarction. *Am J Cardiol*, 1991, 68, 13-20.

[48] Daubert, JP; Zareba, W; Hall, WJ; et al. Predictive value of ventricular arrhythmia inducibility for subsequent ventricular tachycardia or ventricular fibrillation in Multicenter Automatic Defibrillator Implantation Trial (MADIT) II patients. *J Am Coll Cardiol*, 2006, 47, 98-107.

[49] Buxton, AE; Lee, KL; DiCarlo, L; et al. Electrophysiologic testing to identify patients with coronary artery disease who are at risk for sudden death. *N Engl J Med.*, 2000, 342, 1937-45.

[50] Poll, DS; Marchlinski, FE; Buxton, AE; et al. Usefulness of programmed stimulation in idiopathic dilated cardiomyopathy. *Am J Cardiol*, 1986, 58, 992-7.

[51] Meinertz, T; Treese, N; Kasper, W; et al. Determinants of prognosis in idiopathic dilated cardiomyopathy as determined by programmed electrical stimulation. *Am J Cardiol*, 1985, 56, 337-41.

[52] Gehi, AK; Stein, RH; Metz, LD; et al. Microvolt T-wave alternans for the risk stratification of ventricular tachyarrhythmic events a metaanalysis. *J Am Coll Cardiol,* 2005, 46, 75-82.

[53] Gold, MR; Spencer, W. T wave alternans for ventricular arrhythmia risk stratification. *Curr Opin Cardiol,* 2003, 18, 1-5.

[54] Al-Khatib, SM; Sanders, GD; Bigger, JT; et al; Expert panel participating in a Duke's Center for the Prevention of Sudden Cardiac Death conference. Preventing tomorrow's sudden cardiac death today: part I: Current data on risk stratification for sudden cardiac death. *Am Heart J,* 2007, 153, 941-50.

[55] Sanders, GD; Al-Khatib, SM; Berliner, E; et al; Expert panel participating in a Duke Center for the Prevention of Sudden Cardiac Death-sponsored conference. Preventing tomorrow's sudden cardiac death today: part II: Translating sudden cardiac death risk assessment strategies into practice and policy. *Am Heart J,* 2007, 153, 951-9.

[56] Pacemakers and Implantable Defibrillators: A Two Year National Survey for 2003 and 2004. Network Devices Survey Group. http://www.devicesurvey.com/

[57] Scott, PA; Gorman, S; Andrews, NP, et al. Estimation of the requirement for implantable cardioverter defibrillators for the primary prevention of sudden cardiac death post-myocardial infarction based on UK national guidelines (2006)–*Europace* 2008, 10, 453-7.

[58] Plummer, CJ; Irving, J; Mccomb, JM. The incidence of implantable cardioverter defibrillator indications in patients admitted to all coronary care units in a single district. *Europace,* 2005, **7,** 266-72.

[59] Reynolds, MR; Cohen, DJ; Kugelmass, AD; et al. The frequency and incremental cost of major complications among medicare beneficiaries receiving implantable cardioverter-defibrillators. *J Am Coll Cardiol,* 2006, 47, 2493-7.

[60] Gould, PA; Krahn, AD. Complications associated with implantable cardioverter-defibrillator replacement in response to device advisories. *JAMA,* 2006, 295, 1907-11.

[61] TA95 Arrhythmia - implantable cardioverter defibrillators (ICDs): Analysis of cost impact. http://guidance.nice.org.uk/TA95/costtemplate/xls/English

[62] Hlatky MA, Mark DB. The high cost of implantable defibrillators. *Eur Heart J,* 2007, 28, 388-91.

[63] Ridker, PM; Rifai, N; Stampfer, MJ; Hennekens, CH. Plasma concentration of interleukin-6 and the risk of future myocardial infarction among apparently healthy men. *Circulation,* 2000, 101, 1767–72.

[64] Danesh, J; Wheeler, JG; Hirschfield, GM; et al. C-reactive protein and other circulating markers of inf lammation in the prediction of coronary heart disease. *N Engl J Med.,* 2004, 350, 1387-97.

[65] Danesh, J; Lewington, S; Thompson, SG; et al. Plasma fibrinogen level and the risk of major cardiovascular diseases and nonvascular mortality: an individual participant meta-analysis. *JAMA,* 2005, 294, 1799-809.

[66] Cushman, M; Lemaitre, RN; Kuller, LH; et al. Fibrinolytic activation markers predict myocardial infarction in the elderly: the Cardiovascular Health Study. *Arterioscler Thromb Vasc Biol.*, 1999, 19, 493-8.

[67] Hoekstra, T; Geleijnse, JM; Schouten, EG; Kluft, C. Plasminogen activator inhibitor-type 1: its plasma determinants and relation with cardiovascular risk. *Thromb Haemost*, 2004, 91, 861-72.

[68] Doust, JA; Pietrzak, E; Dobson, A; Glasziou, P. How well does B-type natriuretic peptide predict death and cardiac events in patients with heart failure: systematic review. *BMJ*, 2005, 330, 625-8.

[69] Tang, WH; Francis, GS; Morrow, DA; et al; National Academy of Clinical Biochemistry Laboratory Medicine. National Academy of Clinical Biochemistry Laboratory Medicine practice guidelines: Clinical utilization of cardiac biomarker testing in heart failure. *Circulation*, 2007, 116, 99-109.

[70] The Joint ESC/ACC Committee. Myocardial infarction redefined – A consensus document of the Joint ESC/ACC Committee for the Redefinition of Myocardial Infarction. *Eur Heart J*, 2000, 21, 1502-1513.

[71] Wang, TJ; Larson, MG; Levy, D; et al. Plasma natriuretic peptide levels and the risk of cardiovascular events and death. *N Engl J Med.*, 2004, 350, 655-63.

[72] Wang, TJ; Gona, P; Larson, MG; et al. Multiple biomarkers for the prediction of first major cardiovascular events and death. *N Engl J Med.*, 2006, 355, 2631-9.

[73] Sabatine, MS; Morrow, DA; De Lemos, JA; et al. Multimarker approach to risk stratification in non-ST elevation acute coronary syndromes: simultaneous assessment of troponin I, C-reactive protein, and B-type natriuretic peptide. *Circulation*, 2002, 105, 1760-3.

[74] Blankenberg, S; McQueen, MJ; Smieja, M; et al; HOPE Study Investigators. Comparative impact of multiple biomarkers and N-Terminal pro-brain natriuretic peptide in the context of conventional risk factors for the prediction of recurrent cardiovascular events in the Heart Outcomes Prevention Evaluation (HOPE) Study. *Circulation*, 2006 114, 201-8.

[75] Braunwald, E; Bristow, MR. Congestive heart failure: fifty years of progress. *Circulation*, 2000, 102, 14–23.

[76] De Virginy, DR. Novel and potential future biomarkers for assessment of the severity and prognosis of chronic heart failure : a clinical review. *Heart Fail Rev.*, 2006, 11, 333-4.

[77] Maeda, K; Tsutamoto, T; Wada, A; et al. High levels of plasma brain natriuretic peptide and interleukin-6 after optimized treatment for heart failure are independent risk factors for morbidity and mortality in patients with congestive heart failure. *J Am Coll Cardiol*, 2000, 36, 1587-93.

[78] Rauchhaus, M; Doehner, W; Francis, DP; et al. Plasma cytokine parameters and mortality in patients with chronic heart failure. *Circulation*, 2000, 102, 3060-7.

[79] Deswal, A; Petersen, NJ; Feldman, AM; et al. Cytokines and cytokine receptors in advanced heart failure: an analysis of the cytokine database from the Vesnarinone trial (VEST). *Circulation*, 2001, 103, 2055-9

[80] Sato, Y; Yamada, T; Taniguchi, R; et al. Persistently increased serum concentrations of cardiac troponin T in patients with idiopathic dilated cardiomyopathy are predictive of adverse outcomes. *Circulation*, 2001, 103, 369–374.

[81] Horwich, TB; Patel, J; MacLellan, WR; Fonarow, GC. Cardiac troponin I is associated with impaired hemodynamics, progressive left ventricular dysfunction, and increased mortality rates in advanced heart failure. *Circulation*, 2003, 108, 833– 838.

[82] Kuwabara, Y; Sato, Y; Miyamoto, T; et al. Persistently increased serum concentrations of cardiac troponin in patients with acutely decompensated heart failure are predictive of adverse outcomes. *Circ J.*, 2007, 71, 1047-51

[83] Berger, R; Huelsman, M; Strecker, K; et al. B-type natriuretic peptide predicts sudden death in patients with chronic heart failure. *Circulation*, 2002, 105, 2392-7.

[84] Tapanainen, JM; Lindgren, KS; Ma¨kikallio, TH; et al. Natriuretic peptides as predictors of non-sudden and sudden cardiac death after acute myocardial infarction in the beta-blocking era. *J Am Coll Cardiol*, 2004, 43, 757-63.

[85] Christ, M; Sharkova, J; Bayrakcioglu, S; et al. B-type natriuretic peptide levels predict event-free survival in patients with implantable cardioverter defibrillators. *Eur J Heart Fail*, 2007, 9, 272-9.

[86] Verma, A; Kilicaslan, F; Martin, DO; et al. Preimplantation B-type natriuretic peptide concentration is an independent predictor of future appropriate implantable defibrillator therapies. *Heart*, 2006, 92, 190-5.

[87] Manios, EG; Kallergis, EM; Kanoupakis, EM; et al. Amino-terminal pro-brain natriuretic peptide predicts ventricular arrhythmogenesis in patients with ischemic cardiomyopathy and implantable cardioverter-defibrillators. *Chest.*, 2005, 128, 2604-10.

[88] Blangy, H; Sadoul, N; Dousset, B; et al. Serum BNP, hs-C-reactive protein, procollagen to assess the risk of ventricular tachycardia in ICD recipients after myocardial infarction. *Europace*, 2007, 9, 724-9.

[89] Yu, H; Oswald, H; Gardiwal, A; et al. Comparison of N-terminal pro-brain natriuretic peptide versus electrophysiologic study for predicting future outcomes in patients with an implantable cardioverter defibrillator after myocardial infarction. *Am J Cardiol*, 2007, 100, 635-9.

[90] Klingenberg, R; Zugck, C; Becker, R; et al. Raised B-type natriuretic peptide predicts implantable cardioverter-defibrillator therapy in patients with ischaemic cardiomyopathy. *Heart*, 2006, 92, 1323-4.

[91] Biasucci, LM; Giubilato, G; Biondi-Zoccai, G; et al. C reactive protein is associated with malignant ventricular arrhythmias in patients with ischaemia with implantable cardioverter-defibrillator. *Heart*, 2006, 92, 1147-8.

[92] Konstantino, Y; Kusniec, J; Reshef, T; et al. Inflammatory biomarkers are not predictive of intermediate-term risk of ventricular tachyarrhythmias in stable CHF patients. *Clin Cardiol*, 2007, 30, 408-13.

[93] Senges, JC; Becker, R; Schreiner, KD; *et al*. Variability of Holter electrocardiographic findings in patients fulfilling the noninvasive MADIT criteria. Multicenter Automatic Defibrillator Implantation Trial. *Pacing Clin Electrophysiol*, 2002, 25, 183-90.

In: Horizons in World Cardiovascular Research. Volume 1 ISBN: 978-1-60876-447-1
Editor: Eleanor H. Bennington, pp. 117-127

Chapter VII

Pharmacological Therapy in Children with Congenital Long-QT Syndrome

Tarik El Houari*, Rachida Bouhouch, Ibtissam Fellat and Mohamed Arharbi
Department of cardiology B. Ibn Sina Hospital. University of Rabat. Morocco.

Summary

The congenital long QT syndrome (CLQTS) is a genetic channelopathy that affects sodium and calcium kinetics, resulting in prolonged ventricular repolarization. This channelopathy is associated with increased propensity to syncope, malignant ventricular arrhythmias and sudden arrhythmic death in children with normal cardiac structure. Recently, the published data from the International LQTS Registry have established risk factors for sudden cardiac death and aborted cardiac arrest in children. β-blockers are the first-line drug therapy for congenital long-QT syndrome in children. Several β-blockers (propranolol, atenolol, nadolol, metoprolol,..) were used in CLQTS with a significant reduction of cardiac events in patients with LQT1 and LQT2 mutations, but no evident reduction in those with LQT3 mutations. Infrequently, additional Drugs (mexiletine and flecainide) were used in children with CLQTS. The implantable cardioverter defibrillator and left cervicothoracic sympathetic denervation are other therapeutic options in children who remain symptomatic despite β-blocker therapy. Genetic factors may be used to improve risk stratification in genotyped patients and to predict the response to β-blockers.

*Corresponding author: Department of cardiology B. Ibn Sina Hospital, University of Rabat. Morocco. E-mail: drelhouari@yahoo.fr

Introduction

Congenital long QT syndrome (CLQTS) is a genetic disorder caused by mutations that encode cardiac ion channel proteins, which regulate the flux of sodium, potassium, and calcium ions across myocellular membranes [1]. This channelopathy is characterized by the prolongation of the QT interval in the ECG and life-threatening cardiac arrhythmias, occurring especially during conditions of increased sympathetic activity [2]. The genetic disorder is an important cause of sudden cardiac death (SCD) in children without structural heart disease [3].

Recently, the published data from the International LQTS Registry have established risk factors for sudden cardiac death and aborted cardiac arrest in children [4]. Three important implications emerge from the analysis of the registry: 1) Risk factors for aborted cardiac arrest (ACA) or SCD can be assessed from male gender, a history of syncope at any time during childhood, and a QTc duration > 500 ms; 2) Significant interactions exist among the 3 clinical risk factors that can identify risk subsets in children; 3) β-blocker therapy is associated with a significant reduction in the risk of life-threatening cardiac events in CLQTS in children.

Because of technical issues with Intracardiac cardioverter defibrillators implantation, a high incidence of leads dislocation and rupture resulting in inappropriate shocks [5] and their psychological impact in children, Pharmacological therapy remains the first line treatment of CLQTS in this young population.

The application of molecular genetics to cardiovascular disease has allowed the identification of mutations in ion channel genes as the cause of LQTS. Following the identification, in 1995 and 1996, of the first three LQTS genes associated with the most frequently encountered LQTS variants called respectively LQT1, LQT2, and LQT3, there has been a flourishing of identifications of genes proven or just thought to be associated with LQTS [5-7]. This includes the genes for LQT4 through LQT10 (Table 1) [8].

The specific genotype influences the characteristics of the clinical phenotype, including the arrhythmia trigger, frequency of life threatening events, and T-wave morphology [9–11]. The discovery of a distinct molecular basis for LQTS has fostered a hope for specific therapy against the gene defect.

1. β-Blockers

Pharmacological therapy with β-blockers is considered the first choice prophylactic therapy, unless specific contraindications are present. It's recommended to administer β-blockers in all LQTS patients, even those at very low risk [4]. In patients with LQT1 and LQT2 syndrome, life-threatening arrhythmias including torsades de pointes tachyarrhythmia and sudden cardiac death tend to occur with physical or emotional stress [11]. Thus, the attenuation of adrenergic-mediated triggers in LQTS seems to be the mechanism of action of β-blockers, especially in individuals with the LQT1 and LQT2 genotypes [11]. Recent study from the International LQTS Registry has demonstrated that β-blocker therapy is associated with a significant and pronounced reduction in the risk of life-threatening cardiac events in

high-risk LQTS in children [4]. However, despite these beneficial effects, some patients receiving β-blocker therapy had a high rate of residual cardiac events [4,12-15]. In a cohort of 335 genotyped LQTS patients receiving β-blocker therapy [15], cardiac events occurred in 10%, 23%, and 32% of LQT1, LQT2, and LQT3 patients, respectively. Since the HERG channel function is defective in LQT2 patients and HERG channel dependency is increased in LQT1 patients, it is reasonable to consider that β-blockers without HERG channel blocking activity are more preferable for the treatment of these patients. These disparities between LQTS genotype and response to β-blockers seem to be attributed to different practices with respect to type of β-blocker used for the treatment of LQTS. Few data exists about the uniform efficacy between the various β-blockers. Indeed physicians use frequently propranolol, nadolol, atenolol, or metoprolol and make ‘‘lateral’’ substitutions if/when side effects become an issue (Table 2).

Table 1. Long QT syndrome (LQTS) subtypes and mutation-associated genes.

LQTS subtypes	Gene
LQT1 and JLN1 (AR)	KCNQ1
LQT2	*KCNH2*
LQT3	*SCN5A*
LQT4	*ANK2*
LQT5 (RWS) and JLN2	*KCNE1*
LQT6	*KCNE2*
LQT7 (Andersen-Tawil syndrome)	*KCNJ2*
LQT8 (Timothy syndrome)	*CACNA1c*
LQT9	*CAV3*
LQT10	*SCN4B*

Abbreviations:
LQTS = Long QT syndrome;
JLN = Jervell and Lange-Nielsen syndrome;
RWS = Romano-Ward syndrome;
AR = autosomal recessive

1.1. Propranolol

Propranolol is a non-selective β-blockers thus it has nonspecific pharmacological actions, blocking Na+ channels in addition to its β-adrenoceptor blocking effects. Therefore propranolol would be expected to antagonize any residual adrenergic tone caused by spontaneous release of catecholamines from nerve endings in addition to blocking Na+ channels [16]. The advantages of propranolol are its lipophilicity that allows it to cross the blood-brain barrier, and its well-known tolerability for chronic therapy. The disadvantages are the need of multiple daily administrations, the contraindications for patients with asthma and diabetes and the lipid solubility of propranolol causes side effects involving the central nervous system, such as depression. Propranolol is used at daily dosage of 2 to 3 mg/kg; sometime the dosage is increased to 4 mg/kg. At high dose, propranolol seems to prolong QT

interval [17]. More recently, propranolol has been reported to have an inhibitory effect on HERG current by binding to the putative common binding site [18,19]. Thus, propranolol might have a less powerful effect on QT interval at a clinically relevant concentration as reported previously [20-22]. For these reasons propranolol may not be the treatment of choice of patients with LQT1 and LTQ2.

Table 2. The frequency use of pharmacological therapy in children from the International LQTS Registry. [4]

β-Blockers (n= 643/3015 pts)	Propranolol	397 Pts	61.7%
	Atenolol	242 Pts	37.6%
	Nadolol	162 Pts	25.2%
	Metoprolol	27 Pts	4.2%
	Other β-Blockers	19 Pts	2.9%
Sodium channel blockers (n= 34/3015 pts)	Flecainide	5 Pts	0.2%
	Mexiletine	29 Pts	1%

Table 3. Dosages and frequency administration of the main drugs used in the LQTS.

Drug	Dosages (mg/kg/day)	Frequency administration
Propranolol	2.5 - 5	Twice a day
Atenolol	1 - 1.5	One to twice a day
Nadolol	0.75 – 1.5	Twice a day
Metoprolol	1 - 4	One to twice a day
Flecainide	2 - 5	Twice a day
Mexiletine	6 - 8	Four times a day
Spironolactone	2 - 5	Twice a day

1.2. Nadolol

This drug is a non-selective β-adrenoceptor antagonist characterized by its longer half-life, thus it's used twice a-day usually at 1 mg/kg/day.

1.3. Atenolol and Metoprolol

Atenolol and metoprolol did not inhibit HERG currents significantly at least in clinically relevant concentrations. Thus, these drugs are suitable for treatment of LQT1 and LQT2 patients [23]. But atenolol has been reported to be associated with clinical failures more often than propranolol or nadolol thus is used less frequently [9].

1.4. Carvedilol

Carvedilol has been reported to inhibit HERG by Cheng [24] and Karle [25] and has class III antiarrhythmic effect. In the COMET study [26] and its subanalysis [27], carvedilol reduced the mortality rate and sudden cardiac death rate more effectively than metoprolol. The mechanisms for this favourable clinical outcome for patients treated with Carvedilol are not clear. Kawakami and al compared the class III antiarrhythmic effects of multiple b-blockers by estimating HERG channel blocking activity. The class III antiarrhythmic effects were significantly potent for carvedilol compared with other β-blockers. Carvedilol might provide favourable outcome via class III antiarrhythmic effects, in the context of β-blockade, in chronic heart failure patients. These results seem to provide a hypothetical molecular explanation for the favourable outcome in carvedilol treated patients in the COMET study. Carvedilol directly inhibited HERG channels at clinically relevant concentrations. Thus, carvedilol might not be recommended in the treatment of patients with LQT1 and LQT2 [23].

2. Sodium Channel Blockers

Na+ channel blockers such as mexiletine and flecainide are effective in treating LQT-3 patients due to preferential inhibition of mutant Na + channel activity [28,29]

2.1. Flecainide

Flecainide is a class IC sodium channel blocker. It may be of therapeutic benefit in HERG phenotype and in acquired LQT [30]. Flecainide is reported to be effective in abbreviating QT interval in LQT3 patients with a specific mutation (D1790G) in *SCN5A* [31]. Other study [32] indicates that low-dose flecainide could be a promising therapeutic agent for LQT patients with the SCN5A: DeltaKPQ sodium channel mutation. No adverse side effects or proarrhythmias were observed with flecainide in this study. However, class IC sodium channel blockers might elicit a Brugada phenotype in LQT3 patients [33], therefore should not be used in general in LQT3 syndrome except for that with the specific *SCN5A* mutation.

2.2. Mexiletine

Mexiletine is a class Ib antiarrhythmic drug used for ventricular arrhythmias but is also found to be effective for long QT 3 syndrome. The potential utility of mexiletine for the treatment of drug-induced LQT has been studied in vivo in dogs, where it decreased the electrical vulnerability of the heart during cisapride overdose, suggesting that it may become a potential pharmacological strategy for drug-induced LQT [34]. Experimental data from wedge studies indicates that mexiletine is more effective in abbreviating the QT interval in the LQT3 model than in the LQT1 or LQT2 model [21,35], but that the drug reduces

transmural dispersion of repolarization and suppresses the development of Torsade de Pointes equally in the LQT1, LQT2 and LQT3 models [21,35]. This effect of mexiletine to reduce TDR in all three models is attributable to the intrinsically larger late INa in M cells than in epicardial or endocardial cells [36]. Mexiletine blocks late INa, abbreviates the QT interval in LQT3 patients more effectively than in LQT2 patients [37]. This data suggest that mexiletine may be used as first line therapy in LQT3 patients. However, because of a lack of prospective clinical trials mainly due to small number of LQT3 patients, mexiletine should be used at the moment in the presence of β-blockers or under the backup of an implantable cardioverter-defibrillator even in LQT3 patients [38].

3. Potassium and Spironolactone

In HERG genotypes of inherited LQT patients (LQTS 2), the increasing serum potassium levels by potassium loading may be a benefit therapeutic [39-41]. Impaired IKr function could be improved by exogenously administered potassium, resulting in increased outward potassium current and shortening of repolarization. An increase in serum potassium corrected abnormalities of repolarization duration, T-wave morphology, QT-RR slope, and QT dispersion in patients with HERG genotype of LQT [40]. Although raising serum potassium by increased potassium intake and potassium-sparing drugs reverses the ECG abnormalities in HERG genotype of LQT, a long lasting rise of serum potassium is only partially achievable because in the presence of normal renal function, potassium homeostasis limits the amount of serum potassium increase [41]. Etheridge et al [42] demonstrated that a sustainable, mild increase in serum K+ can be safely maintained by oral potassium supplementation and spironolactone. The increase in serum K+ was associated with a significant reduction in QTc and QT dispersion in all subjects, as well as normalization of the T-wave morphology in one-half of the subjects. A dramatic decrease in QTc with elevated serum K+ was observed in three individuals. The improvement in repolarization parameters achieved in this study suggests that oral KCl and spironolactone may be effective adjunctive therapy, together with β-blockers, for the treatment of LQTS. It is unlikely that the improvement in repolarization parameters was due to a direct effect of spironolactone, given that spironolactone derivatives prolong the action potential duration in isolated cardiac preparations [43]. Further studies are warranted to determine whether this will reduce the incidence of life-threatening events in LQTS patients.

4. Calcium Channel Blockers

Early afterdepolarizations have been suggested to play a significant role in QT prolongation and ventricular arrhythmias in congenital long QT syndrome. Calcium channel blocking agents (e.g., verapamil) have been reported [44-47] to be effective in the suppression of early afterdepolarizations and ventricular arrhythmias in some patients with the congenital long QT syndrome. Shimizu et al [48] used monophasic action potentials to investigate the effects of verapamil and propranolol on epinephrine induced repolarization

abnormalities in congenital long QT syndrome. This study indicates that both verapamil and propranolol were effective in suppressing early afterdepolarizations and epinephrine-induced ventricular arrhythmias, in shortening the 90% monophasic action potential duration and the QT interval and in decreasing the dispersion of 90% monophasic action potential duration. *A* prospective study with oral verapamil is needed to confirm *these findings.*

Conclusions

Pharmacological therapy, especially b blockers, holds a very important place in the treatment of long QT syndrome in children. However, there is a lack of studies comparing the efficacy between b blockers due to the poor prevalence of this syndrome. Choice of a B blocker will depend on the availability of the drug, its tolerance by the patient and the physician's own practice.

References

[1] Splawski, I; Shen, J; Timothy, KW; Lehmann, MH; Priori, S; Robinson, JL; Moss, AJ; Schwartz, PJ; Towbin, JA; Vincent, GM; Keating, MT. Spectrum of mutations in long-QT syndrome genes KVLQT1, HERG, SCN5A, KCNE1, and KCNE2. *Circulation*. 2000, 102, 1178 –1185.

[2] Schwartz, PJ. Management of long QT syndrome. Nat Clin Pract Cardiovasc Med 2005, 2(7), 346–351.

[3] Vincent, GM. The long QT and Brugada syndromes: causes of unexpected syncope and sudden cardiac death in children and young adults. *Semin Pediatr Neurol*. 2005, 1, 15–24.

[4] Goldenberg, I; Moss, AJ; Peterson, DR; McNitt, S; Zareba, W; Andrews, ML; Robinson, JL; Locati, EH; Ackerman, MJ; Benhorin, J; Kaufman, ES; Napolitano, C; Priori, SG; Qi, M; Schwartz, PJ; Towbin, JA; Vincent, GM; Zhang, L. Risk factors for aborted cardiac arrest and sudden cardiac death in children with the congenital long-QT syndrome. *Circulation*, 2008, 29, 117(17), 2184-2191.

[5] Eicken, A; Kolb, C; Lange, S; et al. Implantable cardioverter defibrillator (ICD) in children. *Int J Cardiol*, Feb 8 2006, 107(1), 30–5.

[6] Wang, Q; Shen, J; Splawski, I; Atkinson, D; Li, Z, Robinson, JL; Moss, AJ; Towbin, JA; Keating, MT. SCN5A mutations associated with an inherited cardiac arrhythmia, long QT syndrome. *Cell*, 1995, 80, 805-811.

[7] Curran, ME; Splawski, I; Timothy, KW; Vincent, GM; Green, ED; Keating, MT. A molecular basis for cardiac arrhythmia: HERG mutations cause long QT syndrome. *Cell*, 1995, 80, 795-803.

[8] Wang, Q; Curran, ME; Splawski, I; Burn, TC; Millholland, JM; VanRaay, TJ; Shen, J; Timothy, KW; Vincent, GM; De Jager, T; Schwartz, PJ; Towbin, JA; Moss, AJ; Atkinson, DL; Landes, GM; Connors, TD; Keating, MT. Positional cloning of a novel

potassium channel gene: KVLQT1 mutations cause cardiac arrhythmias. *Nat Genet* 1996, 12, 17-23.

[9] Crotti, L; Celano, G; Dagradi, F; Schwartz, PJ. Congenital long QT syndrome. *Orphanet J Rare Dis.*, 2008, 7, 3, 18.

[10] Zhang, L; Timothy, KW; Vincent, GM; et al. Spectrum of ST-T-wave patterns and repolarization parameters in congenital long-QT syndrome: ECG findings identify genotypes. *Circulation*, 2000, 102, 2849–2855.

[11] Zareba, W; Moss, AJ; Schwartz, PJ; et al., the International Long-QT Syndrome Registry Research Group. Influence of genotype on the clinical course of the long-QT syndrome. *N Engl J Med*., 1998, 339, 960–965.

[12] Schwartz, PJ; Priori, SG; Spazzolini, C; et al. Genotype-phenotype correlation in the long-QT syndrome: gene-specific triggers for lifethreatening arrhythmias. *Circulation*, 2001, 103, 89–95.

[13] Hobbs, JB; Peterson, DR; Moss, AJ; et al. Risk of aborted cardiac arrest or sudden cardiac death during adolescence in the long-QT syndrome. *JAMA*, 2006, 296, 1249 – 1254.

[14] Sauer, AJ; Moss, AJ; McNitt, S; et al. Long QT syndrome in adults. *J Am Coll Cardiol*, 2007, 49, 329 –337.

[15] Moss, AJ; Zareba, W; Hall, WJ; et al. Effectiveness and limitations of β-blocker therapy in congenital long-QT syndrome. *Circulation*, 2000, 101, 616 –623.

[16] Priori, SG; Napolitano, C; Schwartz, PJ; et al. Association of long QT syndrome loci and cardiac events among patients treated with βblockers. *JAMA*, 2004, 292, 1341–1344.

[17] Thomas, G; Killeen, MJ; Grac, AA; Huang, CLH. Pharmacological separation of early after depolarizations from arrhythmogenic substrate in DKPQ SCN5A murine hearts modelling human long QT 3 syndrome. *Acta Physiol*, 2008, 192, 505–517.

[18] Farhangi, V; Sansone, RA. QTc prolongation due to propranolol overdose. *Int. J. Psychiatry Med.*, 2003, 33, 201–202.

[19] Kawakami, K; Nagatomo, T; Abe, H; Kikuchi, K; Takemasa, H; Anson, BD;. Delisle, BP; January, CT; Nakashima, Y. Comparison of HERG channel blocking effects of various b-blockers – implication for clinical strategy. *British Journal of Pharmacology*, 2006, 147, 642–652.

[20] Yao, X; Mcintyre, MS; Lang, DG; Song, IH; Becherer, JD; & Hashim, MA. Propranolol inhibits the human ethera- go-go-related gene potassium channels. *Eur. J. Pharmacol*, 2005, 519, 208–211.

[21] Linker, NJ; Colonna, P; Kekwick, CA; Till, J; Camm, AJ; & Ward, DE. Assessment of QT dispersion in symptomatic patients with congenital long QT syndromes. *Am. J. Cardiol.*, .1992, 69, 634–638.

[22] Shimizu, W; Antzelevitch, C. Cellular basis for the electrocardiographic features of the LQT1 form of the long QT syndrome: Effects of β-adrenergic agonists, antagonists and sodium channel blockers on transmural dispersion of repolarization and torsade de pointes. *Circulation*, 1998, 98, 2314–2322.

[23] Shimizu, W; Tanabe, Y; Aiba, T; Inagaki, M; Kurita, T; Suyama, K; Nagaya, N; Taguchi, A; Aihara, N; Sungawa, K; Nakamura, K; Ohe, T; Towbin, JA; Priori, SG; &

Kamakura, S. Differential effects of β-blockade on dispersion of repolarization in the absence and presence of sympathetic stimulation between the LQT1 and LQT2 forms of congenital long QT syndrome. *J. Am. Coll. Cardiol,* 2002, 39, 1984–1991.

[24] Kawakami, K; Nagatomo, T; Abe, H; Kikuchi, K; Takemasa, H;. Anson, BD; Delisle, BP; January, CT & Nakashima Y. Comparison of HERG channel blocking effects of various b-blockers – implication for clinical strategy. *British Journal of Pharmacology*, 2006, 147, 642–652

[25] Cheng, J; Niwa, R; Kamiya, K; Toyama, J; Kodama, I. Carvedilol blocks the repolarizing K+ currents and the L-type Ca2+ current in rabbit ventricular myocytes. *Eur. J. Pharmacol,* 1999, 376, 189–201.

[26] Karle, CA; Kreye, VA; Thomas, D; Rockl, K; Kathofer, S, Zhang, W; & Kiehn, J. Antiarrhythmic drug Carvedilol inhibits HERG potassium channels. *Cardiovasc. Res.*, 2001, 49, 361–370.

[27] Poole-Wilson, PA; Swedberg, K; Cleland, JG; Di Lanarda, A; Hanrath, P; Komajda, M; Lubsen, J; Lutiger, B; Metra, M; Remme, WJ; Torp-Pedersen, C; Scherhag, A; Skene, A. Comparison of carvedilol and metoprolol on clinical outcomes in patients with chronic heart failure in the Carvedilol Or Metoprolol European Trial (COMET): randomised controlled trial. *Lancet*. 2003, 362, 7–13.

[28] Remme, WJ; Cleland, JG; Di Lenarda, A; Hnarath, P; Lutiger, B; Komajda, M; Metra, M; Scherhag, A; Charlesworth, A; & Torp-Pedersen, C. Carvedilol better protects against vascular events than metoprolol in heart failure: results from COMET. *J. Am. Coll. Cardiol.*, 2004, 43, A205–A206.

[29] Moss, AJ; Windle, JR; Hall, WJ; Zareba, W; Robinson, JL; et al. Safety and efficacy of flecainide in subjects with Long QT-3 syndrome (DeltaKPQ mutation): a randomized, double-blind, placebo-controlled clinical trial. *Ann Noninvasive Electrocardiol,* 2005, 10, 59–66.

[30] Kass, RS; Moss, AJ. Mutation-specific pharmacology of the long QT syndrome. *Handb Exp Pharmacol,* 2006, 287–304.

[31] Hallman, K; Carlsson, L. Prevention of class III induced proarrhythmias by flecainide in an animal model of the acquired long QT syndrome. *Pharmacol Toxicol,* 1995, 77, 250– 254.

[32] Benhorin, J; Taub, R; Goldmit, M; Kerem, B; Kass, RS; Windman, I; et al. Effects of flecainide in patients with new SCN5A mutation: mutation-specific therapy for long-QT syndrome? *Circulation*, 2000, 101, 1698–1706.

[33] Windle, JR; Geletka, RC; Moss, AJ; Zareba, W; Atkins, DL. Normalization of ventricular repolarization with flecainide in long QT syndrome patients with SCN5A: DeltaKPQ mutation. *Ann Noninvasive Electrocardiol,* 2001, 6, 153–158.

[34] Priori, SG; Napolitano, C; Schwartz, PJ; Bloise, R; Crotti, L; Ronchetti, E. The elusive link between LQT3 and Brugada syndrome: the role of flecainide challenge. *Circulation*, 2000, 102, 945–947.

[35] Satoh, Y; Sugiyama, A; Tamura, K; Hashimoto, K. Effects of mexiletine on the canine cardiovascular system complicating cisapride overdose: potential utility of mexiletine for the treatment of drug-induced long QT syndrome. *Jpn J Pharmacol*, 2000, 83, 327– 334.

[36] Shimizu, W; Antzelevitch, C. Sodium channel block with mexiletine is effective in reducing dispersion of repolarization and preventing torsade de pointes in LQT2 and LQT3 models of the long-QT syndrome. *Circulation*, 1997, 96, 2038–2047.

[37] Zygmunt, AC; Eddlestone, GT; Thomas, GP; Nesterenko, VV; Antzelevitch, C. Larger late sodium conductance in M cells contributes to electrical heterogeneity in canine ventricle. *Am J Physiol Heart Circ Physiol*, 2001, 281, 689–697.

[38] Schwartz, PJ; Priori, SG; Locati, EH; Napolitano, C; Cantu, F; Towbin, JA; et al. Long QT syndrome patients with mutations of the SCN5A and HERG genes have differential responses to Na+ channel blockade and to increases in heart rate: Implications for gene-specific therapy. *Circulation*, 1995, 92, 3381–3386.

[39] Shimlizul, W; Aiba, T; Antzelevitch, C. Specific Therapy Based on the Genotype and Cellular Mechanism in Inherited Cardiac Arrhythmias. Long QT Syndrome and Brugada Syndrome. *Curr Pharm Des*, 2005, 11, 1561–1572.

[40] Khan, IA. Long-QT syndrome: diagnosis and management. *Am Heart J*, 2002, 143, 7 – 14.

[41] Compton, SJ; Lux, RL; Ramsey, MR; et al. Genetically defined therapy of inherited long QT syndrome. Correction of abnormal repolarization by potassium. *Circulation*, 1996, 94, 1018– 1022.

[42] Tan, HL; Alings, M; Van Olden, RW; Wilde, AA. Long-term (subacute) potassium treatment in congenital HERG-related long QT syndrome (LQTS2). *J Cardiovasc Electrophysiol*, 1999, 10, 229– 233.

[43] Etheridge, SP; Compton, SJ; Tristani-Firouzi, M; Mason, JW. A New Oral Therapy for Long QT Syndrome Long-Term Oral Potassium Improves Repolarization in Patients With *HERG* Mutations. *J Am Coll Cardiol* , 2003, 42, 1777-1782

[44] Coraboeuf, E; Deroubaix, E. Effect of a spirolactone derivative, sodium canrenoate, on mechanical and electrical activities of isolated rat myocardium. *J Pharmacol Exp Ther*, 1974, 191, 128–138.

[45] Jackman, WM; Szabo, B; Friday, KJ; et aL Ventricular tachyarrhythmias related to early afterdepolarizations and triggered firing: relationship to QT interval prolongation and potential therapeutic role for calcium channel blocking agents. *J Cardiovasc Electrophysiol*, 1990, 1, 170-195.

[46] Shimizu, W; Ohe, T; Kurita, T; Tokuda, T; Shimomura, K. Epinephrineinduced ventricular premature complexes due to early aflerdepolarizations and effects of verapamil and propranolol in a patient with congenital long QT syndrome. *J Cardiovasc Electrophysiol*, 1994, 5, 438-444.

[47] Krause, PC; Rardon, DP; Miles, WM; et al. Characteristics of Ca^{2+} -activated K^{+} channels isolated from the left ventricle of a patient with idiopathic long QT syndrome. *Am Heart J.*, 1993, 126, 1134-1141.

[48] Malfatto, G; Rosen, MR; Foresti, A; Schwartz, PJ. Idiopathic long QT syndrome exacerbated by β-adrenergic blockade and responsive to left cardiac sympathetic denervation: implications regarding electrophysiologic substrate and adrenergic modulation. *J Cardiovase Electrophysiot*, 1992, 3, 295-305.

[49] Shimizu, W; Ohe, T; Kurita, T; Kawade, M; Arakaki, Y; Aihara, N; Kamakura, S; Kamiya, T; Shimomura, K. Effects of Verapamil and Propranoloi on Early

Afterdepolarizations and Ventricular Arrhythmias Induced by Epinephrine in Congenital Long QT Syndrome. *J Am Coll Cardiol, 1995, 26, 1299-1309.*

In: Horizons in World Cardiovascular Research. Volume 1 ISBN: 978-1-60876-447-1
Editor: Eleanor H. Bennington, pp. 129-136

Chapter VIII

The Promise of Biological Pacemakers

Alistair Lindsay
Dept. of Cardiology, Harefield Hospital, England

Introduction

In modern day cardiology practice the insertion of electrical pacemaker devices is routine, with an estimated 434 devices being inserted per million people in the United States each year. Although the development of modern pacing devices revolutionised cardiology towards the end of the 20th century, electrical devices remain a palliation, rather than a cure, to an underlying disorder of cardiac rhythm. Thus in recent years the idea of a "biological" pacemaker, whereby artificial electrical components are replaced by cellular and genetic elements capable of producing intrinsic electrical activity, has taken several steps towards becoming a realistic therapeutic goal.

What advantages would such a development have over an already well-established method of treatment? Biological systems offer the promise of being more sensitive to the body's autonomic nervous system, thus providing a more natural control of physiological heart rate compared to current rate sensing pacemakers. Implantation of biological systems into the correct anatomical location would also allow electrical conduction to mimic the heart's intrinsic conduction system, such as the bundle of His, as closely as possible. Thirdly, many of downfalls of electrical pacemaker insertion, such as infection, battery replacement, and the induction of cardiac failure, could be reduced significantly, if not eliminated. For paediatric patients in particular, who face a lifetime of device changes, a biological pacemaker could prove to be a very effective cure.

What properties should a biological pacemaker have? Two main caveats would include; 1) the ability to initiate a cardiac impulse proximal enough in the conducting system to allow physiological depolarisation of the ventricles and 2) to have the ability to last as long as and be as reliable as current electrical pacemaker devices(Plotnikov, Sosunov et al. 2004).

Several different molecular approaches have been successfully shown to initiate spontaneous electrical activity in mammalian hearts, thus raising the initial question as to

which method is the best. Of course, developing a suitable molecular pacing strategy will in part mean developing a suitable method of delivery. A final hurdle involves examining the efficacy, reliability and safety of the new technique. This article will review all the above areas with particular emphasis being made on outlining future challenges to be faced before this ambitious therapy can become a reality.

Background & Preliminary Work

Several different methods of developing an intrinsic pacing system at the molecular level have been attempted to date.

An initial approach to molecular manipulation of the pacing system of the heart was performed by Edelberg in 2001(Edelberg, Huang et al. 2001). By injecting plasmids encoding a beta-2 adrenergic receptor into the atria of pigs (at the site of earliest atrial potential found) faster mean heart rates were demonstrated in the days following plasmid than occurred in control animals. Unfortunately, such an increase in beta-adrenergic receptors also makes the heart more prone to arrhythmias(Rosen, Brink et al. 2004).

In 2002 Miake and colleagues demonstrated an alternative method of biological manipulation of the pacing system(Miake, Marban et al. 2002). Building on the fact that all cardiac cells possess pacemaker activity in the early embryonic heart, quiescent heart muscle cells were altered by adenoviral gene transfer of a dominant-negative form of Kir2. This gene family codes for an inward-rectifier potassium current (I_{K1}) that normally hyperpolarises the cell membrane of ventricular myocytes and suppresses spontaneous electrical activity. Their simple paradigm proved true; by inhibiting the I_{K1} current spontaneous electrical activity was produced. However, as is common with all potassium channel modifications, this also resulted in a prolonged action potential which can increase the potential for arrhythmias.

More recent reports have aimed at altering the inward pacemaker current I_f, which flows only at diastolic potentials and thus should not affect the duration of the action-potential(Qu, Plotnikov et al. 2003). This can be done by overexpressing the HCN gene (hyperpolarization-activated cyclic nucleotide-gated channel), which allows inward sodium current and thus membrane depolarisation. By injecting adenoviral constructs containing the HCN2 gene, Rosen's group was able to establish an I_f-based pacemaker in the atria of dogs(Qu, Plotnikov et al. 2003). This method has since been explored by other groups in more recent reports due to its improved safety profile (Kashiwakura, Cho et al. 2006; Tse, Xue et al. 2006).

Mesenchymal or stem cells with electrical activity have also been successfully transferred and shown to have spontaneous electrical activity in vivo(Xue, Cho et al. 2005). Xue et al. used a lentivirus vector to transfect human embryonic stem cells, which were then injected subepicardially into the left ventricular wall of guinea pig hearts. The integrated syncitium was responsive to the beta-adrenergic agonist isoproterenol, and optical mapping confirmed successful depolarisation from the site of myocardial injection. Rosen's group have also loaded adult human mesenchymal stem cells with the HCN2 gene via electroporation, avoiding the need for viral vectors(Potapova, Plotnikov et al. 2004).

An alternative strategy could involve the use of fetal and/or neonatal cell transplants (Cai, Lin et al. 2006), or the use of human embryonic stem cells forced into a cardiogenic

lineage. When injected into the myocardium of pigs with heart block, the cells have been shown to create an adequate pacemaker current and produce stable idioventricular rhythms(Kehat, Khimovich et al. 2004).

Once an optimal biological strategy has been formulated, cells must be delivered to the appropriate area. Naked DNA has been successfully transfected into the human heart, but it is technically difficult, inefficient and the effects are often very short lived. While more efficient, viral vectors also have problems in that they may cause allergic reactions. Furthermore, persistent viruses such as retrovirus may be complicated by the possibility of malignancy, while the safer adenovirus is less permanent. A third option involves the direct introduction of cells, either embryonic stem cells or human mesenchymal stem cells (hMSCs) which are derived from bone marrow. In fact, technically any cell type which expresses the HCN genes and cardiac connexin genes could serve as a cellular delivery system.

Of course a good delivery system must be accurate, and it is yet to be seen where in the intrinsic conducting system any cell therapy is best placed. Exactly how this is best achieved also remains to be seen; focal delivery with catheters and needles may be needed, or cells could be cultured on a matrix designed to adhere to cardiomyocytes. Most importantly, it will be necessary to prove that any implanted cells remain where they are inserted, and do not migrate to other areas of the heart, or indeed the body, where they may cause harm.

Finally, it is possible that implanted cells may be rejected, and that some form of immunosupression may become necessary. This leads to further obvious concerns about neoplastic transformation.

Future Challenges

In addition to the issues raised above, two main challenges emerge for the future: safety and cost. Introduction of any new electrical system into the heart could in theory precipitate arrhythmia, and the absence of any malignant arrhythmia will be a necessary precursor for any biological pacemaker. Furthermore, viral vectors have the ability to trigger neoplasia, and must only localise to the areas targeted if they are to be used comfortably.

When a reliable, accurate and efficient biological pacing system is formulated, the next step will be to test its efficiency in small animal models, before finally moving on to human clinical trials. In both these cases, initial introduction is likely to be in combination with traditional electrical systems, thus allowing a backup mechanism in the event of failure of the biological system.

Would a biological pacemaker be cost effective? It is far too early to answer this question. The field of gene therapy itself faces many challenges over the coming years; the development of a biological pacemaker is but one of them.

References

Cai, J., Lin, G., et al. (2006). "Transplanted neonatal cardiomyocytes as a potential biological pacemaker in pigs with complete atrioventricular block." *Transplantation, 81(7),* 1022-6.

Edelberg, J. M., Huang, D. T., et al. (2001). "Molecular enhancement of porcine cardiac chronotropy." *Heart, 86(5),* 559-62.

Kashiwakura, Y., H. Cho, C., et al. (2006). "Gene transfer of a synthetic pacemaker channel into the heart: a novel strategy for biological pacing." *Circulation, 114(16),* 1682-6.

Kehat, I., Khimovich, L., et al. (2004). "Electromechanical integration of cardiomyocytes derived from human embryonic stem cells." *Nat Biotechnol, 22(10),* 1282-9.

Miake, J., Marban, E., et al. (2002). "Biological pacemaker created by gene transfer." *Nature, 419(6903),* 132-3.

Plotnikov, A. N., Sosunov, E. A., et al. (2004). "Biological pacemaker implanted in canine left bundle branch provides ventricular escape rhythms that have physiologically acceptable rates." *Circulation, 109(4),* 506-12.

Potapova, I., Plotnikov, A., et al. (2004). "Human mesenchymal stem cells as a gene delivery system to create cardiac pacemakers." *Circ Res., 94(7),* 952-9.

Qu, J., Plotnikov, A. N., et al. (2003). "Expression and function of a biological pacemaker in canine heart." *Circulation, 107(8),* 1106-9.

Rosen, M. R., Brink, P. R., et al. (2004). "Genes, stem cells and biological pacemakers." *Cardiovasc Res., 64(1),* 12-23.

Tse, H. F., Xue, T., et al. (2006). "Bioartificial sinus node constructed via in vivo gene transfer of an engineered pacemaker HCN Channel reduces the dependence on electronic pacemaker in a sick-sinus syndrome model." *Circulation, 114(10),* 1000-11.

Xue, T., Cho, H. C., et al. (2005). "Functional integration of electrically active cardiac derivatives from genetically engineered human embryonic stem cells with quiescent recipient ventricular cardiomyocytes: insights into the development of cell-based pacemakers." Circulation, *111(1),* 11-20.

.

In: Horizons in World Cardiovascular Research. Volume 1 ISBN: 978-1-60876-447-1
Editor: Eleanor H. Bennington, pp. 133-138

Chapter IX

Stem Cells and Repair of the Heart-Current Limitations and Future Perspectives of Cell-Releasing Epicardial Scaffolds

Vizzardi Enrico, Lorusso Roberto*, De Cicco Giuseppe*, Zanini Gregoriana, Faggiano Pompilio and Dei Cas Livio
Department of Cardiology, University of Brescia, Italy
*Experimental Cardiac Surgery Laboratory, Cardiac Surgery Unit, Civic Hospital,Brescia

Chronic heart failure(CHF) has emerged as a major worldwide epidemic. Recently, a fundamental shift in the underlying etiology of CHF is becoming evident, in which the most common cause is no longer hypertension or valvular disease, but rather long-term survival after acute myocardial infarction (AMI)[1,2].

The costs of this syndrome, both in economic and personal terms, are considerable [3]. American Heart Association statistics indicate that CHF affects 4.7 million patients in the United States and is responsible for approximately one million hospitalizations and 300,000 deaths annually.

The total annual costs associated with this disorder have been estimated to exceed $22 billion. The societal impact of CHF is also remarkable. Patients with CHF often suffer a greatly compromised quality of life. About 30% of diagnosed individuals (i.e.,1.5 million in U.S.) experience difficulty breathing with little or no physical exertion, and are very restricted in their daily functions. This forced sedentary lifestyle inevitably leads to further physical and mental distress.

The CHF problem is growing worse. While CHF already represents one of our greatest health care problems, it is expected to become even more severe in the future. By 2010, the number of patients suffering from HF will have grown to nearly 7 million, a more than 40% increase.

Coronary artery disease (CAD) is the cause of CHF in the majority of patients, and CHF is the only mode of CAD presentation associated with increasing incidence and mortality.

However, it is evident, running through the different therapeutical strategies of CHF, that the appropriate treatment of patients with ischemic heart failure is still unknown [4,5].

After myocardial infarction, injured cardiomyocytes are replaced by fibrotic tissue promoting the development of heart failure. Cell transplantation has emerged as a potential therapy and stem cells may be an important and powerful cellular source.

Cell transplantation represents the last frontier within the treatment of cardiac diseases. Cell transplantation is currently generating a great deal of interest since the replacement of akinetic scar tissue by viable myocardium should improve cardiac function, impede progressive LV remodelling, and revascularize ischemic area. The goals of cell therapy are multiple and non exclusive, leading to the formation of a new tissue.

One should expect to replace a scar tissue by living cells and/or to block or reverse the remodelling process or change its nature and/or to restore the contractility of the cardiac tissue and/or to induce neoangiogenesis that would favour the recruitment of hibernating cardiomyocytes or to enhance transplanted cell engraftment, survival, function, and, ultimately, synergistic interaction with resident cells.

From the first paper published in 1992 that has documented the potentials of the transplantation of autologous skeletal muscle to treat the damage induced by acute myocardial infarction [5], innumerable techniques, types of cells, myocardial pathologies, and techniques of implantation have been reported, greatly expanding this innovative and appealing field of search in cardiovascular medicine.

Different stem cell populations have been intensively studied in the last decade as a potential source of new cardiomyocytes to ameliorate the injured myocardium, compensate for the loss of ventricular mass and contractility and eventually restore cardiac function. An array of cell types has been explored in this respect, including skeletal muscle, bone marrow derived stem cells, embryonic stem cells (ESC) and more recently cardiac progenitor cells. The best-studied cell types are mouse and human ESC cells, which have undisputedly been demonstrated to differentiate into cardiomyocyte and vascular lineages and have been of great help to understand the differentiation process of pluripotent cells. However, due to their immunogenicity, risk of tumor development and the ethical challenge arising from their embryonic origin, they do not provide a suitable cell source for a regenerative therapy approach.

Embryonic stem cells can differentiate into true cardiomyocytes, making them in principle an unlimited source of transplantable cells for cardiac repair, although immunological and ethical constraints exist. Somatic stem cells are an attractive option to explore for transplantation as they are autologous, but their differentiation potential is more restricted than embryonic stem cells. Currently, the major sources of somatic cells used for basic research and in clinical trials originate from the bone marrow. The differentiation capacity of different populations of bone marrow-derived stem cells into cardiomyocytes has been studied intensively. Only mesenchymal stem cells seem to form cardiomyocytes, and only a small percentage of this population will do so in vitro or in vivo. A newly identified cell population isolated from cardiac tissue, called cardiac progenitor cells, holds great potential for cardiac regeneration.

New approaches for cardiac repair have been enabled by the discovery that the heart contains its own reservoir of stem cells. These cells are positive for various stem/progenitor cell markers, are self-renewing, and exhibit multilineage differentiation potential. Recently has been developed a method for ex vivo expansion of cardiac-derived stem cells from human myocardial biopsies with a view to subsequent autologous transplantation for myocardial regeneration.

Despite original promises and expectations, current evidences of stem cell transplantation are still weak and controversial. The use of trypsin to detach the cells from the culture dish disrupts their microintercellular communication and extracellular matrix, restricts cell survival and growth, and thus appears deleterious to cell transplantation theraphy. Intercellular communication factors play a key role in cell adhesion, migration, proliferation, differentiation, and death and must be maintained for optimal cellular benefits. Therefore, alternative line of research are being explored, particularly in the field of techniques of cell implantation and engraftment.

Besides direct implantation or myocardial colonization by bone marrow stimulation, epicardial application of cell-delivering systems (scaffold and patches) have gained popularity due to the possibility to apply selectively a cell-containing device which may gradually release the chosen cell type, alone or in combination with trophic substances.

The scaffolds have proven to be successful in this respect and may represent a valid alternative to coronary, intra-myocardial, or venous injection of stem cells, or to stem cell stimulating factors.

Several materials have been assessed for generate scaffold.Li and associates produced 3-D contractile cardiac grafts using gelatin sponges and synthetic biodegradable polymers [6]. Leor and colleagues reported the formation of bioengineered cardiac grafts with 3-D alginate scaffolds [7] Eschenhagen and coworkers engineered 3-D heart tissue by gelling a mixture of cardiomyocytes and collagen [8]. Robinson et al experimented urinary bladder matrix (UBM) and demonstrated UBM superiority to synthetic material for cardiac patching and trends toward myocardial replacement at 3 months [9].

Biological patches may, moreover, show enormous advantages, particularly in congenital diseases, where the existence of a growing tissue might reduce or limit the postoperative complications linked to not-growing material, ultimately leading to stenosis or patient/material mismatch with the need of replacement with all the risks related to redo surgery.

The engineered heart tissue survived and matured after implantation on uninjured hearts. Shimizu and colleagues have developed a novel approach of culturing cell sheets without scaffolds using a temperature-responsive polymer [10]. Several cell sheets were layered on top of each other to create thicker grafts. Ishii et al as an alternative approach, developed an in vitro system for creating sheets of cardiomyocytes on a mesh consisting of ultrafine fibers. This device consists of a thin, highly porous, nonwoven fibrous mesh stretched across a wire ring. This novel scaffold can be fabricated in specific shapes and is easy to handle. However, thicker grafts are required to obtain sufficient function. It is hypothesized that a clinically relevant cardiac graft will require a vasculature to provide sufficient perfusion of oxygenated blood. As an intermediate step toward a thick, vascularized cardiac graft, it is important to assess the ability to increasing the thickness without a vasculature and determine the maximum thickness

before core ischemia is observed in the graft. So isessential the development of a multilayer system as an intermediate step toward functional cardiac grafts.

Kochupura et al matured a novel finding that a tissue-engineered myocardialpatch (TEMP) derived from extra cellular matrix (ECM) contributes to regional function 8 weeks after implantation in the canine heart [11]. In addition, they confirmed cardiomyocyte population of ECM. The etiology of these cells has been under investigation, with possible explanations including the deposition of circulating bone marrow-derived progenitor cells and the fusion of cardiac progenitor cells with host cells.

The regional mechanical benefit with ECM patch report an active contraction of the ECM and not passive elastic recoil. This contraction is also in synchrony with native myocardium. Microscopic evaluation of Dacron patches did not demonstrate the presence of cardiomyocytes nor do the mechanical data indicate that Dacron implantation contributes to regional function. Increasing the number of ECM layers could be an alternative, but it is unclear if the physiological benefit, ie, cardiomyocyte population, would still be evident. Grossly, Dacron elicited far greater fibrosis than ECM, correlating with more mediastinal adhesions and epicardial connective tissue deposition. On placement, the Dacron patch was clearly under tension. In sharp contrast, ECM triggered far less fibrosis. The patch was neither wrinkled nor aneurismal and appeared to share the same surface tension as adjacent native myocardium. Finally, after removal of adhesions, it was difficult to grossly distinguish ECM from native myocardium The quantitative and qualitative differences between ECM and Dacron could be explained by an inherent ability of ECM to house cellular elements that facilitate remodeling.

The modulus of elasticity of Dacron is at least 4 orders of magnitude greater than healthy myocardium, ie, Dacron is stiffer than myocardium. Thus, the use of Dacron as a myocardial patch may have a “tethering effect” that would reduce the mechanical function of surrounding myocardium. Furthermore, the cellular response to Dacron was primarily diffuse fibroblast proliferation, an observation also seen with remodeling after myocardial infarction. In contrast, ECM stimulated less fibrosis and was populated by different cell types, including cardiomyocytes.

Atkins et al have shown that the reduction of infarct stiffness via cell transplantation leads to increased diastolic function [12]. Similarly, Quarterman et al created a detailed finite element model to show that cell transplantation alone will result in changes in compliance that result in mechanical benefit [13]. The potential clinical applications of ECM as a scaffold are many and would have a powerful impact on the management of cardiac disease. These would include instances in which Dacron is presently used as a myocardial patch: repair of ventricular aneurysms, repair of congenital heart defects, and most recently, surgical restoration of a dyskinetic or akinetic ventricle. By its contribution to regional systolic function, ECM provides true restoration of the ventricle rather than nonfunctional substitution of defective tissue, as is the case with Dacron.

Limits and Perspectives

The use of scaffold for tissue engineering is supportive for myocardial regeneration but subject to biocompatibility, biodegradability, and cytotoxicity, including inflammatory response and surface adhesion molecule loss issues, and this limits its efficacy. Eliminating such disadvantages, is necessary to establish cell sheet engineering technology without using scaffolds. The engineered cell sheets from this technique showed preserved cellular communication junctions, endogenous extracellular matrix, and integrative adhesive agents. Nonligature implantation of these engineered neonatal cardiomyocyte sheets to infarcted myocardium showed their integration with impaired myocardium and improved cardiac performance. For clinical application, use of skeletal myoblasts averts ethical and cell source issues. Recent findings suggested that locally or transgenically delivered stromal-derived factor 1 (SDF-1) expression plays a role in mobilizing and recruiting stem cells with neovascularization [14]. Because SDF-1 is secreted in skeletal muscle tissue, grafted myoblasts might beneficially attract hematopoietic stem cells (HSCs) to home in the infarct heart area for heart regeneration and angiogenesis [15].

The use of engineered patches, therefore, represents an appealing frontier which, in several formats, may provide material and solutions for some complex and inoperable disease. These devices, appropriately designed, may also allow the release of any kind of compounds and material, from cells to drugs, from factor to solution, ad programmed speed, ranging from transient and quick release (high biodegradability) to slow release (low degradability, several months). Last, but not least, the material chosen for realising such a device may also represent a containing structure, variably ranging from pure passive to slightly active action, which may play a role in the mechanical effect on cardiac dilatation in the case of heart containment procedure.

Finally, some treatments, particularly drug-related, showed promising results, but the potential disadvantages of systemic administration hampered a clinical or wider application. The possibility to deliver a specifc agent only locally, with obvious reduction in systemic effects, might be appealing and allow higher and focused concentration only to the target organ, that is the heart, or area of the heart.

References

[1] Ansari, M; Massie, BM. Heart failure : how big is the problem ? Who are the patients ? What does the future hold ? *Am Heart J*, 2003, 146, 1-4.

[2] Berry, C; Murdoch, DR; McMurray, JJ. Economics of chronic heart failure. *Eur J Heart Fail.*, 2001, 3, 283-91.

[3] Doenst, T; Velazquez, EJ; Beyerdorf, F; Michler, R; Menicanti, L; Di Donato Gradinac, S; Sun, B; Rao, V. (STITCH Investigators). To STITCH or not to STITCH : we know the answer, but do we understand the question ? *J Thorac Cardiovasc Surg*, 2005, 129, 246-9.

[4] Buckberg, GD. Early and late results of left ventricular reconstruction in thin-walled chambers : is this our patient population ? *J Thorac Cardiovasc Surg*, 2004, 128, 21-6.

[5] Marelli, D; Desrosiers, C; El-Alfy, M; Kao, RL; Chiu, RC. Cell transplantation for myocardial repair: an experimental approach. *Cell Transplant*, 1992, 1, 383-90.

[6] Li, RK; Jia, ZQ; Weisel, RD; Mickle, DA; Choi, A; Yau, TM. Survival and function of bioengineered cardiac grafts. *Circulation*, 1999, 100(suppl II), II63-9.

[7] Leor, J; Aboulafia-Etzion, S; Dar, A; Shapiro, L; Barbash, IM; Battler, A; et al. Bioengineered cardiac grafts: a new approach to repair the infarcted myocardium? *Circulation*, 2000, 102 (suppl 3), III56-61.

[8] Eschenhagen, T; Fink, C; Remmers, U; Scholz, H; Wattchow, J; Weil, J; et al. Three-dimensional reconstitution of embryonic cardiomyocytes in a collagen matrix: a new heart muscle model system. *FASEB J.*, 1997, 11, 683-94.

[9] Robinson, K; Li, J; Mathison, M; Redkar, A; Cui, J; Chronos, F; Matheny, RG; Badylak, S. Extracellular Matrix Scaffold for Cardiac Repair. *Circulation*, 2005, 112[suppl I], I-135–I-143.)

[10] Shimizu, T; Yamato, M; Isoi, Y; Akutsu, T; Setomaru, T; Abe, K; et al. Fabrication of pulsatile cardiac tissue grafts using a novel 3-dimensional cell sheet manipulation technique and temperature responsive cell culture surfaces. *Circ Res*., 2002, 90, e40.

[11] Kochupura, Azeloglu, E; Kelly, D; Doronin, S; Badylak, S, Krukenkamp, I; et al. Tissue-Engineered Myocardial Patch Derived FromExtracellular Matrix Provides Regional Mechanical Function. *Circulation*, 2005, 112[suppl I], I-144–I-149.

[12] Atkins, BZ; Hueman, MT; Meuchel, J; Hutcheson, KA; Glower, DD; Taylor, DA. Cellular cardiomyoplasty improves diastolic properties of injured heart. *J Surg Res.*, 1999, 85, 234 –242.

[13] Quarterman, RL; Moonly, S; Wallace, AW; Guccione, J; Ratcliffe, MB. A finite element model of left ventricular cellular transplantation in dilated cardiomyopathy. *ASAIO J*, 2002, 48, 508 –513.

[14] Askari, AT; Unzek, S; Penn, MS; et al. Effect of stromal-cell-derived factor 1 on stem-cell homing and tissue regeneration in ischemic cardiomyopathy. *Lancet*. 2003, 362, 697-703.

[15] Ratajczak, MZ; Peiper, S; Janowska, WA; et al. Expression of functional CXCR4 by muscle satellite cells and secretion of SDF-1 by musclederived fibroblasts is associated with the presence of both muscle progenitors in bone marrow and hematopoietic stem/progenitor cells in muscles. *Stem Cells*, 2003, 21, 363-71.

In: Horizons in World Cardiovascular Research. Volume 1 ISBN: 978-1-60876-447-1
Editor: Eleanor H. Bennington, pp. 139-175

Chapter X

Cardiovascular Abnormalities as a Consequence of Cerebral Hypoxia-Ischaemia

***Andrew N. Clarkson*[1,2,*]**

[1]Department of Neurology, David Geffen School of Medicine at UCLA, NRB, 635 Charles Young Drive South, Los Angeles, CA 90095, United States & [2]Department of Anatomy and Structural Biology, University of Otago, PO Box 913, Dunedin, New Zealand

Abstract

Cerebral hypoxia-ischaemia (HI) results in a multi-faceted complex cascade of events causing cell death and neurological dawmage to the central nervous system. Furthermore, cerebral ischaemia results in cardiovascular complications that can further confound the prognostic outcome of patients. This chapter addresses the cardiovascular changes that occur subsequent to an ischaemic insult, regulation of the insular cortex, changes in the autonomic nervous system and the role of various circulating cytokines (both pro-inflammatory and anti-inflammatory) and chemokines. In addition, markers of oxidative stress and cardiac enzyme release following an ischaemic insult are also discussed. Given that lack of treatment options available, the use of beta-blockers and pre- and post-conditioning paradigms as possible treatment option to prevent the occurrence of secondary cardiac abnormalities in addition to CNS injuries have also been addressed.

Keywords: autonomic nervous system, inflammation, oxidative stress, cardiac enzymes.

* Corresponding author: Dr Andrew N. Clarkson; Department of Anatomy and Structural Biology, University of Otago, PO Box 913, Dunedin, New Zealand; Tel: - 0064 3 479 7318; Fax: - 0064 3 479 7254; Email: - andrew.clarkson@stonebow.otago.ac.nz

Abbreviations

8-*epi*$PGF_{2\alpha}$	8-isoprostane;
ANS	autonomic nervous system;
APCs	antigen-presenting cells;
BP	blood pressure;
CBF	cerebral blood flow;
CF	coronary flow;
CK-MB	creatine-kinase-myocardial-band;
CMZ	clomethiazole;
CNS	central nervous system;
CRP	C-reactive protein;
CSF	cerebrospinal fluid;
CP	cerebral palsy;
ECG	electrocardiogram;
GABA	gamma-aminobutyric acid;
GM-CSF	granulocyte macrophage-colony stimulating factor;
GPx	glutathione peroxidase;
GTP	guanosine triphosphate;
HI	hypoxia-ischaemia;
HIE	hypoxia-ischaemia encephalopathy;
HMGB1	high mobility group box 1;
ICAM-1	intracellular adhesion molecule-1;
IL	interleukin;
iNOS	inducible nitric oxide synthase;
IR	ischaemic reperfusion;
LDH	lactate dehydrogenase;
LVDP	left ventricular developed pressure;
LPS	lipopolysaccharides;
MCAo	middle cerebral artery occlusion;
MCP	monocyte chemoattractant protein;
MOD	multi-organ dysfunction;
MMP	matrix metalloproteinase;
MI	myocardial infarction;
MIP	macrophage inflammatory protein;
NMDA	N-methyl-D-aspartic acid;
OGD	oxygen glucose deprivation;
PG	prostaglandin;
PMNs	polymorphonuclear neutrophils;
RANTES	regulated on activation normal T cell expressed and secreted;
ROS	reactive oxygen species;
SOD	superoxide dismutase;
TF	tissue factor;
TNF	tumour necrosis factor;
TNFR	tumour necrosis factor receptor;
TX	thromboxane

Cardiovascular Abnormalities Following Cerebral Complications

Cardiovascular abnormalities are a well-known predisposing factor for cerebral ischaemia. However, the associated effects of an ischaemic stroke on cardiac function are not as well recognised. The first findings of cardiovascular complications secondary to an ischaemic episode were reported over half a century ago. Since these reports, a relatively small body of literature has shown the deleterious effects of cerebral ischaemia on the cardiovascular system in both clinical and experimental settings.

Cerebral Ischaemia

During the process of acute cerebral ischaemia, cardiovascular abnormalities such as elevated blood pressure (BP), arrhythmias and ischaemic cardiac damage are evident and can hinder the final prognostic clinical outcome [131; 144; 146; 198]. Even though cardiovascular abnormalities are known to occur as a consequence of cerebral ischaemia, the underlying pathophysiological mechanisms have not been fully elucidated and characterised. Cardiovascular complications secondary to an ischaemic event were first observed by Byer *et al.*, and Burch *et al.*, who noted previously undiagnosed electrocardiogram (ECG) abnormalities such as upright T waves and prolonged Q-T intervals [20; 21]. Since this finding others have found clinically and experimentally arrhythmias, increased BP, increased plasma catecholamine levels, increased serum cardiac enzyme levels, decreased heart rate variability and increased rates of cardiovascular-related sudden death commonly associated with cerebral ischaemia [134; 137; 140; 141; 184; 215]. Myocardial dysfunction has been shown to occur during the acute stages of either ischaemic or haemorrhagic strokes, contributing to increased morbidity and mortality [73].

It has been estimated that as many as 27% of mortalities in stroke patients, occur as a result of cardiac failure where no prior history of cardiovascular complications has been reported [184]. In addition, 15-40% of stroke patients experience ECG changes following cerebral ischaemia [146]. Furthermore, Orlandi *et al.*, found that 76.2% of patients suffering from right hemispheric cerebral ischaemia developed arrhythmias within 24 hours of the initial insult [149]. Within 20 minutes of the initial insult, 75% of all stroke patients experience fluctuations in BP, with 30% still remaining hypertensive after 1 week [19; 215]. The development of arrhythmias, including paroxysmal supraventricular tachycardia, atrial flutter and ventricular fibrillation, are associated with an increased (1.5-3.0 fold) mortality in patients with cerebral ischaemia compared to those who do not develop any form of arrhythmia [173]. Assessment of heart rate dynamics revealed that long-term abnormalities can be used as a predictor of stroke-related mortality [122]. In addition, reduced baroreceptor reflex sensitivity and prolonged hypertension worsen the prognosis of stroke morbidity and mortality [44; 169]. Post-mortem analysis of otherwise healthy patients who perish from an ischaemic stroke, illustrates myocardial necrosis (myocytolysis), myofibrillar degeneration and subendocardial congestion [38]. Furthermore, cerebral ischaemia-induced myocardial damage is characterised pathologically by scattered foci of microlesions [68; 103]. Reducing

the incidence of cardiovascular complications subsequent to cerebral ischaemia would clearly result in better prognosis and a long-term improvement in the quality of life.

Hypoxia-Ischaemia

In addition to neuronal damage as a consequence of hypoxia-ischaemia (HI), multi-organ dysfunction (MOD) has also been documented [81; 180]. However, unlike the brain where extensive work has been carried out to elucidate the underlying mechanisms of damage, little is know about the cardiovascular response following HI-induced injury. One mechanism that may cause the MOD associated with HI, is a process that is similar to the diving reflex. The diving reflex re-distributes blood flow away from the periphery and splanchnic area, to increase delivery to vital organs such as the heart, adrenals glands and brain, to protect these organ against ischaemic-injury [90]. However, prolonged redistribution of blood flow and diminished oxygen and nutrient supply associated with the hypoxia, will ultimately causes damage to these vital organs.

The development of ECG abnormalities and injuries to systemic organs in infants having suffered from acute birth asphyxia has been documented [160; 162]. These studies attributed the systemic organ injury to hypo-perfusion resulting from a decrease in cardiac output. In addition, it has been shown that as many as 78% of neonates sustain some degree of cardiovascular impairment following an hypoxic episode at birth, as indicated by elevated cardiac enzymes or requiring pressor/volume support [81]. In this report, Hankins *et al*. showed maximal cardiac impairments 5-days post-insult in humans [81]. In addition, Yang *et al*., have demonstrated injury to myocardial mitochondria following HI induced brain damage; associated with calcium overload and a concurrent decrease in mitochondrial complex IV activity [231]. Furthermore, maturation of the myocardial enzyme, lactate dehydrogenase (LDH) was also shown to be decreased following HI-induced brain damage in young rats [42].

Recent work has shown that cardiac haemodynamics (left ventricular developed pressure (LVDP) and coronary flow (CF); see Figure 1A and B) are impaired following an HI-insult and are most pronounced 7-days post-HI (Clarkson, Kapoor, Harrison, Jackson and Sammut, unpublished data), which is consistent with Hankins findings in 2002 where maximal findings were seen 5-days post hypoxic insult in humans [81]. Furthermore, we show impaired mitochondrial enzyme kinetics, and increased cardiac caspase-3 activity coupled with increased LDH leakage into the Langendorff perfusate (see Figure 1C and D) 7-days post-HI. We also demonstrate that following an HI-induced insult, there is a significant increase in circulating interleukin (IL)-1β and tumour necrosis factor (TNF)-α levels (see below [31]). The increases in pro-inflammatory cytokine levels occur prior to the impairment in both cardiac haemodynamics and mitochondrial energetics. These findings of an HI-induced increase in cardiac caspase-3 activity driven either through an extrinsic pathway involving inflammation or through an intrinsic mitochondrial-channelled pathway, confirms the presence of myocardial apoptosis, which may consequently play an important role in cardiac damage following cerebral HI (see Figure 2).

A clear body of evidence has accumulated that illustrates cardiovascular abnormalities following an ischaemic-insult. However, the underlying mechanisms associated with this damage have not been fully elucidated. Outlined below are a few mechanisms that may contribute in part to the cardiac damage seen post-cerebral-insult.

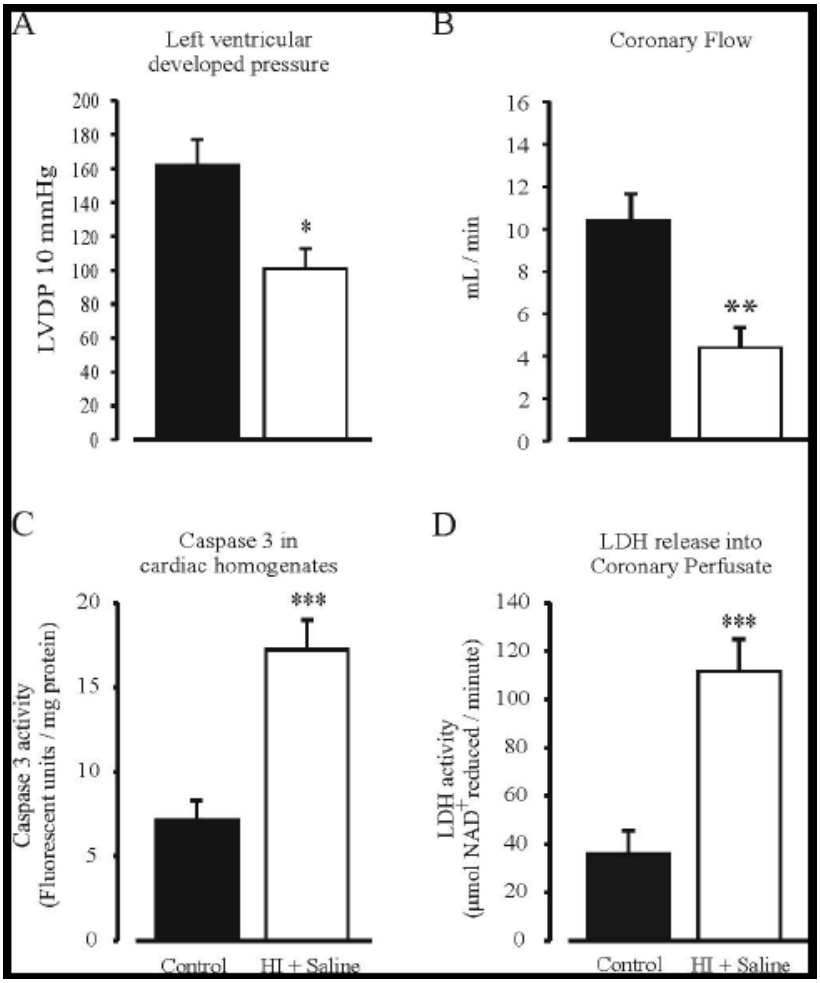


Figure 1. The effects of non-intervention control (black) and HI + saline (white) on LVDP at 10 mmHg (Panel A), sinus coronary flow (Panel B), Caspase 3 activity (Panel C) and LDH leakage into coronary perfusate (Panel D) were assessed from hearts isolated 7-days post-HI. Panel A shows an impairment in LVDP at 10 mmHg following HI. Panel B shows a decrease in sinus coronary flow following an HI-insult. Panel C shows increased caspase 3 activity following an HI-insult. Panel D shows increased LDH leakage from hearts following HI + saline treatment. * = $P<0.05$, ** = $P<0.01$, *** = $P<0.001$ versus non-intervention control.

Release of Cardiac Enzymes

Membrane encapsulated cellular constituents are externalized after cellular damage, enabling assessment of tissue injury through analysis of tissue-specific enzyme levels within

the circulation. A number of studies have shown that creatine-kinase-myocardial-band (CK-MB), a cardiac specific enzyme, is elevated in certain patients following cerebral ischaemia, subarachnoid haemorrhage and in patients with head trauma [50; 77; 141]. However, it should be noted that these changes are in the absence of any clinical evidence of underlying cardiovascular complications. Assessment of post-stroke serum levels has been proposed as an indicator of early ischaemia-mediated death [141].

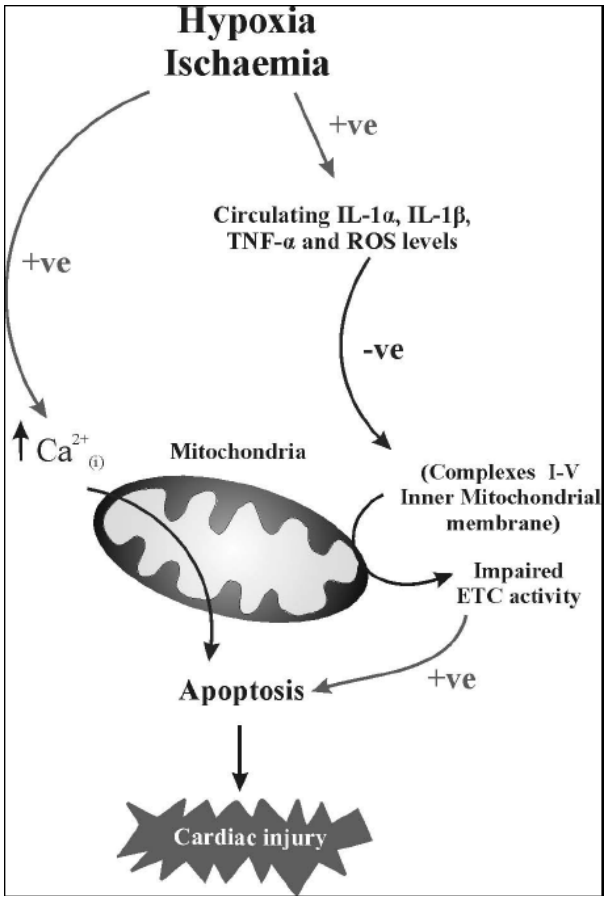


Figure 2. Schematic diagram showing proposed mechanisms contributing cardiac damage following cerebral hypoxia-ischemia. –ve = negative effect; +ve = positive effect.

Compared to the abrupt rise and fall (24 hours) of CK-MB following myocardial infarction (MI), the temporal pattern of CK-MB elevation after stroke is gradual and sustained for a period of days, implying a prolonged state of cardiac deterioration [11; 166]. The myocytolysis accompanying cerebral ischaemia is believed to be the cause of raised CK-MB levels. Two early reports showed differential CK-MB enzyme expression patterns between stroke and non-stroke patients suffering acute MI [139; 141]. These reports demonstrated sharp increases in CK-MB levels within the first 2 days following an MI, however, in stroke patients, CK-MB enzyme levels exhibited a slow, yet gradual increase which did not peak until the 6th day. The authors described this process as reflecting a sub-

acute process that is compatible with diffuse myocardial injury occurring during the first week following a stroke and mirrors the changes seen in catecholamine levels [139].

Troponin T, which is regarded as a more specific cardiac enzyme, was not elevated following cerebral ischaemia in one stroke study, suggesting CK-MB elevations following a stroke are not cardiac in origin [11]. However, Ay *et al*, may have failed to study an appropriate patient population when assessing secondary cardiac dysfunction following cerebral ischaemia. The inclusion in their cohort of relatively young patients with left hemispheric lesions and the absence of sympathetic activity does not parallel what is encountered in patients with cardiac complications following cerebral ischaemia. As stated previously, secondary cardiac dysfunction following cerebral ischaemia commonly results from right hemispheric insular cortex lesions in elderly patients. In a different study, James and co-workers established the importance of these parameters by demonstrating 17% of aged ischaemic stroke patients had raised troponin T levels 12-72 hours following the initial insult, and that this elevation was associated with a 3-fold increased likelihood of mortality [89]. In addition, Moller and colleagues reported a significant elevation in troponin T levels following asphyxiation in neonatal infants [129]. Furthermore, these same authors reported that asphyxiated infants who subsequently develop heart failure have higher troponin T levels than asphyxiated infants who do not develop heart failure. We suggest that cardiac specific Troponin T may indeed serve as a reliable indicator of stroke-induced myocardial damage.

Release of Oxidative Stress Markers

Anti-oxidant defence mechanisms play an important role in the maintenance of cellular function and survival; aberrations in oxidative capacity have been implicated in conditions of ageing, inflammation and ischaemic reperfusion (IR) injury [53; 92]. The production of free radicals has been recognised to occur following cerebral ischaemia and known anti-oxidants have been shown to offer neuroprotection against free radical-mediated injury [32; 84; 195].

Markers of oxidative stress have also been found in the periphery including increased plasma 8-isoprostane (8-*epi*$PGF_{2\alpha}$) levels [171]. Plasma 8-*epi*$PGF_{2\alpha}$ is a reliable marker of *in vivo* oxidative damage and is produced as a stable by-product following the non-enzymatic oxidation of tissue phospholipids by oxygen radicals [164]. Plasma malondialdehyde, which is another marker of oxidative damage, has also been found to be elevated for a period of 48 hours in patients following ischaemic stroke [182].

In stark contrast, decreased intrinsic anti-oxidant enzyme levels (such as superoxide dismutase (SOD)) have been found 48 hours following the onset of cerebral ischaemia. Specifically, lower serum and erythrocyte SOD and glutathione peroxidase (GPx) activities have been shown to occur in stroke patients [27; 192]. In addition, plasma levels of non-enzymatic anti-oxidants, α-tocopherol, ascorbic acid and uric acid are also decreased 24 hours post-ischaemia with all returning to control levels after 1 week with the exception of ascorbic acid and SOD [27]. A poor serum anti-oxidant profile following cerebral ischaemia has been shown to be associated with potentiation of neurological degeneration [114]. It is, therefore, possible that free radical production during and / or following cerebral ischaemia,

whether derived centrally or peripherally, may account for some of the damage seen remote to the initial site of infarction.

Neural Regulation of the Heart

The ability for the brain to regulate cardiac function has been well established with many central nervous system (CNS) regions and mechanisms being implicated. The anatomical locations that are involved in the neural regulation of cardiac function, extends from the spinal cord to the cortex. Within the cortex, the insular cortex has received the most recent attention and will be the man focus of this section. In addition, changes in the cardiovascular autonomic system, which is comprised of a complex network of interconnections throughout the neural axis, will also be discussed.

Involvement of the Insular Cortex

Early reports suggested elderly patients who had suffered ischaemic lesions to the right hemisphere were at greatest risk of developing cardiovascular complications [74; 76]. In addition, patients with hemispheric lesions as opposed to lesions of the brainstem are more likely to develop arrhythmias [194]. The clinical symptoms of cardiovascular abnormalities following cerebral ischaemia are indicative of sympathetic hyper-function and / or parasympathetic hypo-function [13]. Indeed, cardiovascular abnormalities similar to those seen following cerebral ischaemia may be induced by intra-cerebral or systemic catecholamine infusion [26].

The right insular cortex has now been identified as representing the cortical site, which when subjected to ischaemia both experimentally and clinically, induces cardiovascular complications [127; 190]. The insular cortex lies within the region which receives its blood supply from the middle cerebral artery and is involved in the regulation of autonomic control of the heart [233]. Prolonged stimulation of this region produces ECG and myocardial impairment similar to that seen following cerebral ischaemia [145]. In addition, it has been demonstrated that the insular cortex has a role in cardiac chronotropic organization, with stimulation of the rostral posterior insular cortex in chronically anaesthetised rats resulting in tachycardia while stimulation of the caudal posterior insular cortex producing bradycardia [145]. An ischaemic lesion to the right insular cortex also induces specific patterns of neurochemical change. Assessment 5-days post-insult has revealed increased staining for tyrosine hydroxylase, an enzyme involved in catecholamine synthesis, and neuropeptide Y, a neurotransmitter that potentiates the post-synaptic effects of noradrenaline [5]. It is also believed that disinhibition of the insular cortex caused by excitotoxicity triggers an increased sympathetic output and cardiac complications ensue [28].

Involvement of the Autonomic Nervous System

Irregularities in the autonomic nervous system (ANS) can be associated with changes in both the sympathetic and parasympathetic systems [13; 102]. Changes in the ANS are reflected by increases in plasma and urinary catecholamine [134] and corticosteroid levels [143]. In addition, fluctuations in the ANS have also been associated with variable heart rate patterns [101; 137].

From animal and human studies, it is well established that anatomic and functional asymmetries exist in autonomic cardiac innervation. The parasympathetic and sympathetic nerves to the heart have parallel courses [85], with the right side mainly innervating the sinoatrial node (with antagonistic influences on its chronotropic function), while the left side mainly innervates the atrioventricular node and the ventricles. The nerves on the left side (left vagal branch) influence atrioventricular conduction, ventricular fibrillation threshold, QT timing and ST segments of ECG's [80; 85; 132; 178; 179]. This led to the development of clinical corrective measures such as left-sided stellectomy used for the treatment of malignant ventricular arrhythmias with a long QT syndrome [132; 178]. Clinical observations have also suggested an association between right hemispheric lesions and the development of paroxysmal supraventricular tachycardia [111].

Since central autonomic pathways to the heart probably descend uncrossed [4], one should expect a corresponding asymmetry in the CNS control over cardiovascular function. This is supported by animal studies of experimental stroke showing that, right hemispheric lesions induce more pronounced sympathetic effects than lesions in the left hemisphere [24; 74]. It is established that CNS lesions in humans may induce ECG changes [147], cardiac arrhythmias [18], and disturbed cardiovascular reflexes [102], but whether lesions in the left as opposed to the right side of the brain have different consequences for autonomic heart rate control in humans is less well known. The involvement of the insular cortex and changes in the ANS have both been implicated in functional cardiac changes following an ischaemic-insult. Whether these changes and more importantly changes that occur on the right hemisphere compared to the left hemisphere are capable of producing lasting effects by themselves, is not known. It is most likely, however, that these changes work in synergism with other mechanistic changes that occur.

Inflammatory Mediated Response

For a chronic immune response to be initiated post-inflammation, antigen is processed and presented to lymphocytes, which is achieved via antigen-presenting cells (APCs; [34]. In the periphery, APCs are primarily either dendritic cells in the skin or monocytes in the circulation.

C-reactive protein (CRP) is an acute phase protein that is produced in the liver in response to a number of pro-inflammatory cytokines such as, IL-1β, IL-6 and TNF-α [152]. Previously, elevated levels of CRP have been associated with an increased risk of developing cardiovascular abnormalities in apparently healthy patients [168]. In addition, CRP has been used as a predictor of long-term cardiovascular events and an independent prognostic factor

of poor outcome following stroke and has been shown to be significantly elevated for a period of 5 days following cerebral ischaemia [46; 171]. In addition, Smith *et al*., showed a significant correlation between both IL-6 and CRP and the severity of cerebral ischaemia [189]. CRP is also known to activate the complement system, of which components of this important self-defence immune system are involved in chemotaxis and opsonisation, and have been found to be elevated following cerebral ischaemia [154]. However, the complement pathway, if inappropriately activated can cause tissue injury due to activation of inflammatory pathways. Pedersen *et al*. reported a significant systemic increase in the terminal SC5b-9 complement complex that was preceded by an increase in CRP, and suggested that CRP may activate the complement system and subsequent activation of inflammation after stroke [154].

Neopterin is a guanosine triphosphate (GTP) metabolite, though its physiological function is not yet fully defined. Production of neopterin is thought to be only in monocytes and macrophages and has, therefore, been considered as a specific marker for the activation of these cells [117]. Previously, increased levels of neopterin have been shown to occur in patients with carotid atherosclerosis [226]. In addition, Grau *et al*., reported an increase in neopterin following cerebral ischaemia with peak levels seen on days 3 and 7 post-insult [67]. The production of monocytes and macrophages have been shown to have the ability to amplify thrombogenic events, by expressing tissue factor (TF; [47]. In addition, the expression of TF has been shown to induce thrombus formation on ruptured atherosclerotic plaques and has also been shown to play a role in MI and ischaemic stroke [205]. Tissue factor is a cellular receptor and cofactor for plasma factor VII(a), which has been shown to initiate the coagulation protease cascade that results in the stimulation of thrombin and fibrin [167]. Furthermore, it has been shown that TF-dependent coagulation activity is increased in the presence of arachidonic acid stimulated cyclo-oxygenase-1 metabolites prostaglandin $(PG)G_2$ and thromboxane $(TX)A_2$ and inhibited when in the presence of PGE_2 [22]. Clear evidence has accumulated that demonstrates the catastrophic effects of circulating mediators. The expression of TF attributed to circulating monocytes and macrophages could account for the pathological presentations of myocytolysis and myofibrillar degeneration. However, further work is required in order to establish the exact role that TF maybe playing during the process of cardiac damage and also the role that pro- and anti-inflammatory cytokines are having.

Circulating Pro-Inflammatory Cytokines

Cerebral ischaemia evokes an inflammatory response that is characterised by the activation and release of cytokines, chemokines (chemotactic cytokines), endothelial-leukocyte adhesion molecules and proteolytic enzymes that contribute to tissue injury [51]. Production of pro-inflammatory cytokines and chemokines have been detected in experimental models of cerebral ischaemia and HI as well as human patients following acute ischaemic stroke or hypoxia-ischaemia encephalopathy (HIE; [34; 35; 51] see table 1). In addition, increased levels of pro-inflammatory cytokines are correlated with greater infarct size and poorer clinical outcome in patients following ischaemic injury [51; 200; 212].

Table 1. Comparison of cytokine and chemokine levels after hypoxia-ischaemia and ischaemic stroke in both animals and humans

Cytokine			Chemokine		
	HI	Stroke		HI	Stroke
IL-1α	↑[31; 79] NC[155; 209]	↑[113]	MCP1	↑[88; 138; 230]	↑[66]
IL-1β	↑[12; 16; 31; 52; 57; 79; 83; 138; 151; 196; 209] NC[151; 155; 175]	↑[86; 113]	MCP2	↑[138]	
TNFα	↑[12; 16; 31; 52; 57; 138; 151; 155; 196; 209] NC[151; 175; 183]	↑[69; 107; 201; 235]	MCP3		↑[220]
TNFβ	↑[209]		MIP1α	↑[16; 39; 138]	↑[16]
IL-2	NC[209] ↓[138]	↓[95]	MIP1β	↑[16; 138]	↑[16]
IL-3	NC[209] ↓[138]		MIP2	↑[16; 138]	
IL-4	NC[31; 138; 209]	↑[95] NC[156]	RANTES	↑[16; 138] NC[155]	↑[16]
IL-5	NC[138; 209]		gro	↑[16]	↑[14; 201]+
IL-6	↑[29; 52; 57; 79; 123; 138; 155; 175; 183] NC[209]	↑[95; 107; 113; 158]	IL-8	↑[57; 138; 175]	↑[217]
IL-7	NC[138]				
IL-9	↑[57; 138]				
IL-10	NC[12; 31; 138; 175; 175; 209]	↑[156; 201] ↓[210]** ↑↓[158]* NC[107]			
IL-11	↑[138]				
IL-13	↑[138]				
IL-18	↑[83]				
TGFβ	↑[99; 138; 155]				
IFNγ	NC[209]	NC[95]			
GM-CSF	↑[31; 138] NC[175]	↑[201]			
M-CSF	↑[138]				
G-CSF	↑[138]				

NC, no change in the level of that cytokine/chemokine in HI animals/humans compared to control animals/humans. ↑/↓, increase/decrease in the level of that cytokine/chemokine in HI animals/humans compared to control animals/humans. + indicates an increase in IL-8 levels in the ischemic lesion, but not significantly. * Indicates that the levels were subject to changes with time in comparison to controls. ** This study correlated the levels of IL-10 and the incidence of stroke. (adapted from references [34; 35]).

Markers of inflammation are not confined to the ischaemic tissue and evidence now suggests that inflammatory responses, even though initiated within the CNS, are also present

systemically. Several studies have found elevated levels of pro-inflammatory cytokines in the periphery following cerebral ischaemia [54; 171]. The potential cardiac consequences of the increased peripheral pro-inflammatory mediators are seldom recognised, but they may play a pivotal role in the development of secondary cardiac dysfunction subsequent to cerebral ischaemia and HI. Indeed, a robust inflammatory response following cerebral ischaemia is associated with a higher occurrence of subsequent cardiovascular events [46].

TNF-α, a potent inflammatory cytokine is produced by mononuclear leukocytes (primarily macrophages; [2] and is known to induce myocyte apoptosis, and is thought to be an important mediator in the pathogenesis of MI [37; 221]. In addition, serum and cerebrospinal fluid (CSF) levels of TNF-α have been shown to be elevated following cerebral ischaemia and are correlated with a deterioration in neurological outcome [48; 212].

In human asphyxiated babies and newborn infants with HIE, CSF and plasma IL-1β and TNF-α levels were shown to be elevated within 48 hours [57; 151; 183]. In addition, asphyxiated babies that develop cerebral palsy (CP) or neurological deficits within 1 year have increased IL-1β and TNF-α levels within 48 hours after birth [57; 151]. In addition, following HI in neonatal rats, IL-1α, IL-1β and TNF-α have all been shown to be elevated 3-days post-insult ([31] see Figure 3) This suggests that both IL-1β and TNF-α play an important role in the acute stages of inflammation following HI, akin to their actions in peripheral inflammatory responses.

Interleukin-6 (IL-6) has both neurodegenerative and neuroprotective effects [61] and may hence play a dual role in pathologies of cerebral injury. The expression of IL-6 has been shown to occur as a result of other pro-inflammatory cytokines, in particular IL-1β and TNF-α [181]. Expression of IL-6 mRNA and bioactivity has been shown to increase following focal cerebral ischaemia in the rat [115]. Elevated CSF IL-6 levels in acute ischaemic stroke patients has been shown to correlate with the volume of infarction [200]. In patients with acute ischaemic stroke, some studies have reported an association between circulating IL-6 concentrations and brain infarct volume, stroke severity, or outcome up to 6 months [51; 158; 212]. Conversely, other studies have reported no association between serum IL-6 concentrations and infarct volume or stroke severity at 3 months [54; 200]. Previous studies have reported peak values of serum IL-6 within the first 10 days of stroke [51; 54; 158]. The question arises as to whether IL-6 might directly contribute to infarct pathogenesis, or is simply a marker of CNS or other inflammatory injury? In contrast, IL-6 has several pro-inflammatory effects [91; 208] which may contribute to the induction and evolution of early inflammatory injury in the brain and its vasculature. At the same time, IL-6 has been shown to have both neurotrophic [71] and anti-inflammatory properties [176] that may contribute to recovery following cerebral ischaemia.

In humans, CSF, plasma and serum IL-6 levels are up-regulated in asphyxiated newborns and HIE infants within 48-90 hours after birth [29; 57; 123; 175; 183]. Indeed a positive correlation between IL-6 levels and HIE severity / clinical outcome has been shown, suggesting that IL-6 is pro-inflammatory in this setting [123; 175].

Circulating Chemokines

The β-chemokines (which are primarily chemoattractants for mononuclear cells) consist of macrophage inflammatory protein (MIP)-1α, MIP-1β, monocyte chemoattractant protein (MCP)-1, MCP-2 and regulated on activation normal T cell expressed and secreted (RANTES). Recently, elevated levels of MCP-1 have been seen in the CSF 24 hours following ischaemic stroke, however, these levels did not correlate with the corresponding levels seen in the plasma [116]. Plasma concentrations of intracellular adhesion molecule-1 (ICAM-1) and MCP-1, have also been shown to be elevated following cerebral ischaemia [171].

Interleukin-8 (the archetype chemokine) is a member of the α-chemokine family and plays a central role in neutrophil accumulation and activation. Grau *et al*., reported a significant increase in IL-8 plasma levels on day 1 following an ischaemic event, with levels still remaining elevated 3 and 7-days post-insult [67]. Circulating monocytes have been shown to be an important source of IL-8, which is a strong chemoattractant for polymorphonuclear neutrophils (PMNs) that can cause tissue damage by vessel plugging and release of oxygen-derived free radicals and proteinases [100]. In addition, IL-8 has also been shown to contribute to atherogenesis due to its mitogenic and chemoattractant effects on T lymphocytes and smooth muscle cells [204] and to plaque rupture by interference with matrix metalloproteinase-1 (MMP-1) expression [9; 130]. These results have been shown to be similar to results obtained from MI [1] as well as from stroke patients [105]. Recently, studies have shown both IL-8 and granulocyte macrophage-colony stimulating factor (GM-CSF) CSF levels are increased following cerebral ischaemia, peaking 2-days post-insult [201]. Furthermore, IL-8 levels have been shown to be higher in CSF than in plasma [105]. Studies have also shown an increased number of circulating IL-8 mRNA expressing PMNs (primarily neutrophils) after stroke and plasma IL-8 levels were correlated with the expression of IL-8 mRNA [104; 105].

In asphyxiated newborns (including those that develop CP), IL-8 concentrations in the CSF and serum were increased compared to control newborns and there was a positive correlation between the severity of HIE and the level of IL-8 [57; 175]. Therefore, IL-8 is crucial to the development of acute inflammation such that the neutrophil chemoattractant function of IL-8 may in fact be important to the initial development of HI-induced neuronal damage. These findings strengthen the hypothesis that monocytes and activated microglia are important contributors of increased IL-8 levels following cerebral ischaemia and cells in the peripheral circulation contribute to these increases. Recent work has also shown an increase in GM-CSF plasma levels following HI ([31] see Figure 3). In addition, clear evidence demonstrates that pro-inflammatory cytokines and chemokines present in the periphery may contribute to cardiovascular injury following cerebral ischaemia.

Circulating Anti-Inflammatory Cytokines

Interleukin-4 and IL-10 are mainly secreted by Th2 lymphocytes and monocytes / macrophages and have anti-inflammatory properties [203], possibly by providing a negative feedback mechanism to limit the production of pro-inflammatory cytokines following

cerebral ischaemia and HI. Previously, IL-10 has been shown to inhibit monocyte / macrophage synthesis of IL-6 and TNF-α by blocking gene transcription and down regulating the release of ICAM-1 and MMP-9 [45; 185].

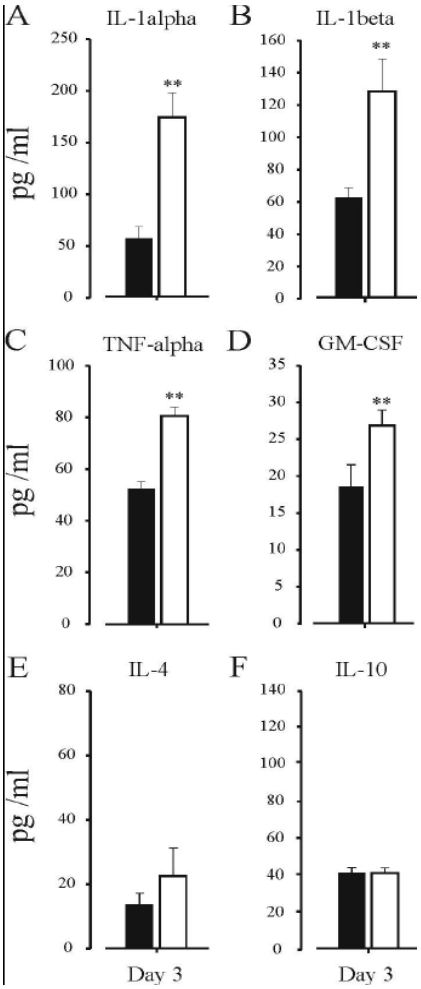


Figure 3. The effects of non-intervention control (black) and HI + saline (white) on circulating IL-1α (Panel A), IL-1β (Panel B), TNF-α (Panel C) GM-CSF (Panel D), IL-4 (Panel E) and IL-10 levels (Panel F) were assessed from plasma collected on day 3 post-HI. An increase in circulating levels of the pro-inflammatory cytokines, IL-1α, IL-1β, TNF-α, and GM-CSF, were seen following HI + saline treatment 3-days post-insult. No Change was seen in circulating levels of the anti-inflammatory cytokines, IL-4 and IL-10 following HI + saline treatment. ** = $p<0.01$, versus non-intervention control. (Modified from [31]).

In human stroke patients, increased levels of IL-10 have been seen in both the CSF and plasma, with levels peaking between 3 and 7-days post-stroke onset [158; 201]. Patients with acute cerebral ischaemia have increased IL-10 secreting monocytes compared with non-stroke controls [156]. However, unlike IL-10, no differences in the levels of IL-4 were detected in patients with or without neurological deterioration [213]. Pelidou and co-workers also failed to detect any differences in IL-4 secreting monocytes in ischaemic stroke patients

compared to non-stroke controls [156]. These results suggest that IL-4, despite any inhibitory effects on pro-inflammatory cytokines, is less important than IL-10 in the acute period (12-24 hours) of cerebral ischaemia. Recently, Vila *et al*, reported that lower levels of the anti-inflammatory cytokine IL-10 and not IL-4 is associated with the onset of neurological deterioration in ischaemic stroke patients [213]. In this study, lower plasma levels of IL-10 were detected within 24 hours following stroke onset and were correlated with early deterioration in neurological symptoms.

The exact role that anti-inflammatory cytokines play in HI-mediated injury, therefore, is still to be fully established. IL-4 and IL-10 were not detected 12 hours post-HI in the rat [209]. Additional studies have also shown that, IL-10 levels were not significantly different from control groups in both asphyxiated newborns [175] and HI animals [12]. Furthermore, Clarkson and colleagues reported that there were no changes in either IL-4 or IL10 plasma levels 3-days post HI ([31] see Figure 3). These findings imply that anti-inflammatory cytokines, such as IL-4 and IL-10 do not contribute to the initial inflammatory response following HI. However, exogenous administration of IL-10 (i.v.) prevented the damage seen after endotoxin administration post-HI, suggesting that IL-10 has a therapeutic effect [60].

The beneficial effects of IL-10 may also stem from anti-inflammatory actions. That is, IL-10 has been shown to regulate soluble apoptotic proteins, such as sFas/APO-1 and sbcl-2, detected in CSF of human patients following cerebral ischaemia [199]. In addition, IL-10 has been shown to modulate neuronal vulnerability to excitotoxic ischaemic injury [70], as well as inhibit inducible nitric oxide synthase (iNOS; [72], which is a key enzyme involved in propagating pro-inflammatory pathways. Finally, animals deficient in the IL-10 gene exhibited larger infarcts; increased neutrophil infiltration; and increased levels of TNF-α, ICAM-1, MMP-2, MMP-9 and iNOS compared to their wild type controls [45; 70; 72; 185]. Evidence suggests that IL-10 rather than IL-4 is the important anti-inflammatory cytokine in ameliorating ischaemic-induced injury. The only drawback, however, is that peak levels occur either 3 days post cerebral ischaemia or not at all following HI, which is possibly too late to afford any significant protection against circulating pro-inflammatory cytokines, which are elevated within the first 48 hours.

Inflammatory Mediated Myocardial Damage

Recent evidence has shown that the pro-inflammatory cytokines, TNF-α and IL-1β synergistically impair human myocardial function through a mechanism associated with sphingosine [23]. Sphingosine is rapidly produced as a result of sphingomyelin hydrolysis by sphingomyelinases that results in the formation of the ceramide intermediate when cardiac myocytes are exposed to TNF-α [124]. In rat myocyte cultures, the ceramidase inhibitor, N-oleoyl ethanolamine, has been shown to inhibit the production of sphingosine and reverse the ionotropic effects associated with TNF-α [148]. TNF-α and IL-6 have been shown to attenuate myocardial contractility directly (which is reversible) via the immediate reductions in systolic cytosolic [Ca^{2+}] associated with alterations in sarcoplasmic reticulum function [236]. In addition, TNF-α has been shown to also decrease myocardial contractility indirectly through nitric oxide-dependent attenuation of myofilament Ca^{2+} sensitivity [64].

Alternatively, TNF-α has been shown to provoke negative ionotropic effects in myocytes partially through the neutral sphingomyelinase pathway. This was shown within minutes following cardiomyocyte injury, where TNF-α decreased systolic function by alterations in Ca^{2+}-induced Ca^{2+}-release from the sarcoplasmic reticulum and also by disruption of the L-type calcium channels [109]. In this, the binding of TNF-α to the TNF-receptor type 1 (TNFR1) leads to the release of the sphingolipid metabolite, which is a stress-induced second messenger, via sphingomyelin degeneration. Oral and co-workers reported that production of sphingosine correlates directly with the imbalance in Ca^{2+} homeostasis, while blockade of sphingosine production negatively regulates TNF-α–induced contractile dysfunction [148]. This is due to the fact that sphingosine has been shown to decrease Ca^{2+} transients via the blockade of the ryanodine receptor, which impedes the Ca^{2+}-induced Ca^{2+}-release from the sarcoplasmic reticulum [125]. The exact mechanisms by which TNF-α exerts its pathophysiological effects are as yet not fully understood. It is suggested that TNF-α triggers the apoptotic pathway [108], which is possibly linked to the myocyte membrane TNFR1 and TNFR2 sites [126]. This is associated with the so called "death domain" that is found in the TNFRI, and suggests that TNF-α acting via the TNFRI site could mediate myocardial cell death via apoptosis [211].

Parasympathetic Nervous System and Inflammation

It has been well established that autonomic dysfunction is a strong correlate of morbidity and mortality resulting from cardiovascular disease, and recent work in humans has shown a correlation between abnormal heart rate variability and elevated levels of inflammatory cytokines such IL-6 and CRP [10]. However, the exact involvement of the ANS and inflammation are still being investigated. The vagus nerve has been shown to innervate the cardiovascular system in addition to other visceral organs such as the liver, spleen, and gut. Recent work by Tracey and colleagues demonstrated that by injecting lipopolysaccharides (LPS) into animals undergoing vagus nerve stimulation, resulted in a marked decrease in macrophage-mediated release of inflammatory cytokines (TNF-α, IL-1β, IL-18, and IL-6) and decreased incidence of death without affecting the release of the anti-inflammatory cytokine IL-10 [17], and these results were reversed following transsection of the vagal nerve. These results indicated that stimulation of the vagus nerve may play a functional role in regulating an anti-inflammatory response [17].

More recently, the mechanisms by which vagal nerve stimulation results in an anti-inflammatory response have been described by Tracey and colleagues [153; 207; 216]. In nicotinic α7 subunit knockout mice, electrical stimulation of the vagal nerve no longer prevented release of inflammatory cytokines, indicating that the α7 subunit of nicotinic receptors plays an important role in regulating the vagal nerve-mediated anti-inflammatory response [153]. In addition, stimulation of α7 subunit knockout mice with LPS resulted in a greater release of inflammatory cytokines compared to wild-type mice [216]. Macrophages have been shown to express nicotinic (cholinergic) receptors comprising of five α7 subunits, which are thought to be involved in the cholinergic anti-inflammatory reflex [216]. Inhibition of the nicotinic receptors in primary human macrophages resulted in a marked dose-

dependent reduction in high mobility group box 1 (HMGB1) inflammatory cytokines following stimulated with endotoxin (200 ng/mL) [216]. These results were unable to be inhibited with the muscarinic antagonist, atropine, however, the nicotinic antagonist, α-conotoxin, inhibited the action of acetylcholine on this receptor [216], indicating that acetylcholine inhibits HMGB1 release via the α7 nicotinic receptors.

Treatment Strategies

The cascade of events that occurs following neuronal injuries is complex and it is unlikely that any single intervention will prevent the entire cascade from being initiated. This damage not only resides within the hemisphere of damage but can also propagate to the contralateral hemisphere (see [31; 33; 35]. In addition to the neural component of damage, clear evidence as outlined above illustrates damage to the cardiovascular system. Therapies that target multiple steps in the cascade may limit neuronal injury more effectively than interventions with single modes of action. Outlined here are a couple of therapeutic interventions that have received growing attention over recent years, anaesthetic pre-conditioning and more recently post-conditioning and the use of beta-blockers.

Pre-Conditioning

Previous work has shown that exposing organs, such as the brain and heart to brief periods of sub-lethal ischaemia, initiates ischaemic tolerance via a pre-conditioning phenomenon [136; 206]. Two distinct patterns of ischaemic tolerance have been noted: the acute phase, whereby effects are seen within minutes and then subsequently disappear after 2-3 hours and the late phase, whereby effects develop over a period of several hours and can last up to several days or weeks [136; 206].

One of the pioneering publications in the field of pre-conditioning came in 1986 when Murry and colleagues reported that myocardial damage as a result of a coronary artery occlusion, is markedly ameliorated if the heart had prior exposure to brief periods of sub-lethal ischaemia [133]. Similarly, these pre-conditioning effects have also been observed in human subjects [234]. In addition, a recent report has also suggested that a transient ischaemic attack can induce ischaemic pre-conditioning within the brain [225]. The CNS has been highlighted as being the most vulnerable organ system in the body to an ischaemic insult. For instance, a brief disruption (5 minutes) to cerebral blood flow (CBF) has been shown to cause neuronal injury, while cardio-myocytes and kidney cells require 20-40 minutes of ischaemia to induce cellular damage [112].

Cerebral ischaemia-induced pre-conditioning was first reported by Kitagawa and co-workers using a model of global ischaemia, whereby 2 minutes of transient ischaemia provided significant protection against subsequent global ischaemia 24 hours after the initial insult [97; 98]. Since these findings, others have shown in a rat model of unilateral carotid artery ligation coupled with hypoxia, that hypoxic-pre-conditioning can induced significant protection to both striatal and hippocampal regions [15; 62].

Anaesthetics and Pre-Conditioning

Early work in the 1960s showed clear evidence that general anaesthetics can offer tolerance against cerebral ischaemia that is induced during periods of temporary carotid occlusion [227]. This was further backed up in 1966 by Goldstein and colleagues who reported that pentobarbital can offer tolerance to cerebral anoxia [65]. Since these two early findings, a considerable amount of work has been carried out illustrating that intravenous and volatile anaesthetics can decrease the amount of neuronal injury during periods of insult. For instance, in a rat model of spinal cord injury, halothane, fentanyl/nitrous oxide and lidocaine all provided significant protection against injury [36]. Furthermore, halothane, sevoflurane and pentobarbital have all been shown to afford protection in a rat model of focal cerebral ischaemia [223; 224]. Clear evidence exists that demonstrates exposing adult rats to volatile anaesthetics (i.e. isoflurane or halothane), can trigger both acute and late phases of ischaemic tolerance within the brain [93; 241; 242].

Over the past decade, the volatile anaesthetics halothane, isoflurane, sevoflurane and desflurane have all been shown to provide significant protection against focal cerebral ischaemia and also provide significant improvement in neurological outcome [30; 49; 223; 228]. Both halothane and desflurane have been shown to afford significant neuroprotection following 2 hours intraluminal middle cerebral artery occlusion (MCAo) and 22 hours of reperfusion [78]. In this model of focal cerebral ischaemia, treatment with desflurane provided greater protection than halothane. The anaesthetic-induced neuroprotection with halothane has also been shown to be maintained even when the pericranial temperature is controlled [222].

Isoflurane has also been shown to prevent hippocampal neuronal injury in an *in vitro* model of cerebral ischaemia due to oxygen glucose derivation (OGD; [163]. In addition, 30 minutes of isoflurane pre-treatment provided significant protection against HI-induced neurodegeneration 24 hours later [239]. However, the protection afforded by isoflurane *in vitro* only delays and does not prevent neuronal damage in an MCAo model of focal cerebral ischaemia *in vivo* [94].

During periods of anaesthesia, auditory, visual and tactile stimuli reach the CNS, however, processing of this information is disturbed [8]. It is thus generally thought that anaesthetics preferentially act on the CNS. It is considered that ion channels, particularly gamma-aminobutyric acid $(GABA)_A$ receptors, are the most-likely target for anaesthetics within the CNS [59; 128]. However, understanding the exact mode of action of anaesthetics is plagued by the fact that most general anaesthetics act on numerous ion channels sites, with limited selectivity at a variety of lipophilic sites associated with neural membranes [30].

Over the past 50 years the noble gas, xenon, has been studied for its anaesthetic properties, which has revealing many salubrious qualities [41]. In addition to antagonising the N-methyl-D-aspartic acid (NMDA) receptor [58], xenon has been shown to have several advantages over many other volatile anaesthetics in use today. For instance, xenon has an extremely low blood/gas partition coefficient which allows for rapid a induction and emergence [135]. In addition, xenon has been shown to exert minimal effects on heart rate, mean arterial pressure and cardiac contractility [193], thus providing ideal haemodynamic stability.

In a series of *in vitro* studies xenon has been shown to reduced injury in a mouse neuronal-glial cell culture induced by either NMDA, glutamate, oxygen deprivation or OGD [229]. In addition, the neuroprotective effects of xenon have been assessed in *in vivo* models of acute neuronal injury involving administration of excitotoxins to rats [120; 161], cardiopulmonary bypass in rats [121], MCAo in mice [87], cardiac arrest in pigs [177], and HI in neonatal rats [119]. Furthermore xenon has also been shown to induce pre-conditioning in many organs, including both the brain and heart [165], which would make xenon an ideal candidate to offer protection against secondary cardiac damage in addition to affording significant neuroprotection.

Involvement of Inflammation in Pre-Conditioning

A growing body of evidence has accumulated over recent years, highlighting the immune system, and more importantly cytokines and chemokines as key mediators, not just in HI [16; 35; 159; 170], but also in other neurodegenerative disorders such as Alzheimer's disease [34]. In addition, these mediators have been shown to be closely inter-related in a complex and often vicious positive feedback cycle, in that pro-inflammatory cytokines are known to induce reactive oxygen species (ROS) and vice versa [3; 56; 63].

Previous studies have shown that following ischaemic pre-conditioning, TNF-α and IL-1β mRNA levels are increased as measured using real-time PCR [218; 219]. The peak levels of IL-1β expression following ischaemic pre-conditioning, however, was significantly less compared to the permanent occlusion of the MCA, 87 copies versus 546 [218]. In addition, others have shown that pre-treatment of rats with low doses of bacterial LPS induces cytokines and subsequently protects against later ischaemic injury [202]. Furthermore, IL-1β has also been shown afford significant protection following direct administration just prior to cerebral ischaemia, which was negated by co-administration of the endogenous IL-1 antagonist, IL-1ra [142].

The use of anaesthetics to interact with inflammatory pathways is not well characterised. The release of leukotriene B4 and IL-1 from activated human monocytes has been shown to be dose-dependently inhibited following treatment with lidocanine and bupivacaine *in vitro* [188]. In a study assessing acute hyperoxic lung injury in rabbits, pre-treatment with an intravenous lidocaine infusion at clinically relevant concentrations markedly decreased the release of IL-1β and TNF-α from the injured lung and also negated the influx and activation of neutrophils [197]. In addition, local anaesthetics such as lidocaine, bupivacaine and amethocaine have been shown to inhibit both the spontaneous and also the TNF-α-induced stimulation of IL-1β and IL-8 with lidocaine also stimulating the secretion of the anti-inflammatory molecule IL-1ra [110].

Recent work has shown that clomethiazole (CMZ), in addition to modulating the GABA receptor offers protection via anti-inflammatory mechanisms [31; 82; 186; 187]. For example, CMZ has been shown to inhibit p38 mitogen-activated protein kinase, in turn attenuating the induction of the immediately early genes c-fos and c-jun in LPS-stimulated cortical glial cultures [186]. More recently, CMZ has been shown to inhibit the IL-1β-induced expression of glial c-fos and iNOS mRNA levels in vitro [187]. Likewise, in a model of experimental

extracorporeal circulation, plasma concentrations of IL-6, IL-8 and TNF-α were reduced by CMZ [82]. Furthermore, CMZ-treatment decreased the HI-induced increase in iNOS activity in a model of HI [33]. Most recently, CMZ has also been shown to decrease the HI-induced increase in circulating pro-inflammatory mediators (IL-1α, IL-1β and TNF-α) and stimulate an increase in the anti-inflammatory cytokine IL-10, in turn providing significant protection to mitochondrial energetics both contralateral and ipsilateral to the occlusion [31]. Ample evidence now supports the view that CMZ has both GABAmimeting and anti-inflammatory properties, and these properties together provide neuroprotection. We have also more recently shown that CMZ is able to offer protection against secondary cardiac damage following HI (Clarkson, Kapoor, Harrison, Jackson and Sammut, unpublished data)

Post-Conditioning

Significant work has highlighted anaesthetic pre-conditioning as a means for offering protection to the CNS against injury. Over resent years the term post-conditioning, which has significant clinical benefits over pre-conditioning, has been coined and shown to afford significant protection that is similar to that seen with pre-conditioning paradigms [40; 214]. In 2003 Zhao and colleagues introduced ischaemic post-conditioning, which is defined by a series of intermittent ischaemic episodes during the reperfusion phase which they showed offered significant protection against myocardial injury following the ligation of the left anterior descending artery [240]. Since this study, ischaemic post-conditioning has been shown to involve the release of adenosine [96] and also the activation of ERK, production of nitric oxide and opening of mitochondrial adenosine-triphosphate sensitive potassium channels [232]. With the exception of one recent study showing that LPS, which is well-known to induce preconditioning, can decrease the recruitment of leukocytes post both cerebral and spinal cord injuries [43], all work to date has been carried out on the heart. Whether the same post-conditioning mechanisms that have been elucidated following injury to the heart are the same following cerebral HI, is yet to be examined.

Use of Beta-Blockers

Increased sympathetic activity has been found after acute stroke [25; 75; 134] and is associated with poor neurological prognosis [172]. Beta-blockers, which have an anti-sympathetic effect, are neuroprotective during cardiac surgery [6]. In an animal model of cerebral ischaemia, pre-treatment with the beta-blocker, carvedilol, decreased the infarct volume and neurological deficits by ≥40% [174]. Possible mechanisms for the neuroprotection include shifting the haemoglobin-oxygen dissociation curve to the right [157], decreasing TNF-α and IL-1β levels [174], offering membrane-stabilisation and antioxidant effect [7], blocking sodium and calcium channels [150], and inhibiting protein kinase C [191] and phosphatidate hydrolase [106].

Clear evidence now exists illustrating that carvedilol, which is classically known for its actions on hypertension and congestive heart failure, has multiple modes of action. Recent

evidence shows that carvedilol is neuroprotective in both *in vivo* global [118] and focal [174] models of cerebral ischaemia. Following focal ischaemia, carvedilol was shown to significantly attenuate the ischaemia-induced increase in TNF-α and IL-1β mRNA levels. Furthermore, the metabolites of carvedilol, SB 211475 and SB 209995, have been shown to be more potent than carvedilol for inhibiting lipid peroxidation in rat brain homogenates [55; 238]. Therefore, these metabolites may also be responsible for some of the neuroprotective effects associated with carvedilol treatment.

Carvedilol is also thought to exert its neuroprotection via antioxidant properties, as carvedilol offers significant protection in *in vitro* model of free radical mediated neuronal injury [118; 238]. In addition, carvedilol has been also shown to prevent apoptosis following myocardial ischaemia–reperfusion injury [237]. Based on these findings, clear evidence exists the highlights the use of carvedilol or possibly other beta-blockers as novel agents in preventing either cerebral ischaemia or HI-induced neural injury, as well as preventing the secondary cardiovascular events that occur subsequent to the neural insult.

Conclusion

Following a cerebrovascular accident, a multi-faceted cascade of events occurs leading to cell death and neurological impairments of the CNS. It is well known clinically that clipping off cerebral aneurysms, carotid endarterectomy and cardiopulmonary bypass present a high risk for transient focal cerebral ischaemia. And one of the most imminent predisposing factors of cerebral ischaemia is cardiovascular complications. However, as outlined in this review, considerable evidence exists that clearly highlights cardiovascular complications subsequent to cerebral ischaemia and HI. These cardiovascular impairments pose a clinical threat and can confound neurological outcome and survival. The heart is tightly regulated by the brain, and damage to the insular cortex and ANS, clearly result in irregularities in cardiac function. One of the major pathways of damage following cerebral ischaemia is inflammatory mediated damage that precipitates primarily from a peripheral origin. And clear evidence exists that illustrates that circulating inflammatory mediators are capable of directly inhibiting cardiovascular function resulting in damage.

At this point, current therapeutic strategies do no more than alleviate the symptomatic presentations and cardiovascular monitoring plays no role, other than to possibly minimize any further neurological damage. Resent evidence however, clearly illustrates that pre-conditioning paradigms, the use of beta-blockers and possibly post-conditioning paradigms offers significant neuroprotection that might also stem to preventing subsequent cardiac impairments. Even though anaesthetic pre-conditioning has been shown to be beneficial in preventing neuronal injury, only a few groups are actually addressing this subject matter. Given the complexity of anaesthetic-mediated pre-conditioning and or post-conditioning in regards to neuronal protection, clearly more work needs to be carried out in order to validate this mechanism as a possible treatment protocol. However, given the data collected to date highlighting the putative beneficial effects of anaesthetics in protecting the CNS from injury, this in it self should be enough reason to further explore this topic at hand. It is a clear possibility the controlled anaesthetic treatment may be used as a treatment not only for

patients with acute ischaemic stroke and HIE, but also for patients who sustain traumatic head injury and also patients undergoing cardiovascular surgery where there is a prevalence of adverse sequelae associated with thrombosis. Furthermore the synergistic activities of that may occur between anaesthetic treatments and other putative neuroprotectants, such as beta-blockers, could be a possible means for treating cerebral ischaemia and HI.

Acknowledgments

This manuscript was completed during tenure of a Postdoctoral Fellowship from the Neurological Foundation of New Zealand. I would like to thank Dr Ivan Sammut for his comments on an earlier version of this manuscript.

References

Abe, Y., Kawakami, M., Kuroki, M., Yamamoto, T., Fujii, M., Kobayashi, H., et al. (1993). Transient rise in serum interleukin-8 concentration during acute myocardial infarction. *Br.Heart J., 70,* 132-134.

Aggarwal, B. B., Kohr, W. J., Hass, P. E., Moffat, B., Spencer, S. A., Henzel, W. J., et al. (1985). Human tumor necrosis factor. Production, purification, and characterization. *J.Biol.Chem., 260,* 2345-2354.

Ali, M. H., Schlidt, S. A., Chandel, N. S., Hynes, K. L., Schumacker, P. T. & Gewertz, B. L. (1999). Endothelial permeability and IL-6 production during hypoxia: role of ROS in signal transduction. *Am.J.Physiol, 277,* L1057-L1065.

Allen, G. V. & Cechetto, D. F. (1992). Functional and anatomical organization of cardiovascular pressor and depressor sites in the lateral hypothalamic area: I. Descending projections. *J.Comp Neurol., 315,* 313-332.

Allen, G. V., Cheung, R. T. & Cechetto, D. F. (1995). Neurochemical changes following occlusion of the middle cerebral artery in rats. *Neuroscience, 68,* 1037-1050.

Amory, D. W., Grigore, A., Amory, J. K., Gerhardt, M. A., White, W. D., Smith, P. K., et al. (2002). Neuroprotection is associated with beta-adrenergic receptor antagonists during cardiac surgery: evidence from 2,575 patients. *J.Cardiothorac.Vasc.Anesth., 16,* 270-277.

Anderson, R., Ramafi, G. & Theron, A. J. (1996). Membrane stabilizing, anti-oxidative interactions of propranolol and dexpropranolol with neutrophils. *Biochem.Pharmacol., 52,* 341-349.

Antkowiak, B. (2001). How do general anaesthetics work? *Naturwissenschaften, 88,* 201-213.

Apostolopoulos, J., Davenport, P. & Tipping, P. G. (1996). Interleukin-8 production by macrophages from atheromatous plaques. *Arterioscler.Thromb.Vasc.Biol., 16,* 1007-1012.

Aronson, D., Mittleman, M. A. & Burger, A. J. (2001). Interleukin-6 levels are inversely correlated with heart rate variability in patients with decompensated heart failure. *J.Cardiovasc.Electrophysiol., 12,* 294-300.

Ay, H., Arsava, E. M. & Saribas, O. (2002). Creatine kinase-MB elevation after stroke is not cardiac in origin: comparison with troponin T levels. *Stroke, 33,* 286-289.

Balduini, W., Mazzoni, E., Carloni, S., De Simoni, M. G., Perego, C., Sironi, L., et al. (2003). Prophylactic but not delayed administration of simvastatin protects against long-lasting cognitive and morphological consequences of neonatal hypoxic-ischemic brain injury, reduces interleukin-1beta and tumor necrosis factor-alpha mRNA induction, and does not affect endothelial nitric oxide synthase expression. *Stroke, 34,* 2007-2012.

Barron, S. A., Rogovski, Z. & Hemli, J. (1994). Autonomic consequences of cerebral hemisphere infarction. *Stroke, 25,* 113-116.

Beech, J. S., Reckless, J., Mosedale, D. E., Grainger, D. J., Williams, S. C. & Menon, D. K. (2001). Neuroprotection in ischemia-reperfusion injury: an antiinflammatory approach using a novel broad-spectrum chemokine inhibitor. *J.Cereb.Blood Flow Metab, 21,* 683-689.

Bergeron, M., Gidday, J. M., Yu, A. Y., Semenza, G. L., Ferriero, D. M. & Sharp, F. R. (2000). Role of hypoxia-inducible factor-1 in hypoxia-induced ischemic tolerance in neonatal rat brain. *Ann.Neurol., 48,* 285-296.

Bona, E., Andersson, A. L., Blomgren, K., Gilland, E., Puka-Sundvall, M., Gustafson, K., et al. (1999). Chemokine and inflammatory cell response to hypoxia-ischemia in immature rats. *Pediatr.Res., 45,* 500-509.

Borovikova, L. V., Ivanova, S., Zhang, M., Yang, H., Botchkina, G. I., Watkins, L. R., et al. (2000). Vagus nerve stimulation attenuates the systemic inflammatory response to endotoxin. *Nature, 405,* 458-462.

Britton, M., de, F. U., Helmers, C., Miah, K., Ryding, C. & Wester, P. O. (1979). Arrhythmias in patients with acute cerebrovascular disease. *Acta Med.Scand., 205,* 425-428.

Broderick, J., Brott, T., Barsan, W., Haley, E. C., Levy, D., Marler, J., et al. (1993). Blood pressure during the first minutes of focal cerebral ischemia. *Ann.Emerg.Med., 22,* 1438-1443.

Burch, G. E., Meyers, R. & Abildskov, J. A. (1954). A new electrocardiographic pattern observed in cerebrovascular accidents. *Circulation, 9,* 719-723.

Byer, E., Ashman, R. & Toth, L. A. (1947). Electrocardiagrams with large upright T-waves and Q-T intervals. *Am.Heart J., 33,* 796-799.

Cadroy, Y., Dupouy, D. & Boneu, B. (1998). Arachidonic acid enhances the tissue factor expression of mononuclear cells by the cyclo-oxygenase-1 pathway: beneficial effect of n-3 fatty acids. *J.Immunol., 160,* 6145-6150.

Cain, B. S., Meldrum, D. R., Dinarello, C. A., Meng, X., Joo, K. S., Banerjee, A., et al. (1999). Tumor necrosis factor-alpha and interleukin-1beta synergistically depress human myocardial function. *Crit. Care Med., 27,* 1309-1318.

Cechetto, D. F. (1993). Experimental cerebral ischemic lesions and autonomic and cardiac effects in cats and rats. *Stroke, 24,* I6-I9.

Cechetto, D. F., Wilson, J. X., Smith, K. E., Wolski, D., Silver, M. D. & Hachinski, V. C. (1989). Autonomic and myocardial changes in middle cerebral artery occlusion: stroke models in the rat. *Brain Res., 502,* 296-305.

Chapel, C. I., Rona, G., Balazs, T. & Gaudry, R. (1959). Comparison of cardiotoxic actions of certain sympathomimetic amines. *Can.J.Biochem.Physiol., 37,* 42.

Cherubini, A., Polidori, M. C., Bregnocchi, M., Pezzuto, S., Cecchetti, R., Ingegni, T., et al. (2000). Antioxidant profile and early outcome in stroke patients. *Stroke, 31,* 2295-2300.

Cheung, R. T. & Hachinski, V. (2004). Cardiac Effects of Stroke. *Curr.Treat.Options.Cardiovasc.Med., 6,* 199-207.

Chiesa, C., Pellegrini, G., Panero, A., De, L. T., Assumma, M., Signore, F., et al. (2003). Umbilical cord interleukin-6 levels are elevated in term neonates with perinatal asphyxia. *Eur.J.Clin.Invest, 33,* 352-358.

Clarkson, A. N. (2007). Anesthetic-mediated protection/preconditioning during cerebral ischemia. *Life Sci., 80,* 1157-1175.

Clarkson, A. N., Clarkson, J., Jackson, D. M. & Sammut, I. A. (2007). Mitochondrial involvement in transhemispheric diaschisis following hypoxia-ischemia: Clomethiazole-mediated amelioration. *Neuroscience, 144,* 547-561.

Clarkson, A. N., Liu, H., Pearson, L., Kapoor, M., Harrison, J. C., Sammut, I. A., et al. (2004). Neuroprotective effects of spermine following hypoxic-ischemic-induced brain damage: a mechanistic study. *FASEB J., 18,* 1114-1116.

Clarkson, A. N., Liu, H., Rahman, R., Jackson, D. M., Appleton, I. & Kerr, D. S. (2005). Clomethiazole: mechanisms underlying lasting neuroprotection following hypoxia-ischemia. *FASEB J., 19,* 1036-1038.

Clarkson, A. N., Rahman, R. & Appleton, I. (2004). Inflammation and autoimmunity as a central theme in neurodegenerative disorders: fact or fiction? *Curr.Opin.Investig.Drugs, 5,* 706-713.

Clarkson, A. N., Sutherland, B. A. & Appleton, I. (2005). The biology and pathology of hypoxia-ischemia: an update. *Arch.Immunol.Ther.Exp.(Warsz.), 53,* 213-225.

Cole, D. J., Shapiro, H. M., Drummond, J. C. & Zivin, J. A. (1989). Halothane, fentanyl/nitrous oxide, and spinal lidocaine protect against spinal cord injury in the rat. *Anesthesiology, 70,* 967-972.

Condorelli, G., Morisco, C., Latronico, M. V., Claudio, P. P., Dent, P., Tsichlis, P., et al. (2002). TNF-alpha signal transduction in rat neonatal cardiac myocytes: definition of pathways generating from the TNF-alpha receptor. *FASEB J., 16,* 1732-1737.

Connor, R. C. (1968). Heart damage associated with intracranial lesions. *Br.Med.J., 3,* 29-31.

Cowell, R. M., Xu, H., Galasso, J. M. & Silverstein, F. S. (2002). Hypoxic-ischemic injury induces macrophage inflammatory protein-1alpha expression in immature rat brain. *Stroke, 33,* 795-801.

Crisostomo, P. R., Wairiuko, G. M., Wang, M., Tsai, B. M., Morrell, E. D. & Meldrum, D. R. (2006). Preconditioning versus postconditioning: mechanisms and therapeutic potentials. *J.Am.Coll.Surg., 202,* 797-812.

Cullen, S. C. & Gross, E. G. (1951). The anesthetic properties of xenon in animals and human beings, with additional observations on krypton. *Science, 113,* 580-582.

Daneshrad, Z., Verdys, M., Birot, O., Troff, F., Bigard, A. X. & Rossi, A. (2003). Chronic hypoxia delays myocardial lactate dehydrogenase maturation in young rats. *Exp.Physiol, 88,* 405-413.

Davis, A. E., Campbell, S. J., Wilainam, P. & Anthony, D. C. (2005). Post-conditioning with lipopolysaccharide reduces the inflammatory infiltrate to the injured brain and spinal cord: a potential neuroprotective treatment. *Eur.J.Neurosci., 22,* 2441-2450.

Dawson, S. L., Manktelow, B. N., Robinson, T. G., Panerai, R. B. & Potter, J. F. (2000). Which parameters of beat-to-beat blood pressure and variability best predict early outcome after acute ischemic stroke? *Stroke, 31,* 463-468.

De Waal Malefyt, R., Abrams, J., Bennett, B., Figdor, C. G. & De Vries, J. E. (1991). Interleukin 10(IL-10) inhibits cytokine synthesis by human monocytes: an autoregulatory role of IL-10 produced by monocytes. *J.Exp.Med., 174,* 1209-1220.

Di Napoli, M., Papa, F. & Bocola, V. (2001). C-reactive protein in ischemic stroke: an independent prognostic factor. *Stroke, 32,* 917-924.

Edwards, R. L., Rickles, F. R. & Bobrove, A. M. (1979). Mononuclear cell tissue factor: cell of origin and requirements for activation. *Blood, 54,* 359-370.

Elneihoum, A. M., Falke, P., Axelsson, L., Lundberg, E., Lindgarde, F. & Ohlsson, K. (1996). Leukocyte activation detected by increased plasma levels of inflammatory mediators in patients with ischemic cerebrovascular diseases. *Stroke, 27,* 1734-1738.

Engelhard, K., Werner, C., Reeker, W., Lu, H., Mollenberg, O., Mielke, L., et al. (1999). Desflurane and isoflurane improve neurological outcome after incomplete cerebral ischaemia in rats. *Br.J.Anaesth., 83,* 415-421.

Fabinyi, G., Hunt, D. & McKinley, L. (1977). Myocardial creatine kinase isoenzyme in serum after subarachnoid haemorrhage. *J.Neurol.Neurosurg.Psychiatry, 40,* 818-820.

Fassbender, K., Rossol, S., Kammer, T., Daffertshofer, M., Wirth, S., Dollman, M., et al. (1994). Proinflammatory cytokines in serum of patients with acute cerebral ischemia: kinetics of secretion and relation to the extent of brain damage and outcome of disease. *J.Neurol.Sci., 122,* 135-139.

Feng, Y. & LeBlanc, M. H. (2003). Effect of agmatine on the time course of brain inflammatory cytokines after injury in rat pups. *Ann.N.Y.Acad.Sci., 1009,* 152-156.

Ferdinandy, P. & Schulz, R. (2003). Nitric oxide, superoxide, and peroxynitrite in myocardial ischaemia-reperfusion injury and preconditioning. *Br.J.Pharmacol., 138,* 532-543.

Ferrarese, C., Mascarucci, P., Zoia, C., Cavarretta, R., Frigo, M., Begni, B., et al. (1999). Increased cytokine release from peripheral blood cells after acute stroke. *J.Cereb.Blood Flow Metab, 19,* 1004-1009.

Feuerstein, R. & Yue, T. L. (1994). A potent antioxidant, SB209995, inhibits oxygen-radical-mediated lipid peroxidation and cytotoxicity. *Pharmacology, 48,* 385-391.

Floyd, R. A. (1999). Neuroinflammatory processes are important in neurodegenerative diseases: an hypothesis to explain the increased formation of reactive oxygen and nitrogen species as major factors involved in neurodegenerative disease development. *Free Radic.Biol.Med., 26,* 1346-1355.

Foster-Barber, A., Dickens, B. & Ferriero, D. M. (2001). Human perinatal asphyxia: correlation of neonatal cytokines with MRI and outcome. *Dev.Neurosci., 23,* 213-218.

Franks, N. P., Dickinson, R., de Sousa, S. L., Hall, A. C. & Lieb, W. R. (1998). How does xenon produce anaesthesia? *Nature, 396,* 324.

Franks, N. P. & Lieb, W. R. (1994). Molecular and cellular mechanisms of general anaesthesia. *Nature, 367,* 607-614.

Froen, J. F., Munkeby, B. H., Stray-Pedersen, B. & Saugstad, O. D. (2002). Interleukin-10 reverses acute detrimental effects of endotoxin-induced inflammation on perinatal cerebral hypoxia-ischemia. *Brain Res., 942,* 87-94.

Gadient, R. A. & Otten, U. H. (1997). Interleukin-6 (IL-6)--a molecule with both beneficial and destructive potentials. *Prog.Neurobiol., 52,* 379-390.

Gidday, J. M., Fitzgibbons, J. C., Shah, A. R. & Park, T. S. (1994). Neuroprotection from ischemic brain injury by hypoxic preconditioning in the neonatal rat. *Neurosci.Lett., 168,* 221-224.

Ginsburg, I. (1998). Could synergistic interactions among reactive oxygen species, proteinases, membrane-perforating enzymes, hydrolases, microbial hemolysins and cytokines be the main cause of tissue damage in infectious and inflammatory conditions? *Med.Hypotheses, 51,* 337-346.

Goldhaber, J. I. (1996). Free radicals enhance Na+/Ca2+ exchange in ventricular myocytes. *Am.J.Physiol, 271,* H823-H833.

Goldstein, A., Jr., Wells, B. A. & Keats, A. S. (1966). Increased tolerance to cerebral anoxia by pentobarbital. *Arch.Int.Pharmacodyn.Ther., 161,* 138-143.

Gourmala, N. G., Buttini, M., Limonta, S., Sauter, A. & Boddeke, H. W. (1997). Differential and time-dependent expression of monocyte chemoattractant protein-1 mRNA by astrocytes and macrophages in rat brain: effects of ischemia and peripheral lipopolysaccharide administration. *J.Neuroimmunol., 74,* 35-44.

Grau, A. J., Reis, A., Buggle, F., Al-Khalaf, A., Werle, E., Valois, N., et al. (2001). Monocyte function and plasma levels of interleukin-8 in acute ischemic stroke. *J.Neurol.Sci., 192,* 41-47.

Greenhoot, J. H. & Reichenbach, D. D. (1969). Cardiac injury and subarachnoid hemorrhage. A clinical, pathological, and physiological correlation. *J.Neurosurg., 30,* 521-531.

Gregersen, R., Lambertsen, K. & Finsen, B. (2000). Microglia and macrophages are the major source of tumor necrosis factor in permanent middle cerebral artery occlusion in mice. *J.Cereb.Blood Flow Metab, 20,* 53-65.

Grilli, M., Barbieri, I., Basudev, H., Brusa, R., Casati, C., Lozza, G., et al. (2000). Interleukin-10 modulates neuronal threshold of vulnerability to ischaemic damage. *Eur.J.Neurosci., 12,* 2265-2272.

Gruol, D. L. & Nelson, T. E. (1997). Physiological and pathological roles of interleukin-6 in the central nervous system. *Mol.Neurobiol., 15,* 307-339.

Gunnett, C. A., Berg, D. J., Faraci, F. M. & Feuerstein, G. (1999). Vascular effects of lipopolysaccharide are enhanced in interleukin-10-deficient mice. *Stroke, 30,* 2191-2195.

Hachinski, V. C. (1993). The clinical problem of brain and heart. *Stroke, 24,* I1-I2.

Hachinski, V. C., Oppenheimer, S. M., Wilson, J. X., Guiraudon, C., & Cechetto, D. F. (1992). Asymmetry of sympathetic consequences of experimental stroke. *Arch.Neurol., 49,* 697-702.

Hachinski, V. C., Smith, K. E., Silver, M. D., Gibson, C. J. & Ciriello, J. (1986). Acute myocardial and plasma catecholamine changes in experimental stroke. *Stroke, 17,* 387-390.

Hachinski, V. C., Wilson, J. X., Smith, K. E. & Cechetto, D. F. (1992). Effect of age on autonomic and cardiac responses in a rat stroke model. *Arch.Neurol., 49,* 690-696.

Hackenberry, L. E., Miner, M. E., Rea, G. L., Woo, J. & Graham, S. H. (1982). Biochemical evidence of myocardial injury after severe head trauma. *Crit Care Med., 10,* 641-644.

Haelewyn, B., Yvon, A., Hanouz, J. L., MacKenzie, E. T., Ducouret, P., Gerard, J. L., et al. (2003). Desflurane affords greater protection than halothane against focal cerebral ischaemia in the rat. *Br.J.Anaesth., 91,* 390-396.

Hagberg, H., Gilland, E., Bona, E., Hanson, L. A., Hahin-Zoric, M., Blennow, M., et al. (1996). Enhanced expression of interleukin (IL)-1 and IL-6 messenger RNA and bioactive protein after hypoxia-ischemia in neonatal rats. *Pediatr.Res., 40,* 603-609.

Hamlin, R. L. & Smith, C. R. (1968). Effects of vagal stimulation on S-A and A-V nodes. *Am.J.Physiol, 215,* 560-568.

Hankins, G. D., Koen, S., Gei, A. F., Lopez, S. M., Van Hook, J. W. & Anderson, G. D. (2002). Neonatal organ system injury in acute birth asphyxia sufficient to result in neonatal encephalopathy. *Obstet.Gynecol., 99,* 688-691.

Harmon, D., Coleman, E., Marshall, C., Lan, W. & Shorten, G. (2003). The effect of clomethiazole on plasma concentrations of interleukin-6, -8, -1beta, tumor necrosis factor-alpha, and neutrophil adhesion molecule expression during experimental extracorporeal circulation. *Anesth.Analg., 97,* 13-8, Table.

Hedtjarn, M., Leverin, A. L., Eriksson, K., Blomgren, K., Mallard, C. & Hagberg, H. (2002). Interleukin-18 involvement in hypoxic-ischemic brain injury. *J.Neurosci., 22,* 5910-5919.

Hickenbottom, S. L. & Grotta, J. (1998). Neuroprotective therapy. *Semin.Neurol., 18,* 485-492.

Higgins, C. B., Vatner, S. F. & Braunwald, E. (1973). Parasympathetic control of the heart. *Pharmacol.Rev., 25,* 119-155.

Hill, J. K., Gunion-Rinker, L., Kulhanek, D., Lessov, N., Kim, S., Clark, W. M., et al. (1999). Temporal modulation of cytokine expression following focal cerebral ischemia in mice. *Brain Res., 820,* 45-54.

Homi, H. M., Yokoo, N., Ma, D., Warner, D. S., Franks, N. P., Maze, M., et al. (2003). The neuroprotective effect of xenon administration during transient middle cerebral artery occlusion in mice. *Anesthesiology, 99,* 876-881.

Ivacko, J., Szaflarski, J., Malinak, C., Flory, C., Warren, J. S. & Silverstein, F. S. (1997). Hypoxic-ischemic injury induces monocyte chemoattractant protein-1 expression in neonatal rat brain. *J.Cereb.Blood Flow Metab, 17,* 759-770.

James, P., Ellis, C. J., Whitlock, R. M., McNeil, A. R., Henley, J. & Anderson, N. E. (2000). Relation between troponin T concentration and mortality in patients presenting with an acute stroke: observational study. *BMJ, 320,* 1502-1504.

Jensen, A., Garnier, Y. & Berger, R. (1999). Dynamics of fetal circulatory responses to hypoxia and asphyxia. *Eur.J.Obstet.Gynecol.Reprod.Biol., 84,* 155-172.

Johnson, J. L., Moore, E. E., Tamura, D. Y., Zallen, G., Biffl, W. L. & Silliman, C. C. (1998). Interleukin-6 augments neutrophil cytotoxic potential via selective enhancement of elastase release. *J.Surg.Res., 76,* 91-94.

Junqueira, V. B., Barros, S. B., Chan, S. S., Rodrigues, L., Giavarotti, L., Abud, R. L., et al. (2004). Aging and oxidative stress. *Mol.Aspects Med., 25,* 5-16.

Kapinya, K. J., Lowl, D., Futterer, C., Maurer, M., Waschke, K. F., Isaev, N. K., et al. (2002). Tolerance against ischemic neuronal injury can be induced by volatile anesthetics and is inducible NO synthase dependent. *Stroke, 33*, 1889-1898.

Kawaguchi, M., Kimbro, J. R., Drummond, J. C., Cole, D. J., Kelly, P. J. & Patel, P. M. (2000). Isoflurane delays but does not prevent cerebral infarction in rats subjected to focal ischemia. *Anesthesiology, 92*, 1335-1342.

Kim, H. M., Shin, H. Y., Jeong, H. J., An, H. J., Kim, N. S., Chae, H. J., et al. (2000). Reduced IL-2 but elevated IL-4, IL-6, and IgE serum levels in patients with cerebral infarction during the acute stage. *J.Mol.Neurosci., 14*, 191-196.

Kin, H., Zhao, Z. Q., Sun, H. Y., Wang, N. P., Corvera, J. S., Halkos, M. E., et al. (2004). Postconditioning attenuates myocardial ischemia-reperfusion injury by inhibiting events in the early minutes of reperfusion. *Cardiovasc.Res., 62*, 74-85.

Kitagawa, K., Matsumoto, M., Kuwabara, K., Tagaya, M., Ohtsuki, T., Hata, R., et al. (1991). 'Ischemic tolerance' phenomenon detected in various brain regions. *Brain Res., 561*, 203-211.

Kitagawa, K., Matsumoto, M., Tagaya, M., Hata, R., Ueda, H., Niinobe, M., et al. (1990). 'Ischemic tolerance' phenomenon found in the brain. *Brain Res., 528*, 21-24.

Klempt, N. D., Sirimanne, E., Gunn, A. J., Klempt, M., Singh, K., Williams, C., et al. (1992). Hypoxia-ischemia induces transforming growth factor beta 1 mRNA in the infant rat brain. *Brain Res.Mol.Brain Res., 13*, 93-101.

Kochanek, P. M. & Hallenbeck, J. M. (1992). Polymorphonuclear leukocytes and monocytes/macrophages in the pathogenesis of cerebral ischemia and stroke. *Stroke, 23*, 1367-1379.

Korpelainen, J. T., Sotaniemi, K. A., Makikallio, A., Huikuri, H. V. & Myllyla, V. V. (1999). Dynamic behavior of heart rate in ischemic stroke. *Stroke, 30*, 1008-1013.

Korpelainen, J. T., Sotaniemi, K. A., Suominen, K., Tolonen, U. & Myllyla, V. V. (1994). Cardiovascular autonomic reflexes in brain infarction. *Stroke, 25*, 787-792.

Koskelo, P., Punsar, S. & Sipilae, W. (1964). Subendocardial haemorrhage and E.C.G. changes in intracranial bleeding. *Br.Med.J., 5396*, 1479-1480.

Kostulas, N., Kivisakk, P., Huang, Y., Matusevicius, D., Kostulas, V. & Link, H. (1998). Ischemic stroke is associated with a systemic increase of blood mononuclear cells expressing interleukin-8 mRNA. *Stroke, 29*, 462-466.

Kostulas, N., Pelidou, S. H., Kivisakk, P., Kostulas, V. & Link, H. (1999). Increased IL-1beta, IL-8 & IL-17 mRNA expression in blood mononuclear cells observed in a prospective ischemic stroke study. *Stroke, 30*, 2174-2179.

Koul, O. & Hauser, G. (1987). Modulation of rat brain cytosolic phosphatidate phosphohydrolase: effect of cationic amphiphilic drugs and divalent cations. *Arch.Biochem.Biophys., 253*, 453-461.

Kouwenhoven, M., Carlstrom, C., Ozenci, V. & Link, H. (2001). Matrix metalloproteinase and cytokine profiles in monocytes over the course of stroke. *J.Clin.Immunol., 21*, 365-375.

Krown, K. A., Page, M. T., Nguyen, C., Zechner, D., Gutierrez, V., Comstock, K. L., et al. (1996). Tumor necrosis factor alpha-induced apoptosis in cardiac myocytes. Involvement of the sphingolipid signaling cascade in cardiac cell death. *J.Clin.Invest, 98*, 2854-2865.

Krown, K. A., Yasui, K., Brooker, M. J., Dubin, A. E., Nguyen, C., Harris, G. L., et al. (1995). TNF alpha receptor expression in rat cardiac myocytes: TNF alpha inhibition of L-type Ca2+ current and Ca2+ transients. *FEBS Lett., 376,* 24-30.

Lahav, M., Levite, M., Bassani, L., Lang, A., Fidder, H., Tal, R., et al. (2002). Lidocaine inhibits secretion of IL-8 and IL-1beta and stimulates secretion of IL-1 receptor antagonist by epithelial cells. *Clin.Exp.Immunol., 127,* 226-233.

Lane, R. D., Wallace, J. D., Petrosky, P. P., Schwartz, G. E. & Gradman, A. H. (1992). Supraventricular tachycardia in patients with right hemisphere strokes. *Stroke, 23,* 362-366.

Lee, J. M., Grabb, M. C., Zipfel, G. J. & Choi, D. W. (2000). Brain tissue responses to ischemia. *J.Clin.Invest, 106,* 723-731.

Legos, J. J., Whitmore, R. G., Erhardt, J. A., Parsons, A. A., Tuma, R. F. & Barone, F. C. (2000). Quantitative changes in interleukin proteins following focal stroke in the rat. *Neurosci.Lett., 282,* 189-192.

Leinonen, J. S., Ahonen, J. P., Lonnrot, K., Jehkonen, M., Dastidar, P., Molnar, G., et al. (2000). Low plasma antioxidant activity is associated with high lesion volume and neurological impairment in stroke. *Stroke, 31,* 33-39.

Loddick, S. A., Turnbull, A. V. & Rothwell, N. J. (1998). Cerebral interleukin-6 is neuroprotective during permanent focal cerebral ischemia in the rat. *J.Cereb.Blood Flow Metab, 18,* 176-179.

Losy, J. & Zaremba, J. (2001). Monocyte chemoattractant protein-1 is increased in the cerebrospinal fluid of patients with ischemic stroke. *Stroke, 32,* 2695-2696.

Lucey, D. R., Clevici, M., Eisenstein, E., Fleisher, T. A. & Shearer, G. M. (1996). Assessment of lymphocyte & monocyte function. In R.R.Rich (Ed.), *Clinical immunology. Principles and practice.* (pp. 2124-2140). St. Louis: Mosby-Years Book.

Lysko, P. G., Lysko, K. A., Yue, T. L., Webb, C. L., Gu, J. L. & Feuerstein, G. (1992). Neuroprotective effects of carvedilol, a new antihypertensive agent, in cultured rat cerebellar neurons and in gerbil global brain ischemia. *Stroke, 23,* 1630-1635.

Ma, D., Hossain, M., Chow, A., Arshad, M., Battson, R. M., Sanders, R. D., et al. (2005). Xenon and hypothermia combine to provide neuroprotection from neonatal asphyxia. *Ann.Neurol., 58,* 182-193.

Ma, D., Wilhelm, S., Maze, M. & Franks, N. P. (2002). Neuroprotective and neurotoxic properties of the 'inert' gas, xenon. *Br.J.Anaesth., 89,* 739-746.

Ma, D., Yang, H., Lynch, J., Franks, N. P., Maze, M. & Grocott, H. P. (2003). Xenon attenuates cardiopulmonary bypass-induced neurologic and neurocognitive dysfunction in the rat. *Anesthesiology, 98,* 690-698.

Makikallio, A. M., Makikallio, T. H., Korpelainen, J. T., Sotaniemi, K. A., Huikuri, H. V. & Myllyla, V. V. (2004). Heart rate dynamics predict poststroke mortality. *Neurology, 62,* 1822-1826.

Martin-Ancel, A., Garcia-Alix, A., Pascual-Salcedo, D., Cabanas, F., Valcarce, M. & Quero, J. (1997). Interleukin-6 in the cerebrospinal fluid after perinatal asphyxia is related to early and late neurological manifestations. *Pediatrics, 100,* 789-794.

Mathias, S., Dressler, K. A. & Kolesnick, R. N. (1991). Characterization of a ceramide-activated protein kinase: stimulation by tumor necrosis factor alpha. *Proc.Natl.Acad.Sci.U.S.A, 88,* 10009-10013.

McDonough, P. M., Yasui, K., Betto, R., Salviati, G., Glembotski, C. C., Palade, P. T., et al. (1994). Control of cardiac Ca2+ levels. Inhibitory actions of sphingosine on Ca2+ transients and L-type Ca2+ channel conductance. *Circ.Res., 75,* 981-989.

Meldrum, D. R. (1998). Tumor necrosis factor in the heart. *Am.J.Physiol, 274,* R577-R595.

Meyer, S., Strittmatter, M., Fischer, C., Georg, T. & Schmitz, B. (2004). Lateralization in autonomic dysfunction in ischemic stroke involving the insular cortex. *Neuroreport, 15,* 357-361.

Mihic, S. J., Ye, Q., Wick, M. J., Koltchine, V. V., Krasowski, M. D., Finn, S. E., et al. (1997). Sites of alcohol and volatile anaesthetic action on GABA(A) and glycine receptors. *Nature, 389,* 385-389.

Moller, J. C., Thielsen, B., Schaible, T. F., Reiss, I., Kohl, M., Welp, T., et al. (1998). Value of myocardial hypoxia markers (creatine kinase and its MB-fraction, troponin-T, QT-intervals) and serum creatinine for the retrospective diagnosis of perinatal asphyxia. *Biol.Neonate, 73,* 367-374.

Moreau, M., Brocheriou, I., Petit, L., Ninio, E., Chapman, M. J. & Rouis, M. (1999). Interleukin-8 mediates downregulation of tissue inhibitor of metalloproteinase-1 expression in cholesterol-loaded human macrophages: relevance to stability of atherosclerotic plaque. *Circulation, 99,* 420-426.

Morfis, L., Schwartz, R. S., Poulos, R. & Howes, L. G. (1997). Blood pressure changes in acute cerebral infarction and hemorrhage. *Stroke, 28,* 1401-1405.

Moss, A. J. and McDonald, J. (1971). Unilateral cervicothoracic sympathetic ganglionectomy for the treatment of long QT interval syndrome. *N.Engl.J.Med., 285,* 903-904.

Murry, C. E., Jennings, R. B. & Reimer, K. A. (1986). Preconditioning with ischemia: a delay of lethal cell injury in ischemic myocardium. *Circulation, 74,* 1124-1136.

Myers, M. G., Norris, J. W., Hachniski, V. C. & Sole, M. J. (1981). Plasma norepinephrine in stroke. *Stroke, 12,* 200-204.

Nakata, Y., Goto, T. & Morita, S. (1997). Comparison of inhalation inductions with xenon and sevoflurane. *Acta Anaesthesiol.Scand., 41,* 1157-1161.

Nandagopal, K., Dawson, T. M. & Dawson, V. L. (2001). Critical role for nitric oxide signaling in cardiac and neuronal ischemic preconditioning and tolerance. *J.Pharmacol.Exp.Ther., 297,* 474-478.

Naver, H. K., Blomstrand, C. & Wallin, B. G. (1996). Reduced heart rate variability after right-sided stroke. *Stroke, 27,* 247-251.

Nelson, K. B., Dambrosia, J. M., Grether, J. K. & Phillips, T. M. (1998). Neonatal cytokines and coagulation factors in children with cerebral palsy. *Ann.Neurol., 44,* 665-675.

Norris, J. W. (1983). Effects of cerebrovascular lesions on the heart. *Neurol.Clin., 1,* 87-101.

Norris, J. W., Froggatt, G. M. & Hachinski, V. C. (1978). Cardiac arrhythmias in acute stroke. *Stroke, 9,* 392-396.

Norris, J. W., Hachinski, V. C., Myers, M. G., Callow, J., Wong, T. & Moore, R. W. (1979). Serum cardiac enzymes in stroke. *Stroke, 10,* 548-553.

Ohtsuki, T., Ruetzler, C. A., Tasaki, K. & Hallenbeck, J. M. (1996). Interleukin-1 mediates induction of tolerance to global ischemia in gerbil hippocampal CA1 neurons. *J.Cereb.Blood Flow Metab, 16,* 1137-1142.

Oppenheimer, S. M. (1990). Plasma cortisol as a measure of stress response in acute stroke. *Stroke, 21,* 1376.

Oppenheimer, S. M. (1994). Neurogenic cardiac effects of cerebrovascular disease. *Curr.Opin.Neurol., 7,* 20-24.

Oppenheimer, S. M. & Cechetto, D. F. (1990). Cardiac chronotropic organization of the rat insular cortex. *Brain Res., 533,* 66-72.

Oppenheimer, S. M., Cechetto, D. F. & Hachinski, V. C. (1990). Cerebrogenic cardiac arrhythmias. Cerebral electrocardiographic influences and their role in sudden death. *Arch.Neurol., 47,* 513-519.

Oppenheimer, S. M. & Hachinski, V. C. (1992). The cardiac consequences of stroke. *Neurol.Clin., 10,* 167-176.

Oral, H., Dorn, G. W. & Mann, D. L. (1997). Sphingosine mediates the immediate negative inotropic effects of tumor necrosis factor-alpha in the adult mammalian cardiac myocyte. *J.Biol.Chem., 272,* 4836-4842.

Orlandi, G., Fanucchi, S., Strata, G., Pataleo, L., Landucci, P. L., Prontera, C., et al. (2000). Transient autonomic nervous system dysfunction during hyperacute stroke. *Acta Neurol.Scand., 102,* 317-321.

Osborne, N. N., Wood, J. P., Chidlow, G., Casson, R., DeSantis, L. & Schmidt, K. G. (2004). Effectiveness of levobetaxolol and timolol at blunting retinal ischaemia is related to their calcium and sodium blocking activities: relevance to glaucoma. *Brain Res.Bull., 62,* 525-528.

Oygur, N., Sonmez, O., Saka, O. & Yegin, O. (1998). Predictive value of plasma and cerebrospinal fluid tumour necrosis factor-alpha and interleukin-1 beta concentrations on outcome of full term infants with hypoxic-ischaemic encephalopathy. *Arch.Dis.Child Fetal Neonatal Ed, 79,* F190-F193.

Paoletti, R., Gotto, A. M. & Jr., Hajjar, D. P. (2004). Inflammation in atherosclerosis and implications for therapy. *Circulation, 109,* III20-III26.

Pavlov, V. A. and Tracey, K. J. (2005). The cholinergic anti-inflammatory pathway. *Brain Behav.Immun., 19,* 493-499.

Pedersen, E. D., Waje-Andreassen, U., Vedeler, C. A., Aamodt, G. & Mollnes, T. E. (2004). Systemic complement activation following human acute ischaemic stroke. *Clin.Exp.Immunol., 137,* 117-122.

Peeters-Scholte, C., Koster, J., van den, T. E., Blomgren, K., Hamers, N., Zhu, C., et al. (2002). Effects of selective nitric oxide synthase inhibition on IGF-1, caspases and cytokines in a newborn piglet model of perinatal hypoxia-ischaemia. *Dev.Neurosci., 24,* 396-404.

Pelidou, S. H., Kostulas, N., Matusevicius, D., Kivisakk, P., Kostulas, V. & Link, H. (1999). High levels of IL-10 secreting cells are present in blood in cerebrovascular diseases. *Eur.J.Neurol., 6,* 437-442.

Pendleton, R. G., Newman, D. J., Sherman, S. S., Brann, E. G. & Maya, W. E. (1972). Effect of propranolol upon the hemoglobin-oxygen dissociation curve. *J.Pharmacol.Exp.Ther., 180,* 647-656.

Perini, F., Morra, M., Alecci, M., Galloni, E., Marchi, M. & Toso, V. (2001). Temporal profile of serum anti-inflammatory and pro-inflammatory interleukins in acute ischemic stroke patients. *Neurol.Sci., 22,* 289-296.

Perlman, J. M. (1998). White matter injury in the preterm infant: an important determination of abnormal neurodevelopment outcome. *Early Hum.Dev., 53,* 99-120.

Perlman, J. M., Tack, E. D., Martin, T., Shackelford, G. & Amon, E. (1989). Acute systemic organ injury in term infants after asphyxia. *Am.J.Dis.Child, 143,* 617-620.

Petzelt, C., Blom, P., Schmehl, W., Muller, J. & Kox, W. J. (2003). Prevention of neurotoxicity in hypoxic cortical neurons by the noble gas xenon. *Life Sci., 72,* 1909-1918.

Phelan, J. P., Ahn, M. O., Korst, L., Martin, G. I. & Wang, Y. M. (1998). Intrapartum fetal asphyxial brain injury with absent multiorgan system dysfunction. *J.Matern.Fetal Med., 7,* 19-22.

Popovic, R., Liniger, R. & Bickler, P. E. (2000). Anesthetics and mild hypothermia similarly prevent hippocampal neuron death in an in vitro model of cerebral ischemia. *Anesthesiology, 92,* 1343-1349.

Pratico, D., MY, L., V, Trojanowski, J. Q., Rokach, J. & Fitzgerald, G. A. (1998). Increased F2-isoprostanes in Alzheimer's disease: evidence for enhanced lipid peroxidation in vivo. *FASEB J., 12,* 1777-1783.

Preckel, B., Weber, N. C., Sanders, R. D., Maze, M. & Schlack, W. (2006). Molecular mechanisms transducing the anesthetic, analgesic & organ-protective actions of xenon. *Anesthesiology, 105,* 187-197.

Puleo, P. R., Guadagno, P. A., Roberts, R., Scheel, M. V., Marian, A. J., Churchill, D., et al. (1990). Early diagnosis of acute myocardial infarction based on assay for subforms of creatine kinase-MB. *Circulation, 82,* 759-764.

Rapaport, S. I. & Rao, L. V. (1992). Initiation and regulation of tissue factor-dependent blood coagulation. *Arterioscler.Thromb., 12,* 1111-1121.

Ridker, P. M. (2001). High-sensitivity C-reactive protein: potential adjunct for global risk assessment in the primary prevention of cardiovascular disease. *Circulation, 103,* 1813-1818.

Robinson, T. G., Dawson, S. L., Eames, P. J., Panerai, R. B. & Potter, J. F. (2003). Cardiac baroreceptor sensitivity predicts long-term outcome after acute ischemic stroke. *Stroke, 34,* 705-712.

Saliba, E. & Henrot, A. (2001). Inflammatory mediators and neonatal brain damage. *Biol.Neonate, 79,* 224-227.

Sanchez-Moreno, C., Dashe, J. F., Scott, T., Thaler, D., Folstein, M. F. & Martin, A. (2004). Decreased levels of plasma vitamin C and increased concentrations of inflammatory and oxidative stress markers after stroke. *Stroke, 35,* 163-168.

Sander, D., Winbeck, K., Klingelhofer, J., Etgen, T. & Conrad, B. (2001). Prognostic relevance of pathological sympathetic activation after acute thromboembolic stroke. *Neurology, 57,* 833-838.

Sandercock, P., Bamford, J., Dennis, M., Burn, J., Slattery, J., Jones, L., et al. (1992). Atrial fibrillation and stroke: prevalence in different types of stroke and influence on early and long term prognosis (Oxfordshire community stroke project). *BMJ, 305,* 1460-1465.

Savitz, S. I., Erhardt, J. A., Anthony, J. V., Gupta, G., Li, X., Barone, F. C., et al. (2000). The novel beta-blocker, carvedilol, provides neuroprotection in transient focal stroke. *J.Cereb.Blood Flow Metab, 20,* 1197-1204.

Savman, K., Blennow, M., Gustafson, K., Tarkowski, E. & Hagberg, H. (1998). Cytokine response in cerebrospinal fluid after birth asphyxia. *Pediatr.Res., 43,* 746-751.

Schindler, R., Mancilla, J., Endres, S., Ghorbani, R., Clark, S. C. & Dinarello, C. A. (1990). Correlations and interactions in the production of interleukin-6 (IL-6), IL-1 & tumor necrosis factor (TNF) in human blood mononuclear cells: IL-6 suppresses IL-1 and TNF. *Blood, 75,* 40-47.

Schmidt, M., Marx, T., Gloggl, E., Reinelt, H. & Schirmer, U. (2005). Xenon attenuates cerebral damage after ischemia in pigs. *Anesthesiology, 102,* 929-936.

Schwartz, P. J., Locati, E. H., Moss, A. J., Crampton, R. S., Trazzi, R. & Ruberti, U. (1991). Left cardiac sympathetic denervation in the therapy of congenital long QT syndrome. A worldwide report. *Circulation, 84,* 503-511.

Schwartz, P. J., Snebold, N. G. & Brown, A. M. (1976). Effects of unilateral cardiac sympathetic denervation on the ventricular fibrillation threshold. *Am.J.Cardiol., 37,* 1034-1040.

Shah, P., Riphagen, S., Beyene, J. & Perlman, M. (2004). Multiorgan dysfunction in infants with post-asphyxial hypoxic-ischaemic encephalopathy. *Arch.Dis.Child Fetal Neonatal Ed, 89,* F152-F155.

Shalaby, M. R., Waage, A., Aarden, L. & Espevik, T. (1989). Endotoxin, tumor necrosis factor-alpha and interleukin 1 induce interleukin 6 production in vivo. *Clin.Immunol.Immunopathol., 53,* 488-498.

Sharpe, P. C., Mulholland, C. & Trinick, T. (1994). Ascorbate and malondialdehyde in stroke patients. *Ir.J.Med.Sci., 163,* 488-491.

Silveira, R. C. and Procianoy, R. S. (2003). Interleukin-6 and tumor necrosis factor-alpha levels in plasma and cerebrospinal fluid of term newborn infants with hypoxic-ischemic encephalopathy. *J.Pediatr., 143,* 625-629.

Silver, F. L., Norris, J. W., Lewis, A. J. & Hachinski, V. C. (1984). Early mortality following stroke: a prospective review. *Stroke, 15,* 492-496.

Silvestre, J. S., Mallat, Z., Tamarat, R., Duriez, M., Tedgui, A. & Levy, B. I. (2001). Regulation of matrix metalloproteinase activity in ischemic tissue by interleukin-10: role in ischemia-induced angiogenesis. *Circ.Res., 89,* 259-264.

Simi, A., Ingelman-Sundberg, M. & Tindberg, N. (2000). Neuroprotective agent chlomethiazole attenuates c-fos, c-jun & AP-1 activation through inhibition of p38 MAP kinase. *J.Cereb.Blood Flow Metab, 20,* 1077-1088.

Simi, A., Porsmyr-Palmertz, M., Hjerten, A., Ingelman-Sundberg, M. & Tindberg, N. (2002). The neuroprotective agents chlomethiazole and SB203580 inhibit IL-1beta signalling but not its biosynthesis in rat cortical glial cells. *J.Neurochem., 83,* 727-737.

Sinclair, R., Eriksson, A. S., Gretzer, C., Cassuto, J. & Thomsen, P. (1993). Inhibitory effects of amide local anaesthetics on stimulus-induced human leukocyte metabolic activation, LTB4 release and IL-1 secretion in vitro. *Acta Anaesthesiol.Scand., 37,* 159-165.

Smith, C. J., Emsley, H. C., Gavin, C. M., Georgiou, R. F., Vail, A., Barberan, E. M., et al. (2004). Peak plasma interleukin-6 and other peripheral markers of inflammation in the first week of ischaemic stroke correlate with brain infarct volume, stroke severity and long-term outcome. *BMC.Neurol., 4,* 2.

Smith, K. E., Hachinski, V. C., Gibson, C. J. & Ciriello, J. (1986). Changes in plasma catecholamine levels after insula damage in experimental stroke. *Brain Res., 375,* 182-185.

Sozzani, S., Agwu, D. E., McCall, C. E., O'Flaherty, J. T., Schmitt, J. D., Kent, J. D., et al. (1992). Propranolol, a phosphatidate phosphohydrolase inhibitor, also inhibits protein kinase C. *J.Biol.Chem., 267,* 20481-20488.

Spranger, M., Krempien, S., Schwab, S., Donneberg, S. & Hacke, W. (1997). Superoxide dismutase activity in serum of patients with acute cerebral ischemic injury. Correlation with clinical course and infarct size. *Stroke, 28,* 2425-2428.

Stowe, D. F., Rehmert, G. C., Kwok, W. M., Weigt, H. U., Georgieff, M. & Bosnjak, Z. J. (2000). Xenon does not alter cardiac function or major cation currents in isolated guinea pig hearts or myocytes. *Anesthesiology, 92,* 516-522.

Strittmatter, M., Meyer, S., Fischer, C., Georg, T. & Schmitz, B. (2003). Location-dependent patterns in cardio-autonomic dysfunction in ischaemic stroke. *Eur.Neurol., 50,* 30-38.

Sutherland, B. A., Shaw, O. M., Clarkson, A. N., Jackson, D. N., Sammut, I. A. & Appleton, I. (2005). Neuroprotective effects of (-)-epigallocatechin gallate following hypoxia-ischemia-induced brain damage: novel mechanisms of action. *FASEB J., 19,* 258-260.

Szaflarski, J., Burtrum, D. & Silverstein, F. S. (1995). Cerebral hypoxia-ischemia stimulates cytokine gene expression in perinatal rats. *Stroke, 26,* 1093-1100.

Takao, Y., Mikawa, K., Nishina, K., Maekawa, N. & Obara, H. (1996). Lidocaine attenuates hyperoxic lung injury in rabbits. *Acta Anaesthesiol.Scand., 40,* 318-325.

Talman, W. T. (1985). Cardiovascular regulation and lesions of the central nervous system. *Ann.Neurol., 18,* 1-13.

Tarkowski, E., Rosengren, L., Blomstrand, C., Jensen, C., Ekholm, S. & Tarkowski, A. (1999). Intrathecal expression of proteins regulating apoptosis in acute stroke. *Stroke, 30,* 321-327.

Tarkowski, E., Rosengren, L., Blomstrand, C., Wikkelso, C., Jensen, C., Ekholm, S., et al. (1995). Early intrathecal production of interleukin-6 predicts the size of brain lesion in stroke. *Stroke, 26,* 1393-1398.

Tarkowski, E., Rosengren, L., Blomstrand, C., Wikkelso, C., Jensen, C., Ekholm, S., et al. (1997). Intrathecal release of pro- and anti-inflammatory cytokines during stroke. *Clin.Exp.Immunol., 110,* 492-499.

Tasaki, K., Ruetzler, C. A., Ohtsuki, T., Martin, D., Nawashiro, H. & Hallenbeck, J. M. (1997). Lipopolysaccharide pre-treatment induces resistance against subsequent focal cerebral ischemic damage in spontaneously hypertensive rats. *Brain Res., 748,* 267-270.

Tedgui, A. and Mallat, Z. (2001). Anti-inflammatory mechanisms in the vascular wall. *Circ.Res., 88,* 877-887.

Terkeltaub, R., Boisvert, W. A. & Curtiss, L. K. (1998). Chemokines and atherosclerosis. *Curr.Opin.Lipidol., 9,* 397-405.

Tipping, P. G., Malliaros, J. & Holdsworth, S. R. (1989). Procoagulant activity expression by macrophages from atheromatous vascular plaques. *Atherosclerosis, 79,* 237-243.

Tomai, F., Crea, F., Chiariello, L. & Gioffre, P. A. (1999). Ischemic preconditioning in humans: models, mediators & clinical relevance. *Circulation, 100,* 559-563.

Tracey, K. J. (2002). The inflammatory reflex. *Nature, 420,* 853-859.

Ulich, T. R., del, C. J. & Guo, K. Z. (1989). In vivo hematologic effects of recombinant interleukin-6 on hematopoiesis and circulating numbers of RBCs and WBCs. *Blood, 73,* 108-110.

van den Tweel, E. R., Peeters-Scholte, C. M., van, B. F., Heijnen, C. J. & Groenendaal, F. (2002). Inhibition of nNOS and iNOS following hypoxia-ischaemia improves long-term outcome but does not influence the inflammatory response in the neonatal rat brain. *Dev.Neurosci., 24,* 389-395.

van, E. E., Gussekloo, J., de Craen, A. J., Bootsma-van der, W. A., Frolich, M. & Westendorp, R. G. (2002). Inflammation and stroke: the Leiden 85-Plus Study. *Stroke, 33,* 1135-1138.

Vandenabeele, P., Declercq, W., Beyaert, R. & Fiers, W. (1995). Two tumour necrosis factor receptors: structure and function. *Trends Cell Biol., 5,* 392-399.

Vila, N., Castillo, J., Davalos, A. & Chamorro, A. (2000). Proinflammatory cytokines and early neurological worsening in ischemic stroke. *Stroke, 31,* 2325-2329.

Vila, N., Castillo, J., Davalos, A., Esteve, A., Planas, A. M. & Chamorro, A. (2003). Levels of anti-inflammatory cytokines and neurological worsening in acute ischemic stroke. *Stroke, 34,* 671-675.

Vinten-Johansen, J., Zhao, Z. Q., Zatta, A. J., Kin, H., Halkos, M. E. & Kerendi, F. (2005). Postconditioning--A new link in nature's armor against myocardial ischemia-reperfusion injury. *Basic Res.Cardiol., 100,* 295-310.

Wallace, J. D. and Levy, L. L. (1981). Blood pressure after stroke. *JAMA, 246,* 2177-2180.

Wang, H., Yu, M., Ochani, M., Amella, C. A., Tanovic, M., Susarla, S., et al. (2003). Nicotinic acetylcholine receptor alpha7 subunit is an essential regulator of inflammation. *Nature, 421,* 384-388.

Wang, X., Ellison, J. A., Siren, A. L., Lysko, P. G., Yue, T. L., Barone, F. C., et al. (1998). Prolonged expression of interferon-inducible protein-10 in ischemic cortex after permanent occlusion of the middle cerebral artery in rat. *J.Neurochem., 71,* 1194-1204.

Wang, X., Li, X., Currie, R. W., Willette, R. N., Barone, F. C. & Feuerstein, G. Z. (2000). Application of real-time polymerase chain reaction to quantitate induced expression of interleukin-1beta mRNA in ischemic brain tolerance. *J.Neurosci.Res., 59,* 238-246.

Wang, X., Li, X., Erhardt, J. A., Barone, F. C. & Feuerstein, G. Z. (2000). Detection of tumor necrosis factor-alpha mRNA induction in ischemic brain tolerance by means of real-time polymerase chain reaction. *J.Cereb.Blood Flow Metab, 20,* 15-20.

Wang, X., Li, X., Yaish-Ohad, S., Sarau, H. M., Barone, F. C. & Feuerstein, G. Z. (1999). Molecular cloning and expression of the rat monocyte chemotactic protein-3 gene: a possible role in stroke. *Brain Res.Mol.Brain Res., 71,* 304-312.

Wang, Y. N., Che, S. M. & Ma, A. Q. (2004). Clinical significance of serum cytokines IL-1beta, sIL-2R, IL-6, TNF-alpha & IFN-v in acute coronary syndrome. *Chin Med.Sci.J., 19,* 120-124.

Warner, D. S., Ludwig, P. S., Pearlstein, R. & Brinkhous, A. D. (1995). Halothane reduces focal ischemic injury in the rat when brain temperature is controlled. *Anesthesiology, 82,* 1237-1245.

Warner, D. S., McFarlane, C., Todd, M. M., Ludwig, P. & McAllister, A. M. (1993). Sevoflurane and halothane reduce focal ischemic brain damage in the rat. Possible influence on thermoregulation. *Anesthesiology, 79,* 985-992.

Warner, D. S., Takaoka, S., Wu, B., Ludwig, P. S., Pearlstein, R. D., Brinkhous, A. D., et al. (1996). Electroencephalographic burst suppression is not required to elicit maximal neuroprotection from pentobarbital in a rat model of focal cerebral ischemia. *Anesthesiology, 84,* 1475-1484.

Wegener, S., Gottschalk, B., Jovanovic, V., Knab, R., Fiebach, J. B., Schellinger, P. D., et al. (2004). Transient ischemic attacks before ischemic stroke: preconditioning the human brain? A multicenter magnetic resonance imaging study. *Stroke, 35,* 616-621.

Weiss, G., Willeit, J., Kiechl, S., Fuchs, D., Jarosch, E., Oberhollenzer, F., et al. (1994). Increased concentrations of neopterin in carotid atherosclerosis. *Atherosclerosis, 106,* 263-271.

Wells, B. A., Keats, A. S. & Cooley, D. A. (1963). Increased tolerance to cerebral ischemia produced by general anesthesia during temporary carotid occlusion. *Surgery, 54,* 216-223.

Werner, C., Mollenberg, O., Kochs, E. & Schulte, J. a. E. (1995). Sevoflurane improves neurological outcome after incomplete cerebral ischaemia in rats. *Br.J.Anaesth., 75,* 756-760.

Wilhelm, S., Ma, D., Maze, M. & Franks, N. P. (2002). Effects of xenon on in vitro and in vivo models of neuronal injury. *Anesthesiology, 96,* 1485-1491.

Xu, H., Barks, J. D., Schielke, G. P. & Silverstein, F. S. (2001). Attenuation of hypoxia-ischemia-induced monocyte chemoattractant protein-1 expression in brain of neonatal mice deficient in interleukin-1 converting enzyme. *Brain Res.Mol.Brain Res., 90,* 57-67.

Yang, H. M., Yao, Y. J. & Li, W. R. (2004). [Injury of myocardial mitochondria in neonatal swines with hypoxic-ischemic brain damage]. *Sichuan.Da.Xue.Xue. Bao.Yi.Xue.Ban., 35,* 39-41.

Yang, X. M., Proctor, J. B., Cui, L., Krieg, T., Downey, J. M. & Cohen, M. V. (2004). Multiple, brief coronary occlusions during early reperfusion protect rabbit hearts by targeting cell signaling pathways. *J.Am.Coll.Cardiol., 44,* 1103-1110.

Yasui, Y., Breder, C. D., Saper, C. B. & Cechetto, D. F. (1991). Autonomic responses and efferent pathways from the insular cortex in the rat. *J.Comp Neurol., 303,* 355-374.

Yellon, D. M. & Dana, A. (2000). The preconditioning phenomenon: A tool for the scientist or a clinical reality? *Circ.Res., 87,* 543-550.

Yin, L., Ohtaki, H., Nakamachi, T., Dohi, K., Iwai, Y., Funahashi, H., et al. (2003). Expression of tumor necrosis factor alpha (TNFalpha) following transient cerebral ischemia. *Acta Neurochir.Suppl, 86,* 93-96.

Yokoyama, T., Vaca, L., Rossen, R. D., Durante, W., Hazarika, P. & Mann, D. L. (1993). Cellular basis for the negative inotropic effects of tumor necrosis factor-alpha in the adult mammalian heart. *J.Clin.Invest, 92,* 2303-2312.

Yue, T. L., Ma, X. L., Wang, X., Romanic, A. M., Liu, G. L., Louden, C., et al. (1998). Possible involvement of stress-activated protein kinase signaling pathway and Fas receptor expression in prevention of ischemia/reperfusion-induced cardiomyocyte apoptosis by carvedilol. *Circ.Res., 82,* 166-174.

Yue, T. L., McKenna, P. J., Lysko, P. G., Gu, J. L., Lysko, K. A., Ruffolo, Jr., R. R., et al. (1994). SB 211475, a metabolite of carvedilol, a novel antihypertensive agent, is a potent antioxidant. *Eur.J.Pharmacol., 251,* 237-243.

Zhao, P. & Zuo, Z. (2004). Isoflurane preconditioning induces neuroprotection that is inducible nitric oxide synthase-dependent in neonatal rats. *Anesthesiology, 101,* 695-703.

Zhao, Z. Q., Corvera, J. S., Halkos, M. E., Kerendi, F., Wang, N. P., Guyton, R. A., et al. (2003). Inhibition of myocardial injury by ischemic postconditioning during reperfusion: comparison with ischemic preconditioning. *Am.J.Physiol Heart Circ.Physiol, 285,* H579-H588.

Zheng, S. & Zuo, Z. (2004). Isoflurane preconditioning induces neuroprotection against ischemia via activation of P38 mitogen-activated protein kinases. *Mol.Pharmacol., 65,* 1172-1180.

Zheng, S. & Zuo, Z. (2003). Isoflurane preconditioning reduces purkinje cell death in an in vitro model of rat cerebellar ischemia. *Neuroscience, 118,* 99-106.

In: Horizons in World Cardiovascular Research. Volume 1 ISBN: 978-1-60876-447-1
Editor: Eleanor H. Bennington, pp. 177-207

Chapter XI

The Origin and Role of N-Homocysteinylated Proteins in Cardiovascular Disease

Hieronim Jakubowski[2]
Department of Microbiology and Molecular Genetics, UMDNJ-New Jersey Medical School, International Center for Public Health, Newark, NJ 07101, USA, and Institute of Bioorganic Chemistry, Polish Academy of Sciences, Poznań, Poland

Abstract

The non-protein amino acid homocysteine (Hcy), a metabolite of the essential amino acid methionine, is implicated in the pathology of human cardiovascular and neurodegenerative diseases. In addition to its elimination by the remethylation and transsulfuration pathways, Hcy is also metabolized to the thioester Hcy-thiolactone in an error-editing reaction in protein biosynthesis when Hcy is mistakenly selected in place of methionine by methionyl-tRNA synthetase. In humans, the accumulation of Hcy-thiolactone can be detrimental because of its intrinsic ability to modify proteins by forming *N*-Hcy-protein adducts, in which a carboxyl group of Hcy is *N*-linked to ε-amino group of a protein lysine residue. *N*-linked Hcy occurs in each protein examined and constitutes a significant pool of Hcy in human blood. *N*-Hcy proteins are recognized as neo-self antigens and induce an auto-immune response. As a result, IgG and IgM anti-*N*-Hcy-protein auto-antibodies, are produced in humans. Serum levels of anti-*N*-Hcy-protein IgG auto-antibodies are positively correlated with plasma total Hcy, but not with plasma cysteine or methionine levels, which is consistent with the etiology of these auto-antibodies. In a group of male patients with stroke, the levels of anti-*N*-Hcy-protein IgG auto-antibodies and total Hcy are significantly higher than in a group of healthy subjects. In a group of male patients with angiographically documented coronary artery disease, seropositivity for anti-*N*-Hcy-protein IgG auto-antibodies occurs 5-times more frequently

[2] Corresponding author: Hieronim Jakubowski, Ph. D. Department of Microbiology and Molecular Genetics UMDNJ-New Jersey Medical School International Center for Public Health 225 Warren Street Newark, NJ 07101-1709, USA, Phone: 973-972-4483 Fax: 973-972-8981 E-mail:jakubows@umdnj.edu.

than in controls and is an independent predictor of coronary artery disease. These findings show that an auto-immune response against *N*-Hcy-proteins is a general feature of atherosclerosis and provide support for a hypothesis that *N*-Hcy-protein is a neo-self antigen, which contributes to immune activation, an important modulator of atherogenesis. Plasma Hcy lowering by folic acid administration leads to significant decreases in anti-*N*-Hcy-protein IgG auto-antibody levels in control subjects, but not in coronary artery disease patients. The results of these Hcy-lowering treatments suggest that, while primary Hcy-lowering intervention is beneficial, secondary Hcy-lowering intervention in coronary artery disease patients may be ineffective in reducing the advanced damage caused by Hcy, and may explain at least in part the failure of vitamin therapy to lower cardiovascular events in recent Hcy-lowering trials. Chronic activation of immune responses towards *N*-Hcy-protein associated with hyperhomocysteinemia over many years would lead to vascular disease.

Keywords: autoantibodies; atherosclerosis; coronary artery disease; Hyperhomocysteinemia; homocysteine thiolactone hypothesis; protein N-homocysteinylation; stroke

Introduction

Cardiovascular disease is a major cause of morbidity and mortality in industrial nations.

Despite advances in our understanding of cardiovascular disease, traditional risk factors such as hyperlipidemia, hypertension, smoking, and diabetes do not accurately predict cardiovascular events and over half of all coronary events occur in persons without overt hyperlipidemia [1; 2; 3; 4]. Thus, a search continues for new markers and strategies to guide the development of novel antiatherosclerotic therapies beyond low-density lipoprotein (LDL) cholesterol reduction. Although atherosclerosis has been viewed as a lipid storage disease [5], the growing body of evidence suggests that inflammation participates in all stages of atherosclerosis from the initial lesion to the end-stage thrombotic complications [2; 6; 7; 8; 9; 10; 11]. The principal culprits responsible for the initiation of inflammation appear to be proteins modified by products of lipid peroxidation or by glucose, particularly oxidized or glycated LDL. Modified LDL induces both innate and adaptive immune responses, and autoantibodies against modified LDL are present in atherosclerotic plaques and in circulation [12; 13]. Among other inducers of inflammation is homocysteine (Hcy) [14], a non-traditional risk factor for vascular disease [15]. A mechanism by which Hcy induces an adaptive immune response is a topic of this chapter.

Severe hyperhomocysteinemia secondary to mutations in the *CBS*, *MTHFR*, or *MS* gene causes pathologies in multiple organs, including the cardiovascular system and the brain, and leads to premature death due to vascular complications [16; 17; 18]. McCully observed advanced arterial lesions in children with inborn errors in Hcy metabolism and proposed that Hcy causes vascular disease [19]. Although severe hyperhomocysteinemia is rare, mild hyperhomocysteinemia is quite prevalent in the general population and is associated with an increased risk of vascular [20] and neurological complications [21; 22], and predicts mortality in heart disease patients [23]. The strongest evidence that Hcy plays a causal role in atherothrombosis comes from the studies of severe genetic hyperhomocysteinemia in humans and the finding that Hcy-lowering by vitamin-B supplementation greatly improves vascular

outcomes in CBS deficient patients [16; 17; 18]. For example, untreated CBS-deficient patients suffer 1 vascular event per 25 patient-years [16] while vitamin-B-treated CBS-deficient patients suffer only 1 vasular event per 263 patient-years (relative risk 0.091, $p<0.001$) [18]. Hcy-lowering therapy started early in life also prevents brain disease from severe MTHFR deficiency [24]. Furthermore, studies of genetic and nutritional hyperhomocysteinemia in animal models also provide a strong support for a causative role of Hcy [14; 25; 26]. In humans, lowering plasma Hcy by vitamin-B supplementation improves cognitive function in the general population [27] and leads to a 21-24% reduction of vascular outcomes in high risk stroke patients [28; 29], but not in myocardial infarction (MI) patients [29; 30]. Hcy-lowering trials are currently ongoing, and the results of these trials are required before making recommendations on the use of vitamins for prevention of vascular disease [31].

Atherosclerosis, a disease of the vascular wall, is initiated by endothelial damage. Endothelial dysfunction, immune activation, and thrombosis, characteristic features of vascular disease [8], are all observed in hyperhomocysteinemia in humans [16] and experimental animals [26]. The degree of impairment in endothelial function during hyperhomocysteinemia is similar to that observed with hypercholesterolemia. Multiple mechanisms, such as protein homocysteinylation, unfolded protein response, decreased bioavailability of nitric oxide, oxidative stress, altered cellular methylation and epigenetic regulation, and the induction of innate and adaptive immune responses appear to contribute to Hcy pathobiology in cardiovascular disease [14; 25; 26; 32; 33; 34; 35; 36; 37]. The Hcy-thiolactone hypothesis [36][37a, 37b] states that metabolic conversion of Hcy to Hcy-thiolactone, catalyzed by methionyl-tRNA synthetase (MetRS) (*Eq.* 1), followed by protein N-homocysteinylation by Hcy-thiolactone (*Eq*. 2), causes a variety of pathophysiological consequences including protein [38] and cell damage [39; 40; 41], enhanced thrombosis [42; 43], and induction of auto-immune responses [14; 35; 36]. In this chapter, I will discus the mechanism of formation of *N*-Hcy-proteins, new neo-self antigens derived from Hcy, summarize evidence for their presence in the human body, and describe their antigenic properties and emerging evidence for an important role of anti-*N*-Hcy-protein autoantibodies in vascular disease.

Overview of Homocysteine Metabolism

Homocysteine (Hcy) is a sulfur-containing amino acid that is found as an intermediary metabolite in all living organisms. In mammals Hcy is formed from dietary methionine (Met) as a result of cellular methylation reactions [16]. In this pathway, dietary Met is taken up by cells and then activated by ATP to yield S-adenosylmethionine (AdoMet), a universal methyl donor (Figure 1). As a result of the transfer of its methyl group to an acceptor, AdoMet is converted to S-adenosylhomocysteine (AdoHcy). The reversible enzymatic hydrolysis of AdoHcy is the only known source of Hcy in the human body. Levels of Hcy are regulated by remethylation to Met, catalyzed by the enzyme Met synthase (MS), and transsulfuration to cysteine, the first step of which is catalyzed by the enzyme cystathionine β-synthase (CBS). The remethylation requires vitamin B_{12} and 5,10-methyl-tetrahydrofolate (CH_3-THF),

generated by 5,10-methylene-THF reductase (MTHFR). The transsulfuration requires vitamin B_6.

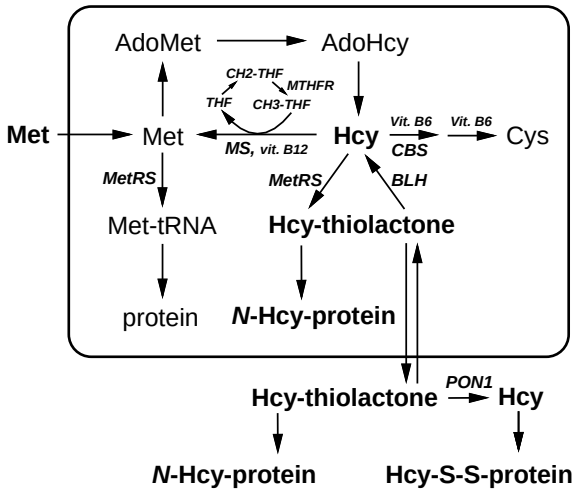


Figure 1. Neo-self antigen, protein N-linked Hcy (N-Hcy-protein), is a byproduct of Hcy metabolism in humans.

A fraction of Hcy is also metabolized by MetRS to a thioester, Hcy-thiolactone (Figure 1), in an error-editing reaction in protein biosynthesis when Hcy is mistakenly selected in place of Met [44; 45; 46; 47; 48]. The flow through the Hcy-thiolactone pathway is increased by a high-Met diet [49], inadequate supply of CH_3-THF [49; 50; 51], or impairment of re-methylation or trans-sulfuration reactions by genetic alterations of enzymes, such as CBS [49; 50; 52; 53], MS [52; 53], and MTHFR [49]. Because of its exceptionally low pK_a value (Table 1), Hcy-thiolactone is neutral at physiological pH and thus can diffuse out of the cell (Figure 1) and accumulate in the extracellular fluids [49; 50; 51; 54; 55]. Hcy-thiolactone is hydrolyzed to Hcy by intracellular [56] and extracellular Hcy-thiolactonases [57; 58; 59; 60], previously known as bleomycin hydrolase (BLH) and paraoxonase 1 (PON1), respectively. Because of the oxidative environment in the blood, extracellular Hcy forms disulfides, mostly with serum proteins [38; 57] such as albumin [34] and globulins [61], and only ~1% of plasma total Hcy exists in a free reduced form in humans [62]. Furthermore, as discussed in a greater detail in the following sections of this chapter, Hcy-thiolactone reacts spontaneously with proteins, forming *N*-Hcy-proteins (Figure 1), which are recognized as neo-self antigens by the immune system.

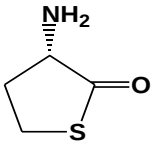
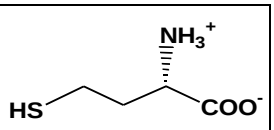
The Mechanism of Protein N-Homocysteinylation

Fundamental physical-chemical properties of Hcy (Table 1) underlie its ability to undergo metabolic conversion to Hcy-thiolactone. During protein biosynthesis Hcy is often mistakenly selected in place of Met by MetRS and metabolized to Hcy-thiolactone in an error-editing reaction according to Equation (1) [44; 55; 66].

$$\text{MetRS} + \text{Hcy} + \text{ATP} \Leftrightarrow \text{MetRS} \bullet \text{Hcy}\sim\text{AMP} \Rightarrow \text{Hcy-thiolactone} + \text{MetRS} \quad (1)$$

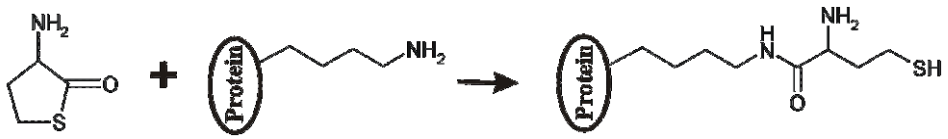
$-PP_i$ $-AMP$

Table 1. Physical-chemical properties of L-Hcy-thiolactone and L-Hcy [46]

Property	*L*-Hcy-thiolactone 	*L*-Hcy 
Chemical character	Aminoacyl-thioester	Mercaptoamino acid
UV spectrum	Yes, λ_{max} = 240 nm, ε = 5,000 M^{-1} cm^{-1}	No significant absorption at λ >220nm
Stability at 37°C, $t_{0.5}$ phosphate-saline human serum	 ~30 h ~1 h	 2 h 2 h
pK_a of amino group	6.67 [a]	9.04, 9.71 [b] 9.02, 9.69 (thiol group) [b]
Chemical reactivity	Acylates amino groups of protein lysine residues [c] Reacts with aldehydes to afford tetrahydrothiazines [a] Resistant to oxidation Base-hydrolyzed to Hcy	Condenses to Hcy-thiolactone Reacts with aldehydes to afford tetrahydrothiazines [a] Oxidized to disulfides Reacts with nitric oxide to afford *S*-nitroso-Hcy [d]

[a] Ref. [63], [b] Ref. [64], [c] Ref. [38; 50], [d] Ref. [65].

It should be noted that the high energy of the anhydrate bond of ATP is conserved in the thioester bond of Hcy-thiolactone, which is responsible for the chemical reactivity of Hcy-thiolactone (Table 1). Thus, Hcy-thiolactone spontaneously modifies proteins by forming *N*-Hcy-protein adducts, in which Hcy is *N*-linked to the ε-amino group of protein lysine residues as shown in Equation (2) [38; 46; 50].



(2)

These two reactions, studied extensively *in vitro* in model systems and *ex vivo* in cultured cells [38; 46; 47; 48; 50], are relevant *in vivo*, as demonstrated in humans and mice [49; 53; 54; 61; 67]. Protein N-homocysteinylation is a novel example of protein modification reaction that expands the biological repertoire of known protein modifications by other metabolites, such as glucose, products of lipid peroxidation, or certain drugs, such as penicillin or aspirin [38]. These protein modification reactions have two common aspects: a) each involves protein lysine residues as sites of modifications, and b) are linked to human pathological conditions, including diabetes, vascular disease, Alzheimer's disease, or drug

allergy or intolerance [38]. The primary focus of this chapter is on the mechanism of generation of protein N-linked Hcy epitopes in humans and their immunogenic properties.

The Molecular Mechanism of Hcy-Thiolactone Synthesis

In living organisms, the formation of Hcy-thiolactone is a consequence of error-editing reactions of aminoacyl-tRNA synthetases [44; 45; 47; 48; 68; 69; 70; 71]. Because of its similarity to protein amino acids Met, leucine, and isoleucine, the non-protein amino acid Hcy poses a selectivity problem in protein biosynthesis. Indeed, Hcy enters the first step of protein biosynthesis and forms Hcy-AMP with methionyl-, leucyl-, and isoleucyl-tRNA synthetases [72] [72a]. However, misactivated Hcy is not transferred to tRNA [65], and thus cannot enter the genetic code. Instead, Hcy-AMP is destroyed by editing activities of these aminoacyl-tRNA synthetases [44; 66], as shown in Equation (1). Hcy editing is universal, occurs in all organisms investigated, including bacteria [53; 72; 73; 74] [72a, yeast [52; 53; 75], plants [76], mice [36; 49], and humans [36; 49; 53; 54; 67], and prevents direct access of Hcy to the genetic code [44; 45; 46; 47; 48].

Although studied in several systems [77], molecular mechanism of Hcy editing is best understood for *E. coli* MetRS [78; 79; 80]. The Hcy editing reaction occurs in the synthetic/editing active site [78]], whose major function is to carry out the synthesis of Met-tRNA [80]. Whether an amino acid completes the synthetic or editing pathway is determined by the partitioning of its side chain between the specificity and thiol-binding sub-sites of the synthetic/editing active site [79]. A sub-site that binds carboxyl and α-amino groups of cognate or non-cognate substrates does not appear to contribute to specificity [78].

Methionine completes the synthetic pathway because its side chain is firmly bound by the hydrophobic and hydrogen bonding interactions with the specificity sub-site (Figure 2). Crystal structure of MetRS-Met complex [80] reveals that hydrophobic interactions involve side chains of Tyr15, Trp253, Pro257, and Tyr260; Trp305 closes the bottom of the hydrophobic pocket, but is not in the contact with the methyl group of the substrate methionine. The sulfur of the substrate methionine makes two hydrogen bonds: one with the hydroxyl of Tyr260 and the other with the backbone amide of Leu13.

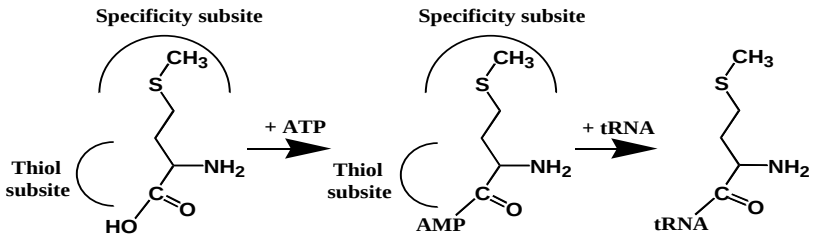


Figure 2. The aminoacylation of tRNA with Met catalyzed by MetRS.

The non-cognate substrate Hcy, missing the methyl group of methionine, cannot interact with the specificity sub-site as effectively as cognate methionine does. This allows the side

chain of Hcy to move to the thiol-binding sub-site, which promotes the synthesis of the thioester bond during editing (Figure 3). Mutations of Tyr15 and Trp305 affect Hcy/Met discrimination by the enzyme [78]. Asp52, which forms a hydrogen bond with the α-amino group of the substrate methionine, deduced from the crystal structure of MetRS·Met complex [80], is involved in the catalysis of both synthetic and editing reactions, but does not contribute to substrate specificity of the enzyme. The substitution Asp52Ala inactivates the synthetic and editing functions of MetRS [65; 78; 79].

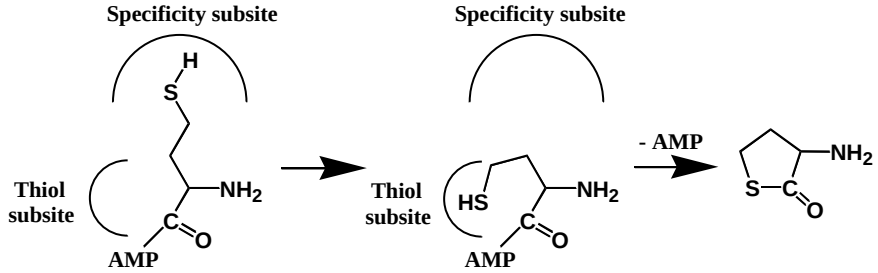


Figure 3. The formation of Hcy-thiolactone during Hcy editing catalyzed by MetRS.

Futrhermore, the thiol-binding sub-site also supports the ability of MetRS to edit in trans, *i.e.*, to catalyze thioester bond formation between a thiol and the cognate methionine (Figure 4). With CoA-SH or cysteine as a thiol substrate, MetRS catalyzes the formation of Met-*S*-CoA thioesters [81] and Met-Cys di-peptides [79], respectively. The formation of Met-Cys di-peptide proceeds *via* a Met-*S*-Cys thioester intermediate, which spontaneously rearranges to the Met-Cys di-peptide. Remarkably, the formation of Met-Cys di-peptide as a result of editing in trans, is as fast as the formation of Hcy-thiolactone during Hcy editing.

Hcy-Thiolactone is Synthesized by Methionyl-tRNA Synthetase in Human Cells

As discussed above, the biosynthesis of Hcy-thiolactone *via* the Hcy editing pathway has been originally discovered in microorganisms, such as *Escherichia coli* [73] and the yeast *Saccharomyces cerevisiae* [52]. The first indication that Hcy-thiolactone is a significant component of Hcy metabolism in mammals, including humans, came with the discovery that Hcy-thiolactone is synthesized by cultured mammalian cells, such as human cervical carcinoma (HeLa), mouse adenocarcinoma (RAG), and Chinese hamster ovary (CHO) [55]. We also demonstrated that a temperature-sensitive MetRS mutant of CHO cells fails to synthesize Hcy-thiolactone at the non-permissive temperature, which indicates that MetRS is involved in Hcy-thiolactone formation in CHO cells [55].

Subsequent work has shown that human diploid fibroblasts in which Hcy metabolism has been deregulated by mutations in the *CBS* gene produced more Hcy-thiolactone than wild type fibroblasts [50]. Furthermore, supplementation of CBS-deficient and wild type human fibroblasts, and human breast cancer (HTB-132) cells with the anti-folate drug aminopterin, which prevents remethylation of Hcy to methionine by methionine synthase, greatly enhances

Hcy-thiolactone synthesis. In general, human cancer cells produce more Hcy-thiolactone than normal cells [50; 55; 69].

Further experiments with cultured human umbilical vein vascular endothelial cells (HUVEC) suggest that Hcy-thiolactone synthesis is important in human vascular tissues [51]. These experiments have shown that in the presence of physiological concentrations of Hcy, methionine, and folic acid, HUVEC efficiently metabolize Hcy to Hcy-thiolactone. The extent of Hcy-thiolactone synthesis in human endothelial cells is directly proportional to Hcy, and inversely proportional to methionine, concentrations, consistent with the involvement of MetRS.

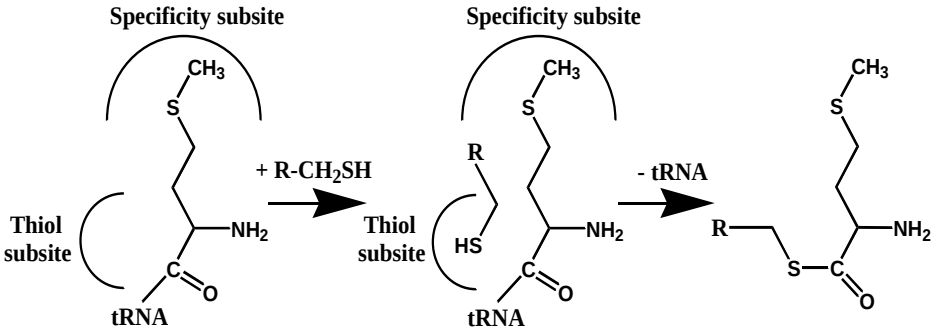


Figure 4. Editing in *trans*: The formation of methionyl thioesters catalyzed by MetRS.

Although folates are utilized in Hcy metabolism and DNA synthesis, it appears that folic acid limitation predominantly impacts Hcy metabolism, but not DNA metabolism, in endothelial cells. For example, physiological levels of folic acid (26 nM) present in the M199 media used in our studies are insufficient for transmethylation of Hcy to methionine and, as a result, Hcy is mostly converted to Hcy-thiolactone, while very little methionine is synthesized in these cells [51]. However, these levels of folic acid support endothelial cells growth when methionine is also present, which means that they are sufficient for DNA synthesis. Supplementation of endothelial cell cultures with folic acid redirects Hcy to the transmethylation pathway, which results in lower synthesis of Hcy-thiolactone and greater synthesis of methionine. The synthesis of Hcy-thiolactone in endothelial cell cultures is also inhibited by the supplementation with high-density lipoprotein (HDL) [51], which carries PON1 protein exhibiting Hcy-thiolactone hydrolyzing activity [57; 58; 59; 60].

Hcy-Thiolactone is Elevated in Hyperhomocysteinemic Humans and Mice

The findings that cultured human cells, including vascular endothelial cells, have the ability to metabolize Hcy to Hcy-thiolactone suggest that Hcy-thiolactone is likely to be synthesized *in vivo* in humans and animals. With the recent developments of highly selective and sensitive HPLC-based assays [53; 67], the demonstration of the *in vivo* relevance of Hcy-thiolactone became possible. In particular, the Hcy-thiolactone hypothesis [36] predicts that

Hcy-thiolactone will be elevated under conditions predisposing to vascular disease, such as hyperhomocysteinemia. As described in the following sections, this prediction has recently been confirmed *in vivo* in humans and mice.

Human Genetic Hyperhomocysteinemia

It is well established that genetic deficiencies in the *CBS* or *MTHFR* gene lead to great elevation of plasma tHcy levels in humans and mice [16]. However, it was not known whether these genetic deficiencies affect Hcy-thiolactone levels. To answer this question we studied 14 patients with homocystinuria due to homozygous mutations in the CBS gene, 4 patients with hyperhomocysteinemia due to a homozygous mutation in the *MTHFR* gene, 6 unaffected siblings heterozygous for the *MTHFR* mutation, and 9 healthy unrelated subjects. We found that the CBS deficiency in humans leads to elevation of Hcy-thiolactone levels: mean plasma Hcy-thiolactone concentration in CBS deficient patients (14.4 nM) was 72-fold higher than in normal subjects [49]. This finding is consistent with my previous *ex vivo* observations that cultured human CBS-deficient fibroblasts synthesize more Hcy-thiolactone than normal fibroblasts [50].

We also found that 5-methyltetrahydofolate deficiency, caused by the MTHFR mutation leads to elevation of Hcy-thiolactone levels in humans: plasma Hcy-thiolactone in MTHFR-deficient patients (11.8 nM) was 24- or 59-fold higher than in MTHFR heterozygous or normal individuals, respectively [49]. This *in vivo* finding is consistent with our previous *ex vivo* observations that limiting availability of folic acid greatly enhances Hcy-thiolactone synthesis in human fibroblasts [50] and vascular endothelial cells [51]. It should be noted that, because MTHFR-deficient patients, like CBS-deficient patients, were on Hcy-lowering therapy, their Hcy-thiolactone concentrations represent minimal values. In one patient for whom samples were obtained before therapy, the therapy resulted in lowering plasma Hcy-thiolactone from 47.3 nM to 16.6 nM (tHcy was lowered from 208 μM before therapy to 66.2 μM after therapy) [49].

Mouse Dietary Hyperhomocysteinemia

Feeding a high methionine diet over extended periods of time is often used as a useful model of experimental hyperhomocysteinemia and atherosclerosis [14; 25; 26]. We found that plasma and urinary Hcy-thiolactone levels in mice fed a normal diet have a mean value of 3.7 nM and 140 nM, respectively [49]. We also found that a high methionine diet causes 3.7-fold and 25-fold increases in plasma and urinary Hcy-thiolactone, respectively, in mice. The distributions of Hcy-thiolactone between plasma and urine in mice fed a normal diet and humans are similar: much higher Hcy-thiolactone concentrations accumulate in urine than in plasma (urinary/plasma Hcy-thiolactone is 37 in mice [49] and 100 in humans [54]). This shows that urinary clearances of Hcy-thiolactone in mice and humans are similar, and that in mice, like in humans [54], >95% of the filtered Hcy-thiolactone is excreted in the urine. Furthermore, significantly higher urinary/plasma Hcy-thiolactone ratios are found in mice fed

hyperhomocysteinemic diets than in the animals fed a normal diet. This finding suggests that urinary clearance of Hcy-thiolactone is much more efficient in hyperhomocysteinemic mice, compared to animals with normal tHcy levels.

Protein Lysine Residues are Targets for the Modification by Hcy-Thiolactone

Hcy-thiolactone is a novel Hcy metabolite, discovered in living organisms in the 1990's. Thus, although its propensity to react with primary amino groups has been recognized shortly after its chemical synthesis in the 1930's, the reactions of Hcy-thiolactone with proteins remained virtually unexplored, until the end of 1990's [46].

The discovery that Hcy-thiolactone and proteins containing N-linked Hcy (*N*-Hcy-protein) are formed by cultured mammalian, including human, cells has led to a hypothesis that the chemical reactivity of Hcy-thiolactone may underlie the involvement of Hcy in the pathology of human vascular disease [50]. This in turn prompted detailed studies of the reactions of Hcy-thiolactone with proteins [35; 36; 38; 46; 50; 70; 71; 82].

Initial studies have established that [^{35}S]Hcy-thiolactone added to human or animal serum disappears with a half-life of is from 0.25-1.5 hours, depending on the source of serum. I found that the disappearance of Hcy-thiolactone in serum is due to two major reactions: the formation of an *N*-Hcy-protein adduct, in which Hcy is attached *via* an isopeptide bond to the ε-amino group of a protein lysine residue (Equation 2) [38; 50], and enzymatic hydrolysis by serum paraoxonase/Hcy-thiolactonase to Hcy, which then forms a mixed protein-S-S-Hcy disulfide, mostly with the Cys34 of serum albumin (Figure 1) [38; 57; 58; 71; 82]. In the presence of [^{35}S]Hcy-thiolactone, each individual human or rabbit serum protein becomes *N*-homocysteinylated in proportion to its abundance in serum [38].

Hcy-thiolactone has a propensity to modify amino groups of free amino acids, albeit less efficiently than free lysine [50]. However, only the side chain amino groups of lysine residues in proteins, but not any other amino acid residues, are modified by Hcy-thioalctone [38; 71; 82]. In particular, Hcy-thiolactone does not appreciably react with the side chains of arginine, histidine, serine, or thereonine. Moreover, the N-terminal α-amino group in human serum albumin, hemoglobin, cytochrome *c*, or fibrinogen does not appear to react with Hcy-thiolactone. Using proteomic approaches only internal lysine residues were identified as targets for Hcy-thiolactone modification [42; 83; 84].

Second order rate constants for reactions of Hcy-thiolactone with individual purified proteins indicate that *N*-homocysteinylation is relatively robust and goes to completion within a few hours at physiological conditions of pH and temperature. A major determinant of the reactivity of most proteins with Hcy-thiolactone is their lysine content. For proteins that vary in size from 104 to 698 amino acid residues there is a very good correlation ($r = 0.97$) between protein's lysine content and its reactivity with Hcy-thiolactone. Larger proteins, such as fibrinogen (3588 amino acid residues) and low-density lipoproteins (LDL) (~5,000 amino acid residues), react with Hcy-thiolactone ~6-fold less efficiently than expected from their lysine contents. Of many lysine residues present in a protein only a few are predominant sites

for the modification by Hcy-thiolactone, as has been shown for albumin [83], hemoglobin (R. Glowacki, H. Jakubowski, unpublished data), fibrinogen [42], and cytochrome c [84].

Protein *N*-Linked Hcy is a By-Product of Human Hcy Metabolism

Evidence from Tissue Culture Studies

The first indication that protein N-linked Hcy is likely to be an important component of Hcy metabolism in humans came from studies of Hcy-thiolactone metabolism in human tissue cultures [50]. Proteins from normal and CBS-deficient fibroblasts and breast cancer cells have been shown to contain small amounts of protein N-linked Hcy (0.4 to 2.4% relative to protein methionine). When metabolic conversion of Hcy to methionine was inhibited by the anti-folate drug aminopterin, the amounts of Hcy, Hcy-thiolactone, *and* protein N-linked Hcy increased [50].

Further experiments with cultured human umbilical vein endothelial cells provide evidence that the formation protein N-linked Hcy is likely to be important in human vascular tissues [51]. These experiments show that the formation of protein N-linked Hcy occurs concomitantly with the synthesis of Hcy-thiolactone in the presence of physiological concentrations of Hcy, methionine, and folic acid. Like the levels of Hcy-thiolactone, levels of protein N-linked Hcy are directly proportional to Hcy, and inversely proportional to methionine concentrations. Supplementation of endothelial cell cultures with folic acid inhibits the synthesis of extracellular and intracellular protein N-linked Hcy by facilitating the conversion of Hcy to methionine, thereby indirectly preventing synthesis of Hcy-thiolactone by methionyl-tRNA synthetase. The formation of extracellular, but not intracellular, protein N-linked Hcy in endothelial cell cultures is inhibited by supplementation with HDL [51], which carries an Hcy-thiolactone-hydrolyzing enzyme, paraoxonase 1 [57; 58; 59; 60].

The mode of Hcy incorporation into endothelial cell protein has been established by using Edman degradation, a classic protein chemistry procedure which releases from proteins amino acids having free α-amino group. About half of total Hcy incorporated into protein was found to be sensitive to Edman degradation [45; 51], suggesting that Hcy incorporation is due to reactions of Hcy-thiolactone with protein lysine residues (Equation 2) [38; 50]. The presence of a fraction of *N*-Hcy-protein that is resistant to Edman degradation suggests that translational, *S-nitroso*-Hcy-mediated, incorporation of Hcy into protein [65] also occurs in endothelial cell cultures.

Protein N-Linked Hcy is Present in Humans

To examine a possibility that N-Hcy-protein is relevant *in vivo* in the human body, I have developed a highly selective and sensitive HPLC-based methods for the determination of protein N-linked Hcy [61] 61a]. The initial sample workup removes free and disulfide-linked

Hcy by extensive treatments with the reducing agent dithiothreitol. The method is based on a quantitative conversion of protein N-linked Hcy to Hcy-thiolactone, which is achieved by acid hydrolysis under reducing conditions (in the presence of dithiothreitol). Hcy-thiolactone is then purified and quantified by HPLC on a cation exchange column with multi-wavelength diode array UV detection, including A_{240} [61] or fluorescence detection after post-column derivatization with orthophtaldialdehyde [61a].

That protein N-linked Hcy is present in human plasma proteins was first described in 2000 [82]. Subsequent studies have shown that protein N-linked Hcy is present in serum albumin purified from various organisms, including human. Protein N-linked Hcy occurs in all purified individual human blood proteins examined so far [61]. The highest amounts of protein N-linked Hcy, 50 mol%, are present in human and equine ferritins [61a]. In human blood, 0.36-0.6 mol% of protein N-linked Hcy is present in human hemoglobin, serum albumin, and γ-globulins, respectively. Other serum proteins, such as fibrinogen, LDL, HDL, transferrin, and antitrypsin contain from 0.04 to 0.1 % of protein N-linked Hcy. *N*-Hcy-hemoglobin, present in normal blood at a concentration of 12.7 μM, constitutes a major Hcy pool in the human blood [61]. Interestingly, rodents have more N-linked Hcy in their blood proteins that humans [61a].

Although the levels of protein N-linked Hcy in individual human blood proteins correlate with the reactivity of these proteins toward Hcy-thiolactone [61], protein N-linked Hcy may also arise by *S-nitroso*-Hcy-mediated translational mechanism, in which Hcy substitutes a protein methionine residue [45]. However, the presence of protein N-linked Hcy in pig albumin [61], which does not contain methionine, strongly suggests that Hcy-thiolactone-mediated mechanism is responsible for Hcy incorporation.

Protein N-Linked Hcy is Elevated in Hyperhomocysteinemia and is Associated with Coronary Artery Disease (CAD) in Humans

The Hcy-thiolactone hypothesis [36] predicts that protein N-homocysteinylation will be elevated under conditions conducive to atherosclerosis, such as hyperhomocysteinemia. The verification of this prediction became possible with the development of sensitive chemical [61] and immunological assays [85] for protein N-linked Hcy in humans. Indeed, as predicted by the Hcy-thiolactone hypothesis, protein N-linked Hcy is elevated in subjects with genetic hyperhomocysteinemia [45; 61; 71; 82].

I found that human plasma contains from 0.1 to 13 μM protein N-linked Hcy, which represents up to 25% of plasma total Hcy [61]. Plasma concentrations of protein N-linked Hcy correlate positively with tHcy, suggesting that plasma tHcy level is a determinant of protein N-linked Hcy level. Interestingly, in some subjects, plasma levels of protein N-linked Hcy are lower than expected from their tHcy content; this suggests that factors other than tHcy can affect plasma protein N-linked Hcy levels [61]. A likely candidate for a determinant of plasma protein N-linked Hcy levels, is Hcy-thiolactonase activity [57; 59; 60], which has been shown to affect the formation of protein N-linked Hcy in HUVEC cultures [51] and in human serum *in vitro* [58].

We found that plasma protein N-linked Hcy levels are significantly elevated in CBS- or MTHFR-deficient patients and that CBS-deficient patients have significantly elevated levels of pro-thrombotic *N*-Hcy-fibrinogen [130]. These findings provide an explanation for increased atherothrombosis observed in CBS-deficient patients. Furthermore, plasma protein N-linked Hcy is elevated 10-fold in mice fed a pro-atherogenic high-methionine diet [131]. Inactivation of *Cbs*, *Mthfr*, or the proton coupled folate transporter (*Pcft*) gene in mice results in 19- to 30-fold increase in plasma protein N-linked Hcy levels [131]. These finding provide evidence that protein N-linked Hcy is an important metabolite associated with Hcy pathophysiology in humans and mice.

Other investigators have studied protein N-homocysteinylation in uremic patients [86; 87] to explain a link between hyperhomocysteinemia and higher cardiovascular risk and mortality observed in these patients [88]. Significantly higher protein N-linked Hcy levels were found in hyperhomocysteinemic uremic patients on hemodialysis than in control subjects [86; 87]. Interestingly, protein N-linked Hcy comprises less tHcy in hemodialysis patients than in control subjects [86; 87]. Similarly, protein N-linked Hcy comprises less tHcy in patients with higher plasma tHcy (50-120 μM) than in patients with lower plasma tHcy (5-40 μM) [61]. The lower protein N-linked Hcy/tHcy ratios suggest that the Hcy-thiolactone clearance is more effective at higher tHcy levels. This suggestion is supported by a finding that in mice fed a hyperhomocysteinemic high Met or Hcy diet urinary/plasma Hcy-thiolactone is 7-fold or 4-fold higher, respectively, compared to mice fed a normal diet [49].

Hyperhomocysteinemia in CAD patients is linked with increased mortality in these patients [23]. In one clinical study which examined a relationship between Hcy and coronary heart disease, plasma protein N-linked Hcy levels, like tHcy levels, were significantly higher in coronary heart disease patients than in controls [85]. Furthermore, there was a weak but significant positive correlation between protein N-linked Hcy level and the number of diseased coronary arteries: the higher protein N-linked Hcy level the greater the number of afflicted arteries.

Using polyclonal rabbit anti-*N*-Hcy-protein IgG antibodies [89], we have demonstrated that *N*-Hcy-protein is present in human cardiac tissues [90]. For example, we observed positive immunohistochemical staining of myocardium and aorta samples from cardiac surgery patients. Control experiments have demonstrated that the staining was specific for *N*-Hcy-protein. No immunostaining was observed with rabbit preimmune IgG, with iodoacetamide-treated tissues (which destroys the *Nε*-Hcy-Lys epitope), or with the antibody pre-adsorbed with *N*-Hcy-albumin [90]. Further support for a role of *N*-Hcy-protein in atherogenesis is provided by our finding of increased immunohistochemical staining for *N*-Hcy-protein in aortic lesions from ApoE-/- mice with hyperhomocysteinemia induced by a high methionine diet, relative to the mice fed a control chow diet [90].

Modification by Hcy-Thiolactone Causes Protein Damage

In proteins that were studied thus far, usually a few lysine residues are predominant targets for the modification by Hcy-thiolactone. For example, Lys525 [83] is a predominant

site of albumin N-homocysteinylation *in vitro* and *in vivo*. Four lysine residues of cytochrome c (Lys8 or 13, Lys 86 or 87, Lys 99, and Lys 100) are susceptible to N-homocysteinylation [84]. Twelve lysine residues of fibrinogen (7 in Aα chain, 2 in Bβ chain and 3 in γ chain) were found to be susceptible to the modification by Hcy-thiolactone [42]. Four lysine residues (Lys16, Lys56 in α chain and Lys59, Lys95 in β chain) are predominant sites of N-homocysteinylation in hemoglobin (H. Jakubowski, R. Glowacki, unpublished data).

The acylation of a basic ε-amino group of a protein lysine residue (pK=10.5) by Hcy-thiolactone generates an *N*ε-Hcy-Lys residue containing a much less basic amino group (pK~7) and a free thiol group (*Eq.* 1). This substitution is expected to significantly alter protein structure and function. Indeed, hemoglobin, albumin [83], and cytochrome c [38] are sensitive to *N*-homocysteinylation; incorporation of one Hcy/mol protein induces gross structural alterations in these proteins. For instance, *N*-Hcy-cytochrome c becomes resistant to proteolytic degradation (by trypsin, chymotrypsin, and pronase) [84] and susceptible to aggregation due to intermolecular disulfide bond formation [38], which also interferes with the red-ox state of the heme iron by rendering it reduced [84]. *N*-Hcy-hemoglobin, in contrast to unmodified hemoglobin, is susceptible to further irreversible damage by oxidation. Of the two physiological forms of human albumin, albumin-Cys34-S-S-Cys (containing cysteine in a disulfide linkage with Cys34 of albumin) is modified by Hcy-thiolactone faster than mercaptoalbumin (containing a free thiol at Cys34). Hcy-thiolactone-modified and unmodified forms of albumin exhibit different susceptibilities to proteolytic degradation by trypsin, chymotrypsin, or elastase [83].

Other proteins are inactivated only by incorporation of multiple Hcy residues. For example, complete loss of enzymatic activity occurs after *N*-homocysteinylation of eight lysine residues in MetRS (33% of total lysine residues) or eleven lysine residues in trypsin (88% of total lysine residues) [38]. Furthermore, extensively *N*-homocysteinylated proteins, such as fibrinogen, transferin, globulins, myoglobin, RNase A, and trypsin are prone to multimerization and undergo gross structural changes that lead to their denaturation and precipitation [38]. Chicken egg lysozyme is also denatured by extensive *N*-homocysteinylation [91].

N-Hcy-LDL, in which 10% or 25% lysine residues have been modified (*i. e.*, containing 36 and 89 mol Hcy/mol LDL), is taken up and degraded by human monocyte-derived macrophages significantly faster than native LDL [92]. However, *N*-Hcy-LDL containing eight molecules of Hcy/mol LDL is taken up and degraded by leukemic L2C guinea pig lymphocytes to the same extent as native LDL *via* the high affinity LDL-specific receptor pathway [93].

Hcy-thiolactone may also inactivate enzymes by other mechanisms. For example, lysine oxidase, an important enzyme responsible for post-translational collagen modification essential for the biogenesis of connective tissue matrices, is inactivated by micromolar concentrations of Hcy-thiolactone, which derivatizes the active site tyrosinequinone cofactor with a half-life of 4 min [94].

Chronic Treatments with Hcy-Thiolactone are Harmful

As predicted by the Hcy-thiolactone hypothesis [36] [37a, 37b], chronic treatments of animals with Hcy-thiolactone cause pathophysiological changes similar to those observed in human genetic hyperhomocysteinemia. For example, Hcy-thiolactone infusions in baboons [95] or Hcy-thiolactone-supplemented diet in rats [96] produce atherosclerosis. Treatments with Hcy-thiolactone cause developmental abnormalities in chick embryos [97], including optic lens dislocation [98], a characteristic diagnostic feature present in the CBS-deficient human patients [16; 17; 18]. However, rabbits, which have the highest levels of serum Hcy-thiolactonase/PON1, and thus efficiently detoxify Hcy-thiolactone [57; 58; 71], are resistant to detrimental effects of Hcy-thiolactone infusions [99; 100].

Immunogenic Properties of Hcy-Thiolactone-Modified Proteins

Hcy-thiolactone-mediated incorporation of Hcy into protein (***Eq.* 2**) can impact cellular physiology through many routes. Protein modification by Hcy-thiolactone can disrupt protein folding, and create altered proteins with newly acquired interactions, or can lead to induction of autoimmune responses. During the folding process, proteins form their globular native states in a manner determined by their primary amino acid sequence [101; 102]. Thus, small changes in amino acid sequence caused by Hcy incorporation have the potential to create misfolded protein aggregates. Indeed, *N*-Hcy-proteins have a propensity to form protein aggregates [38]. Furthermore, the appearance of misfolded/aggregated proteins in the endoplasmic reticulum (ER) activates an unfolded protein response (UPR) signaling pathway, that, when overwhelmed, leads to cell death *via* apoptosis. Protein aggregates are known to be inherently toxic [103]. The toxicity of *N*-Hcy-LDL, which in contrast to native LDL, has the propensity to aggregate [92] and induces cell death in cultured human endothelial cells [40], is consistent with this concept. These pathways can be induced in cultured human endothelial cells and in mice by elevating Hcy [104; 105; 106; 107], which also elevates Hcy-thiolactone [49; 51]. Moreover, treatments with Hcy-thiolactone induce ER stress and UPR in retinal epithelial cells [108], as well as apoptotic death in cultured human vascular endothelial cells [39; 41]. In this scenario the formation of *N*-Hcy-proteins leads to the UPR and induction of the apoptotic pathway. Proteolytic degradation of *N*-Hcy-proteins can generate potentially antigenic peptides, which can be displayed on cell surface and induce adaptive immune response.

Atherosclerosis is now widely recognized as a chronic inflammatory disease that involves innate and adaptive immunity [7; 10; 11]. That inflammation is important is supported by studies showing that increased plasma concentration of markers of inflammation, such as C-reactive protein, interleukin-1, serum amyloid A, and soluble adhesion molecules are independent predictors of vascular events [9]. Autoantibodies against modified LDL were found to be elevated in vascular disease patients in some, but not all

studies [12; 13]. Lipid peroxidation is thought to play a central role in the initiation of both cellular and humoral responses. Reactive aldehydes resulting from phospholipid peroxidation, such as malondialdehyde, 4-hydroxynonenal, and 1-palmitoyl-2-(5-oxovaleroyl)-s*n*-glycero-3-phosphocholine can modify lysine residues in LDL and in other proteins. The resulting oxidized lipids-protein adducts, e.g., malondialdehyde-LDL, carry neo-self epitopes which are recognized by specific innate and adaptive immune responses. As will be discussed in the following sections of this chapter, protein N-homocysteinylation by Hcy-thiolactone [35; 36] also appears to play an important role.

N-Hcy-Proteins are Immunogenic

By generating structurally altered proteins, the modification by Hcy-thiolactone, like other chemical modifications, such as glycation, acetylation, methylation, ethylation, carbamylation [7], can render proteins particularly immunogenic. Indeed, intradermal inoculations of rabbits with *N*-Hcy-LDL induces the synthesis of anti-*N*-Hcy-LDL antibodies in these animals [109]. Furthermore, immunization of rabbits with Hcy-thiolactone-modified keyhole limpet hemocyanin (KHL) leads to generation of antibodies that bind to *N*-Hcy-LDL [89; 110]. Of considerable interest are the observations that antisera from such immunizations bound not only to the *N*-Hcy-LDL but to a variety of other human proteins on which the N-linked Hcy epitope was present, such as *N*-Hcy-albumin, *N*-Hcy-hemoglobin, *N*-Hcy-transferrin, *N*-Hcy-antitrypsin, but not to native unmodified proteins. *N*ε-Hcy-*N*α-acetyl-Lys, but not *N*ε-acetyl-*N*α-Hcy-Lys, prevented the rabbit antibodies from binding to human *N*-Hcy-hemoglobin. This shows that the rabbit IgG specifically recognizes Hcy linked by isopeptide bond to ε-amino group of protein lysine residue; Hcy linked by peptide bond to α-amino group is not recognized. The rabbit antibodies bind short peptides containing the *N*ε-Hcy-Lys epitop. Hcy, Hcy-thiolactone, lysine, or unmodified lysine derivatives not are bound by the rabbit anti-N-Hcy-protein antibodies [110]. Furthermore, pre-immune rabbit serum exhibits significant titers of autoantibodies against *N*-Hcy-albumin [H. Jakubowski unpublished], which suggests that endogenous *N*-Hcy-proteins present in rabbit blood [61] are autoantigenic. Taken together, these data suggest that autoantibodies, once formed *in vivo* in response to *N*-Hcy-LDL would be capable of binding to endogenous *N*-Hcy-proteins.

An Auto-Immune Response to *N*-Hcy-Proteins in Humans

To determine whether *N*-Hcy-proteins are autoimmunogenic in humans, human sera were assayed for the presence of antibodies binding to *N*-Hcy-hemoglobin as an antigen. We found that each human serum tested showed some titer of IgG [110; 111; 112] and IgM (J. Perla-Kajan, T. Twardowski, H. Jakubowski, unpublished data) auto-antibodies against *N*-Hcy-hemoglobin or *N*-Hcy-albumin.

The plasma levels of anti-*N*-Hcy-protein autoantibodies [89; 110; 111; 112] and protein *N*-linked Hcy [45; 61; 82; 83] vary considerably among individuals and are strongly correlated with plasma Hcy, but not with Cys or Met [110]. Such correlations can be

explained by the Hcy-thiolactone hypothesis [36]: elevation in Hcy leads to inadvertent elevation in Hcy-thiolactone, observed *ex vivo* in human fibroblasts [50] and endothelial cells [51; 53], and *in vivo* in humans [49; 53; 54; 67] and mice [36; 49]. Hcy-Thiolactone mediates Hcy incorporation into proteins and the formation of neo-self antigens, *Nε*-Hcy-Lys-protein (***Eq.* 1**). Raising levels of neo-self *Nε*-Hcy-Lys epitopes on proteins trigger an autoimmune response. The presence of IgM and IgG autoantibodies against *N*-Hcy-proteins in human blood [35; 36] suggest that Hcy incorporation into proteins triggers both innate and an adaptive immune response in humans.

Antigen Specificity of the Human Anti-*N*-Hcy-Proteins Autoantibodies

The anti-*N*-Hcy-protein IgG autoantibodies specifically recognize an *Nε*-Hcy-Lys epitope on N-homocysteinylated human proteins, such as *N*-Hcy-hemoglobin, *N*-Hcy-albumin, *N*-Hcy-transferrin, and *N*-Hcy-antitrypsin. The thiol group of N-linked Hcy is important for binding and proteins containing the *Nε*-Hcy-Lys epitope with its thiol blocked by the thiol reagent iodoacetamide are not bound by these autoantibaodies. Small molecules, such as *Nε*-Hcy-Lys, *Nε*-Hcy-*Nα*-acetyl-Lys, and *Nε*-Hcy-*Nα*-acetyl-LysAla are also bound by these autoantibodies, as demonstrated by their effective competition for autoantibody binding to antigen-coated microtiter plate wells [110]. High specificity of these autoantibodies is further demonstrated by our finding that *Nε*-acetyl-*Nα*-Hcy-Lys, in which Hcy is attached to the α-amino group of lysine instead of the ε-amino group, did not compete with the human IgG binding. Lysine, LysAla, *Nα*-acetyl-Lys, Hcy or Hcy-thiolactone also did not compete with the human IgG binding. Taken together, these data suggest that human IgG specifically recognizes *N*ε-Hcy-Lys epitope on an *N*ε-Hcy-Lys-protein and that the antigen specificity of the human anti-*N*-Hcy-protein autoantibodies is essentially identical to the specificity of the rabbit anti-*N*-Hcy-protein antibodies generated by inoculations with *N*-Hcy-LDL or *N*-Hcy-KLH [110].

Anti-*N*-Hcy-Protein Autoantibodies are Associated with Stroke

Innate and adaptive immune responses directed against modified LDL are known to modulate the progression of vascular disease and increased plasma levels of markers of these responses are independent predictors of coronary events [6]. Although plasma levels of autoantibodies against oxidized or glycated LDL are often associated with vascular disease [12; 13], the role of anti-*N*-Hcy-protein auto-antibodies was unknown. In a case-control study [110], we examined the relation between anti-*N*-Hcy-protein auto-antibodies and stroke.

Our cohorts of 54 stroke patients (63.4 years old) and 74 healthy controls (66.3 years old) did not differ with respect to triglycerides, total cholesterol and LDL cholesterol levels, whereas HDL cholesterol was lower in stroke patients than in controls. We found significant differences in levels of anti-*N*-Hcy-protein IgG autoantibodies between the group of 39 male patients with stroke and the group of 29 healthy subjects. Male stroke patients had higher serum anti-*N*-Hcy-protein IgG levels than healthy controls [110]. Male stroke patients had

also higher plasma tHcy than controls, consistent with earlier studies. Thus, both plasma tHcy and anti-*N*-Hcy-protein IgGs are associated with stroke in male subjects. Plasma levels of tHcy and anti-*N*-Hcy-protein IgG autoantibody in a group of 17 female stroke patients were similar to corresponding levels in a group of 45 female controls, suggesting that stroke in the female patients may have been caused by factors other than elevated Hcy or cholesterol. Furthermore, we found no differences in plasma cysteine or methionine concentrations between stroke patients and controls both for males and females. Thus, the high levels of anti-*N*-Hcy-protein autoantibodies in male stroke patients reflect high Hcy levels in these patients.

Anti-*N*-Hcy-Protein Autoantibodies are Associated with CAD

To test a concept that anti-*N*-Hcy-protein autoantibodies are an important feature of atherosclerosis, we examined the relation between anti-*N*-Hcy-protein autoantibodies and CAD in male subjects [111]. Our cohort of 88 male patients (45 years old) with angiographically documented CAD had significantly higher plasma levels of triglycerides, total cholesterol and LDL cholesterol, and lower levels of HDL cholesterol, compared to a cohort of 100 healthy male controls (43.5 years old). Significant differences in mean levels of anti-*N*-Hcy-protein IgG autoantibodies were found between a group of CAD male patients and a group of age-matched controls. Male CAD patients had 47 % higher serum levels of anti-*N*-Hcy-protein IgG autoantibodies than healthy controls. Levels of anti-*N*-Hcy-protein IgG were not associated with traditional risk factors. However, there was a weak positive correlation between the autoantibodies and plasma tHcy. Male CAD patients had also higher levels of plasma tHcy than controls, consistent with earlier studies. Thus, the higher levels of anti-*N*-Hcy-protein autoantibodies that are present in CAD male patients, like in stroke patients, reflect the higher levels of Hcy in these patients.

An age-adjusted risk for CAD related to seropositivity for anti-*N*-Hcy-protein IgG autoantibodies is 9.87 (95% CI 4.50-21.59, $p<10^{-5}$). In multivariate logistic regression analysis, seropositivity to anti-*N*-Hcy-protein IgG autoantibodies (OR, 14.82; 95% CI, 4.47 to 49.19; p=0.00002), smoking (OR, 8.84; 95% CI, 2.46 to 31.72; p=0.001), hypertension (OR, 43.45; 95% I, 7.91 to 238.7; p=0.0001), and HDL cholesterol (OR, 0.015; 95% CI, 0.002 to 0.098; p=0.00002 for each unit increase) were independent predictors of early CAD in men <50 years old (χ^2=26.17, $p<10^{-5}$ for the increment in goodness of fit as compared to a three variable model employing smoking, HDL cholesterol, and hypertension). These analyses show that elevated levels of anti-*Nε*-Hcy-Lys-protein autoantibodies significantly contribute to the risk of CAD in male patients.

Anti-*N*-Hcy-Protein Autoantibodies are Associated with Uremia

As discussed above, the levels of *N*-Hcy-protein are elevated in uremic patients on hemodialysis [86; 87]. These finding suggests that an autoimmune response against *N*-Hcy-protein might also be enhanced in these patients. This possibility was examined in a group of

43 patients (58.8 years old) who were on maintenance hemodialysis for an average of 50 months and an age and sex matched group of 31 apparently healthy individuals [113]. Significantly higher levels of anti-*N*-Hcy-protein IgG autoantibodies were found in the hemodiallysis patients, compared with controls. Like in our previous studies [110], the levels of anti-*N*-Hcy-protein IgG autoantibodies were strongly correlated with plasma total Hcy, both in hemodialysis patients and in controls. Among the hemodialysis patients, a subgroup of survivors of myocardial infarction (n=14) had significantly higher levels of anti-*N*-Hcy-protein IgG autoantibodies than a subgroup of hemodialysis patients without a history of CAD (n=29) [113]. Taken together, these data suggest that an autoimmune response against N-Hcy-proteins contributes to the development of CAD in hemodialysis patients.

Hyperhomocysteinemia, *N*-Hcy-Protein, and an Innate Immune Response

We also found that the levels of anti-*N*-Hcy-protein autoantibodies are weakly, but significantly, correlated with plasma CRP levels (r=0.24, p=0.002) [111]. This finding suggests that *N*-Hcy-protein can also elicit an innate immune response. Many investigators, but not all [114; 115; 116], have linked Hcy to immune responses. For example, a weak, but significant, association between plasma total Hcy and CRP was observed in the Framingham Heart Study [117] and in the Physician's Health Study [118]. Holven *et al.* reported that in humans hyperhomocysteinemia is associated with increased levels of both CRP and interleukin-6 [119]. A similar positive association between Hcy and interleukin-6 was reported in patients with diabetic nephropathy [120]. Importantly, in the Holven *et al*. study, elevated level of interleukin-6 is observed in hyperhomocysteinemic individuals in the absence of hypercholesterolemia. Plasma total Hcy was positively associated with soluble tumor necrosis factor receptor in the Nurses' Health Study [121]. A positive correlation is observed between plasma tHcy and neopterin (a marker of Th1 type immune response) in Parkinson's disease patients [122]. Elevated Hcy is associated with elevated monocyte chemotactic protein-1 and increased expression of vascular adhesion molecules in humans [123; 124] and rats [125; 126; 127; 128]. Plasma Hcy is a determinant of TNF-α in hypertensive patients [129]. Furthermore, in mice dietary hyperhomocysteinemia is known to trigger atherosclerosis and enhance vascular inflammation, manifested by increased activation of NF-κB in the aorta and kidney, enhanced expression of VCAM-1 and RAGE in the aorta and TNF-α in plasma [14].

How Hcy can trigger these innate inflammatory responses is unknown, However, given that hyperhomocysteinemia causes elevation of Hcy-thiolactone and *N*-Hcy-protein levels in humans and mice [49], these responses are likely to be caused by *N*-Hcy-protein, particularly by *N*-Hcy-LDL. Consistent with this suggestion are the observations that *N*-Hcy-LDL is highly immunogenic [109], is present in human blood [61], and is taken up by macrophages faster than unmodified LDL [92]. Further studies are needed to elucidate the mechanism of Hcy-induced innate immune responses.

Possible Roles of Anti-*N*-Hcy-Protein Autoantibodies in Atherosclerosis

Our findings that anti-*N*-Hcy-protein autoantibodies are elevated in stroke and CAD patients suggest that an autoimmune response against *N*-Hcy-proteins is an important feature of atherosclerosis [35]. In general, antibodies protect against exogenous pathogens and endogenous altered neo-self molecules to maintain homeostasis by neutralization and clearance. Like autoantibodies against oxidatively modified LDL [7], the anti-*N*-Hcy-protein autoantibodies can be beneficial or deleterious. For example, the clearing of *N*-Hcy-protein proteins from circulation by the autoantibodies would be beneficial. On the other hand, binding of the anti-*N*-Hcy-protein autoantibodies to *N*-Hcy-proteins [35; 89] in tissues may contribute to the deleterious effects of hyperhomocysteinemia on many organs [16; 17; 18]. For instance, if the neo-self *Nε*-Hcy-Lys epitopes were present on endothelial cell membrane proteins, anti-*N*-Hcy-protein autoantibodies would form antigen-antibody complexes on the surface of the vascular wall. Endothelial cells coated with anti-*N*-Hcy-protein autoantibodies would be taken up by the macrophage *via* the Fc receptor, resulting in injury to the vascular surface. Under chronic exposures to excess Hcy, the neo-self epitopes *Nε*-Hcy-Lys, which initiate the injury, are formed continuously, and the repeating attempts to repair the damaged vascular wall would lead to an atherosclerotic lesion [35; 36].

Hcy-Lowering Therapy and Anti-*N*-Hcy-Protein Autoantibodies

If anti-*N*-Hcy-protein autoantibodies reflect plasma tHcy levels and arise through the mechanisms postulated by the Hcy-thiolactone hypothesis [36], then lowering plasma tHcy by folic acid supplementation should also lower plasma levels of anti-*N*-Hcy-protein autoantibodies. This prediction was tested in groups of hyperhomocysteinemic (plasma tHcy>15 μM) male patients (n=12) with angiographically documented CAD and healthy men (n=20) [112]. At baseline, the two groups did not differ with respect to age, tHcy, folate, lipid profile, and CRP. As in our two previous studies [110; 111], the baseline levels of anti-*N*-Hcy-protein autoantibodies were significantly higher in CAD patients than in healthy subjects and plasma tHcy was positively correlated with anti-*N*-Hcy-protein autoantibodies in both groups ($r=0.77$ to 0.85, $p<0.0001$ to 0.002) [112]. Furthermore, folate levels measured prior to folic acid supplementation correlated negatively with anti-*N*-Hcy-protein autoantibodies in healthy subjects ($r=-0.58$, p0.008) and in CAD patients ($r=-0.9$, $p<0.0001$).

Folic acid supplementation for 3 months or 6 months resulted in significant lowering of plasma tHcy (by 30%) and increased plasma folate levels (by 230%) in our CAD patients and controls, consistent with other Hcy-lowering studies [27; 28; 29; 30]. In healthy subjects, plasma levels of anti-*N*-Hcy-protein autoantibodies fell significantly ($p<0.001$) following 3 months (by 38%), and remained at a lower level at 6 months (by 48%), of folic acid supplementation. However, in CAD patients, surprisingly, plasma levels of anti-*N*-Hcy-protein autoantibodies fell by only 8.5-12% at 3 or 6 months of folic acid supplementation, but this effect was not significant [112]. The effects of Hcy-lowering therapy on anti-*N*-Hcy-protein autoantibodies suggest that the neo-self *N*-Hcy-protein antigens respond relatively quickly to changes in Hcy levels and can be cleared in healthy subjects. In contrast, the neo-

self *N*-Hcy-protein antigens appear to persist in CAD patients and not to respond to Hcy lowering therapy. Interestingly, in another study the levels of anti-*N*-Hcy-protein IgG autoantibodies were found to be similar in groups of uremic patients on hemodialysis who were taking (n=37) or not taking (n=6) folic acid supplementation [113]. These findings suggest that the immune activation caused by protein N-homocysteinylation in uremia and in CAD patients cannot be easily reversed.

Taken together, the effects of Hcy-lowering therapy on anti-*N*-Hcy-protein autoantibodies support the involvement of Hcy in the synthesis of these autoantibodies according to a mechanism postulated by the Hcy-thiolactone hypothesis [36] (Figure 5).

Furthermore, our findings that lowering plasma Hcy by folic acid supplementation lowers anti-*N*-Hcy-protein autoantibodies in control subjects, but not in patients with CAD, support the involvement of an autoimmune response in CAD [112]. These findings also suggest that, while primary Hcy-lowering intervention by vitamin supplementation is beneficial, secondary intervention may be ineffective, and may explain at least in part the failure of vitamin therapy to lower cardiovascular events in MI patients [29; 30].

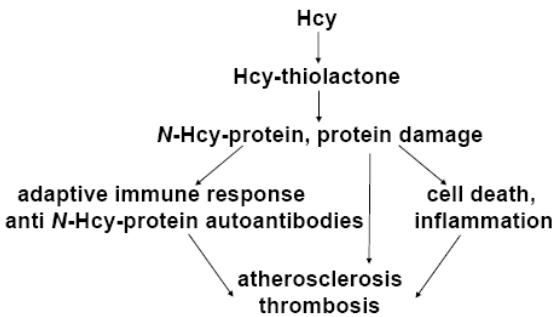


Figure 5. Hcy-thiolactone-mediated incorporation of Hcy into proteins leads to the induction of anti-N-Hcy-protein autoantibodies and is associated with atherosclerosis and thrombosis in humans.

Conclusion

Accumulating evidence suggests that elevated Hcy contributes to adaptive and innate immune responses in atherosclerosis in humans and experimental animals. In this chapter, I have discussed the evidence supporting a concept that the incorporation of Hcy into protein *via* isopetide linkages, causes alterations in the protein's structure and the formation of neo-self antigens that elicit anti-*N*-Hcy-protein autoantibodies, and emphasized their potential importance in vascular disease (Figure 5). Of many known natural Hcy metabolites, only the thioester Hcy-thiolactone can mediate the incorporation of Hcy into proteins *via* stable isopeptide bonds. Protein N-homocysteinylation creates altered proteins with newly acquired interactions, including immunogenic properties. Elevated levels of Hcy-thiolactone and protein N-linked Hcy are observed in genetic and dietary hyperhomocysteinemia in humans and mice. Levels of protein N-linked Hcy are also elevated in CAD patients. Protein N-homocysteinylation leads to the formation of neo-self protein N-linked Hcy epitopes, which cause an immune response in humans, manifested by the induction of anti-*N*-Hcy-protein

autoantibodies. Levels of these autoantibodies correlate with plasma total Hcy, are elevated in stroke and CAD patients, and thus may play an important role in atherosclerosis. Primary Hcy-lowering vitamin therapy lowers the levels of anti-*N*-Hcy-protein autoantibodies in healthy subjects. In contrast, secondary vitamin intervention appears to be ineffective in reducing an autoimmune response: it lowers plasma tHcy, but not anti-*N*-Hcy-protein autoantibodies in CAD patients. These results support the Hcy-thiolactone hypothesis, which states that the metabolic conversion of Hcy to Hcy-thiolactone followed by the non-enzymatic protein modification by Hcy-thiolactone is an underlying mechanism that contributes to the pathophysiology of hyperhomocysteinemia. We are only beginning to understand pathophysiological consequences of *N*-Hcy-protein accumulation. Along with other aspects of protein N-homocysteinylation, identifying anti-*N*-Hcy-protein autoantibodies, and understanding their roles in health and disease are likely to yield an understanding of the basic mechanisms that evolved to deal with the consequences of Hcy-thiolactone formation.

References

[1] Braunwald, E. Shattuck lecture--cardiovascular medicine at the turn of the millennium: triumphs, concerns, and opportunities. *N. Engl. J. Med.*, 337, 1997, 1360-9.

[2] Willerson, JT; Ridker, PM. Inflammation as a cardiovascular risk factor. *Circulation*, 2004, 109, II2-10.

[3] Libby, P. The forgotten majority: unfinished business in cardiovascular risk reduction. *J. Am. Coll. Cardiol.*, 2005, 46, 1225-8.

[4] Tardif, JC; Heinonen, T; Orloff, D; Libby, P. Vascular biomarkers and surrogates in cardiovascular disease. *Circulation*, 2006, 113, 2936-42.

[5] Lusis, AJ. Atherosclerosis. *Nature*, 2000, 407, 233-41.

[6] Binder, CJ; Chang, MK; Shaw, PX; Miller, YI; Hartvigsen, K; Dewan, A; Witztum, JL. Innate and acquired immunity in atherogenesis. *Nat. Med.*, 2002, 8, 1218-26.

[7] Binder, CJ; Shaw, PX; Chang, MK; Boullier, A; Hartvigsen, K; Horkko, S; Miller, YI; Woelkers, DA; Corr, M; Witztum, JL. The role of natural antibodies in atherogenesis. *J. Lipid. Res.*, 2005, 46, 1353-63.

[8] Croce, K; Libby, P; Intertwining of thrombosis and inflammation in atherosclerosis. *Curr. Opin. Hematol.*, 2007, 14, 55-61.

[9] Libby, P. Inflammation in atherosclerosis. *Nature*, 2002, 420, 868-74.

[10] Libby, P; Ridker, PM. Inflammation and atherothrombosis from population biology and bench research to clinical practice. *J. Am. Coll. Cardiol.*, 2006, 48, A33-46.

[11] Forrester, JS; Libby, P. The inflammation hypothesis and its potential relevance to statin therapy. *Am. J. Cardiol.*, 2007, 99, 732-8.

[12] Virella, G; Lopes-Virella, MF. Lipoprotein autoantibodies: measurement and significance. *Clin. Diagn. Lab. Immunol.*, 2003, 10, 499-505.

[13] Virella, G; Thorpe, SR; Alderson, NL; Derrick, MB; Chassereau, C; Rhett, JM; Lopes-Virella, MF. Definition of the immunogenic forms of modified human LDL recognized

by human autoantibodies and by rabbit hyperimmune antibodies. *J. Lipid. Res*., 2004, 45, 1859-67.

[14] Hofmann, MA; Lalla, E; Lu, Y; Gleason, MR; Wolf, BM; Tanji, N; Ferran, LJ; Jr., Kohl, B; Rao, V; Kisiel, W; Stern, DM; Schmidt, AM. Hyperhomocysteinemia enhances vascular inflammation and accelerates atherosclerosis in a murine model. *J. Clin. Invest.*, 2001, 107, 675-83.

[15] Refsum, H; Nurk, E; Smith, AD; Ueland, PM; Gjesdal, CG; Bjelland, I; Tverdal, A; Tell, GS; Nygard, O; Vollset, SE. The Hordaland Homocysteine Study: a community-based study of homocysteine, its determinants, and associations with disease. *J. Nutr.*, 2006, 136, 1731S-1740S.

[16] Mudd, SH; Levy, HL; KJP. Disorders of transsulfuration. in: Scriver, CR; Beaudet, AL; Sly, WS; et al; (Eds.), The metabolic and molecular bases of inherited disease, Mc Graw-Hill, New York, 2001, pp. 2007-2056.

[17] Kluijtmans, LA; Boers, GH; Kraus, JP; Van Den Heuvel, LP; Cruysberg, JR; Trijbels, FJ; Blom, HJ. The molecular basis of cystathionine beta-synthase deficiency in Dutch patients with homocystinuria: effect of CBS genotype on biochemical and clinical phenotype and on response to treatment. *Am. J. Hum. Genet.*, 1999, 65, 59-67.

[18] Yap, S; Boers, GH; Wilcken, B; Wilcken, DE; Brenton, DP; Lee, PJ; Walter, JH; Howard, PM; Naughten, ER. Vascular outcome in patients with homocystinuria due to cystathionine beta-synthase deficiency treated chronically: a multicenter observational study. *Arterioscler. Thromb. Vasc. Biol.*, 2001, 21, 2080-5.

[19] McCully, KS. Vascular pathology of homocysteinemia: implications for the pathogenesis of arteriosclerosis. *Am. J. Pathol.*, 1969, 56, 111-28.

[20] Wald, DS; Law, M; Morris, JK. Homocysteine and cardiovascular disease: evidence on causality from a meta-analysis. *Bmj*, 2002, 325, 1202.

[21] Seshadri, S. Elevated plasma homocysteine levels: risk factor or risk marker for the development of dementia and Alzheimer's disease? *J. Alzheimers Dis.*, 2006, 9, 393-8.

[22] Seshadri, S; Beiser, A; Selhub, J; Jacques, PF; Rosenberg, IH; D'Agostino, RB; Wilson, PW; Wolf, PA. Plasma homocysteine as a risk factor for dementia and Alzheimer's disease. *N. Engl. J. Med.*, 2002, 346, 476-83.

[23] Anderson, JL; Muhlestein, JB; Horne, BD; Carlquist, JF; Bair, TL; Madsen, TE; Pearson, RR. Plasma homocysteine predicts mortality independently of traditional risk factors and C-reactive protein in patients with angiographically defined coronary artery disease. *Circulation*, 2000, 102, 1227-32.

[24] Strauss, KA; Morton, DH; Puffenberger, EG; Hendrickson, C; Robinson, DL; Wagner, C; Stabler, SP; Allen, RH; Chwatko, G; Jakubowski, H; Niculescu, MD; Mudd, SH. Prevention of brain disease from severe 5,10-methylenetetrahydrofolate reductase deficiency. *Mol. Genet. Metab.*, 2007, 91, 165-75.

[25] Lawrence De Koning, AB; Werstuck, GH; Zhou, J; Austin, RC. Hyperhomocysteinemia and its role in the development of atherosclerosis. *Clin. Biochem.*, 2003, 36, 431-41.

[26] Lentz, SR. Mechanisms of homocysteine-induced atherothrombosis. *J. Thromb Haemost.*, 2005, 3, 1646-54.

[27] Durga, J; Van Boxtel, MP; Schouten, EG; Kok, FJ; Jolles, J; Katan, MB; Verhoef, P. Effect of 3-year folic acid supplementation on cognitive function in older adults in the FACIT trial: a randomised, double blind, controlled trial. *Lancet,* 2007, 369, 208-16.

[28] Spence, JD; Bang, H; Chambless, LE; Stampfer, MJ. Vitamin Intervention For Stroke Prevention trial: an efficacy analysis. *Stroke,* 2005, 36, 2404-9.

[29] Lonn, E; Yusuf, S; Arnold, MJ; Sheridan, P; Pogue, J; Micks, M; McQueen, MJ; Probstfield, J; Fodor, G; Held, C; Jr; Genest, J. Homocysteine lowering with folic acid and B vitamins in vascular disease. *N. Engl. J. Med.,* 2006, 354, 1567-77.

[30] Bonaa, KH; Njolstad, I; Ueland, PM; Schirmer, H; Tverdal, A; Steigen, T; Wang, H; Nordrehaug, JE; Arnesen, E; Rasmussen, K. Homocysteine lowering and cardiovascular events after acute myocardial infarction. *N. Engl. J. Med.,* 2006, 354, 1578-88.

[31] Clarke, R; Lewington, S; Sherliker, P; Armitage, J. Effects of B-vitamins on plasma homocysteine concentrations and on risk of cardiovascular disease and dementia. *Curr. Opin. Clin. Nutr. Metab. Care.,* 2007, 10, 32-9.

[32] Ingrosso, D; Cimmino, A; Perna, AF; Masella, L; De Santo, NG; De Bonis, ML; Vacca, M; D'Esposito, M; D'Urso, M; Galletti, P; Zappia, V. Folate treatment and unbalanced methylation and changes of allelic expression induced by hyperhomocysteinaemia in patients with uraemia. *Lancet,* 2003, 361, 1693-9.

[33] James, SJ; Melnyk, S; Pogribna, M; Pogribny, IP; Caudill, MA. Elevation in S-adenosylhomocysteine and DNA hypomethylation: potential epigenetic mechanism for homocysteine-related pathology. *J. Nutr.,* 2002, 132, 2361S-2366S.

[34] Jacobsen, DW; Catanescu, O; Dibello, PM; Barbato, JC. Molecular targeting by homocysteine: a mechanism for vascular pathogenesis. *Clin. Chem. Lab. Med.,* 2005, 43, 1076-83.

[35] Jakubowski, H. Anti-N-homocysteinylated protein autoantibodies and cardiovascular disease. *Clin. Chem. Lab. Med.,* 2005, 43, 1011-4.

[36] Jakubowski, H. Pathophysiological consequences of homocysteine excess. *J. Nutr.,* 2006, 136, 1741S-1749S.

[37] Perla-Kajan, J; Twardowski, T; Jakubowski, H. Mechanisms of homocysteine toxicity in humans. *Amino Acids,* 2007, 32, 561-72.

[37a] H. Jakubowski. The molecular basis of homocysteine thiolactone-mediated vascular disease. *Clin. Chem. Lab. Med.* 45 (2007) 1704-16.

[37b] H. Jakubowski. The pathophysiological hypothesis of homocysteine thiolactone mediated vascular disease. *J Physiol Pharmacol.* 59 Suppl 9 (2008) 155-67.

[38] Jakubowski, H. Protein homocysteinylation: possible mechanism underlying pathological consequences of elevated homocysteine levels. *Faseb.,* 1999, J 13, 2277-83.

[39] Mercie, P ; Garnier, O; Lascoste, L; Renard, M; Closse, C; Durrieu, F; Marit, G; Boisseau, RM; Belloc, F. Homocysteine-thiolactone induces caspase-independent vascular endothelial cell death with apoptotic features. *Apoptosis,* 2000, 5, 403-11.

[40] Ferretti, G; Bacchetti, T; Moroni, C; Vignini, A; Nanetti, L; Curatola, G. Effect of homocysteinylation of low density lipoproteins on lipid peroxidation of human endothelial cells. *J. Cell Biochem.,* 2004, 92, 351-60.

[41] Kerkeni, M; Tnani, M; Chuniaud, L; Miled, A; Maaroufi, K; Trivin, F. Comparative study on in vitro effects of homocysteine thiolactone and homocysteine on HUVEC cells: evidence for a stronger proapoptotic and proinflammative homocysteine thiolactone. *Mol. Cell Biochem.,* 2006, 291, 119-26.

[42] Sauls, DL; Lockhart, E; Warren, ME; Lenkowski, A; Wilhelm, SE; Hoffman, M. Modification of fibrinogen by homocysteine thiolactone increases resistance to fibrinolysis: a potential mechanism of the thrombotic tendency in hyperhomocysteinemia. *Biochemistry*, 2006, 45, 2480-7.

[43] Undas, A; Brozek, J; Jankowski, M; Siudak, Z; Szczeklik, A; Jakubowski, H. Plasma homocysteine affects fibrin clot permeability and resistance to lysis in human subjects. *Arterioscler. Thromb. Vasc. Biol*., 2006, 26, 1397-404.

[44] Jakubowski, H; Goldman, E. Editing of errors in selection of amino acids for protein synthesis. *Microbiol. Rev.,* 1992, 56, 412-29.

[45] Jakubowski, H. Translational accuracy of aminoacyl-tRNA synthetases: implications for atherosclerosis. *J. Nutr.* 2001, 131, 2983S-7S.

[46] Jakubowski, H. Molecular basis of homocysteine toxicity in humans. *Cell Mol. Life Sci.,* 2004, 61, 470-87.

[47] Jakubowski, H. tRNA synthetase editing of amino acids, Encyclopedia of Life Sciences, John Wiley and Sons, Ltd, Chichester, UK, 2005, pp. http://www.els.net /doi:10.1038 /npg.els.0003933.

[48] Jakubowski, H. Accuracy of Aminoacyl-tRNA Synthetases: Proofreading of Amino Acids. in: M. Ibba, C. Francklyn, and S. Cusack, (Eds.), The Aminoacyl-tRNA Synthetases, Landes Bioscience/Eurekah.com Georgetown, TX, 2005, pp. 384-396.

[49] Chwatko, G; Boers, GH; Strauss, KA; Shih, DM; Jakubowski, H. Mutations in methylenetetrahydrofolate reductase or cystathionine {beta}-syntase gene, or a high-methionine diet, increase homocysteine thiolactone levels in humans and mice. *Faseb. J.* 2007, 21, 1707-13.

[50] Jakubowski, H. Metabolism of homocysteine thiolactone in human cell cultures. Possible mechanism for pathological consequences of elevated homocysteine levels. *J. Biol. Chem.,* 1997, 272, 1935-42.

[51] Jakubowski, H; Zhang, L; Bardeguez, A; Aviv, A. Homocysteine thiolactone and protein homocysteinylation in human endothelial cells: implications for atherosclerosis. *Circ. Res.,* 2000, 87, 45-51.

[52] Jakubowski, H. Proofreading in vivo: editing of homocysteine by methionyl-tRNA synthetase in the yeast Saccharomyces cerevisiae. *Embo. J.,* 1991, 10, 593-8.

[53] Jakubowski, H. The determination of homocysteine-thiolactone in biological samples. *Anal. Biochem.,* 2002, 308, 112-9.

[54] Chwatko, G; Jakubowski, H. Urinary excretion of homocysteine-thiolactone in humans. *Clin. Chem.,* 2005, 51, 408-15.

[55] Jakubowski, H; Goldman, E. Synthesis of homocysteine thiolactone by methionyl-tRNA synthetase in cultured mammalian cells. *FEBS Lett.,* 1993, 317, 237-40.

[56] Zimny, J; Sikora, M; Guranowski, A; Jakubowski, H. Protective mechanisms against homocysteine toxicity: the role of bleomycin hydrolase. *J. Biol. Chem.,* 2006, 281, 22485-92.

[57] Jakubowski, H. Calcium-dependent human serum homocysteine thiolactone hydrolase. A protective mechanism against protein N-homocysteinylation. *J. Biol. Chem.,* 2000, 275, 3957-62.

[58] Jakubowski, H; Ambrosius, WT; Pratt, JH. Genetic determinants of homocysteine thiolactonase activity in humans: implications for atherosclerosis. *FEBS Lett.,* 2001, 491, 35-9.

[59] Lacinski, M; Skorupski, W; Cieslinski, A; Sokolowska, J; Trzeciak, WH; Jakubowski, H. Determinants of homocysteine-thiolactonase activity of the paraoxonase-1 (PON1) protein in humans. *Cell Mol. Biol.,* (Noisy-le-grand), 2004, 50, 885-93.

[60] Domagała, TB; Łacinski, M; Trzeciak, WH; Mackness, B; Mackness, MI; Jakubowski, H. The correlation of homocysteine-thiolactonase activity of the paraoxonase (PON1) protein with coronary heart disease status. *Cell Mol. Biol.,* (Noisy-le-grand), 2006, 52, 4-10.

[61] Jakubowski, H. Homocysteine is a protein amino acid in humans. Implications for homocysteine-linked disease. *J. Biol. Chem.,* 2002, 277, 30425-8.

[61a] Jakubowski H. New method for the determination of protein N-linked homocysteine. *Anal. Biochem.* 380 (2008) 257-61.

[62] Mudd, SH; Finkelstein, JD; Refsum, H; Ueland, PM; Malinow, MR; Lentz, SR; Jacobsen, DW; Brattstrom, L; Wilcken, B; Wilcken, DE; Blom, HJ; Stabler, SP; Allen, RH; Selhub, J; Rosenberg, IH. Homocysteine and its disulfide derivatives: a suggested consensus terminology. *Arterioscler. Thromb. Vasc. Biol.*, 2000, 20, 1704-6.

[63] Jakubowski, H. Mechanism of the condensation of homocysteine thiolactone with aldehydes. *Chemistry*, 2006, 12, 8039-43.

[64] Reuben, DM; Bruice, TC. Reaction of thiol anions with benzene oxide and malachite green. *J. Am. Chem .Soc*., 1976, 98, 114-121.

[65] Jakubowski, H. Translational incorporation of S-nitrosohomocysteine into protein. *J. Biol. Chem.,* 2000, 275, 21813-6.

[66] Jakubowski, H; Fersht, AR. Alternative pathways for editing non-cognate amino acids by aminoacyl-tRNA synthetases. *Nucleic Acids Res.,* 1981, 9, 3105-17.

[67] Chwatko, G; Jakubowski, H. The determination of homocysteine-thiolactone in human plasma. *Anal. Biochem.* 2005, 337, 271-7.

[68] Jakubowski, H. Energy cost of translational proofreading in vivo. The aminoacylation of transfer RNA in Escherichia coli. *Ann. N Y Acad. Sci.*, 1994, 745, 4-20.

[69] Jakubowski, H. Synthesis of homocysteine thiolactone in normal and malignant cells. in: Rosenberg, IH; Graham, I.; Ueland, PM; Refsum, H. (Eds.); Homocysteine Metabolism: From Basic Science to Clinical Medicine, Kluwer Academic Publishers, Norwell, MA, 1997, pp. 157-165.

[70] Jakubowski, H. Protein N-homocysteinylation: implications for atherosclerosis. *Biomed. Pharmacother.,* 2001, 55, 443-7.

[71] Jakubowski, H. Biosynthesis and reactions of homocysteine thiolactone. in: Jacobson, D; Carmel, R; (Eds.), Homocysteine in Health and Disease, Cambridge University Press, *Cambridge*, UK, 2001, pp. 21-31.

[72] Jakubowski, H. Proofreading in vivo. Editing of homocysteine by aminoacyl-tRNA synthetases in Escherichia coli. *J. Biol. Chem.,* 1995, 270, 17672-3.

[72a] Sikora M, Jakubowski H. Homocysteine editing and growth inhibition in *Escherichia coli*. *Microbiology*. 155 (2009) 1858-65.

[73] Jakubowski, H. Proofreading in vivo: editing of homocysteine by methionyl-tRNA synthetase in Escherichia coli. *Proc. Natl. Acad. Sci. U S A*, 1990, 87, 4504-8.

[74] Gao, W; Goldman, E; Jakubowski, H. Role of carboxy-terminal region in proofreading function of methionyl-tRNA synthetase in Escherichia coli. *Biochemistry*, 1994, 33, 11528-35.

[75] Senger, B; Despons, L; Walter, P; Jakubowski, H; Fasiolo, F. Yeast cytoplasmic and mitochondrial methionyl-tRNA synthetases: two structural frameworks for identical functions. *J. Mol. Biol.*, 2001, 311, 205-16.

[76] Jakubowski, H; Guranowski, A. Metabolism of homocysteine-thiolactone in plants. *J. Biol. Chem.*, 2003, 278, 6765-70.

[77] Jakubowski, H. Proofreading in trans by an aminoacyl-tRNA synthetase: a model for single site editing by isoleucyl-tRNA synthetase. *Nucleic Acids Res.*, 1996, 24, 2505-10.

[78] Kim, HY; Ghosh, G; Schulman, LH; Brunie, S. Jakubowski, H. The relationship between synthetic and editing functions of the active site of an aminoacyl-tRNA synthetase. *Proc. Natl. Acad. Sci. U S A*, 1993, 90, 11553-7.

[79] Jakubowski, H. The synthetic/editing active site of an aminoacyl-tRNA synthetase: evidence for binding of thiols in the editing subsite. *Biochemistry*, 1996, 35, 8252-9.

[80] Serre, L; Verdon, G; Choinowski, T; Hervouet, N; Risler, JL; Zelwer, C. How methionyl-tRNA synthetase creates its amino acid recognition pocket upon L-methionine binding. *J. Mol. Biol.*, 2001, 306, 863-76.

[81] Jakubowski, H. Aminoacylation of coenzyme A and pantetheine by aminoacyl-tRNA synthetases: possible link between noncoded and coded peptide synthesis. *Biochemistry*, 1998, 37, 5147-53.

[82] Jakubowski, H. Homocysteine thiolactone: metabolic origin and protein homocysteinylation in humans. *J. Nutr.*, 2000, 130, 377S-381S.

[83] Glowacki, R; Jakubowski, H. Cross-talk between Cys34 and lysine residues in human serum albumin revealed by N-homocysteinylation. *J. Biol. Chem.*, 2004, 279, 10864-71.

[84] Perla-Kajan, J; Marczak, L; Kajan, L; Skowronek, P; Twardowski, T; Jakubowski, H. Modification by Homocysteine Thiolactone Affects Redox Status of Cytochrome c. *Biochemistry*, 2007, 46, 6225-31.

[85] Yang, X; Gao, Y; Zhou, J; Zhen, Y; Yang, Y; Wang, J; Song, L; Liu, Y; Xu, H; Chen, Z; Hui, R. Plasma homocysteine thiolactone adducts associated with risk of coronary heart disease. *Clin .Chim. Acta.*, 2006, 364, 230-4.

[86] Uji, Y; Motomiya, Y; Hanyu, N; Ukaji, F; Okabe, H. Protein-bound homocystamide measured in human plasma by HPLC. *Clin. Chem*., 2002, 48, 941-4.

[87] Perna, AF; Satta, E; Acanfora, F; Lombardi, C; Ingrosso, D; De Santo, NG. Increased plasma protein homocysteinylation in hemodialysis patients. *Kidney Int.*, 2006, 69, 869-76.

[88] Mallamaci, F; Zoccali, C; Tripepi, G; Fermo, I. Benedetto, FA. Cataliotti, A; Bellanuova, I; Malatino, LS; Soldarini, A. Hyperhomocysteinemia predicts cardiovascular outcomes in hemodialysis patients. *Kidney Int.*, 2002, 61, 609-14.

[89] Perla, J; Undas, A; Twardowski, T; Jakubowski, H. Purification of antibodies against N-homocysteinylated proteins by affinity chromatography on Nomega-homocysteinyl-aminohexyl-Agarose. *J. Chromatogr. B Analyt. Technol. Biomed. Life Sci.*, 2004, 807, 257-61.

[90] Perła-Kaján, J; Stanger, O; Ziółkowska, A; Melandowicz, LK; Twardowski, T; Jakubowski, H. Immunohistochemical detection of N-homocysteinylated proteins in cardiac surgery patients. *Clin. Chem. Lab.* Med., 2007, 45, in press.

[91] Hop, CE; Bakhtiar, R. Homocysteine thiolactone and protein homocysteinylation: mechanistic studies with model peptides and proteins. *Rapid Commun. Mass Spectrom*, 2002, 16, 1049-53.

[92] Naruszewicz, M; Mirkiewicz, E; Olszewski, AJ; McCully, KS. Thiolation of low density lipoproteins by homocysteine thiolactone causes increased aggregation and altered interaction with cultured macrophages. *Nutr. Metab. Cardiovasc. Dis.*, 1991, 4, 70-77.

[93] Vidal, M; Sainte-Marie, J; Philippot, J; Bienvenue, A. Thiolation of low-density lipoproteins and their interaction with L2C leukemic lymphocytes. *Biochimie*, 1986, 68, 723-30.

[94] Liu, G; Nellaiappan, K; Kagan, HM. Irreversible inhibition of lysyl oxidase by homocysteine thiolactone and its selenium and oxygen analogues. Implications for homocystinuria. *J. Biol. Chem.*, 1997, 272, 32370-7.

[95] Harker, LA; Slichter, SJ; Scott, CR; Ross, R. Homocystinemia. Vascular injury and arterial thrombosis. *N. Engl. J. Med.*, 1974, 291, 537-43.

[96] Endo, N; Nishiyama, K; Otsuka, A; Kanouchi, H; Taga, M; Oka, T. Antioxidant activity of vitamin B6 delays homocysteine-induced atherosclerosis in rats. *Br. J. Nutr.*, 2006, 95, 1088-93.

[97] Rosenquist, TH; Ratashak, SA; Selhub, J. Homocysteine induces congenital defects of the heart and neural tube: effect of folic acid. *Proc. Natl. Acad .Sci. U S A*, 1996, 93, 15227-32.

[98] Maestro De Las Casas, C; Epeldegui, M; Tudela, C; Varela-Moreiras, G; Perez-Miguelsanz, J. High exogenous homocysteine modifies eye development in early chick embryos. *Birth Defects Res. A Clin. .Mol. Teratol.* 2003, 67, 35-40.

[99] Donahue, S; Struman, JA; Gaull, G. Arteriosclerosis due to homocyst (e) inemia. Failure to reproduce the model in weanling rabbits. *Am. J. Pathol.* 1974, 77, 167-3.

[100] Makheja, AN; Bombard, AT; Randazzo, RL; Bailey, JM. Anti-inflammatory drugs in experimental atherosclerosis. Part 3. Evaluation of the atherogenicity of homocystine in rabbits. *Atherosclerosis*, 1978, 29, 105-12.

[101] Anfinsen, CB. Principles that govern the folding of protein chains. *Science*, 1973, 181, 223-30.

[102] Fersht, A. Structure and mechansim in protein science, WH Freeman and Company, New York, 2000.

[103] Stefani, M. Protein misfolding and aggregation: new examples in medicine and biology of the dark side of the protein world. *Biochim. Biophys. Acta,* 2004, 1739, 5-25.

[104] Werstuck, GH; Lentz, SR; Dayal, S; Hossain, GS; Sood, SK; Shi, YY; Zhou, J; Maeda, N; Krisans, SK; Malinow, MR; Austin, RC. Homocysteine-induced endoplasmic reticulum stress causes dysregulation of the cholesterol and triglyceride biosynthetic pathways. *J. Clin. Invest.,* 2001, 107, 1263-73.

[105] Zhang, C; Cai, Y; Adachi, MT; Oshiro, S; Aso, T; Kaufman, RJ; Kitajima, S. Homocysteine induces programmed cell death in human vascular endothelial cells through activation of the unfolded protein response. *J. Biol. Chem.,* 2001, 276, 35867-74.

[106] Zhou, J; Moller, J; Danielsen, CC; Bentzon, J; Ravn, HB; Austin, RC; Falk, E. Dietary supplementation with methionine and homocysteine promotes early atherosclerosis but not plaque rupture in ApoE-deficient mice. *Arterioscler. Thromb Vasc. Biol.,* 2001, 21, 1470-6.

[107] Hossain, GS; Van Thienen, JV; Werstuck, GH; Zhou, J; Sood, SK; Dickhout, JG; De Koning, AB; Tang, D; Wu, D; Falk, E; Poddar, R; Jacobsen, DW; Zhang, K; Kaufman, RJ; Austin, RC. TDAG51 is induced by homocysteine, promotes detachment-mediated programmed cell death, and contributes to the cevelopment of atherosclerosis in hyperhomocysteinemia. *J. Biol. Chem.,* 2003, 278, 30317-27.

[108] Roybal, CN; Yang, S; Sun, CW; Hurtado, D; Vander Jagt, DL; Townes, TM; Abcouwer, SF. Homocysteine increases the expression of vascular endothelial growth factor by a mechanism involving endoplasmic reticulum stress and transcription factor ATF4. *J. Biol. Chem.,* 2004, 279, 14844-52.

[109] Ferguson, E; Parthasarathy, S; Joseph, J; Kalyanaraman, B. Generation and initial characterization of a novel polyclonal antibody directed against homocysteine thiolactone-modified low density lipoprotein. *J. Lipid. Res.,* 1998, 39, 925-33.

[110] Undas, A; Perla, J; Lacinski, M; Trzeciak, W; Kazmierski, R; Jakubowski, H. Autoantibodies against N-homocysteinylated proteins in humans: implications for atherosclerosis. *Stroke,* 2004, 35, 1299-304.

[111] Undas, A; Jankowski, M; Twardowska, M; Padjas, A; Jakubowski, H; Szczeklik, A. Antibodies to N-homocysteinylated albumin as a marker for early-onset coronary artery disease in men. *Thromb Haemost.,* 2005, 93, 346-50.

[112] Undas, A; Stepien, E; Glowacki, R; Tisonczyk, J; Tracz, W; Jakubowski, H. Folic acid administration and antibodies against homocysteinylated proteins in subjects with hyperhomocysteinemia. T*hromb Haemost.* 2006, 96, 342-7.

[113] Undas, A; Kolarz, M; Kopec, G; Glowacki, R; Placzkiewicz-Jankowska, E; Tracz, W. Autoantibodies against N-homocysteinylated proteins in patients on long-term haemodialysis. *Nephrol. Dial.Transplant.,* 2007, 22, 1685-9.

[114] Folsom, AR; Desvarieux, M; Nieto, FJ; Boland, LL; Ballantyne, CM; Chambless, LE. B vitamin status and inflammatory markers. *Atherosclerosis,* 2003, 169, 169-74.

[115] Ravaglia, G; Forti, P; Maioli, F; Servadei, L; Martelli, M; Arnone, G; Talerico, T; Zoli, M; Mariani, E. Plasma homocysteine and inflammation in elderly patients with cardiovascular disease and dementia. *Exp. Gerontol*., 2004, 39, 443-50.

[116] Peeters, AC; Van Aken, BE; Blom, HJ; Reitsma, PH; Den Heijer, M. The effect of homocysteine reduction by B-vitamin supplementation on inflammatory markers. *Clin. Chem. Lab. Med.*, 2007, 45, 54-8.

[117] Friso, S; Jacques, PF; Wilson, PW; Rosenberg, IH; Selhub, J. Low circulating vitamin B(6) is associated with elevation of the inflammation marker C-reactive protein independently of plasma homocysteine levels. *Circulation*, 2001, 103, 2788-91.

[118] Rohde, LE; Hennekens, CH; Ridker, PM. Survey of C-reactive protein and cardiovascular risk factors in apparently healthy men. *Am. J. Cardiol.* 1999, 84, 1018-22.

[119] Holven, KB; Aukrust, P; Retterstol, K; Hagve, TA; Morkrid, L; Ose, L. Nenseter, MS. Increased levels of C-reactive protein and interleukin-6 in hyperhomocysteinemic subjects. *Scand. J. Clin. Lab. Invest.*, 2006, 66, 45-54.

[120] Aso, Y; Yoshida, N; Okumura, K; Wakabayashi, S; Matsutomo, R; Takebayashi, K; Inukai, T. Coagulation and inflammation in overt diabetic nephropathy: association with hyperhomocysteinemia. *Clin. Chim. Acta*, 2004, 348, 139-45.

[121] Shai, I; Stampfer, MJ; Ma, J; Manson, JE; Hankinson, SE; Cannuscio, C; Selhub, J; Curhan, G; Rimm, EB. Homocysteine as a risk factor for coronary heart diseases and its association with inflammatory biomarkers, lipids and dietary factors. *Atherosclerosis*, 2004, 177, 375-81.

[122] Widner, B; Leblhuber, F; Frick, B; Laich, A; Artner-Dworzak, E; Fuchs, D. Moderate hyperhomocysteinaemia and immune activation in Parkinson's disease. *J. Neural Transm.*, 2002, 109, 1445-52.

[123] Holven, KB; Scholz, H; Halvorsen, B; Aukrust, P; Ose, L; Nenseter, MS. Hyperhomocysteinemic subjects have enhanced expression of lectin-like oxidized LDL receptor-1 in mononuclear cells. *J. Nutr.*, 2003, 133, 3588-91.

[124] Powers, RW; Majors, AK; Cerula, SL; Huber, HA; Schmidt, BP; Roberts, JM. Changes in markers of vascular injury in response to transient hyperhomocysteinemia. *Metabolism*, 2003, 52, 501-7.

[125] Wang, G; Woo, CW; Sung, FL; Siow, YL; K. O. Increased monocyte adhesion to aortic endothelium in rats with hyperhomocysteinemia: role of chemokine and adhesion molecules. *Arterioscler. Thromb. Vasc. Biol.*, 2002, 22, 1777-83.

[126] Zhang, R; Ma, J; Xia, M; Zhu, H. Ling, W. Mild hyperhomocysteinemia induced by feeding rats diets rich in methionine or deficient in folate promotes early atherosclerotic inflammatory processes. *J. Nutr.*, 2004, 134, 825-30.

[127] Lee, H; Kim, HJ; Kim, JM; Chang, N. Effects of dietary folic acid supplementation on cerebrovascular endothelial dysfunction in rats with induced hyperhomocysteinemia. *Brain Res.*, 2004, 996, 139-47.

[128] Lee, H; Kim, JM; Kim, HJ; Lee, I; Chang, N. Folic acid supplementation can reduce the endothelial damage in rat brain microvasculature due to hyperhomocysteinemia. *J. Nutr*., 2005, 135, 544-8.

[129] Bogdanski, P; Pupek-Musialik, D; Dytfeld, J; Lacinski, M; Jablecka, A; Jakubowski, H. Plasma homocysteine is a determinant of tissue necrosis factor-alpha in hypertensive patients. *Biomed. Pharmacother.* 62 (2008) 360-5.

[130] Jakubowski H, Boers GH, Strauss KA. Mutations in cystathionine beta-synthase or methylenetetrahydrofolate reductase gene increase N-homocysteinylated protein levels in humans. *FASEB J* 22 (2008) 4071-6.

[131] Jakubowski H, Perla-Kajan J, Finnell RH, Cabrera RM, Wang H, Gupta S, Kruger WD, Kraus JP, Shih DM. Genetic or nutritional disorders in homocysteine or folate metabolism increase protein N-homocysteinylation in mice. *FASEB J.* 23 (2009) 1721-7.

Index

A

B

C

D

E

F

G

H

I

J

K

L

M

N

O

P

Q

R

S

T

U

V

W

X

Y