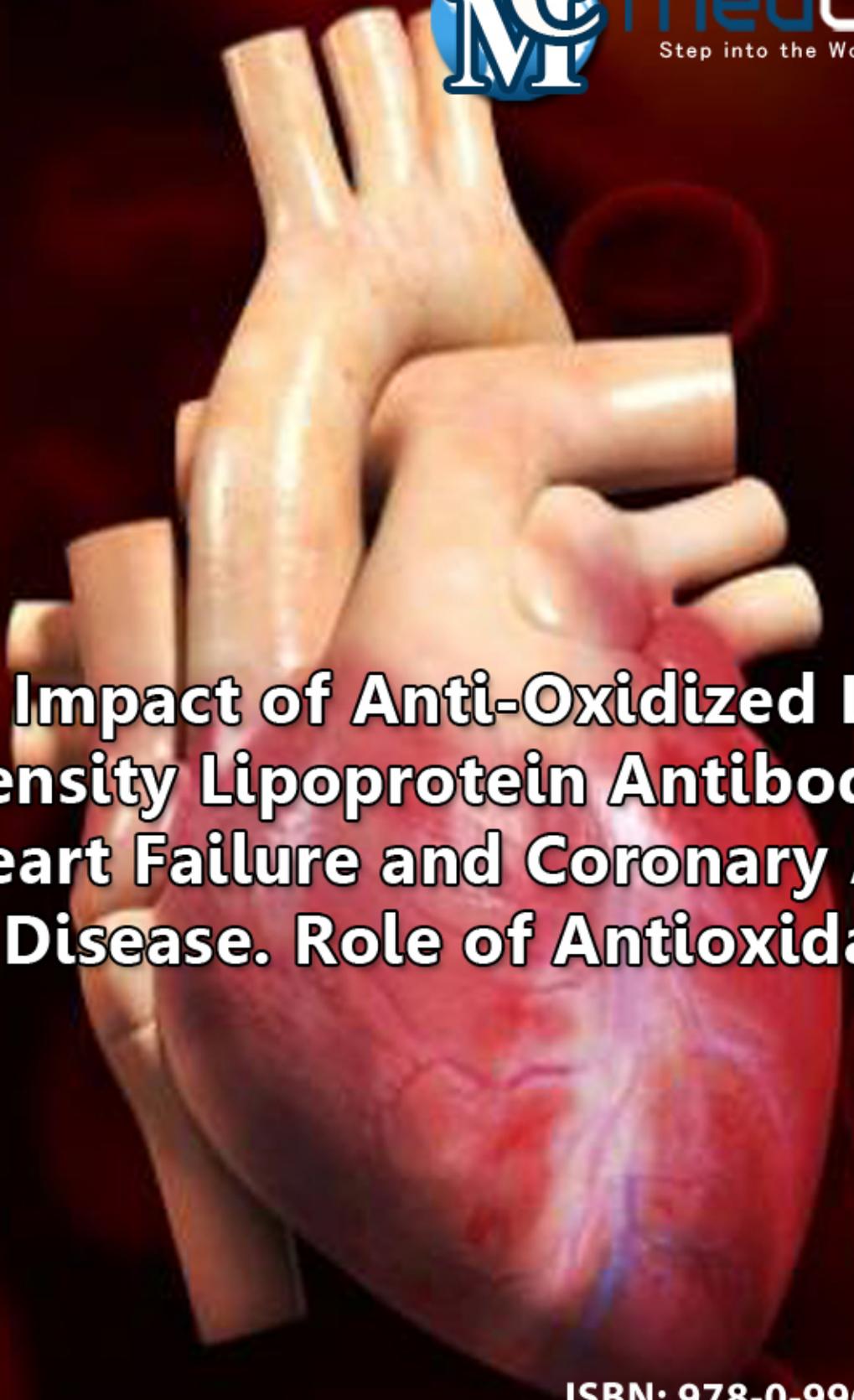




MedCrave

Step into the World of Research



Impact of Anti-Oxidized Low Density Lipoprotein Antibodies in Heart Failure and Coronary Artery Disease. Role of Antioxidants

ISBN: 978-0-9967956-4-7

Gideon Charach

Impact of Anti - Oxidized Low Density Lipoprotein Antibodies in Heart Failure and Coronary Artery Disease. Role of Antioxidants



Gideon Charach, MD

The Department of Internal Medicine "C", Affiliated to Sackler Medical School, Tel Aviv University Tel Aviv Sourasky Medical Center, 6 Weizman Street, Tel Aviv 6423906, Israel, Tel: +972 524 266752; Fax: +972-3-6974990; Email: drcharach@012.net.il

Ori Argov, Ori Rogowsky and Itamar Grosskopf

The Department of Internal Medicine "C", Tel Aviv Sourasky Medical Center, 6 Weizman Street, Tel Aviv 6423906, Israel

Contents

Abbreviations	1
Declaration	2
Acknowledgement	3
Chapter 1	4
Abstract	5
Keywords	5
Chapter 2	6
Pathophysiology of LDL Oxidation	7
Oxygen Radicals	7
LDL oxidation	7
Ox LDL Antibodies	8
Immune Defense	8
Immune Regulation of Anti Ox LDL Abs	8
Lectin like Ox LDL Scavenger Receptor - 1 (LOX-1)	8
Atherosclerosis	9
Role of Ox LDL in Atherosclerosis	9
Ox LDL Abs Destructive and Protective	10
Ox LDL in Endothelial Atheroma	10
Endothelial Dysfunction	11
Oxidative Stress and Diseases not Associated with Artherosclerosis	11
Cardiovascular Disease - Animal Studies	11
Cardiovascular Disease - Human Studies , Atherosclerosis	12
Anti Ox LDL Abs - Predictor of Morbidity and Mortality in CAD	12
Myocardial Insulin Resistance	12
Heart Failure	12
Anti-Ox LDL Abs and B-type Natriuretic Peptide (BNP)	13
Clinical Impact of Ox LDL Abs on Rehabilitation and Prognosis	14
Antioxidants	14
Cancer and Antioxidants	15
Age Related Eye Disease and Antioxidants	15
Antioxidants and Heart Disease	15
Chapter 3	18
Conclusion	19
References	20

Abbreviations

AMI: Acute Myocardial Infarction

OFR: Oxygen Free Radicals

Ox LDL AB: Antibodies against Oxidized Low Density Lipoprotein

Apo: Apolipoprotein

CAD: Coronary Artery Disease

ROS: Reactive Oxygen Species

ELISA: Enzyme-Linked Immunosorbent Assay

HDL: High-Density Lipoprotein

LDL: Low-Density Lipoprotein

Lp (a): Lipoprotein (a)

MDA: Malonic Dialdehyde

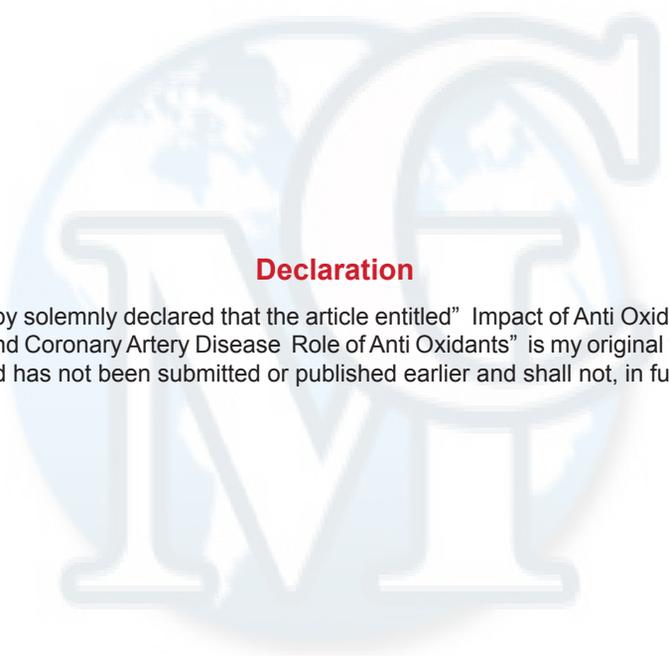
NO: Nitric Oxide

ATP: Adenosine Tri Phosphate

NOS: Nitric Oxide Synthetase

AGE: Advanced Glycation Products





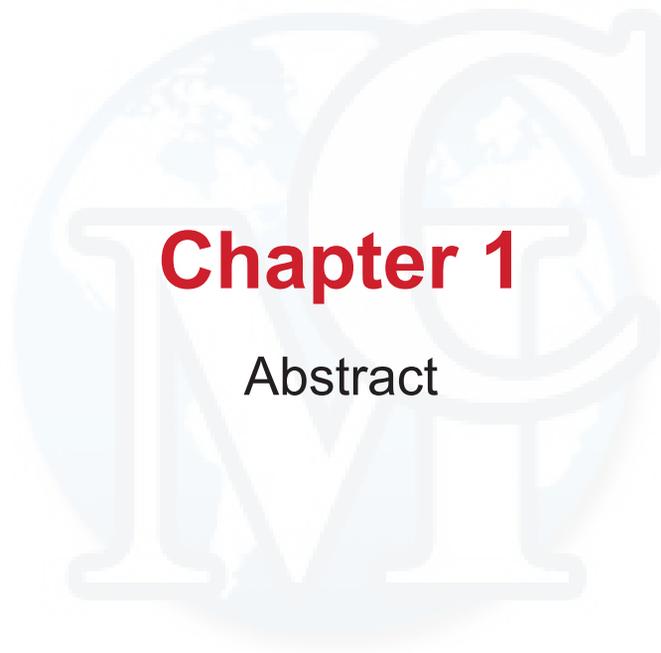
Declaration

I, Dr. Gideon Charach , hereby solemnly declared that the article entitled” Impact of Anti Oxidized Low Density Lipoprotein Antibodies in Heart Failure and Coronary Artery Disease Role of Anti Oxidants” is my original work except where otherwise acknowledged in the text and has not been submitted or published earlier and shall not, in future, be submitted by me.



Acknowledgement

Heartfelt thanks to my friend - Professor Jacob George - Kaplan Medical Center, Rehovot, Israel who introduced me to the theme - oxidized LDL antibodies - and made writing of this book possible, he had been supportive in the research and writing of several articles.



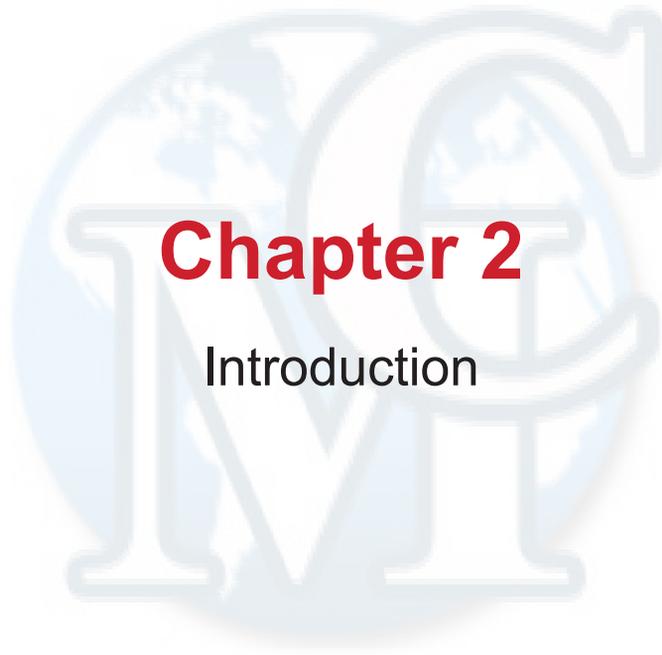
Chapter 1

Abstract

Abstract

Oxidative stress may play a significant role in the pathogenesis of heart failure (HF). Antibodies to oxidized low-density lipoprotein (Ox LDL Abs) reflect an immune response to LDL over a prolonged period and may represent long-term oxidative stress in HF. The Ox LDL plasma level is a useful predictor of mortality in HF and coronary artery disease (CAD) patients, and measurement of the Ox LDL Abs level may allow better management of those patients. Antibodies to Ox LDL also significantly correlate with the New York Heart Association (NYHA) score. Smoking, hypercholesterolemia, hypertension and obesity are known risk factors for atherosclerotic coronary artery disease (CAD) leading to heart failure, but these factors account for only 50% of all cases and our understanding of the pathogenesis underlying HF remains incomplete. Nutrients with antioxidant properties can reduce the susceptibility of LDL to oxidation. Treatment by antioxidants may be an adjunct to lipid-lowering, angiotensin converting enzyme inhibition and metformin (in diabetes) therapy due to the greatest impact on CAD and HF. There are many reports that suggest a protective effect of antioxidant supplementation on the incidence of HD. This review summarizes the data on Ox LDL Abs as a predictor of morbidity and mortality in HF patients.

Keywords: Heart Failure; Oxidized Low-Density Lipoproteins; Antibodies; Antioxidants



Chapter 2

Introduction

Introduction

The clinical syndrome of chronic heart failure (HF) as characterized by abnormalities of left ventricular function and neurohormonal regulation, which are accompanied by effort intolerance, fluid retention and decreased longevity, were described for the first time by Milton Packer [1]. Dysfunction of vascular endothelium in patients with HF is a main component in the characteristic systemic vasoconstriction and reduced peripheral perfusion. Regulation of vascular endothelial tonus is mediated mainly by nitric oxide (NO) [2]. Oxidative stress is a term that determinates the imbalance between different factors that promote production of reactive oxygen species (ROS) and the ability to scavenge and neutralize the toxic byproducts of these reactive free radicals [3-5]. Normally ROS react with NO in the setting of decreased antioxidant defenses that would clear these radicals, culminating in attenuated endothelium-dependent vasodilatation in patients with heart failure [2,3-5].

Several studies suggested that oxidative stress could be involved in the pathogenesis of HF. Reactive free radicals have a pathogenetic role in the progressive deterioration of the decompensating myocardium [5,6] as well. Infusion of oxidized free radicals produces a marked decrease in myocardial contractility [2,3,6-10]. Immunoglobulins to oxidized low-density lipoprotein (Ox LDL) were discovered by chance by Beaumont in a patient with multiple myeloma and hyperlipidemia [9]. Antibodies (Ab) against Ox LDL were found in several diseases other than atherosclerosis, among them HF, diabetes mellitus, renovascular syndrome, uremia, rheumatic fever, ankylosing spondylitis and lupus erythematosus [2,3,11,12]. Moreover, antibody levels of Ox LDL antibodies were reported to correlate significantly with the clinical status of HF patients, as defined by their New York Heart Association (NYHA) score [8]. Measurements of Ox LDL Abs also reflect the status of lipoprotein oxidation over a prolonged period [3,10].

Assessment of oxidative stress in humans is complex since there is no standardized, reproducible methodology [7,8,10]. The purpose of this book is to acquaint the reader with the new investigations on Ox LDL Abs and their use and determination in clinical practice. Current review also cites recent studies on antioxidants and shows their implications in the treatment in HF and CAD emphasizing that antioxidants may contribute to better treatment and longevity [11-17].

Pathophysiology of LDL oxidation

Oxygen radicals

Oxygen free radicals have a crucial role in the origin of life and biological evolution, bestowing beneficial effects on the organisms [18]. The ROS are involved in many biochemical activities of cells, such as signal transduction, gene transcription and regulation of soluble guanylate cyclase activity. People are constantly exposed to free radicals created by electromagnetic radiation from the human-made environment, such as pollutants and cigarette smoke.

Natural resources, such as radon, cosmic radiation, as well as cellular metabolisms (respiratory burst, enzyme reactions) also add oxygen free radicals to the environment. The most common reported cellular free radicals are hydroxyl ($\text{OH}\cdot$), superoxide ($\text{O}_2\cdot^-$) and nitric monoxide (NO). Even some other molecules, such as hydrogen peroxide (H_2O_2) and peroxynitrite (ONOO^-), are not free radicals, and they are often reported to generate free radicals through various chemical reactions [10]. The most known is NO which is an important signaling molecule that efficiently regulates the relaxation and proliferation of smooth, vascular muscle cells, leukocytes adhesion, platelets aggregation, angiogenesis, thrombosis, vascular tone and hemodynamics [11-17]. Cells exposed to environment fortified with oxygen continuously generate oxygen free radicals (OFR). Defense systems of antioxidants co-evolved along with aerobic metabolism to counteract oxidative damage from OFR [21]. The human body produces oxygen free radicals and other ROS as by products through numerous physiological and biochemical reactions. Oxygen-related free radicals (superoxide and hydroxyl radicals) and reactive species (hydrogen peroxide, nitric oxide, peroxynitrite and hypochlorous acid) are produced in the body, primarily as a result of aerobic metabolism [22,23].

Creation of ROS is a particularly destructive mechanism of oxidative stress. Such species include oxygen free radicals and peroxides. Some of these species (such as superoxide) can be converted by oxidoreduction reactions with transition metals or other redox cycling compounds (including quinones) into more aggressive radical species that can cause severe cellular damage [24-26]. Majority of long-term effects are caused by damage to DNA [24-29]. This lesion of DNA induced by ionizing radiation is similar to oxidative stress, and these lesions have been implicated in the aging process and in cancer. Biological effects of single-base damage by radiation or oxidation, such as 8-oxoguanine and thymine glycol, have been extensively studied. The main interest has recently shifted to some of the more complex lesions. DNA damages are formed at substantial frequency by ionizing radiation and metal-catalyzed H_2O_2 reactions. In anoxic conditions, the predominant double-base lesion is a species in which C8 of guanine is linked to the 5-methyl group of an adjacent 3'-thymine [24-26]. The majority of these ROS species are produced at a low level by normal aerobic metabolism, and normal defense mechanisms of the cells destroy most of them. Normally, any lesion to cells is constantly repaired. However, under the extensive levels of oxidative stress that cause necrosis, the damage causes ATP depletion, preventing controlled apoptotic death and causing the cell to simply fall apart [24-26].

LDL Oxidation

Oxidation of LDL is a complex process that takes place in both the extra- and intracellular space [3,10,12-15]. It plays an important role in endothelial dysfunction as follows. Modification of LDL particles due to oxidation, glycation and binding of advanced glycation end-products

or malondialdehyde (MDA, a final product of lipid peroxidation) is considered as being crucial in the process of atherogenesis [4,7]. Oxidatively modified LDL particles are distinguished by another receptor type, which was discovered on the surface of macrophages and termed “the scavenger receptor [3,10,13,14]. Massive intake of LDL converts macrophages to foam cells, and their accumulation under the vascular endothelium is involved in the initiation of atherosclerosis [7,13,14].

Ox LDL Antibodies

Modified LDL particles show chemotactic, cytotoxic and immunogenic properties at the end of this oxidative process. The Ox LDL particles express a large number of epitopes and cause the production of a polyclonal mixture of Abs (isoantibodies IgA and IgG) caused by HDL and LDL polymorphism against these products, especially the lipid phase of LDL, against apoB100 modified by MDA and 4-hydroxynonenal [3,12-14].

Immune defense

The immune system uses the lethal effects of oxidants by making the production of oxidizing species a central part of its mechanism of killing pathogens, with activated phagocytes producing both ROS and reactive nitrogen species. These include superoxide (O₂⁻), NO and their particularly reactive product, peroxynitrite (ONOO⁻) [27]. However, Abs can also stimulate other immune effects, such as immune opsonin-associated antigen binding to phagocytes, complement activation, and presentation of antigens to T lymphocytes, to trigger an adaptive immune response. Antibodies to Ox LDL playing an important role in these immune functions can induce an immune injury [17,18,21-29].

Antibodies to Ox LDL belong to different classes and subclasses, including IgA, IgG1, IgG2, IgG3, and IgM, as well as specific idiotypes [5,6,24-33]. The multiple classes and subclasses of anti-Ox LDL Abs may result in heterogeneous effects: namely, Ox LDL (not antibodies) contains phospholipids; IgG2 is the dominant subclass to respond to epitopes containing phospholipids; and the IgG3 subclass fixes, complements better, and binds Fc receptors more avidly and thus has more pathogenic properties than the IgG2 subclass [24-33]. However, these differences in Ox LDL Abs characteristics and their correlation with clinical manifestations have not yet been analyzed [26,28,30-36].

Molecules of Ox LDL may act as an auto antigen. It is frequently present in the sera of patients with autoimmune conditions, acute coronary syndrome, or stable coronary artery disease (CAD) [15-18]. Ox LDL molecules have been associated with both subclinical atherosclerosis and inflammatory processes [19,20]. A large amount of Ox LDL accumulates in atherosclerotic plaques, [29-31] and the serum concentrations of circulating Ox LDL may correlate with the severity of coronary diseases [23] and unstable angina [24]. Ox LDL is an immunogenic molecule that stimulates production of anti-Ox LDL Abs. Therefore, it is not surprising that an association was found between

the presence of anti-Ox LDL Abs, atherosclerosis and cardiovascular disease (CVD) [26,29-36].

Immune regulation of anti Ox LDL Abs

The Ox LDL Abs in healthy people seem to be native Abs [6-8,24-26,29-36] that neutralize and catabolize Ox LDL [6-8,26,29-38]. Native Abs and anti-idiotypic Abs with intravenous immunoglobulin (IVIg) could affect atherosclerosis and CAD progression. The administration of IVIg to apoE-deficient mice modulated both the development of fatty streaks and the progression to fibro fatty atherosclerotic plaques and resulted in reduced atherosclerosis [24-38]. The treatment by intravenous immunoglobulin was associated with T-cell energy and reduction of IgM anti-Ox LDL Abs titers. Human IVIg from different manufacturers contains both protective anti-Ox LDL Abs and anti-idiotypic Abs to anti-Ox LDL Ab [6-8,26, 29-36].

Ox LDL Abs bind Ox LDL and create immune complexes. Circulating immune complexes are not pathogenic. They cause damage only if they are deposited in tissues. The pathogenesis of atherosclerosis and CAD involves different mechanisms, including the response of the vessel wall to injury, toxic effects of immune complexes, and the effects of Ox LDL [32-38]. These mechanisms supplement each other: Ox LDL induce an immune reaction, with one of the consequences being the production of immune complexes [24-28,30-35].

Immunoglobulins to Ox LDL (Abs against Ox LDL) can be found directly in intimal lesions or as a component of circulating immune complexes [2,12-14]. Increased generation of ROS reportedly promoted patients exercise intolerance and decreased tissue perfusion due to increased peripheral resistance in patients with HF and CAD [2]. Moreover, Ox LDL Abs levels correlated with the quality of HF treatment control, as reflected by the number of hospital admissions recorded in the year prior to enrolment [4,8]. The changes and correlations of Ox LDL Abs, anti-beta-2-glycoprotein I IgG and antiphospholipid antibodies explain the immunological mechanism between thrombotic and atherosclerotic processes in the human body [3,13,14], therefore indicating that the increased concentration of Ox LDL Abs correlates with the severity of HF and CAD.

Lectin-like Ox LDL scavenger receptor-1 (LOX-1)

The biological effects of Ox LDL are mediated via its receptors. A number of scavenger receptors for Ox LDL, such as SR-A1/II, CD36, SR-B1, and CD68, have been identified on smooth muscle cells monocytes and macrophages [34-37]. However, these receptors are not present on vascular endothelial cells in any significant amount. It has been suggested that vascular endothelial cells in culture and in vivo internalize and degrade Ox LDL through a receptor-mediated pathway that does not involve the macrophage scavenger receptors [3-6,32-39]. LOX-1 as a very important molecule that is responsible for Ox LDL uptake by vascular endothelial cells. Ox LDL

uptake via LOX-1 causes endothelial activation [38-45]. Uptake of Ox-LDL in endothelial cells (internalization) and subsequent extrusion may be a mechanism by which Ox-LDL is transported to the sub endothelium [35-37,40-43].

Ox LDL plays a crucial role in a many of cardiovascular diseases, including atherosclerosis, hypertension and ischemia of the myocard. Encoded by the OLR1 gene, LOX-1 is the major receptor for Ox LDL at the surface of the cells. LOX-1 activation by Ox LDL, ROS, angiotensin II and inflammatory signals causes many pathological processes: cell proliferation, apoptosis and autophagy, which are hallmarks of the atherosclerotic lesions. LOX-1 is also involved in mitochondrial DNA damage-mediated inflammatory response [38-43]. All these experimental observations support the possible contribution of LOX-1 to the pathogenesis of CVD, particularly atherosclerosis, indicating that targeting LOX-1 may be an effective strategy for the treatment of CVD. These data summarize current knowledge of LOX-1 function and possible therapeutic options targeting LOX-1 in CAD [35-45].

It has been observed that Ox LDL increases intracellular free radical production and activates transcription factor NF- κ B in bovine endothelial cells [38-40]. It should be noted that Ox LDL might stimulate different signal pathways, which interact with each other. These mechanisms may reflect the complicated cross-processes between intracellular signaling pathways induced by Ox-LDL and other pro-atherogenic signals [40-55].

Atherosclerosis is an established etiology of myocardial ischemia, peripheral vascular disease, cerebrovascular disease and several other CVD. It was proven that Ox LDL is more potent in the development of atherosclerosis than native LDL [34-43]. This finding strongly suggest that Ox LDL starts and sustains atherogenesis by LOX-1 activation. We previously mentioned that LOX-1 deletion may prevent atherogenesis in LDLR knockout mice fed a high cholesterol diet [34-43]. In human LOX-1 gene has been also confirmed as being associated with several other CVD [37-39]. Reperfusion injury results in temporary cessation of coronary blood circulation, needed treatment by thrombolysis, percutaneous coronary interventions, and coronary bypass grafting. In mice with myocardial ischemia-reperfusion injury, Kataoka et al. [42] found that, compared with normal IgG or saline administration, LOX-1 inhibition by its Ab treatment resulted in a nearly 50% reduction in myocardial infarct size. Animal studies using an ischemia-reperfusion model in which rats received pretreatment with LOX-1 Ab, Li et al. [40] reported that LOX-1 inhibition prevented adhesion molecule expression and leukocyte activation and accumulation [41-45]. For determination of the role of LOX-1 in ischemia-reperfusion injury to the heart, Hu et al. [44] subjected wild-type and LOX-1 knockout mice to one hour of left coronary artery occlusion followed by another one hour min of reperfusion. This investigation showed that LOX-1 knockout resulted in a significant reduction of myocardial damage and in the recruitment of inflammatory cells [39,45-51].

Atherosclerosis

The etiology of atherosclerosis is complex and multifactorial, but there is extensive evidence indicating that oxidized lipoproteins and Ab against Ox LDL may play a critical role. At present, the site and mechanism by which lipoproteins are oxidized are not resolved, and it is not clear if oxidized lipoproteins form locally in the artery wall and/or are sequestered in atherosclerotic lesions following the uptake of circulating oxidized lipoproteins [32-39,46-56]. The diet-derived oxidized fatty acids in chylomicron remnants and oxidized cholesterol in remnants and LDL accelerate atherosclerosis by increasing oxidized lipid levels in circulating LDL and chylomicron remnants. This hypothesis is supported by the experiments in feeding animals. In rabbits fed oxidized fatty acids or oxidized cholesterol, the fatty streak lesions in the aorta were increased by one hundred percent!! Furthermore, dietary oxidized cholesterol significantly increased aortic plucks in apo-E and LDL receptor-deficient mice. A typical Western diet is rich in oxidized fats and therefore could contribute to the increased arterial atherosclerosis in the population of developed countries [26,33,37-38,53-58].

Role of Ox LDL in atherosclerosis

The importance of Ox LDL in atherosclerosis was first established through the use of the antioxidant probucol in atherosclerosis-prone hyperlipidemic (WHHL) rabbits [26,38-40,40-49]. Those investigations showed the significance of the oxidative state (stress) in atherogenesis. The pro-oxidant state is present in all stages of atherosclerosis from the beginning to the thrombotic event. Oxidative stress and formation of Ox LDL are potent mitogens for smooth muscle cells [43,48-56]. Ox LDL is absorbed by the macrophages, which become foam cells. To elucidate the role of Ox LDL for plaque instability in CAD, Ehara et al. [49,50] measured plasma Ox LDL levels in patients with acute myocardial infarction, unstable angina pectoris, and stable angina pectoris. Levels of Ox LDL in patients plasma who suffered from acute myocardial infarction were much higher than in patients with unstable or stable angina pectoris. Importantly, serum levels of total, HDL, and LDL cholesterol did not vary among those groups. The post mortem data in patients who died of acute myocardial infarction revealed that the epicenter of coronary lesion contained abundant macrophage-derived foam cells with distinct positivity for Ox LDL and its receptors! Those results strongly suggested an important role for Ox LDL in the development of plaque instability which was found in human coronary atherosclerotic lesions. Increased concentration of Ox LDL in blood were also shown to be higher in patients with diabetes mellitus [50]. As it was mentioned earlier [55] soluble LOX-1 levels are significantly higher in the serum of patients with myocardial ischemia than in those of the control group. Recent studies established that soluble LOX-1 may well become the marker for early diagnosis of acute coronary syndrome [38-42,48-53].

Ox LDL Ab –Destructive and Protective

Pathophysiologic role of Ox LDL Ab

Several studies found associations between anti-Ox LDL Abs and CVD-related conditions [8,47-53]. Immunoglobulins in atherosclerotic plucks [53] - specifically recognize Ox LDL. Elevated anti-Ox LDL Abs concentrations may be a predictor for the development of atherosclerosis, CAD and CVD [48-54]. Those Abs are the most effective indicators in the prediction of the extent of coronary atherosclerosis [48-52]. The presence of high concentrations of Ox LDL Ab is also associated with a higher risk for coronary restenosis after percutaneous transluminal coronary angioplasty [49-55]. Higher levels of the Abs in patients with peripheral occlusive arterial disease portend more extensive atherosclerotic lesions [47-55]. Elevated levels of Ox LDL Abs are related to other diseases as hypertension, systemic vasculitis, peripheral vascular disease, cerebrovascular and other non coronary atherosclerosis [25,28,29,47-51,55-66].

However, there is also a defensive role of Ox LDL Abs in humans. Human anti-Ox LDL Abs play an important role in the regulation of Ox LDL levels. These Abs have been found in children, healthy adults [48,58], and patients with coronary heart disease [47-52]. Circulating Abs that recognize Ox LDL are present in children without cardiac diseases. The levels of the Ab in children are significantly higher than in adults, [48-52,58,59] suggesting that they may not necessarily be related to atherosclerosis and CVD. It is probable that the high levels of Ab in children modulate the antigen and thus protect against the development of atherosclerosis and cardiovascular diseases. One investigation of anti-Ox LDL Abs in patients with early CVD revealed that those Abs were decreased in patients with borderline hypertension [49]. The results of a survey on complications of diabetic CAD supported that finding [33,47-52].

Ox LDL in endothelial atheroma

Atherosclerosis is recognized as a chronic inflammatory disease of the arterial wall that leads to atheromatous plaque formation. There is a consensus based on recent studies that oxidation of LDL in the endothelial wall is an early event in atherosclerosis [24,53]. Indeed, the circulating LDL particles are transported from the vascular space into the arterial endothelium by transcytosis mechanism [41,47-53]. LDL is retained in the extracellular matrix of the subendothelial space through the binding of basic amino acids in a lipoprotein B100 to negatively charged sulphate groups of proteoglycans in the extracellular matrix, where it is prone to be oxidized by oxidative stress, producing Ox LDL [21,33,48-53]. It is known that Ox LDL participates actively in atheromatous plaque formation, and that it is retained there. Multiple studies provided evidence to suggest that Ox LDL contributes to atherosclerotic plaque formation in several ways. In fact, at least four mechanisms complementary to each other have been proposed: a) endothelial dysfunction, b) foam cell formation, c) migration and proliferation of smooth muscle cells and d) induction of platelet adhesion and aggregation [21,33,48-53].

Endothelial Dysfunction

Endothelial dysfunction is a pathological condition in which the endothelium displays an impairment of anti-inflammatory, anti-coagulant and vascular regulatory properties. It is the crucial event in the development of atherosclerosis. Ox LDL formed and retained in the sub-endothelial space activates endothelial cells through the induction of the cell surface adhesion molecules which, in turn, induce the rolling and adhesion of blood monocytes and T cells. It was reported that Ox LDL induces the expression of intercellular adhesion molecule-1 (ICAM-1) and vascular-cell adhesion molecule-1 (VCAM-1), increasing the adhesive properties of endothelium in a similar manner as that of the effect of pro-inflammatory cytokines, as interleukin 1 beta, known as "leukocytic pyrogen", "leukocytic endogenous mediator", "mononuclear cell factor", "lymphocyte activating factor" and stimulates impairment of endothelial barrier function in post capillary venules. It increase pro-coagulation by activation of platelet factors, which stimulates development of thrombus [26,28,30,32,-34,38,42,44,48- 56].

The recruited blood leukocytes migrate into the tunica intima guided by chemokines. In fact, Ox LDL stimulates endothelial cells and smooth muscle cells to secrete monocyte chemoattractant protein-1 (MCP-1), and monocyte colony-stimulating factor (m CSF) to induce the accumulation of monocytes in the endothelium [28,30,32-34,38,47-56]. Moreover, Ox LDL itself can play the role of chemotactic factor for monocytes and T lymphocytes, since it possesses lyso-phosphatidylcholine, and for macrophages as well [28,30,32-34,36,48-52,53-59].

NO is recognized as an important cardiovascular protective molecule because it exerts vasodilator properties and inhibits the adhesion of leucocytes and platelets to endothelium. It is generated in the vasculature by endothelial NO synthetase (e NOS): the impairment of NO (nitric oxide) production and the secretion by endothelial cells is considered one of the most important characteristic of endothelial dysfunction [28,30,32-34,38,50-56].

NO production from endothelial cells is inhibited by Ox LDL, given that Ox LDL may induce cholesterol depletion in the plasma membrane invaginations ("caveolae"), which causes the translocation of the protein caveolin and eNOS from the membrane domains, inhibiting eNOS activity in endothelial cells [26, 28,30,32-34,38,49-53,67-69]. Another mechanism has also been proposed to explain the inhibitory effect of Ox LDL over NO production in endothelial cells. It has been reported that Ox LDL leads to increased oxidative stress in endothelial cells, producing large amounts of superoxide, which chemically inactivates NO by forming peroxynitrite [26,28,30,32,33,47].

The impact of scavenger receptor expression for monocytes and lymphocytes adhesion on the arterial endothelium. Early stages of atherosclerosis are characterized by a critical phenomenon-the focal accumulation of lipid-laden foam cells derived from macrophages. Localized attachment of circulating monocytes to arterial endothelial

cells appeared to precede the formation of foam cells in various cholesterol-fed animal models of atherosclerosis. It was suggested that monocyte recruitment into early lesions depends on the endothelial adhesiveness for monocytes and lymphocytes. Experiments have found molecules, such as ICAM-1, VCAM-1, and P-selectin “in vivo and in vitro”, that can support the adhesion of monocytes and lymphocytes [70]. Moreover, oxidized LDL, lysophosphatidylcholine, and oxidized fatty acids induce the expression not only of those adhesion molecules but also of scavenger receptors, such as CD-36, SR-A, and LOX-1 [41-49]. Receptors for oxidized LDL were recently isolated and characterized, specifically, LOX-1 and SR-PSOX [41-46]. Expression of LOX-1 is found on endothelial cells, smooth muscle cells, and macrophages, whereas SR-PSOX is expressed on macrophages. The oxidized LDL and its receptors, LOX-1 and SR-PSOX, play a significant role in atherosclerosis development [41-45] (see section- Lectin-like Ox LDL scavenger receptor-1 LOX-1 receptor).

Oxidative stress and diseases not associated with atherosclerosis

Oxidative stress is believed to be important in neurodegenerative diseases, including Lou Gehrig's disease (motor neuron disease or amyotrophic lateral sclerosis), Parkinson's disease, Alzheimer's disease, Huntington's disease, and multiple sclerosis [18-27]. Indirect evidence via monitoring biomarkers, such as ROS and reactive nitrogen species production, and decreased antioxidant defense suggests that oxidative damage may be involved in the pathogenesis of these diseases, while cumulative oxidative stress with disrupted mitochondrial respiration and mitochondrial damage are found related to Alzheimer's disease, Parkinson's disease, and others [18-27,30,34,47,51,53].

Oxidative stress has also been associated with chronic fatigue syndrome. Oxidative stress also contributes to tissue injury following irradiation and hyperoxia, as well as to diabetes. It is likely to be involved in age-related development of cancer [18-27]. The ROS produced in oxidative stress can cause direct damage to the DNA and are therefore mutagenic, and they may also suppress apoptosis and promote proliferation, invasiveness and metastasis [22-25]. It was shown that *Helicobacter pylori* infection increases the production of reactive oxygen and nitrogen species in the human stomach, and is also thought to be important in the development of gastric cancer [20-25,30,32,36,37,40-47].

Cardiovascular disease: Animal studies

Ox LDL is an agent in the development of atherosclerosis and CVD [30-33,47-54]. LDL can be oxidatively modified in vivo to become an immunogen associated with the progression of atherosclerosis and CVD [32,33]. In a study using a mouse model of experimental antiphospholipid syndrome, mice immunized with Ox LDL exhibited a significantly more severe form of the disease compared with native LDL-immunized mice, as expressed by lower

platelet counts, longer activated partial thromboplastin time, and higher fetal resorption rates [34]. The interaction of IgG anti- β 2GP-I Ab from the spontaneous mouse APS model (i.e., NZW x BXSB F1 mouse) with the β 2GP-I-OxLDL complexes significantly enhanced Ox LDL uptake by macrophages [35,36]. The results [34] indicated that Ox LDL aggravates the clinical manifestations of APS and suggested that autoantibodies cross-reactive with Ox LDL may provide a pathogenic mechanism for accelerated atherosclerosis in antiphospholipid syndrome [34].

The accumulation of Ox LDL in the vessel wall stimulates the overlying endothelial cells to produce a number of pro-inflammatory molecules, including adhesion molecules, such as the ICAM-1, the VCAM-1, and endothelial selectin (E-selectin), as well as growth factors, such as the macrophage colony-stimulating factor. These active molecules seem to contribute to the recruitment of leukocytes to the affected area [20]. A vast number of T cells, primarily CD4⁺ CD45RO⁺ memory cells (a large proportion of which express HLA-DR and very late activation antigen-1), have been found within the atherosclerotic lesions [32,34-37,47-51]. The presence of those cells in the atherosclerotic plaques is due to a direct response to the Ox LDL accumulation in the arterial wall. The high concentrations of Ox LDL in the vessel wall are recognized and phagocytosed by macrophages, thereby contributing to a cascade of events characterized as immune inflammatory reactions of atherosclerosis [34,28,30,32,46-51].

Oxidation of LDL (oxidative stress) in the vascular endothelium is precursor to plaque formation in CAD. Oxidative stress also plays a role in the ischemic cascade due to oxygen reperfusion injury following hypoxia. This cascade includes both strokes and heart attacks [2-5,7,8,28,30-34,40]. Experimental studies in animal models of cardiac dysfunction, such as those produced by myocardial infarction after left anterior descending artery ligation, doxorubicin administration and pressure overload, all exhibited increased production of free radicals [16,17,35,36,55,56]. Animal studies have addressed the potential importance of the generation of intracellular ROS in the cells that normally comprise the vessel wall. Superoxide anion O₂⁻ was increased in the aortas of rabbits which were fed high cholesterol diets for a period of several weeks, leading to impaired endothelial-dependent relaxation that was reversible by treatment with polyethylene-glycolated superoxide dismutase or probucol [2,54,55]. Antioxidant therapy was shown to attenuate myocardial injury induced by doxorubicin [15,16,19-21]. Increased expression of the antioxidant superoxide dismutase gene has been reported in rats without heart failure (HF) after endurance training that resulted in greater NO activity [2,6,15,56-58].

Depressed vascular endothelial function was observed in rats with experimental HF despite an increase in endothelial NO synthetase (eNOS) gene expression, and was attributed to increased vascular O₂⁻ production [17,26,56-58]. Dhalla et al. [17] suggested that the mechanism by which oxidative stress is increased by hyperlipidemia could involve the

renin-angiotensin system. Both endothelial dysfunction and lesion area were improved by treatment with an angiotensin II receptor antagonist in a rabbit model [55]. Moreover, NAD(P)H oxidase subunit expression and O₂⁻ production doubled in rats made hypertensive by angiotensin II infusion [1-13,28,30,32,33]. Because LDL up-regulates angiotensin II receptor type 1 (AT1) expression, the effects of angiotensin II can be exacerbated by hypercholesterolemia. Finally, angiotensin II causes hypertrophy of the smooth vascular muscle in a ROS-dependent fashion, a process which can play a part in arterial thickening [17,58].

Cardiovascular disease: Human studies, Atherosclerosis

Despite a recent decline, atherosclerosis remains the most common cause of death in the Western world. Atherosclerosis is also the main cause of HF. Cholesterol itself is neither toxic nor antigenic towards the LDL particles that transport cholesterol: they become harmful to the organism if they are altered. This modification due to oxidation, glycation and binding of advanced glycation products (AGEs), is considered most important in the process of atherogenesis. The interaction of modified LDLs with scavenger receptors on the surface of the endothelium represents the first phase of the atherosclerotic process. Lipid peroxidation can be observed *in vitro* as a change in the lag phase of LDL oxidation stimulated by Cu²⁺ ions [2,3,7,11-14]. *In vivo* lipid peroxidation was especially apparent in tissue macrophages, endothelial cells and smooth muscle cells, and hemoglobin, hypochlorous acid, ceruloplasmin, lipoxygenase and peroxidase appeared to be effective oxidants [3,11,12].

Anti-Ox LDL ABS – predictor of morbidity and mortality in CAD

Oxidized LDL is present in atheromatous plaques and correlates with the extent of atherosclerosis [4-6,12-17,20,22-24]. Assessment of Ox LDL Abs may more reliably reflect the level of oxidative stress than plasma Ox LDL. These Abs have already been shown to correlate with the extent of atherosclerosis and predict future myocardial infarction [12,14-17,19-24]. Elevated levels of Abs against Ox LDL were found to be predictive of myocardial infarction in several investigations [7,9, 22,23,58-60]. The correlation was independent of LDL cholesterol levels, although Ox LDL Abs had an additive predictive effect. The mean Ab level, as expressed in optical density units, was significantly higher in cases of myocardial infarction than in controls (0.412 vs 0.356, $P = 0.002$). After adjustment for age, smoking, blood pressure, and HDL cholesterol level, there was a 2.5-fold increased risk (95% confidence interval, 1.3-4.9) of a cardiac endpoint in the highest tertile of Ab level compared to the lowest tertile ($P = 0.005$ for trend) [19]. Thus, elevated Ab levels added to the predictive effects of classic coronary risk factors [5,15-17].

Myocardial Insulin resistance

Recent human studies strongly support the existence

of a link between insulin resistance and non-ischemic HF [60,61]. The occurrence of a specific insulin-resistant cardiomyopathy, independent of vascular abnormalities, has now been recognized. Cardiac insulin resistance is characterized by reduced availability of sarcolemmal Glut4 transporters and consequent lower glucose uptake. A shift away from glycolysis towards fatty acid oxidation for adenosine triphosphate supply is apparent, and it is associated with myocardial oxidative stress.

The pathophysiology of CVD in diabetes involves traditional and novel cardiac risk factors, including hypertension, dyslipidemia, smoking, genetic factors, hyperglycemia, insulin resistance/hyperinsulinemia, metabolic abnormalities, oxidative/glycoxidative stress, inflammation, endothelial dysfunction, a pro coagulant state and myocardial fibrosis. It has been suggested that specific vascular, myopathic and neuropathic alterations are responsible for the cardiovascular events and mortality in diabetes [61]. These alterations manifest themselves clinically as coronary artery disease (CAD) and HF. In order to contain the emerging epidemic of CVD, diabetic patients should have excellent glycemic control, a low normal blood pressure and low levels of LDL cholesterol, and be taking an angiotensin-converting enzyme inhibitor and aspirin, which may prevent CVD [59-63]. Metformin stimulates production of e NOS, increases plasma NO levels, and improves myocardial insulin resistance [61,63-66].

Heart failure

Tsutsui et al. [59] measured the plasma level of Ox LDL by sandwich enzyme-linked immunosorbent assay with a specific monoclonal antibody against Ox LDL, and showed that plasma levels of Ox LDL had a good correlation with HF severity and mortality. In that study, the plasma Ox LDL level was significantly higher in patients with severe HF than in patients with mild HF and healthy subjects. Others found a significant negative correlation between the plasma level of Ox LDL and left ventricular ejection fraction (LVEF), and a significant positive correlation between the Ox LDL plasma level and circulating norepinephrine levels [16,60]. In another study, most patients (mean age 71.5 years) had systolic HF, with a mean NYHA functional class of 2.7 and a mean LVEF of 39.7%. The mean immunoglobulin G (IgG). Ox LDL Abs levels in patients with hospital admissions were 3.4 times higher than those in subjects not hospitalized over the previous year [8]. Assessments of Ox LDL IgG levels were able to discriminate between patients with clinically controlled HF and patients requiring hospital admission [7,8,10,66,67]. Levels of Ox LDL Abs also correlated with the presence of chronic atrial fibrillation, a finding that could be related to more severe HF or to the possible involvement of oxidative stress in the pathogenesis of atrial fibrillation [3,12-16].

There is considerable evidence that oxidative stress is increased in both ischemic and non-ischemic cardiomyopathies.[1-10] Ox LDL is present in atheromatous plaque and correlates with the extent of atherosclerosis and

HF [11-13]. Together with the inverse relation between Ox LDL plasma level and LVEF, this supported the assumption that plasma levels of Ox LDL have value in predicting mortality in HF patients [7,8,12-14]. Steinerova et al. [10] suggested that assessment of Ox LDL Abs may reliably reflect the level of oxidative stress. Those Abs have already been shown to correlate with the extent of atherosclerosis and predict future myocardial infarction [15-21]. Oxidative stress has been reported to increase in subjects aged 65 and over, possibly arising from an uncontrolled production of free radicals by ageing mitochondria and decreased antioxidant defences [7,9,10,12-24].

The aim of this study was to assess the potential applicability of Ox LDL Abs levels in predicting morbidity, mortality and the composite outcome of the two in a cohort of patients with chronic HF [67]. aged 65 and over. LVEF did not contribute to the understanding of the outcome. Ox LDL Abs levels, NYHA class and a history of current smoking were the best predictors of time to hospitalization (HR = 3.16, $p < 0.001$, HR = 3.148, $p < 0.001$ and HR = 3.584, $p < 0.001$, respectively). The Cox regression analysis of hospitalization revealed significant differences in morbidity rates: 66% event free rate regarding hospitalization for patients with Ox LDL Ab levels < 200 units, versus 25% for those with Ox LDL AB levels above 200 units, $p < 0.001$ [67]. The multiple adjusted Hazard ratios (HRs) of the clinical and laboratory parameters and the major risk factors were examined as predictors of composite outcome. The hazard ratio of Ox LDL Abs for the composite outcome was significant, albeit lower than for morbidity. Notably, the Ox LDL Abs level was the best predictor of composite outcome (morbidity and mortality), i.e., better than N-terminal pro-brain natriuretic peptide (NT-pro-BNP), LVEF and NYHA class [8,67].

It was shown for the first time, that the Ox LDL Abs plasma level was a significant and independent predictor of morbidity and of a composite outcome of morbidity and mortality in a population of individuals with HF, 65 years old or over. Among elderly patients, the Ox LDL Abs HR for hospitalization (3.160) was much higher than it was for the general HF population (1.016), as was the Ox LDL Abs HR for composite outcome [8,67]. Those findings expanded the reports from several recent works that showed Ox LDL Abs to have increased with increased severity of HF in patients with systolic and diastolic dysfunction due to ischemic and valvular disease [8,10,59,67]. It was also reported that Ox LDL Abs correlated to past hospital admissions of HF patients [7,8,67]. These data take previous reports one step further by underscoring the predictive value of an Ox LDL Abs level above 200 units/mL with regard to prediction of morbidity alone and of morbidity in combination with mortality [67]. The results of the recent study demonstrated that Ox LDL Abs levels were superior to NT-pro BNP levels as predictors for time to hospitalization. Ox LDL Abs levels were also better predictors of the combined endpoint. In comparison to earlier reports [21,24]. Unfortunately Ox LDL Abs did not correlate with mortality alone in this cohort of patients [67].

The apparent discrepancy in the predictive power of Ox LDL Abs and NT-pro-BNP may be related to the different mechanisms underlying their elevated levels. As such, NT-pro-BNP reflects the activation of the neurohormonal axis, whereas Ox LDL Abs reflects immune response to oxidative stress. The much longer lifespan of the Ab compared to hormones can explain why Ox LDL Abs have better prediction for long-time follow-up, while NT-pro-BNP are more suitable for estimating the short-term outcome during acute events. These two mechanisms governing HF progression may possibly be activated differently among various patients and, therefore, may each predict different endpoints [67].

Lastly, it is significant that the predictive power of Ox LDL Abs levels for the outcome of HF is not dependent on any other parameter, such as total cholesterol, LDL, HDL, triglycerides, ejection fraction, NYHA class main risk factors for CAD thus, defining Ox LDL-Abs level as the single most prominent prognostic factor that can be useful in clinical practice. Overall, although the results of [67] demonstrated that Ox LDL Abs is an important prognostic marker in patients with established HF, aged 65 and over, we called for further data to determine its role in HF diagnosis, its role during the peri-hospitalization period, as well as the role of serial monitoring.

Anti-Ox LDL Abs and B-type natriuretic peptide (BNP)

There is growing necessity in follow up markers for patients with HF [10,59,60,64-67,69]. One of them, brain natriuretic peptides, became an established surrogate follow-up marker which highly correlated with the severity of the disease [7,8,67]. Several studies compared Ox LDL to other established biomarkers. Elevation of the acute phase reactant, C-reactive protein (CRP) was demonstrated as being a major risk factor for CVD [57,60,61,63,67] CRP was reported to bind to Ox LDL but not to native LDL, [60,61,63,67] and to be part of the innate immune response to oxidized phosphorylcholine-bearing phospholipids within this modified lipoprotein. Interestingly, the HR for CRP did not reach a level of significance [67], suggesting that while CRP reflects oxidative stress and may be related to myocardial damage, it is not a good predictor for long-time outcome of HF, in line with previous reports [59,60]. Surprisingly, LVEF ($< 40\%$ versus $\geq 40\%$) did not emerge as a prognostic marker for HF, and the NYHA class was significant only for the prediction of morbidity, but not for the prediction of the composite outcome [67].

Several studies concluded that the discriminative power of anti-Ox LDL Abs was even better than that obtained for serum NT-pro-BNP) in patients admitted for worsening HF [8,60,61,63,67]. These results support the observation of elevated oxidative stress in patients with HF. It is highly significant that there was no association between Nt Pro-B-type and anti-Ox LDL Abs levels, which suggests that determination of the latter may have an incremental value over that provided by the former [8]. Plasma levels of Ox LDL Abs were shown to have increased with the severity of HF

in patients with different etiologies, e.g., systolic, diastolic, ischemic and valvular diseases, in many investigations [2-4,8,14-17,56,57,60].

BNP is an established surrogate follow up marker for patients with chronic heart failure (CHF) [7,8,60-64,67]. The results of a study by our team [8] demonstrated that NT-pro-BNP plasma levels, Ox LDL Abs, LVEF and NYHA class were of prognostic value in terms of outcome in HF patients as assessed by multivariate analysis. However, NT-pro BNP was a better predictor of all-cause mortality, and ox LDL Abs plasma levels were a significant independent predictor of long-term morbidity and mortality in HF. Abs to Ox LDL significantly correlated with the mean NYHA score [8]. The apparent differential predictive power of Ox LDL Abs and NT-pro-BNP may be attributable to the different mechanisms leading to their elevated levels. Thus, NT-pro-BNP represents the neurohormonal axis, whereas Ox LDL Abs mirrors oxidative stress. These two mechanisms governing HF progression can predict different endpoints in the management of patients with HF [8,67]. Ox LDL-Abs levels were superior to NT-pro BNP levels as predictors for time to hospitalization, and they were also better predictors of the combined endpoint.

The apparent disparity in the predictive power of Ox LDL-Abs and NT-pro-BNP may be related to the different mechanisms underlying their elevated levels. As such, NT-pro-BNP reflects the activation of the neurohormonal axis, whereas Ox LDL Abs reflects immune response to oxidative stress. The much longer lifespan of the Ab compared to hormones can explain why Ox LDL Abs have better prediction for long-time follow-up, while NT-pro-BNP are more suitable for estimating short-term outcome during acute events. These two mechanisms governing HF progression may possibly be activated differently among various patients and, therefore, each may predict different endpoints. The results of our earlier work that pertain to HF patients with decreased renal function may or may not represent the general ≥ 65 -year-old population [67]. Moreover, the creatinine level did not emerge as a significant prognostic factor. The explanation for the latter finding could be that being monitored in a specialized HF clinic with very strict attention to renal function by nephrologists might have reduced oxidized stress and contributed to the lowering of the Ox LDL Abs levels. Those results are similar to others studies [60-64,67].

Clinical impact of Ox LDL Abs on rehabilitation and prognosis

Oxidized LDL Ab might prove to be a useful marker for predicting the clinical course and outcome of many patients with HF of different etiologies. There is an urgent need to develop simplified assays that are applicable to high-throughput analysis. The patient's oxidant status could then be assessed and the true efficacy of antioxidant therapies would then be established, thus enabling effective therapy to be provided selectively. Refinement of clinical trial designs to incorporate such indices would ensure recruitment of appropriate patients, identify the most efficient antioxidant

dosing regimens and perform controlled analysis. Better monitoring and prognostic predictors are required in order to achieve further improvement in the management of patients with HF [8,57,64].

Vascular endothelial function and, particularly, NO-mediated vasodilation are clearly enhanced by physical training among HF patients [12,62,64-65,68-70]. The molecular basis for this improvement is unclear. One attractive hypothesis is that training induces NO production by increased expression of the gene encoding eNOS [2,3,5,62-63,65-69]. The NOS promoter contains a cis-acting shear-stress response element [69], and so its expression could be regulated directly by periodic increases in blood flow that occur during physical training. Alternatively, vasodilatation could be enhanced indirectly after training by mechanism that decreases oxygen free radicals that otherwise can inactivate NO-.

Rehabilitation programs involving immersed exercises are more and more frequently recommended for even severe cardiac patients. Laurent et al. [70] studied one group of 24 male stable CHF patients and 24 male CAD patients with preserved left ventricular function who participated in a rehabilitation program performing cycle endurance exercises on land. They also performed gymnastic exercises either on land (the first half of the participants) or in water (the second half). Resting plasma concentration of NO metabolites (nitrates and nitrites) and catecholamines were evaluated, and a symptom-limited exercise test on a cycle ergometer was performed before and after the rehabilitation program [70]. The plasma concentration of nitrates in the groups that performed water-based exercises was significantly increased ($P = 0.035$ for HF and $P = 0.042$ for CAD), whereas it did not significantly change in the groups that performed gymnastic exercises on land. Plasma catecholamine concentration levels did not change, but the cardiorespiratory capacity of all patients was significantly increased after rehabilitation. The water-based exercises seemed to effectively increase the basal level of plasma nitrates. Such changes may be related to an enhancement of endothelial function and may be of importance for the patient's overall health status [67-70].

Antioxidants

There are defense mechanisms against free radicals. The body produces vast quantities of molecules and extracts free-radical fighters from food [71-78]. These defenders are often lumped together as "antioxidants", and they provide electrons to free radicals without turning into electron-scavenging substances themselves. There are many different substances that can act as antioxidants [71-94]. The most familiar ones are vitamin C, vitamin E, beta-carotene and other related carotenoids, as well as the minerals selenium and manganese. Others include glutathione, coenzyme Q10, lipoic acid, flavonoids, phenols, polyphenols, phytoestrogens, and many more.

Tea and coffee are widely consumed beverages worldwide and they are rich sources of various polyphenols.

Polyphenols are also well known to impart antioxidant properties that are beneficial against several oxidative stress-related diseases, such as cancer and CVD, as well as aging [72-76,90-96]. Antioxidants may play a role in the management or prevention of some medical conditions, such as some cancers, macular degeneration, Alzheimer's disease, some arthritis-related conditions, ischemic heart disease and HF [18-27,70-73].

Cancer and Antioxidants

When it comes to cancer prevention, the picture remains inconclusive for some antioxidant supplements. Few trials have gone on long enough to adequately test their place in cancer management. In the long-term Physicians' Health Study, cancer rates were similar among men taking beta-carotene and those taking a placebo [95]. Other trials also have largely shown no effect, including HOPE [95]. The SU.VI.MAX trial [95] demonstrated a reduction in cancer risk and all-cause mortality among men taking an antioxidant cocktail, but no apparent effect in women, possibly because men tended to have low blood levels of beta-carotene and other vitamins at the beginning of the study. A randomized trial of selenium in people with skin cancer demonstrated significant reductions in cancer and cancer mortality at various sites, including colon, lung, and prostate [71,94]. The effects were strongest among those with low selenium levels at baseline.

Age-related eye disease and antioxidants

This is the one bright spot for antioxidant vitamins. A six-year trial, the Age-Related Eye Disease Study (AREDS), found that a combination of vitamin C, vitamin E, beta-carotene, and zinc offered some protection against the development of advanced age-related macular degeneration, but not cataract, in people who were at high risk of the disease [72,85,88,94]. Lutein, a naturally occurring carotenoid found in green, leafy vegetables, such as spinach and kale, may also protect vision. However, relatively short trials of lutein supplementation for age-related macular degeneration have yielded conflicting findings [72]. A new trial of the AREDS supplement regimen plus lutein, zeaxanthin, and fish oil is underway. This trial could yield more definitive information about antioxidants and macular degeneration [72]. As mentioned earlier, free radicals have a role in the progressive deterioration of the decompensating myocardium [7,8]. Antioxidants terminate these chain reactions by removing free radical intermediates and inhibiting other oxidation reactions. They do this by being oxidized themselves, therefore antioxidants, e.g., thiols, ascorbic acid, or polyphenols, often act as reducing agents [69,70,73,74,84,85, 88]. Overall, these low molecular mass antioxidant molecules add significantly to the defense provided by the enzymes superoxide dismutase, catalase and glutathione peroxidase. However, antioxidant vitamin therapy has not been convincingly demonstrated as being beneficial in randomized trials [18,35,74,88]. The data are, however, entirely consistent with the alternative hypothesis, that reduced oxidative stress may account for the increase in vascular NO-mediated vasodilation. An insight into

the mechanism of this process may be relevant when considering therapies for exercise-intolerant HF patients [65,66,68,70,73,75,79]. Investigations of the role of dietary antioxidants suggested that vitamins A and E along with coenzyme Q10, flavonoids, and resveratrol show promise in extending human life [74-89,91]. Many studies examined the impact of antioxidants and their implications in the aging process, with the conclusion that these antioxidants may contribute to longevity [11,74-84-88,91-93].

The possibility of translating the patient's oxidant status into use of effective antioxidant drugs is not, however, supported by current evidence. Notwithstanding promising observational data, prospective, double blind, placebo-controlled trials have not supported a causal relationship between antioxidant therapy, mainly vitamin supplements, and lowering of CAD risk [46,51,70-89]. Antioxidants include some vitamins (e.g., vitamins C and E), some minerals (e.g., selenium), and flavonoids, which are found in plants [70-89]. The best sources of antioxidants are fruits and vegetables and flavonoids can be found in fruits, red wine, and teas.

When performed well, randomized, placebo-controlled trials provide the strongest evidence, but they offer little support to the standpoint that taking vitamin C, vitamin E, beta-carotene, or other single antioxidants provides substantial protection against heart disease, cancer, or other chronic conditions. The results of the largest of such trials have been mostly negative [70-82,85-89,94-96].

Antioxidants and heart disease

Vitamin E, beta-carotene, and other so-called antioxidants are not the ultimate solution to heart disease and stroke that researchers were hoping for, although the final chapter has not been written on vitamin E [18,86-96]. In the Women's Health Study, 39,876 initially healthy women took 600 IU of natural source vitamin E or a placebo every other day for 10 years. At the study's end, the rates of major cardiovascular events and cancer were no lower among those taking vitamin E than they were among those taking the placebo. However, the trial did observe a significant 24% reduction in total cardiovascular mortality. Although this was not a primary endpoint for the trial, it nevertheless represents an extremely important outcome [86].

Earlier large vitamin E trials that had been conducted among individuals with previously diagnosed coronary disease or at high risk for it generally showed no benefit. In the Heart Outcomes Prevention Evaluation (HOPE) trial, the rates of major cardiovascular events were essentially the same in the vitamin E (21.5%) and placebo (20.6%) groups, although participants taking vitamin E had higher risks of HF and hospitalization for HF [96]. In the GISSI trial [98], the results were mixed but mostly showed no preventive effects after more than three years of treatment with vitamin E among 11,000 heart attack survivors [98]. However, some studies suggest potential benefits among certain subgroups. A recent trial of vitamin E in Israel, for example, showed a marked reduction in CHD among people with

type 2 diabetes who have a common genetic predisposition for greater oxidative stress [87]. We reviewed recently published basic research on the protective cardiovascular effects of antioxidants, especially resveratrol, because of their potential to lead to the development of a new treatment in patients with HF [76].

Vitamin A- has been called the “anti-infective” vitamin because of its role in supporting the immune system. Carotenoids, which are pre-formed vitamin A found in plants, were shown to be determinants of longevity [61,74,76,79,85,92-98]. Vitamin A supplementation led to an improvement in the lifespan of mice only when its use was initiated at the beginning of life [51,79]. Ascorbic acid has a multiplicity of antioxidant properties, but it can exert pro-oxidant effects in vitro, usually by interaction with transition metal ions. It is as yet uncertain that these pro-oxidant effects have any biological relevance. Vitamin E. One of the most widely researched antioxidants, vitamin E, was similarly found to extend the lifespan in mice when initiated during the early years of life. Vitamin E may protect older healthy men against atherogenesis (formation of thick plaque of cholesterol and other lipids in arterial walls), improve relearning ability, and reduce cancer formation [74]. However, vitamin E supplementation might be associated with an increase in total mortality, HF, and hemorrhagic stroke [74]. Vitamin E has been shown to increase oxidative resistance in vitro and prevent atherosclerotic plaque formation in mouse models [40,74]. Consumption of foods rich in vitamin E has been associated with a lower risk of CHD in middle-aged to older men and women [74]. However clinical studies have not demonstrated any benefit of vitamin E in the primary and secondary prevention of CVD [35]. The American Heart Association does not support the use of vitamin E supplements to prevent CVD, but it does recommend the consumption of foods abundant in antioxidant vitamins and other nutrients [35,47, 74,88]. Selenium is an essential trace element, and low levels of selenium in humans has been linked to increased risk of cancer and heart disease [71,88,94-99].

Coenzyme Q10 is the only known antioxidant that is synthesized in the body [91]. It extends life by reducing oxidative damage, thereby lowering cardiovascular risk and inflammation. Q10 is the primary homologue found in longer-living mammalian species, including humans. There were non-significant trends towards increased left ventricular ejection fraction and reduced mortality in nine randomized trials of Q10 in HF published up to 2003 [89]. Q10 decreases pro-inflammatory cytokines and blood viscosity, which is helpful in patients with HF and CAD. It also improves ischemia and reperfusion injury of coronary revascularization [89]. It was recently found to be an independent predictor of mortality in congestive HF. Coenzyme Q10 has also been found to be helpful in vertigo and Meniere-like syndrome by improving the immune system [89]. There is ongoing research aimed at establishing its role in the treatment of cardiovascular diseases [89].

Flavonoids, the most common group of polyphenolic compounds in the human diet, are found mostly in plants. Flavonol-rich chocolate acutely improves vascular and platelet function in patients with HF [84]. Green tea supplementation has been found to protect against oxidative stress, and it increased antioxidant ability in the rat brain [76]. Another flavonoid, anthocyanin, has also been shown to be protective against vascular disease [76,84].

Resveratrol- is a polyphenolic compound found in grapes, red wine, purple grape juice, peanuts, and some berries. Evidence from the “French Paradox” and from controlled studies point to its effectiveness in extending life [76]. It has also been associated with improved bone density, motor coordination, and cardiovascular function, as well as with delaying cataracts. Other studies have shown that it offers protection against Alzheimer’s disease, prolongs the human lifespan and retards aging [76]. The cardiovascular protective capacities of resveratrol are associated with multiple molecular targets, and this may lead to the development of novel therapeutic strategies for atherosclerosis, ischemia/reperfusion, metabolic syndrome, and HF [76].

Statins: The pleiotropic effects of statins appear to result from improvements in endothelial function, a reduction in inflammatory mediators, a decline in the development of atheroma through the stabilization of atheromatous plaques, and the inhibition of cardiac hypertrophy through an antioxidant mechanism [77,90]. Long-term statin use may reduce morbidity and mortality rates in a wide range of patients [77]. However, lower LDL cholesterol levels appear to predict a less favorable outcome in patients with HF, particularly those taking statins, thus raising questions about the need for an aggressive LDL cholesterol-lowering strategy in patients with HF, regardless of its etiology [74,90-98]. HF is associated with endothelial dysfunction and increased platelet activation.

Clopidogrel treatment in patients with CAD not only inhibits platelet activation but also improves endothelial function and NO bioavailability. Hu et al. [91] investigated whether treatment with clopidogrel modified endothelial function in HF following myocardial infarction and concluded that endothelial dysfunction and vascular oxidative stress have a negative prognostic impact on cardiovascular events.

Nitrates are very effective anti-ischemic drugs used for the treatment of patients with stable angina, acute myocardial infarction and chronic congestive HF. There are new data on the protective properties of the organic nitrate pentaerythryl tetranitrate, which, in contrast to all other organic nitrates, is able to up-regulate enzymes with a strong anti-oxidative capacity thereby preventing tolerance and the development of endothelial dysfunction [79].

Carvedilol is a beta-blocker with antioxidant properties. In several large clinical trials on patients with mild to severe HF, treatment with carvedilol improved mortality, especially in severe cases with the worst prognosis [2,80]. The beta-blocker nebivolol has been used in Europe for almost 10 years [81]. Like carvedilol, it belongs to the third generation

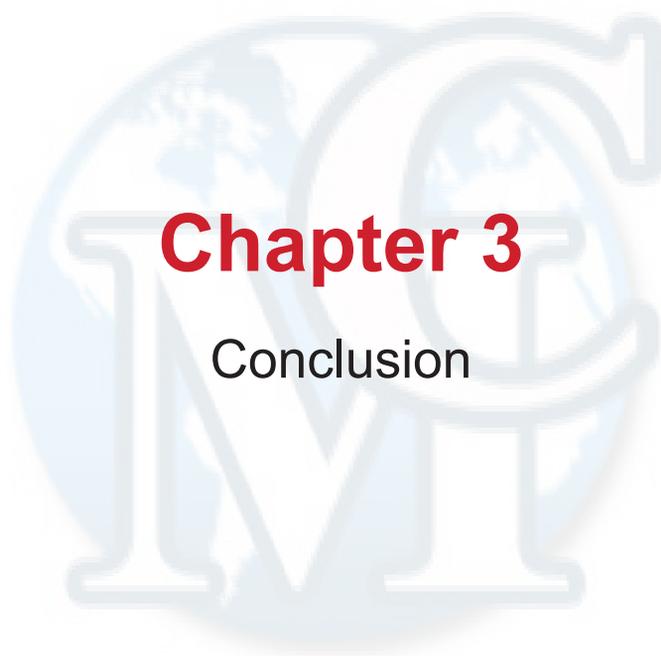
of beta-blockers which possess direct vasodilator properties in addition to their adrenergic blocking characteristics [81]. Nebivolol has the highest beta (1)-receptor affinity among the beta-blockers and, interestingly, it substantially improves endothelial dysfunction via its strong stimulatory effects on the activity of e-NOS and via its antioxidative properties. Because impaired endothelial activity is considered to have a major causal role in the pathophysiology of congestive HF, the endothelium-agonistic properties of nebivolol suggest that this drug might provide additional benefit beyond beta-receptor blockade. Clinically, this compound has been proven to have antihypertensive and anti-ischemic effects as well as beneficial effects on hemodynamics and prognosis in patients with chronic congestive HF [80,81]. Further studies are required to compare the benefit of nebivolol in terms of its prognostic impact in patients with HF [81].

Spirolactone is an aldosterone receptor antagonist that has been shown to decrease mortality in patients with severe HF when added to conventional therapy [82]. Treatment with spironolactone resulted in a significant increase in the forearm blood flow response to acetylcholine ($P < 0.001$) [82]. This demonstration of improvement in endothelial function (caused by oxidative stress) provides a novel mechanism for the beneficial effect of spironolactone in HF patients [82].

Angiotensin-converting enzyme inhibitors (ACEI) and angiotensin receptor blockers are widely used drugs in patients with HF to prevent hypertrophy of the myocardium and smooth vascular muscle caused by angiotensin II in a ROS-dependent fashion, which can contribute to arterial thickening [78,83]. Treatment with ACEI (especially quinaprilate) for patients with HF, CAD and other conditions associated with endothelial dysfunction induced by oxidative stress has been shown to improve endothelium-dependent vasodilation and contribute to increased exercise capacity [78,83].

Metformin: Some studies have shown that metformin activates AMP-activated protein kinase and has a potent cardio protective effect against ischemia/reperfusion injury as result of oxidative stress. Both left ventricular fractional shortening and left ventricular end-diastolic pressure were significantly improved in dogs treated with oral metformin. As a result of these effects, metformin decreased apoptosis and improved cardiac function in failing canine hearts. As such, metformin may be a potential new therapy for HF [92,93].

Free radicals contribute to chronic diseases, from cancer to heart disease and from Alzheimer's disease to loss of vision. This does not automatically mean that substances with antioxidant properties will fix the problem, especially not when they are taken out of their natural context. The studies on free radicals are inconclusive so far, but generally do not provide strong evidence that antioxidant supplements have a substantial impact on disease. The main problem is that most of the trials have had fundamental limitations due to their relatively short duration and for having been conducted on subjects with existing disease. The benefit of beta-carotene on cognitive function was published in the Physicians' Health Follow-up Study only after 18 years of follow-up, and no other trial has continued for so long. At the same time, abundant evidence suggests that eating whole fruits, vegetables, and whole grains-all rich in networks of antioxidants and their helper molecules-provides protection against many of the aging-related diseases.



Chapter 3

Conclusion

Conclusion

The Ox LDL Abs level is a useful predictor of morbidity and mortality in HF patients. Assessment of oxidative stress in humans is a complex undertaking, and there is no reproducible, standardized methodology for evaluating it. Additional prospective data with further determination of Ox LDL Abs levels may prove Ox LDL Abs as a useful marker for predicting exacerbations in patients with HF. Therapies that improve endothelial function caused by oxidative stress have been shown to improve exercise tolerance and outcomes in patients with HF. Dietary antioxidants, such as vitamin A, along with coenzyme Q10, flavonoids, and resveratrol, and medicines, such as spironolactone, pentaerythryl tetranitrate, nebivolol, quinaprilate, clopidogrel and methformin, show promise in extending the life expectancy of patients with HF. Further research is needed to elucidate therapies that will increase NO production, interrupt the pathologic cascade that results in the generation of free radicals, and augment antioxidant defenses in patients with HF.

References

1. Packer M (1988) Survival in patients with chronic heart failure and its potential modification by drug therapy. In: Cohn JN (Ed.), *Drug treatment of heart failure*. (2nd edn), Secaucus, Advanced Therapeutics Communications International, New Jersey, USA, pp. 273.
2. Winlaw D, Smythe G, Keough A, Schyvens C, Spratt P, et al. (1994) Increased nitric oxide production in heart failure. *Lancet* 344(8919): 373-374.
3. Sharma R, Davidoff MN Oxidative stress and endothelial dysfunction in heart failure. *Congest Heart Fail* 8: 165-172.
4. Griendling KK, FitzGerald GA (2003) Oxidative stress and cardiovascular injury: Part II: animal and human studies. *Circulation* 108(17): 2034-2040.
5. McMurray J, Chopra M, Abdullah I, Smith WE, Dargie HJ, et al. (1993) Evidence of oxidative stress in chronic heart failure in humans. *Eur Heart J* 14(11): 1493-1498.
6. Boullier A, Bird DA, Chang MK, Dennis EA, Friedman P, et al. (2001) Scavenger receptors, oxidized LDL, and atherosclerosis. *Ann N Y Acad Sci* 947: 214-222.
7. George J, Wexler D, Roth A, Barak T, Sheps D, et al. (2006) Usefulness of anti-oxidized LDL antibody determination for assessment of clinical control in patients with heart failure. *Eur J Heart Fail* 8(1): 58-62.
8. Charach G, George J, Afek A, Wexler D, Sheps D, et al. (2009) Antibodies to oxidized LDL as predictors of morbidity and mortality in patients with chronic heart failure. *J Card Fail* 15(9): 770-774.
9. Beaumont JL (1965) L'hyperlipidémie par autoanticorps anti-beta-lipoprotéine. Une nouvelle entité pathologique. *C R Acad Sci Paris* 261: 4563-4566.
10. Steinerová A, Racek J, Stozický F, Zima T, Fialová L, et al. (2001) Antibodies against oxidized LDL--theory and clinical use. *Physiol Res* 50(2): 131-141.
11. Chong-Han K (2010) Dietary lipophilic antioxidants: implications and significance in the aging process. *Crit Rev Food Sci Nutr* 50(10): 931-937.
12. Ennezat P, Malendowicz S, Testa M, Colombo P, Cohen Solal A, et al. (2001) Physical training in patients with chronic heart failure enhances the expression of genes encoding antioxidative enzymes. *J Am Coll Cardiol* 38(1): 194-198.
13. Holvoet P, Mertens A, Verhamme P, Bogaerts K, Beyens G, et al. (2001) Circulating oxidized LDL is a useful marker for identifying patients with coronary artery disease. *Arterioscler Thromb Vasc Biol* 21(5): 844-848.
14. Holvoet P, Vanhaecke J, Janssens S, Van de Werf F, Collen D (1998) Oxidized LDL and malondialdehyde-modified LDL in patients with acute coronary syndromes and stable coronary artery disease. *Circulation* 98(15): 1487-1494.
15. Myers CE, McGuire WP, Liss RH, Ifrim I, Grotzinger K, et al. (1977) Adriamycin: the role of lipid peroxidation in cardiac toxicity and tumor response. *Science* 197(4299): 165-167.
16. Singal PK, Kapur N, Dhillon KS, Beamish RE, Dhalla NS (1982) Role of free radicals in catecholamine-induced cardiomyopathy. *Can J Physiol Pharmacol* 60(11): 1390-1397.
17. Dhalla AK, Hill MF, Singal PK (1996) Role of oxidative stress in transition of hypertrophy to heart failure. *J Am Coll Cardiol* 28(2): 506-514.
18. Kontush K, Schekatolina S (2004) "Vitamin E in neurodegenerative disorders: Alzheimer's disease". *Ann N Y Acad Sci* 1031: 249-262.
19. Riahi Y, Cohen G, Shamni O, Sasson S (2010) "Signaling and cytotoxic functions of 4-hydroxyalkenals". *Am J Physiol Endocrinol Metab* 299(6): E879-E886.
20. Liao F, Andalibi A, Qiao JH, Allayee H, Fogelman AM, et al. (1994) Genetic evidence for a common pathway mediating oxidative stress, inflammatory gene induction, and aortic fatty streak formation in mice. *J Clin Invest* 94(2): 877-884.
21. Halliwell B (2007) Oxidative stress and cancer: have we moved forward? *Biochem J* 401(1): 1-11.
22. Halliwell B (1994) Free radicals, antioxidants, and human disease: curiosity, cause, or consequence? *Lancet* 344(8924): 721-724.
23. Halliwell B, Gutteridge JM (1990) Role of free radicals and catalytic metal ions in human disease: an overview. *Methods Enzymol* 186: 1-85.
24. Lelli JL, Becks LL, Dabrowska MI, Hinshaw DB (1998) ATP converts necrosis to apoptosis in oxidant-injured endothelial cells. *Free Radic Biol Med* 25(6): 694-702.
25. Lee YJ, Shacter E (1999) Oxidative stress inhibits apoptosis in human lymphoma cells. *J Biol Chem* 274(28): 19792-19798.
26. Staprans I, Pan XM, Rapp JH, Feingold KR (2005) The role of dietary oxidized cholesterol and oxidized fatty acids in the development of atherosclerosis. *Mol Nutr Food Res* 49(11): 1075-1082.
27. Nathan C, Shiloh MU (2000) Reactive oxygen and nitrogen intermediates in the relationship between mammalian hosts and microbial pathogens. *Proc Natl Acad Sci USA* 97(16): 8841-8848.
28. Wu R, Shoenfeld Y, Sherer Y, Patnaik M, Matsuura E, et al. (2003) Anti-idiotypes oxidized LDL antibodies in intravenous immunoglobulin preparations: possible immunomodulation of atherosclerosis. *Autoimmunity* 36(2): 91-97.
29. Boyd HC, Gown AM, Wolfbauer G, Chait A (1989) Direct evidence for a protein recognized by a monoclonal antibody against oxidatively modified LDL in atherosclerotic lesions from a Watanabe heritable hyperlipidemic rabbit. *Am J Pathol* 135(5): 815-825.
30. Libby P (2002) Inflammation in atherosclerosis. *Nature* 420(6917): 868-874.
31. Lusis AJ (2000) Atherosclerosis. *Nature* 407(6801): 233-241.
32. Maiolino G, Rossitto G, Caielli P, Bisogni V, Rossi GP, et al. (2013) Calò The role of oxidized low-density lipoproteins in atherosclerosis: the myths and the facts. *Mediators Inflamm* 2013: 714653.
33. Yehuda Shoenfeld, Ruihua Wu, Linda DD, Eiji Matsuura (2004) Are anti-oxidized low-density lipoprotein antibodies pathogenic or protective? *Circulation* 110: 2552-2558.
34. Kita T, Kume N, Minami M, Hayashida K, Murayama T, et al.

- (2001) Role of oxidized LDL in atherosclerosis. *Ann N Y Acad Sci* 947: 199-205.
35. Salonen JT, Yla Herttua S, Yamamoto R, Butler S, Korpela H, et al. (1992) Autoantibodies against oxidized LDL and progression of carotid atherosclerosis. *Lancet* 339(8798): 883-887.
 36. Puurunen M, Mänttari M, Manninen V, Tenkanen L, Alfthan G, et al. (1994) Antibody against oxidized low-density lipoprotein predicting myocardial infarction. *Arch Intern Med* 154(22): 2605-2609.
 37. Ding Z, Wang X, Schnackenberg L, Khaidakov M, Liu S, et al. (2013) Regulation of autophagy and apoptosis in response to ox-LDL in vascular smooth muscle cells, and the modulatory effects of the microRNA hsa-let-7g. *Int J Cardiol* 168(2): 1378-1385.
 38. Ding Z, Liu S, Wang X, Dai Y, Khaidakov M, et al. (2014) LOX-1, mtDNA damage, and NLRP3 inflammasome activation in macrophages: implications in atherogenesis. *Cardiovasc Res* 103(4): 619-628.
 39. Ding Z, Liu S, Wang X, Khaidakov M, Dai Y, et al. (2015) Lectin-like ox-LDL receptor-1 (LOX-1)-Toll-like receptor 4 (TLR4) interaction and autophagy in CATH. a differentiated cells exposed to angiotensin II. *Mol Neurobiol* 51(2): 623-632.
 40. Li D, Mehta JL (2005) Oxidized LDL, a critical factor in atherogenesis. *Cardiovasc Res* 68(3): 353-354.
 41. Falconi M, Ciccone S, D Arrigo P, Viani F, Sorge R, et al. (2013) Design of a novel LOX-1 receptor antagonist mimicking the natural substrate. *Biochem Biophys Res Commun* 438(2): 340-345.
 42. Kataoka K, Hasegawa K, Sawamura T, Fujita M, Yanazume T, et al. (2003) LOX-1 pathway affects the extent of myocardial ischemia-reperfusion injury. *Biochem Biophys Res Commun* 300(3): 656-660.
 43. Li D, Williams V, Liu L, Chen H, Sawamura T, et al. (2002) LOX-1 inhibition in myocardial ischemia-reperfusion injury: modulation of MMP-1 and inflammation. *Am J Physiol Heart Circ Physiol* 283(5): H1795-H1801.
 44. Hu C, Chen J, Dandapat A, Fujita Y, Inoue N, et al. (2008) LOX-1 abrogation reduces myocardial ischemia-reperfusion injury in mice. *J Mol Cell Cardiol* 4(1): 76-83.
 45. Mango R, Clementi F, Borgiani P, Forleo GB, Federici M, et al. (2003) Association of single nucleotide polymorphisms in the oxidized LDL receptor 1 (OLR1) gene in patients with acute myocardial infarction. *J Med Genet* 40(12): 933-936.
 46. Berliner JA, Navab M, Fogelman AM, Frank JS, Demer LL, et al. (1995) Atherosclerosis: basic mechanisms oxidation, inflammation, and genetics. *Circulation* 91(9): 2488-2496.
 47. Haller H, Schaper D, Ziegler W, Philipp S, Kuhlmann M, et al. (1995) Low-Density Lipoprotein Induces Vascular Adhesion Molecule Expression on Human Endothelial Cells. *Hypertension* 25(4 Pt 1): 511-516.
 48. Ehara S, Ueda M, Naruko T, Haze K, Itoh A, Otsuka M, et al. (2001) Elevated levels of oxidized low density lipoprotein show a positive relationship with the severity of acute coronary syndromes. *Circulation* 103(5): 1930-1932/47.
 49. Yang CM, Chien CS, Hsiao LD, Pan SL, Wang CC, et al. (2001) Mitogenic effect of oxidized low-density lipoprotein on vascular smooth muscle cells mediated by activation of Ras/Raf/MEK/MAPK pathway. *Br J Pharmacol* 132(7): 1531-154.
 50. Ehara S, Ueda M, Naruko T, Haze K, Matsuo T, et al. (2002) Pathophysiological role of oxidized low-density lipoprotein in plaque instability in coronary artery diseases. *J Diabetes Complications* 16(1): 60-64.
 51. Tribble DL (1999) Antioxidant consumption and risk of coronary heart disease: emphasis on vitamin c, vitamin e, and b-carotene. *Circulation* 99(4): 591-595.
 52. Iughetti L, Volta C, Maggi E, Palladini G, Perugini C, et al. (1999) Circulating antibodies recognizing oxidatively modified low-density lipoprotein in children. *Pediatr Res* 45(1): 94-99.
 53. Shoji T1, Nishizawa Y, Fukumoto M, Shimamura K, Kimura J, et al. (2000) Inverse relationship between circulating oxidized low density lipoprotein (ox LDL) and anti-ox LDL antibody levels in healthy subjects. *Atherosclerosis* 148(1): 171-177.
 54. Pashkow FJ (2011) Oxidative stress and inflammation in heart disease: do antioxidants have a role in treatment and/or prevention? *Int J of Inflam* pp. 9.
 55. Hayashida K, Kume N, Murase T, Minami M, Nakagawa D, et al. (2005) Serum soluble lectin-like oxidized low-density lipoprotein receptor-1 levels are elevated in acute coronary syndrome: a novel marker for early diagnosis. *Circulation* 112(6) 812-818.
 56. Hollander J, Fiebig R, Gore M, Bejma J, Ookawara T, et al. (1999) Superoxide dismutase gene expression in skeletal muscle: fiber-specific adaptation to endurance training. *Am J Physiol* **277(3 Pt 2): R856-R862.**
 57. Katz SD, Yuen J, Bijou R, LeJemtel TH (1997) Training improves endothelium-dependent vasodilation in resistance vessels of patients with heart failure. *J Appl Physiol* 82(5): 1488-1492.
 58. Bauersachs J, Bouloumié A, Fraccarollo D, Hu K, Busse R, et al. (1999) Endothelial dysfunction in chronic myocardial infarction despite increased vascular endothelial nitric oxide synthase and soluble guanylate cyclase expression: role of enhanced vascular superoxide production. *Circulation* **100(3): 292-298.**
 59. Tsutsui T, Tsutamoto T, Wada A, Maeda K, Mabuchi N, et al. (2002) Plasma oxidized low-density lipoprotein as a prognostic predictor in patients with chronic congestive heart failure. *J Am Coll Cardiol* 39(6): 957-962.
 60. Anand IS, Fisher LD, Chiang YT, Latini R, Masson S, et al. (2003) Changes in brain natriuretic peptide and norepinephrine over time and mortality and morbidity in the Valsartan Heart Failure Trial (Val-HeFT). *Circulation* **107: 1278-1283.**
 61. Candido R, Srivastava P, Cooper ME, Burrell LM (2003) Diabetes mellitus: a cardiovascular disease. *Curr Opin Investig Drugs* **4(9): 1088-1094.**
 62. Hambrecht R, Fiehn E, Weigl C, Gielen S, Hamann C, et al. (1998) Regular physical exercise corrects endothelial dysfunction and improves exercise capacity in patients with

- chronic heart failure. *Circulation* **98(24)**: 2709-2715.
63. Hornig B, Maier V, Drexler H (1996) Physical training improves endothelial function in patients with chronic heart failure. *Circulation* **93(2)**: 210-214.
 64. Cambi GE, Lucchese G, Djeokeng MM, Modesti A, Fiaschi T, et al. (2012) Impaired JAK2-induced activation of STAT3 in failing human myocytes. *Mol Biosyst* **8(9)**: 2351-2359.
 65. Koller A, Huang A, Sun D, Kaley G (1995) Exercise training augments flow-dependent dilation in rat skeletal muscle arterioles. Role of endothelial nitric oxide and prostaglandins. *Circ Res* **76(4)**: 544-550.
 66. Sessa WC, Pritchard K, Seyedi N, Wang J, Hintze TH (1994) Chronic exercise in dogs increases coronary vascular nitric oxide production and endothelial cell nitric oxide synthase gene expression. *Circ Res* **74(2)**: 349-353.
 67. Charach G, Michowitz Y, Rogowski O, Charach L, Argov O, et al. (2015) Usefulness of antibodies to oxidized low-density lipoproteins as predictors of morbidity and prognosis in heart failure patients aged ≥ 65 years. *Am J Cardiol* **116(9)**: 1379-1384.
 68. Varin R, Mulder P, Richard V, Tamion F, Devaux C, et al. (1999) Exercise improves flow-mediated vasodilatation of skeletal muscle arteries in rats with chronic heart failure. Role of nitric oxide, prostanoids, and oxidant stress. *Circulation* **99(22)**: 2951-2957.
 69. Resnick N, Gimbrone MA (1995) Hemodynamic forces are complex regulators of endothelial gene expression. *FASEB J* **9(10)**: 874-882.
 70. Laurent M, Daline T, Malika B, Fawzi O, Philippe V, et al. (2009) Training-induced increase in nitric oxide metabolites in chronic heart failure and coronary artery disease: an extra benefit of water-based exercises? *Eur J Cardiovasc Prev Rehabil* **16(2)**: 215-221.
 71. Duffield Lilloco AJ, Reid ME, Turnbull BW, Combs GF, Slate EH, et al. (2002) Baseline characteristics and the effect of selenium supplementation on cancer incidence in a randomized clinical trial: a summary report of the nutritional prevention of cancer trial. *Cancer Epidemiol Biomarkers Prev* **11(7)**: 630-639.
 72. Age-Related Eye Disease Study Research Group (2001) A randomized, placebo-controlled, clinical trial of high-dose supplementation with vitamins C and E, beta-carotene, and zinc for age-related macular degeneration and vision loss: AREDS report no. 8. *Arch Ophthalmol* **119(10)**: 1417-1436.
 73. Heiss C, Keen CL, Kelm M (2010) Flavanols and cardiovascular disease prevention. *Eur Heart J* **31(21)**: 2583-2592.
 74. Saremi A, Arora R (2010) Vitamin E and cardiovascular disease. *Am J Ther* **17(3)**: e56-e65.
 75. Yamagishi S, Matsui T (2011) Nitric oxide, a janus-faced therapeutic target for diabetic microangiopathy-Friend or foe? *Pharmacol Res* **64(3)**: 187-194.
 76. Wang H, Yang YJ, Qian HY, Zhang Q, Xu H, et al. (2012) Resveratrol in cardiovascular disease: what is known from current research? *Heart Fail Rev* **17(3)**: 437-448.
 77. Marzilli M (2010) Pleiotropic effects of statins: evidence for benefits beyond LDL-cholesterol lowering. *Am J Cardiovasc Drugs* **10** (Suppl 1): 3-9.
 78. Sukumaran V, Veeraveedu PT, Gurusamy N, Yamaguchi K, Lakshmanan AP, et al. (2011) Cardioprotective effects of telmisartan against heart failure in rats induced by experimental autoimmune myocarditis through the modulation of angiotensin-converting enzyme-2/angiotensin 1-7/mas receptor axis. *Int J Biol Sci* **7(8)**: 1077-1092.
 79. Daiber A, Wenzel P, Oelze M, Münzel T (2008) New insights into bioactivation of organic nitrates, nitrate tolerance and cross-tolerance. *Clin Res Cardiol* **97(1)**: 12-20.
 80. Matsuda Y, Akita H, Terashima M, Shiga N, Kanazawa K, et al. (2000) Carvedilol improves endothelium-dependent dilatation in patients with coronary artery disease. *Am Heart J* **140(5)**: 753-759.
 81. Münzel T, Gori T (2009) Nebivolol: the somewhat-different beta-adrenergic receptor blocker. *J Am Coll Cardiol* **54(16)**: 1491-1499.
 82. Pitt B, Zannad F, Remme WJ, Cody R, Castaigne A, et al. (1999) The effect of spironolactone on morbidity and mortality in patients with severe heart failure. Randomized Aldactone Evaluation Study Investigators. *N Engl J Med* **341(10)**: 709-717.
 83. Hornig B, Arakawa N, Haussmann D, Drexler H (1998) Differential effects of quinaprilat and enalaprilat on endothelial function of conduit arteries in patients with chronic heart failure. *Circulation* **98(25)**: 2842-2848.
 84. Flammer AJ, Sudano I, Wolfrum M, Thomas R, Enseleit F, et al. (2011) Cardiovascular effects of flavanol-rich chocolate in patients with heart failure. *Eur Heart J* **33(17)**: 2172-2180.
 85. Tinkel J, Hassanain H, Khouri SJ (2012) Cardiovascular antioxidant therapy: a review of supplements, pharmacotherapies, and mechanisms. *Cardiol Rev* **20(7)**: 77-83.
 86. Lee IM, Cook NR, Gaziano JM, Gordon D, Ridker PM, et al. (2005) Vitamin E in the primary prevention of cardiovascular disease and cancer: the Women's Health Study: a randomized controlled trial. *JAMA* **294(1)**: 56-65.
 87. Milman U, Blum S, Shapira C, Aronson D, Lotan MR, et al. (2007) Vitamin E supplementation reduces cardiovascular events in a subgroup of middle-aged individuals with both type 2 diabetes mellitus and the haptoglobin 2-2 genotype. A prospective double-blinded clinical trial. *Arterioscler Thromb Vasc Biol* **28(2)**: 341-347.
 88. Pryor WA (2000) Vitamin E and heart disease: basic science to clinical intervention trials. *Free Radic Biol Med* **28(1)**: 141-164.
 89. Kumar A, Kaur H, Devi P, Mohan V (2009) Role of coenzyme Q10 (CoQ10) in cardiac disease, hypertension and Meniere-like syndrome. *Pharmacol Ther* **124(3)**: 259-268.
 90. Charach G, George J, Roth A, Rogowski O, Wexler D, et al. (2010) Baseline low-density lipoprotein cholesterol levels and outcome in patients with heart failure. *Am J Cardiol* **105(1)**: 100-104.
 91. Hu H, Batteux F, Chereau C, Kaviani N, Marut W, et al. (2011) Clopidogrel protects from cell apoptosis and oxidative

- damage in a mouse model of renal ischaemia-reperfusion injury. *J Pathol* 225(2): 265-275.
92. Mellor KM, Bell JR, Ritchie RH, Delbridge LM (2012) Myocardial insulin resistance, metabolic stress and autophagy in diabetes. *Clin Exp Pharmacol Physiol* 40(1): 56-61.
93. Sasaki H, Asanuma H, Fujita M, Takahama H, Wakeno M, et al. (2009) Metformin prevents progression of heart failure in dogs: role of AMP-activated protein kinase. *Circulation* 119(19): 2568-2577.
94. Pashkow F (2011) Oxidative Stress and Inflammation in Heart Disease: Do Antioxidants Have a Role in Treatment and/or Prevention? *Int J Inflam* 2011: 514623.
95. Christen W, Gaziano J, Hennekens CH (2000) Design of Physicians' Health Study II--a randomized trial of beta-carotene, vitamins E and C, and multivitamins, in prevention of cancer, cardiovascular disease, and eye disease, and review of results of completed trials. *Ann Epidemiol* 10(2): 125-134.
96. Sleight P (2000) The HOPE Study (Heart Outcomes Prevention Evaluation). *J Renin Angiotensin Aldosterone Syst* 1(1): 18-20.
97. Hercberg S, Galan P, Preziosi P, Bertrais S, Mennen L, et al. (2004) The SU.VI.MAX Study: a randomized, placebo-controlled trial of the health effects of antioxidant vitamins and minerals. *Arch Intern Med* 164(21): 2335-2342.
98. Stone N (2000) The Gruppo Italiano per lo Studio della Sopravvivenza nell'Infarto Miocardio (GISSI)-Prevenzione Trial on fish oil and vitamin E supplementation in myocardial infarction survivors. *Curr Cardiol Rep* 2(5): 445-451.
99. Bandyopadhyay P, Ghosh A, Ghosh C (2012) Recent developments on polyphenol-protein interactions: effects on tea and coffee taste, antioxidant properties and the digestive system. *Food Funct* 3(6): 592-605.
100. Tinggi U (2008) Selenium: its role as antioxidant in human health. *Environ Health Prev Med* 13(2): 102-108.