1

Coronary Artery Disease and Oxidative Stress

Mara S. Benfato, Tássia M. Medeiros and Tiago B. Salomon
LEO, Depto. de Biofísica, IBIO,
Programa de Pós-Graduação em Biologia Celular e Molecular,
Universidade Federal do Rio Grande do Sul,
Brazil

1. Introduction

O_2 arose on Earth in about 3.8 x 10^9 years ago due to the photosynthetic process in cyanobacteria hydrolyzed water. But it was only about 2.5 x 10^9 years ago that its levels rose to significant amounts. The increase in atmospheric concentrations of O_2 led to a great selective event, the first great mass extinction, due to stress on organisms that did not adapt to the new conditions. It also helped in the conquest of the land with the formation of O_3 (ozone) in the stratosphere, which filters the most harmful of the ultraviolet radiation (UV-C). In addition, using the O_2 as a substrate, the organisms generated much more energy (about 32 times more) but, in doing so, they started to generate reactive species in the process.

Reactive species (RS) are elements that react with biologically relevant organisms and although they act as cellular messengers, they also damage cellular components. In response to that, the organisms developed defences, which are now called antioxidants. The imbalance of the relation between RS and antioxidants is called oxidative stress. In this chapter we will study diseases related to oxidative stress but, in order to understand them, we first need to comprehend the radicals, their chemistry and the defences against such elements.

1.1 Reactive oxygen species (ROS) and reactive nitrogen species (RNS)

The RS are named according to the principal element in their composition, reactive oxygen species (ROS) and reactive nitrogen species (RNS), and are divided into radicals and non-radicals. Radicals have at least one unpaired electron in an open shell configuration and non-radicals are compounds that can generate radical species. Below we will see a list of the most important reactive species for human health (considered to date).

Note: Radicals are written with a dot attached to the upper right level representing the unpaired electron.

1.1.1 Reactive oxygen species

Hydroxyl radical ($HO^{\bullet}$)

A hydroxyl radical is the most reactive radical known *in vivo* and the most harmful, to which the human body has no defence mechanism. But, because it is so reactive, it reacts immediately after formation (within 5 molecular diameters from its production site).

It can be formed mainly by the Fenton reaction, in which the hydrogen peroxide (see below) reacts with a transition metal (Fe^{2+} or Cu^{+}) forming two hydroxides, one of them a radical and the other just an ion (see equation below).

$$H_2O_2 + Fe^{2+} \rightarrow OH^- + OH^{\bullet} \quad (1)$$

The reaction is faster with Cu^{+} (more than 60 times faster), but since it is not as bioavailable as Fe^{2+}, hydrogen peroxide reacts more with Fe^{2+} than with Cu^{+} (Halliwell & Gutteridge, 2007). It can cause modification of DNA bases and strand breaks, inactivation of proteins and lipid peroxidation. As explained above, $HO^{\bullet}$ is too reactive to be enzymatically removed (it would attack the enzymes), hence the way to control its damages is to reduce its formation and repair the damage.

Superoxide ($O_2^{\bullet -}$)

Superoxide is both an anion and a radical formed when an electron is added to the O_2 molecule. It is produced mainly by the electron leakage in the mitochondrial electron transport chain, but there are also other sources (e.g. endoplasmatic reticulum). Superoxide is quite toxic and is used in the defence systems to control pathogens for being a pro-oxidant and precursor for other species, but this toxicity works both ways, damaging important cellular components, especially inactivate enzymes by oxidation or reduction of its Fe-S sites (Flint et al., 1993), such as in an aconitase enzyme (which converts citrate to isocitrate, in the Krebs cycle) with the superoxide which reduces its $(Fe_4S_4)^{2+}$ to $(Fe_4S_4)^{+}$.

Hydrogen Peroxide (H_2O_2)

Hydrogen peroxide is a covalent, pale-blue, viscous liquid. Mainly produced *in vivo* by superoxide dismutation (see 1.2), but other oxidases may produce it as well, it is also produced by the oxidation of long chain fatty acids in the peroxissome (Titorenko & Terlecky, 2011). It plays a part in the immune response via formation of hydroxyl radicals or via inactivation of the pathogens' enzymes. However, for reacting with transition metals, hydrogen peroxide (see Fenton reaction above) represents a major problem to living organisms.

1.1.2 Reactive nitrogen species

Nitric Oxide ($NO^{\bullet}$)

$NO^{\bullet}$ is a colourless monomeric gas stable in pure water. In physiological conditions the half-life of nitric oxide is only a few seconds. In mammals nitric oxide is produced by the oxidation of L-arginine catalyzed by nitric oxide synthase (NOS) (Mungrue et al., 2003). Nitric oxide has several physiological roles, especially in neural and vascular systems. In the neural system it works as a neurotransmitter, strengthens the most used synapses and has a role in long-term memory but in excess, may cause strokes and epilepsy. In the vascular system it controls the blood pressure (vasodilator), kills foreign organisms (e.g. Leishmania), in excess may cause chronic inflammation, septic shock and transplant rejection. It has a role in bladder control, penile erection and peristaltic movements.

Peroxynitrite

Peroxynitrite is formed by the reaction of the radicals superoxide and nitric oxide, the peroxynitrite is an unstable, short-lived, potent oxidant, non-radical.

$$O_2^{\bullet-} + NO^{\bullet} \rightarrow ONOO^- \quad (2)$$

Peroxynitrite causes damage to proteins (-sulfur groups), hydroxylation and nitration of aromatic compounds. It may damage DNA as well by strand breaks and damages 2-deoxyribose.

1.2 Antioxidant defences

As we observed in the last topic, reactive species play a great role in biological systems, but they tend to cause much damage as well. To defend against such damage organisms developed defences, generically called "antioxidants", and when such defences fail we also have a repair system. The antioxidants may be classified in two major groups, enzymatic and non-enzymatic or endogenous and diet-derived.

1.2.1 Enzymatic

Catalase

Catalase is a very reactive enzyme that dismutates hydrogen peroxide (H_2O_2) into water (H_2O) and O_2, as seen in eq. 3.

$$2H_2O_2 \rightarrow 2H_2O + O_2 \quad (3)$$

Located in intracellular organelles (mostly peroxissomes) that are known as high producers of hydrogen peroxide (H_2O_2), Catalase is a tetramer of four polypeptide chains, each over 500 amino acids long and containing one *Fe(III)-heme* group that allows the enzyme to react with the hydrogen peroxide. As hydrogen peroxide enters the active site, it interacts with the amino acids causing an oxygen transfer between the heme group and the peroxide. The free oxygen is bound to the heme group (eq. 4), later, it reacts with a second hydrogen peroxide molecule and produce water and oxygen (eq. 5).

$$H_2O_2 + \text{CAT-Fe(III)} \rightarrow H_2O + \text{Compound I} \quad (4)$$

$$H_2O_2 + \text{Compound I} \rightarrow 2H_2O_2 + \text{CAT-Fe(III)} + O_2 \quad (5)$$

Superoxide dismutase

Superoxide dismutases (SODs) are enzymes that dismutate superoxide in oxygen and hydrogen peroxide. In humans three forms of superoxide dismutase are present. SOD1 (CuZnSOD) is located in the cytoplasm, SOD2 (MnSOD) in the mitochondria and SOD3 (CuZnSOD) is extracellular. The CuZnSOD contains two protein subunits, each with a metal, a Cu in one and Zn in the other (hence the name CuZnSOD). The copper ions catalyze the dismutation of superoxide and the zinc only helps the stability of the enzyme. Although CuZnSOD, SOD1 and SOD3 are two different proteins encoded by different genes, SOD3 is synthesized containing a signal peptide that directs this enzyme exclusively to extracellular spaces (Halliwell & Gutteridge, 2007).

$$Cu^{2+}\text{-SOD} + O_2^{\bullet-} \rightarrow Cu^{+}\text{-SOD} + O_2 \quad (6)$$

$$Cu^{+}\text{-SOD} + O_2^{\bullet-} + 2H^{+} \rightarrow Cu^{2+}\text{-SOD} + H_2O_2 \quad (7)$$

The MnSOD (SOD2) is quite different from CuZnSOD (not even having similar amino acid sequences), but performs the same reaction. It is more sensitive to denaturation (e.g. by heat) than the CuZnSOD. Each of its four protein subunits contains a manganese ion.

$$Mn^{3+}\text{-SOD} + O_2^{\bullet -} \rightarrow Mn^{2+}\text{-SOD} + O_2 \quad (8)$$

$$Mn^{2+}\text{-SOD} + O_2^{\bullet -} + 2H^+ \rightarrow M^{3+}\text{-SOD} + H_2O_2 \quad (9)$$

Glutathione peroxidise (GPx)

Glutathione peroxidase is the general name of an enzyme family, which consists of eight known human isoforms, whose main role is to protect the organism from oxidative damage. It is more versatile than catalase's action (as seen above) on lipid peroxides and in addition to hydrogen peroxide, is not limited to organelles, but its reaction speed (km) is much slower.

In order to detoxify peroxides it requires glutathione as a cofactor (eq. 9).

$$2GSH + H_2O_2 \rightarrow GSSG + 2H_2O \quad (10)$$

Since this process oxidize glutathione another enzyme is required to reduce the oxidize glutathine, via NADPH spending, the glutathione reductase. This process allows the glutathione to be used again by the peroxidase or another process (see Glutathione).

$$GS\text{–}SG + NADPH + H^+ \rightarrow 2\,GSH + NADP \quad (11)$$

Heme oxygenase

Human heme oxygenase-1 (hHO-1) is a stress protein linked to cytoprotection against oxidative stress. It catalyzes the reaction of heme to biliverdin, Fe^{2+} and carbon monoxide (CO). The carbon monoxide has pro- and antioxidant effects and also pro- or antiapoptotic effects that depend on dose (Piantadosi et al., 2006).

$$\text{Heme} + 3O_2 + 3½NADPH + 3½H^+ + 7e^- \rightarrow \text{biliverdin} + Fe^{2+} + CO + 3½NADP^+ + H_2O \quad (12)$$

The biliverdin reductase acts on biliverdin by reducing its double-bond between the pyrrole rings into a single-bond with $NADPH+H^+$ generating then, biliverdin and $NADP^+$. The biliverdin then takes on antioxidant properties by scavenging peroxyl radicals and limiting the peroxidation of membrane lipids and proteins.

1.2.2 Non-enzymatic

Glutathione

Glutathione (GSH) is a tripeptide, the most ubiquitous peptide found in cells. GSH can be obtained from the diet or can be synthesized *de novo* in the liver. It is the most abundant intracellular antioxidant. It works as a cofactor to GPx (as seen above) and also reacts, *in vitro*, with $HO^{\bullet}$, $ONOO^-$ among others species. It can also chelate copper, reducing its interaction with hydrogen peroxide, decreasing the Fenton reaction, and therefore reducing the formation of $HO^{\bullet}$. Its reaction with $ONOO^-$ leads to the formation of nitrosothiol (GSNO) which can be converted to $NO^{\bullet}$.

Ascorbic acid (vitamin C)

Ascorbic acid is an antioxidant produced by plants and some animals (e.g. rats, some birds) and one of its functions is to maintain redox homeostasis. The animals that do not synthesize ascorbic acid (including humans) must obtain ascorbic acid from the diet. They are unable to synthesize due to the lack of the enzyme gulonolactone oxidase, which catalyzes the final step in the synthesis of ascorbic acid (Yoshihara et al., 2010). Ascorbic acid has two oxidizable -OH groups. At physiological pH, it remains in the ionized form, ascorbate.

Among the many roles of vitamin C, we can highlight it acting as the scavenger of superoxide, hydroxyl, among others, also in the absorption of iron in the intestine (eq. 13) by reducing it from Fe^{3+} to Fe^{2+}, which works as a cofactor for several enzymes but also may be involved in Fenton reaction (see eq. 1) and regenerates the tocopheryl radical in tocopherol (very important).

$$Fe^{3+} + \text{ascorbate} \rightarrow Fe^{2+} + \text{ascorbyl} \quad (13)$$

Lower vitamin C levels found in elderly people, diabetic patients and cigarette smokers are most likely due to increased oxidative stress. Some studies showed that vitamin C supplementation decreased the level of oxidative DNA damage in mononuclear blood cells and also increased the LDL oxidation in patients' hemodialysis, but failed to prevent steady-state levels of lipid peroxidation (Yoshihara et al., 2010). There are some encouraging data to support vitamin C as a protective factor against cardiovascular diseases, but as a matter of fact there are more discouraging data (Collins et al., 2002) on this topic.

Tocopherols (vitamin E)

Tocopherols are a fat-soluble antioxidants (vitamin E is a name used to design several tocopherols) and are the most important inhibitors of lipid peroxidation. It can reduce Fe^{3+} to Fe^{2+} and Cu^{2+} to Cu^{+}. This ability is the basis of colorimetric method for measuring tocopherols. At high concentrations, the tocopherols present pro-oxidant effects, promoting lipid peroxidation. It can also affect blood clotting by interfering with the action of vitamin K. Its supplementation in diet is not recommended (Yoshihara et al., 2010).

1.3 Iron homeostasis

Iron is by far the most abundant transition metal in the human body and essential element for life. It is crucial for DNA synthesis, respiration and key metabolic reactions. It is an important component of enzymes that are involved in oxidation or reduction of biologic substrates, due to its ability to exist in two redox states making it useful at the catalytic centre like in cytochomes. It is also an essential component of oxygen carriers hemoglobin and myoglobin; alternatively, iron can bind to enzymes in a form of non-heme moieties or iron-sulfur (Fe-S) motifs (several mitochondrial enzymes). When iron exceeds the metabolic needs of the cell it may form a low molecular weight pool, tentatively referred to as the labile iron pool, which converts normal by-products of cell respiration, like $O_2^{\bullet -}$ and H_2O_2, into highly damaging hydroxyl radicals or equally aggressive ferryl ions. The redox state that do this is ferrous iron and the reaction that produces $OH^{\bullet -}$ is called Fenton Reaction. Therefore, iron must be chelated in very specific ways that discourage redox cycling. However, iron can have benign or malign effects on the cell, depending on whether it is a

micronutrient or a catalyst of free radical reactions. The average human adult contains approximately 4 g of iron, a little more than 2 g of which is in hemoglobin and 1g in body stores predominantly in the liver, the rest are in other iron-containing proteins, mainly in skeletal muscle (~300mg, most in myoglonbin) and macrophages (~600mg in total). Since total plasma iron turnover is some 35mg/day, iron deficiency can cause cellular growth arrest and death; iron excess can cause damage lipid membranes, proteins and nucleic acids. For example, iron deficiency represents the most common cause of anaemia worldwide and can cause development retardation in children as iron overload in hereditary hemochromatosis and thalassemias leads to potentially fatal liver or heart failure due, in the most part, to the amount of iron deposits.

Iron absorption needs to be tightly controlled due its activity redox which can also lead to the production of ROS. Its absorption occurs in the proximal small intestine and involves many key molecules. Iron absorption occurs in lumen of the duodenum and can be modulated by the size of the body's iron stores, by erythropoietic activity and by recent dietary iron intake. Iron can be absorbed from diet in two forms: as inorganic (non-heme) iron predominantly released from foods such as vegetables or cereals, or as heme iron from the breakdown of hemoglobin and myoglobin contained in red meat. Most iron in food is in ferric form [Fe (III) state], the most stable oxidation state for iron. Iron across is mediated by brush border iron transporter divalent metal transporter 1 (DMT1), which transports iron in the ferrous form [Fe (II))]. Hence, there are agents in gastric juice that solubilize and reduce Fe (III) in Fe (II), such as the ascorbate and hydrochloric acid (Frazer & Anderson, 2005), moreover, there are also in the epithelial surface apical ferric reductases. Heme (protoporphyrin ring that binds ferrous form) is more efficiently absorbed than inorganic iron and taken up by apical heme transporters after being released by proteolysis of hemeproteins in gut lumen is taken up and the iron removed from it in the mucosal cells by the action of heme oxygenase in ferrous form (Figure 1).

Inside the enterocytes, iron can be stored in ferritin in the cytoplasm, utilized in mitochondria or exported to plasma by ferroportin on the basolateral surface. Ferroportin cooperates with the multicopper ferroxidase hephaestin, which converts ferrous to ferric iron for uptake by plasma transferrin and regulated by hepcidin, an inhibitor of iron absorption and releases from macrophages and other cell types. The hepcidin causes ferroportin internalization and degradation, decreasing the transfer of iron to the body. Extracellular iron is bound with high affinity by the serum iron-transport protein transferrin and taken into the circulation (the labile iron pool). The majority of it is destined for nascent erythrocytes in the bone marrow. The cellular uptake of iron occurs through receptor-mediated endocytosis of transferrin (TfR). TfR containing transferrin binds on the cell membrane and is internalized by endocytosis. So, iron is used for cellular processes and excess iron is stored in ferritin (Dunn et al., 2007). It is important to know about these proteins because they have key roles in healthy processes and diseases in relation to iron homeostasis, for example, formation of atherosclerotic lesions, as will be discussed later. The excess of iron is lost by epithelial shedding in the gastrointestinal tract and the skin (approximately 1 to 2 g each day), through blood loss in menses of premenopausal women, in sweat and possibly a small amount excreted by lungs into mucus. The amount of iron absorbed can be affected by several mechanisms like inflammation, hypoxia, anaemia and iron overload. Iron can be recycled or stored as needed. Human erythrocytes undergo

surface alterations that mark them to be phagocyted and digested by macrophages in the spleen and the liver. In macrophages, iron is recovered from heme by the action of heme oxygenase and stored in ferritin, but the major site of iron storage is the liver, into hepatocytes. The capacity of readily exchanging electrons makes iron not only essential for fundamental cell functions, but also a potential catalyst for chemical reactions involving free-radical formation and subsequent oxidative stress and cell damage. Therefore, iron levels are carefully regulated to minimize the pool of potentially toxic "free iron". The majority of proteins described above are posttranscriptional controlled by iron regulatory proteins (IRP-1 and IRP-2). Iron regulatory proteins recognize at the mRNA level non-coding sequences (the iron-responsive elements [IRE]) which have been found in genes that control the iron homeostasis like ferritin and TfR, being that the ferritin synthesis is increased to sequester excess iron and TfR is downregulated in order to stop iron uptake (Cairo & Pietrangelo, 2000).

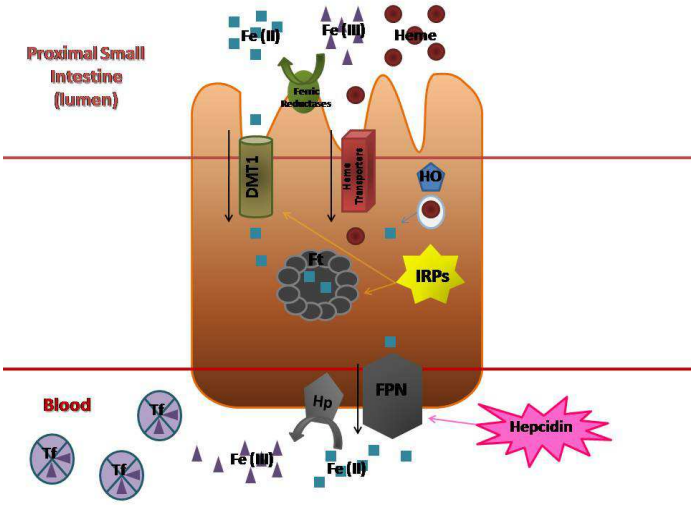


Fig. 1. Intestinal iron absorption. Iron absorption in the proximal small intestine mucosa of the gut requires transport across the apical and basolateral membranes of duodenal enterocytes. The dietary non-heme iron in the duodenal lumen is reduced by a ferric reductases and thus made available for divalent metal transporter 1 (DMT1), which transports ferrous iron across the apical brush border membrane and heme iron is transported by heme transporters. The amount of iron not retained by the cell inside the iron storage protein ferritin (Ft) is transferred to the bloodstream. The basolateral release of non-heme iron (which is also derived from heme catabolized by heme oxygenase [HO]) is mediated by ferroportin (FPN) which transports the metal across the membrane and hephaestin (Hp), which re-oxidizes iron as a necessary step for binding to the plasma carrier protein transferrin (Tf). The hepcidin causes ferroportin internalization and degradation, decreasing the transfer of iron to the body. The main proteins involved in iron absorption are controlled by iron regulatory proteins (IRPs), whose activity is regulated by the levels of the metal in the labile iron pool.

1.4 Oxidative damage

Oxidative stress describes the damage that occurs when oxidants overwhelm the antioxidants' defences; this can cause oxidative damage in macromolecules like DNA and proteins. The progressive and irreversible accumulation of oxidative damage may contribute to impaired physiological function and increased incidence of disease. Oxidative damage to lipids, proteins and DNA occurs primarily via the action of ROS. ROS can be generated by several mechanisms, but the principal source in aerobic cells is mitochondria. In an electron transport chain, oxygen can be reduced in superoxide ($O_2^{\bullet-}$). Superoxide itself does not appear to damage all macromolecules at physiologically relevant concentrations; redox reactions involving $O_2^{\bullet-}$, however, generate other reactive species that damage nucleic acids, proteins and lipids. This process generates the reactive intermediates encompassing a wide spectrum of oxygen-, carbon- or sulfur-centred radicals, originated from oxygen, hydrogen peroxide and lipid peroxides. Such damage is detectable under normal physiological conditions even in young animals, suggesting that endogenous protective mechanisms cannot suppress all oxidative damage even during basal levels of ROS generation (Halliwell & Gutteridge, 2007).

1.4.1 DNA damage

Damage to various macromolecules may not accumulate and therefore may not be critical. DNA, on the other hand, is the prime information molecule of the cell and nuclear DNA, in particular, must last the lifetime of the cell, therefore, DNA damage represents a critical threat to cell function. If DNA damage is severe or its accumulation exceeds its elimination by DNA repair mechanisms, cellular senescence or apoptosis will occur. Oxidative damage to nuclear DNA causes strand breakage that may lead to cell death. Additionally, oxidative damage to DNA causes mutations that can impair protein synthesis and lead to cell dysfunction. The hydroxyl radical ($OH^{\bullet}$) reacts with DNA by addition to double bonds of DNA bases and by hydrogen atom from abstraction the methyl group of thymine and each of the C-H bonds of 2′-deoxyribose. One of the DNA base products of interaction with reactive oxygen and other free radical species is 8-oxo-7,8-dihydro-2′-deoxuguanosine (8-OHdG). This is the oxidative lesion major and its level in DNA has, therefore, been consistently used as a measure of oxidative damage to DNA (Cooke et al., 2003). In addition, with $OH^{\bullet}$, it is important to note that hydrogen peroxide (H_2O_2) can cause massive acute DNA double-strand breaks and is involved in signalling cell stress (Chen et al., 2007).

1.4.2 Protein damage

Damage to proteins can occur by direct attack of reactive species or by secondary damage involving attack by end-products, like lipid peroxidation (Halliwell & Gutteridge, 2007). The importance of protein oxidation towards cellular homeostasis derives from the fact that proteins serve vital roles in regulating cell structure, cell signalling and the various enzymatic processes of the cell. Therefore, protein oxidation can rapidly contribute to oxidative stress by directly affecting cellular functions. Oxidation of proteins can lead to the formation of oxidized amino acids, such as dityrosine, 3-nitrotyrosine, 3-chlorotyrosine, oxohistidine and altered amino acid side-chains containing reactive carbonyls, and result in the loss of catalytic function, increased sensitivity to denaturation and increased susceptibility to proteolysis. One major pathway believed to generate protein carbonyls *in*

vivo is the metal-catalyzed protein oxidation pathway. In addition, there are others modes of inducing protein oxidation, among them are oxidation induced cleavage, amino acid oxidation and the conjugation of lipid peroxidation products. It is important to know that the accumulation of oxidized proteins is often measured by the content of reactive carbonyls. Some protein damage is reversible, such as methionine sulphoxide formation and destruction of Fe-S clusters by $O_2^{\bullet -}$. Other damage, for example of side-chains to carbonyl residues, appears irreversible and the protein is destroyed and replaced. Several mechanisms are activated when a protein undergoes damage by reactive species. This is necessary because accumulation of proteins with incorrect conformation can lead to cell death. When oxidized proteins resist proteolytic attack, they form aggregates which decrease their toxicity by sequestering them in insoluble clumps (Halliwell & Gutteridge, 2007).

1.4.3 Lipid peroxidation

Lipid peroxidation is involved in various and numerous pathological states including inflammation, atherosclerosis, neurodegenerative diseases and cancer. It has been know that lipid peroxidation induces disturbance of fine structures, alteration of integrity, fluidity and permeability, causes functional loss of biomembranes, modifies low density lipoprotein (LDL) to proatherogenic and proinflammatory forms and generates potentially toxic products. However, recently products of lipid peroxidation have been shown to exert various biological functions *in vivo,* such as regulators of gene expression, signalling messengers, activators of receptors and nuclear transcription factors, and inducers of adaptive responses, as well as ROS and RNS. Initiation of lipid peroxidation can be caused by addition of reactive species or, more usually, by hydrogen atom abstraction from a methylene group by reactive species (Halliwell & Gutteridge, 2007). The process of lipid peroxidation occurs by three distinct mechanisms, that is, (1) free radical-mediated oxidation, (2) free radical-independent, non-enzymatic oxidation, and (3) enzymatic oxidation. There are specific antioxidants to inhibit each type of lipid peroxide formed by mechanisms. For example, in the first situation $O_2^{\bullet -}$ and $NO^{\bullet}$ do not activate per se lipid peroxidation directly, but they react quite rapidly at the diffusion-controlled rate to give peroxynitrite ($ONOO^-$), which may initiate lipid peroxidation chain reactions. Both molecules are important to control muscular contraction in endothelium. A non-enzymatic oxidation example is the lipid oxidation by singlet oxygen, which can cause deleterious damage, such as a disease porphyria on the skin for oxidizing unsaturated lipids mainly. The thirst mechanism is another important type. It has been shown that lipoxygenase and ciclooxigenase oxidize arachidonic acid to prostaglandins, prostacyclin, thromboxane and leukotrienes, moreover, lipoxygenase directly oxidizes phospholipids and cholesteryl esters in LDL particles. It is important to cite that cholesterol is oxidized by three mechanisms noted above (Niki, 2009). Various molecular weight aldehydes, such as acrolein, malondialdehyde (MDA) and 4-hydroxy-2-nonenal (HNE) are formed during lipid peroxidation as secondary or decomposition products, and they are highly reactive and readily react with proteins, DNA and phospholipids to cause deleterious effects. MDA and HNE are considered good biomarkers of lipid peroxidation *in vivo*. Lipid peroxide alters chemical characteristics and the physical organization of cellular membranes to induce functional loss and modifies lipoproteins to proatherogenic and proinflammatory forms. It is assumed to be pathogenic and contribute to the etiology of various diseases (Niki, 2009).

Carbon radicals often stabilize by molecular rearrangement to form conjugated dienes, but if two radicals collide within a membrane they cross-link the fatty acid side-chain. When the formation of peroxy radical (by O_2 action) occurs, this can abstract a hydrogen atom from an adjacent fatty-acid side-chain. Thus happen the propagation stage of lipid peroxidation, mainly in membranes.

2. Atherosclerosis

Cardiovascular diseases are the leading cause of death and disability in the Western world. The majority of cardiovascular diseases result from complications of atherosclerosis. Atherosclerosis is a progressive disease that is generally characterized by the accumulation of lipids, fibrous elements and inflammatory cells and molecules within the arterial wall. The lesions of atherosclerosis occur principally in large and medium-sized elastic and muscular arteries and can lead to ischemia of heart, brain or extremities, resulting in infarction.

2.1 Formation and progression

The initiation of atherosclerosis begins with endothelial injury or dysfunction that is characterized by enhanced endothelial permeability and LDL deposition in the intima. LDL is accumulated in the preferred sites for lesion formation and undergoes oxidative modification as a result of its interaction with ROS. The endothelial injury likely is caused by ox-LDL itself, as well as physical or chemical forces and infection. This lesion induces the expression of a number of proinflammatory molecules, like adhesion molecules such as P-selectin, chemotactic and growth factors. These lead to the tethering, activation and attachment of monocytes and T lymphocytes to the endothelial cells. Monocytes ingest lipoproteins and morph into macrophages; macrophages generate ROS, which convert ox-LDL into highly oxidized LDL, which is, in turn, taken up by macrophages to form foam cells. Foam cells combine with leukocytes to become the fatty streak and as the process continues foam cells secrete growth factors that induce smooth muscle cells' migration into the intima. Endothelial cells, macrophages and smooth muscle cells highly oxidize LDL by the action of ROS produced. The foam cells secrete more growth factors that induce smooth muscle cells' migration into the intima and proliferation forming the fibrous plaques. Later, calcification can occur and cause plaque stabilization. In plaques that are not calcified the fibrous plaques may rupture and form thrombi that may ultimately occlude vessels, for example in the case of acute coronary syndromes that lead to myocardial infarction. Possible causes of endothelial dysfunction leading to atherosclerosis include elevated and modified LDL; free radicals caused by cigarette smoking, hypertension and diabetes mellitus; genetic alterations; elevated plasma homocysteine concentrations (toxic to endothelium and prothrombotic); infections microorganisms; and combinations of these or other factors. The process of atherosclerosis occurs primarily in certain arteries, such as coronary and carotid arteries (Ross, 1999).

2.2 Oxidative stress and inflammation

Oxidative stress plays an important role in the formation of atherosclerosis plaque. The oxidation hypothesis suggests multiple mechanism(s) by which oxidation of LDL might

promote atherosclerosis. LDL retained within the artery can be oxidized by a number of cell types present within arteries, including endothelial cells, smooth muscle cells, monocytes and macrophages, and lymphocytes. HDL can also be oxidized by endothelial cells and by chemical means. Oxidation of these lipoproteins can be blocked by antioxidants. Ox-LDL also has potentially atherogenic affects, inhibits the mobility of tissue macrophages, enhances production of chemotatic factors and adhesion molecules, induces smooth muscle cells' migration and both proliferation and apoptosis in endothelial cells, smooth muscle cells and macrophages (Schwenke, 1998). In the vasculature, production of reactive species occurs that are used to control physiological functions. Oxygen undergoes reduction to $O_2^{\bullet-}$ by means of enzymes, such as the nicotinamine adenine dinucleotide (phosphate) (NADH/NAD(P)H) oxidases and xantine oxidases (XO). The $O_2^{\bullet-}$ is used to promote vasoconstriction and can form H_2O_2 that can react with other radicals, such as transition metal Fe^{2+} to produce $OH^{\bullet}$ (Fenton reaction). Myeloperoxidase, a heme protein secreted by phagocytes, can amplify the oxidative potential of H_2O_2 by production of hypoclorous acid (HOCl) that can react with $O_2^{\bullet-}$ to produce $OH^{\bullet}$. Other sources of ROS in the vessel wall include mitochondria, ciclooxygenase (COX), lipoxygenase and uncoupled endothelial nitric oxide synthase (eNOS). This last, in normal conditions, generates nitric oxide ($NO^{\bullet}$), but if there is availability of precursors, eNOS become uncoupled generating $O_2^{\bullet-}$. Although $NO^{\bullet}$ is a reactive species, it is thought be antiatherosclerotic because it is a vasodilator potent and inhibits LDL peroxidation by scavenging peroxil radicals. These reactive species ($O_2^{\bullet-}$, H_2O_2 and $NO^{\bullet}$) cannot oxidize LDL, but form other reactive species that can do this, like $OH^{\bullet}$ and $ONOO^-$ (described above) (Madamanchi et al., 2005, Halliwell & Gutteridge, 2007). But how can free ferrous iron in the body be a catalyst for the formation of $OH^{\bullet}$, powerful pro-oxidants and promote lipid oxidation (increased formation of ox-LDL)? In 1981 Sullivan created The Iron Heart Hypothesis suggesting that increased body iron stores are a risk factor for coronary heart disease and thus that iron depletion though phlebotomy or other means can reduce risk (Sullivan, 1981). In addition to enhancing oxidative stress, increased iron stores are believed to adversely affect cardiovascular disease through other mechanisms, including alteration of endothelial function, decreased vascular reactivity and reperfusion injury by iron-induced free radicals (Hu, 2007). Furthermore, iron can contribute to the signalling in inflammatory pathways and hypoxia response. Atherosclerosis is an inflammatory disease and inflammatory mechanisms have emerged as playing a pivotal role in all stages of atherosclerotic plaque formation. Systemic inflammation occurs in the vasculature as a response to injury, lipid peroxidation and perhaps infection. A number of inflammatory mediators are released by cells involved in the lesion, including tumour necrosis factor α (TNFα) or interleukin 1 (IL-1), chemokines, such as IL-8 or monocyte chemoattractant protein-1 and adhesion molecules, such as intercellular adhesion molecule 1 (ICAM-1) or selectins. In particular, smooth muscle cells also release IL-6 which is the main hepatic stimulus for the acute phase reactant, C-reactive protein (CRP), which causes expression of adhesion molecules and also stimulates hepcidin. The ferritin also has synthesis regulated by cytokines, such as TNFα and IL-1, at various levels (transcriptional, posttranscriptional and translational) (You & Wang, 2005).

Abnormal ferritin levels or iron homeostasis have been linked to atherosclerosis. To prove the iron hypothesis, many epidemiological studies have been performed. Most studies testing the hypothesis of iron measured levels of ferritin. The ferritin level rises with iron loading and declines with depletion of tissue iron stores. Salonen et al. first reported a

significant association between the serum ferritin levels and the risk of myocardial infarction of 1,931 middle-aged men during an average follow-up of three years (2.2 higher risk of myocardial infarction in men with higher serum ferritin levels) (Salonen et al., 1992). A study from our laboratory compared patients with coronary heart disease and sleep apnea (also inflammation disease) showing that serum ferritin levels increased in coronary heart disease patients and positively correlated with sleep apnea. These studies are supported by the evidences that show iron deposition in human atherosclerotic lesions, suggesting that iron may play a role in the development of atherosclerosis (Hower et al., 2009).

With regard to sleep apnea, studies have demonstrated sleep disordered breathing to be associated with cardiovascular disease, include coronary artery disease, heart failure, hypertension, cardiac arrhythmias and stroke, which further increase morbidity and mortality in the sleep disordered breathing population (Flemons et al., 1999). Hypoxia events, endothelial dysfunction, coagulopathy, impaired sympathetic drive, oxidative and inflammatory stress are the pathophysiological pathways suggested for the development of cardiovascular disease in sleep disordered breathing (Butt et al., 2010). Increase in ROS in endothelial cells exposed to hypoxia has been evidenced. Among the possible sources of ROS by hypoxia are the mitochondria, leukocytes (NADPH oxidase pathway) and epithelial tissue enzymes, such as xanthine oxidase, cyclooxygenase, lipooxygenase, NO-synthase and hemeoxygenases (Lavie, 2003). As discussed above, hepcidin is a peptide also involved in iron homeostasis and has impact in inflammatory hypoferrimia because inflammation is mediated by citokine-driven increase in hepcidin production, causing release and recycled iron from macrophages and and blocking the passage of iron from enterocytes to plasma. Hepcidin production is controlled by inflammatory citokines like ferritin. The main citokines are IL-6 and TNFα. In addition to cytokines, hepcidin is downregulated under hypoxia conditions and little is known of the involvement of ROS in this mechanism. A study suggested that ROS (produced by hypoxia) repress the hepcidin gene (Choi et al., 2007).

The same recent study in our lab that verified the serum ferritin levels in coronary heart disease (CHD) and sleep apnea patients also verified the serum prohepcidin levels (the precursor of hepcidin). The study was performed in 56 patients with stable coronary heart disease referred for angiography (male gender 54%). Exclusion criteria, to avoid potent oxidative stress factors, were: smoking, age older than 65 years, morbid obesity, diabetes. Patients underwent a portable polysomnography to verify the apnea-hypopnea index (AHI) and determination of hemoglobin, hematocrit, ferritin, prohepcidin and high-sensitivity C-reactive protein (hs-CRP) levels. Patients were divided into controls and cases at the median AHI, 28 controls with an AHI low and 28 cases with moderate to severe AHI. The mean ferritin levels are significantly higher in cases than the control and this is the first report of such findings in sleep apnea (170±140.1 vs. 285±194.5; $p < 0.05$). There were a significantly greater percentage of subjects with CHD in the group with moderate to severe sleep apnea (72%; p 0.001). The Pearson's correlation coefficients showed positive significance between ferritin and AHI ($r = 0.398$, $P = 0.002$), prohepcidin and ferritin ($r = 0.432$, $P = 0.001$) and iron and ferritin ($r = 0.346$; $P = 0.009$); between AHI and prohepicidin was $r= -0.15$ ($P = 0.3$) (figure 2). How hypoxia could be affecting the ferritin and hepcidin levels is not known. In a multivariate regression, however, controlling for age, sex, body mass index and coronary heart disease, the AHI and ferritin explain 30.4% of the variance of prohepcidin. Thus, it is

suggested that hypoxia-reoxygenation in obstructive sleep apnea may influence prohepcidin in human and, consequently, iron homeostasis, aggravating oxidative stress and contributing to the emergence of coronary heart disease (increased ferritin levels).

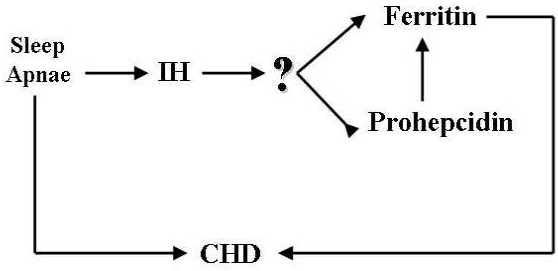


Fig. 2. Scheme of regressions and correlations found among studied parameters. The sleep apnea through intermittent hypoxia (IH) events activates some unknown route (?) generating decreased prohepcidin levels and increased ferritin levels. The hypoxia interferes positively in the ferritin levels and negatively in prohepcidin levels. Prohepcidin already induces the ferritin synthesis. It is suggested that the hypoxia induction by ferritin levels overlaps the prohepcidin inhibition by hypoxia, because there was increased ferritin levels, as well as increased AHI. A relationship was found between OSAS and CHD, as well as ferritin and CHD corroborating with literature data.

2.3 Oxidative stress biomarkers

Many experimental and observational studies showed the relationship between oxidative stress biomarkers and cardiovascular disease. Among the most used are ox-LDL, myeloperoxidase, lipid peroxidation products and protein oxidation. The ability of oxidative stress biomarkers to predict cardiovascular disease has yet to be established. Some of them have already been examined, now we will look in more detail at these and comment about new markers. As described above, ox-LDL is believed to play an intrinsic role within atherosclerosis plaque formation and progression of atherosclerosis. In the same study that showed a relationship between ferritin and hepcidin with coronary heart disease, the levels of ox-LDL and paraoxanase-1 (enzyme present in HDL that reduces ox-LDL accumulation) were also analyzed, indicating that they are important predictors of coronary heart disease (intern communication). Paraoxanase-1 possess antioxidant and anti-inflammatory properties and protect against atherogenesis, and for this, can be associated with the action of HDL (Jayakumari & Thejaseebai, 2009). The measurement of F_2-isoprostanes (a prostaglandin-like compound formed from radical catalyzed peroxidation of fatty acids, like arachidonic acid, without the direct action of enzymes) has emerged as one of most sensitive and reliable biomarkers of lipid peroxidation *in vivo* (Davies & Roberts, 2011). As previously indicated, damage to proteins by ROS produces carbonyls and other amino acid modifications. Some studies used protein oxidation as a predictor of cardiovascular disease endpoints (Strobel et al., 2011). For example, the study that analyzed the cardiovascular disease linked to sleep apnea verify that the carbonylation of erythrocytic proteins associated with sleep apnea is a predictor of cardiovascular disease (Klein et al., 2010).

The 8-OHdG is typical biomarker of oxidative stress. Increased 8-OHdG levels are frequently related to cardiovascular disease. The level of 8-OHdG has been demonstrated to be very high in aorta fragments taken at surgery from patients suffering from severe atherosclerosis lesion. An increase 8-OHdG levels in DNA isolated from lymphocytes are related to cardiovascular disease (Gackowski et al., 2001).

3. Hypercholesterolemia

Cardiovascular disease is a complex and multifactorial disease; there can be no doubt now that elevated plasma cholesterol levels play a dominant role. Hypercholesterolemia is associated with an increased risk of atherosclerosis. Fatty streaks can even be found in the foetus, to an extent increasing with maternal plasma cholesterol levels. There are genetic disorders that may have a relationship with hypercholesterolemia, such as a disease familial hypercholesterolemia, in which the LDL receptors are defective or absent, so that blood LDL (and hence cholesterol) levels become very high and these people have high atherosclerosis incidence (Halliwell & Gutteridge, 2007).

3.1 Hypercholesterolemia and oxidative stress

There are many possible factors involved in the atherosclerosis process; the oxidation hypothesis has been the central focus on the pathogenesis of atherosclerosis for almost 30 years. This hypothesis states that the oxidative modification of LDL, or other lipoproteins and polyunsaturated fatty acids, is central, if not obligatory to the atherogenic process. The important issue is that inhibition of such oxidation should reduce the progression of atherosclerosis, independent of reduction of other risk factors, such as elevated LDL levels. The interest in ox-LDL is based on the fact that ox-LDL is cytotoxic to endothelial and other cells, and thus, could directly cause damage to arterials cells and, in addition it can activate an immune and proinflammatory response. There are many potential mechanisms by which oxidized forms of LDL may influence atherogenesis, these include uptake of ox-LDL by macrophages leading to foam cell formation; ox-LDL products are chemotactic for monocytes and T-cells, they can inhibit the motility of tissue macrophages and induce apoptosis; ox-LDL or its products are mitogenic for smooth muscle cells and macrophages, for example, they can induce proinflammatory genes and macrophage scavenger receptors, thereby enhancing its own uptake; ox-LDL is immunogenic and elicits autoantibody formation and activated T-cells; ox-LDL may enhance procoagulant pathways (induction of tissue factor and platelet aggregation, and can adversely impact arterial vasomotor properties (Witztum & Steinberg, 2001). We already know that ox-LDL is proatherogenic, but how is it generated *in vivo*? There are lingering uncertainties about the mechanism of LDL oxidation *in vivo*. The LDL is not necessarily oxidized within the plasma compartment, the LDL could undergo oxidative modification on the artery wall or in fact in any extravascular, extracellular site and then return to the plasma compartment (Chisolm & Steinberg, 2000). Oxidation of LDL results in the generation of a variety of modifications to the lipid and protein moieties, including the covalent modification of apolipoprotein B (ApoB) with reactive products of the decomposition of oxidized lipids, yielding MDA and HNE. Remembering that core lipid particles are composed of cholesterol ester and triglyceride, an outer monolayer is composed of free cholesterol and phospholipid including phosphatidilcholine, and on molecule of ApoB surrounds LDL particles (Yoshida & Kisugi,

2010). In addition, the residual oxidized phospholipid containing aldehyde terminate fatty acids. These, and presumably many other changes, generate immunogenic neo-epitopes on the modified LDL and are important to the atherosclerotic process. A variety of oxidized lipids' products, with similar characteristics of ox-LDL, are found in human plasma, atherosclerotic tissue and urine. Although some of these may indeed arise from oxidation of LDL, they could equally derive from oxidation of lipids at other sites and that oxidation may or may not parallel the rate at which LDL itself is undergoing oxidative modification (Witztum & Steinberg, 2001). Some of these proatherogenic effects of ox-LDL could also be induced by organic phase extracts of ox-LDL, suggesting that oxidized lipids themselves were proatherogenics, in addition to oxidatively modified ApoB (Davies & Roberts, 2011).

Still addressing LDL, it can be oxidized non-enzymatically by transition metal ions, heme and other catalysis. On the other hand, there are many postulated mechanisms by which LDL could become oxidized via several enzymes within the artery wall. Transitions metals are important to lipid oxidation. Most cells present in the arterial intima can promote LDL oxidation by its enzymes that mediated LDL oxidation, but it arguably requires the presence of transitions metals, iron or copper microconcentration. Elevated levels of metal ions are present in the advanced atherosclerotic lesions. Tissue homogenates prepared from atherosclerotic lesions contain catalytically active metal ions, indicating that these metals may stimulate LDL oxidation in the artery wall. One mechanism that has now gained strong support is the enzimatic. Lypoxygenase is one intracellular enzyme that directly oxygenates polyunsaturated fatty acids. The enzyme initiates the seeding of LDL with hydroperoxides, leading to the subsequent initiation of lipid peroxidation. These lipid peroxides could be released from cells and might translocate to LDL. Leucocytes-released myeloperoxidase catalyzes the formation of reactive substance species (HOCl) and generates a series of secondary radical or non-radical oxidants that may provide lipid peroxidation, oxidized LDL, advanced glycation end products and nitrating species. Among the mechanisms protein glycation is included. The last mechanism cited refers to $NO^{\bullet}$ (which has already been mentioned here). Although $NO^{\bullet}$ is a stable radical that fails to oxidized LDL at physiological pH, it is rapidly inactivated by $O_2^{\bullet-}$ to form peroxynitrite, a potent oxidant, implicating in LDL oxidation. This mechanism should be important *in vivo* since endothelial cells, smooth muscle cells and macrophages generate $O_2^{\bullet-}$ (Yoshida & Kisugi, 2010). There are lipid peroxidation products in the vasculature that do not arise directly from LDL and could contribute to atherogenesis. These oxidation products create proinflammatory mediators that drive a chronic inflammatory state, such as isoprostanes. It is important to know that well-established risk factors as causes of cardiovascular disease may have lipid peroxidation as part of its mechanism, such as smoking and diabetes (Davies and Roberts, 2011). Therefore, the evidence shows us clearly that hypercholesterolemia plus other risk factors increase the disease process and progression.

The oxidant hypothesis makes us question whether or not administration of antioxidants significantly slows the formation of atherosclerotic lesion. In a large number of epidemiologic studies, the dietary intake or plasma levels of antioxidant nutrients correlates negatively with risk of clinical cardiovascular disease. The user in clinical trials is tocopherols and beta-carotene because they are naturally occurring nutrients which would pose no toxicological problems. The relevance of vitamin C is as a potent trap for singlet oxygen, but much less effective in terminating free radical chain reactions and the vitamin E

is an excellent terminating free radical chain reaction. The protect effect against LDL oxidation is more effective with use of vitamin E than C. This difference may be because vitamin C is distributed exclusively in the aqueous phase, whereas vitamin E takes up residence predominantly in lipoprotein (Witztum and Steinberg, 2001).

HDL normally plays an anti-atherogenic role, unlike LDL. The protective capacity of HDL has been ascribed primarily to its ability to remove excess cholesterol from peripheral tissues in the reverse cholesterol transport pathway. However, recent studies have suggested more mechanisms. For example, HDL can inhibit LDL oxidation and this may contribute to inverse association between plasma HDL levels and risk of developing atherosclerosis. These protective effects of HDL have been attributed to the various proteins associated with HDL. Paraoxonase-1 is an enzyme associated with HDL in blood and has been reported to posse antioxidant and anti-inflammatory properties. This enzyme is able to hydrolyze oxidized phospholipids and to destroy the biologically active lipids in ox-LDL. There is growing evidence that reduced activity of HDL-associated paraoxonase-1 is predictive of vascular disease (Jayakumari & Thejaseebai, 2009).

4. Hypertension

Previous studies have indicated that hypertension and hypercholesterolemia frequently co-exist, causing what is known as "dyslipidemic hypertension". The combination of these factors more than additively increases the risk of cardiovascular disease events compared with the occurrence of one alone (Wong et al., 2006). The resultant oxidative stress is considered a unifying mechanism for hypertension and atherosclerosis.

Hypertension development is intrinsically linked with vascular function and structural changes, including endothelial dysfunction, altered contractility and vascular remodelling. One of the key characteristics of hypertension is increased peripheral resistance, due largely to a reduced lumen diameter of the resistance vessel, and a small change in diameter can significantly impact on vascular resistance. The small arteries and arterioles that determine peripheral resistance undergo both structural and functional changes in hypertension. Examples of these changes include endothelial function, vascular smooth muscle growth, extracellular matrix deposition and vascular inflammation, altering contractility and vascular remodelling (Paravicini & Touyz, 2006).

4.1 Hypertension and oxidative stress

Within the cardiovascular system the ROS have a key role including regulation of cell growth and differentiation, modulation of extracellular matrix production and breakdown, $NO^{\bullet}$ inactivation and stimulation of many kinases. Many of this effects are associated with pathological changes observed in hypertension (Madamanchi et al., 2005).

Patients with hypertension demonstrate increased levels of oxidative stress by-products together with decreased activity of endogenous antioxidants enzymes, oxidative DNA damage and higher levels of $O_2^{\bullet-}$ production. ROS are produced by all vascular types of cells and can be formed by numerous enzymes, such as xanthine oxidase, uncoupled endothelial NO synthase and NAD(P)H oxidase, that are the most relevant in vascular disease and hypertension. It is worth keeping in mind the function of this enzymes, the

xanthine oxidase catalyses the oxidation of hypoxanthine and xanthine to form $O_2^{\bullet-}$, and is known to be present in vascular endothelium. Although xanthine oxidase-derived $O_2^{\bullet-}$ has been primarily studied in the context of ischemia-reperfusion injury and heart failure, there is also some evidence to suggest involvement in the endothelial dysfunction seen in hypertension. Nitric oxide synthase (NOS) can also contribute to ROS production, as all three NOS isoforms have been shown to be susceptible to the uncoupling that leads to the formation of $O_2^{\bullet-}$ (rather than $NO^{\bullet}$) under certain conditions. Many studies have shown that the major source of ROS in the vascular wall is nonphagocytic NAD(P)H oxidase, which utilizes NADH/NADPH as the electron donor to reduce molecular oxygen and produce $O_2^{\bullet-}$. Activation of this enzyme is regulated by many vasoactive hormones, growth factors and mechanical stimuli (shear stress and stretch) (Higashi et al., 2009).

The biomechanical forces influence the redox signalling. Two main forces acting on the blood vessel wall are shear stress (movement of blood) and stretch (luminal pressure). Shear stress and cyclic mechanical stretch influence vascular function and structure, in part, by stimulating production of $NO^{\bullet}$ and ROS. Summarizing, the biomechanical forces increase activation and expression of endothelial NOS and stimulate production of $O_2^{\bullet-}$ and H_2O_2 (Paravicini & Touyz, 2006). Again, remembering that $O_2^{\bullet-}$ and $NO^{\bullet}$ can form $ONOO^-$; increased vascular pressure in hypertension is associated with stretch of endothelial and vascular smooth muscle cells, which can directly activate NAD(P)H oxidase to generate ROS. This effect may be amplified by activation of the rennin-angiotensin system. Increased oxidative stress in response to stretch contributes to activation of pro-inflammatory transcription factors, activation of growth-promoting MAP kinases, upregulation of profibrogenic mediators and altered vascular tone, important processes contributing to the vascular phenotype associated with hypertension (Paravicini & Touyz, 2006).

As discussed before, the excessive ROS have a central common pathway by which disparate influences may induce and exacerbate hypertension. Furthermore, a significant number of epidemiological and clinical trial data suggest that diets known to contain significant concentrations of naturally occurring antioxidants appear to reduce blood pressure and may reduce cardiovascular risk. Because of this, there is much interest in identifying key, naturally occurring antioxidants to both prevent and treat hypertension (Madamanchi et al., 2005). As in hypercholesterolemia, the focus is on vitamins E and C, and also vitamin A. The interest in vitamin A derivates has turned to lycopene, a potent antioxidant found in tomatoes. One small study has shown a reduction in blood pressure with tomato extract-based intervention. Vitamin C antihypertensive efficacy has been evaluated in small studies, showing modest reductions in blood pressure in both normotensive and hypertensive populations. With regard to vitamin E, small studies show either no effect or a pressor effect from supplementation. It is important to take care with the use of higher doses of supplements, since there is the risk of an antioxidant becoming pro-oxidant when used at high doses, for example, the ascorbate increase the risk of forming oxalate renal calculi (Kizhakekuttu & Widlansky, 2010). The addition of vitamins, the L-arginine, flavonoids and mitochondria-targeted agents are part of a target group of studies. L-arginine is an amino acid and the main substrate for the production of $NO^{\bullet}$ from NOS, and reduced levels lead to uncoupling of NOS resulting in the generation $O_2^{\bullet-}$ (low levels could contribute to hypertension). L-arginine supplementation could reduce blood pressure allowing for a restoration of normal $NO^{\bullet}$ bioavailability. There are studies demonstrating that flavonoids can inhibit NADPH oxidase and increase NOS-specific $NO^{\bullet}$ production, but investigation

into the antihypertensive effects of flavonoids are inconclusive. The mitochondria-target agents include mainly coenzyme Q10 (CoQ) and lipoic acid. CoQ levels have been shown to be lower in hypertensive patients. CoQ may reduce mitochondrial $O_2^{\bullet-}$ production and reduce lipid peroxidation in plasma; CoQ supplementation was also demonstrated to reduce blood pressure. The potential beneficial effects of lipoic acid supplementation is given because it may improve coupling of NOS and has anti-inflammatory actions (Kizhakekuttu & Widlansky, 2010).

5. Conclusion

Throughout this chapter we have seen the numerous connections between heart disease, associated diseases and oxidative. Therefore, we cannot talk of homeostasis or change it without talking about redox balance. Any event that alters the delicate balance between defences and ROS moves the scales and triggers oxidative stress. Luckily our bodies are adapted to these constant changes, but only to a limited extent. Minor damage accumulates over the years. The fittest survive and we must be aware that not escaping natural selection, it continues to act upon us.

An alert on the evaluation of data involving oxidative stress: strict criteria are needed. For example, studies of ascorbic acid supplementation in rats and mice should be evaluated very carefully since these species synthesize vitamin C, while humans do not. Extrapolation of this data type for the human species must be carefully evaluated if it is to have any value. In the case of human data it must not be forgotten that the effect of an antioxidant that shows promise for a patient group cannot be extrapolated to healthy humans for example. In addition, dietary supplements that may be beneficial for the chronically ill should not be recommended for healthy people. What may be an antioxidant to one group can be pro-oxidant to another. A simple explanation of why: chemical reactions are reversible. The direction of the reaction in one group may be different from the direction of the reaction in the other group. The inclusion of a reactant or product may mean the reactions favouring or inhibiting the reactions that follow.

As we have learned over the past years for various diseases that afflict humanity, coronary heart disease can be triggered by many environmental and genetic factors. The disease in itself can trigger numerous other changes altering the homeostasis of the organism. Where is oxidative stress involved? Is it a cause or consequence? These questions are difficult to answer as we cannot address this issue without being aware of the chemistry of reactive species; we only know them with a solid knowledge of basic chemistry, which leads us to basic biochemistry, a deep knowledge of cell biology, physiology and so on. Molecular biology and genetics will help us with information no less important. Therefore, we need many more research groups than in the past century and in the clinical area, multidisciplinary cooperation. Maybe this is the biggest challenge for the 21st century.

6. References

Arens, R. & Marcus, C. L. (2004). Pathophysiology of upper airway obstruction: a developmental perspective. *Sleep,* 27, 997-1019. ISSN 0161-8105

Bradley, T. D. & Floras, J. S. (2009). Obstructive sleep apnoea and its cardiovascular consequences. *Lancet,* 373, 82-93. ISSN 0140-6736

Butt, M., Dwivedi, G., Khair, O. & Lip, G. Y. H. (2010). Obstructive sleep apnea and cardiovascular disease. *International Journal of Cardiology,* 139, 7-16. ISSN 0167-5273

Cairo, G. & Pietrangelo, A. (2000). Iron regulatory proteins in pathobiology. *Biochemical Journal,* 352, 241-250. ISSN 0264-6021

Chisolm, G. M. & Steinberg, D. (2000). The oxidative modification hypothesis of atherogenesis: An overview. *Free Radical Biology and Medicine,* 28, 1815-1826. ISSN 0891-5849

Choi, S. O., Cho, Y. S., Kim, H. L. & Park, J. W. (2007). ROS mediate the hypoxic repression of the hepcidin gene by inhibiting C/EBP alpha and STAT-3. *Biochemical and Biophysical Research Communications,* 356, 312-317. ISSN 0006-291X

Christou, K., Moulas, A. N., Pastaka, C. & Gourgoulianis, K. I. (2003). Antioxidant capacity in obstructive sleep apnea patients. *Sleep Medicine,* 4, 225-228. ISSN 1389-9457

Collins, R., Armitage, J., Parish, S., Sleight, P., Peto, R. & Heart protection study, C. (2002). MRC/BHF Heart Protection Study of antioxidant vitamin supplementation in 20536 high-risk individuals: a randomised placebo-controlled trial. *Lancet,* 360, 23-33. ISSN 0140-6736

Cooke, M. S., Evans, M. D., Dizdaroglu, M. & Lunec, J. (2003). Oxidative DNA damage: mechanisms, mutation, and disease. *Faseb Journal,* 17, 1195-1214. ISSN 0892-6638

CousE, J. F. & Korach, K. S. (1999). Estrogen receptor null mice: What have we learned and where will they lead us? *Endocrine Reviews,* 20, 358-417. ISSN 0163-769X

Davies, S. S. & Roberts, L. J. (2011). F-2-isoprostanes as an indicator and risk factor for coronary heart disease. *Free Radical Biology and Medicine,* 50, 559-566. ISSN 0891-5849

Dunn, L. L., Rahmanto, Y. S. & Richardson, D. R. (2007). Iron uptake and metabolism in the new millennium. *Trends in Cell Biology,* 17, 93-100. ISSN 0962-8924

Fadini, G. P., Albiero, M., Cignarella, A., Bolego, C., Pinna, C., Boscaro, E., Pagnin, E., De toni, R., DE Kreutzenberg, S., Agostini, C. & Avogaro, A. (2009). Effects of androgens on endothelial progenitor cells in vitro and in vivo. *Clinical Science,* 117, 355-364. ISSN 0143-5221

Flemons, W. W., Buysse, D., Redline, S., Pack, A., Strohl, K., Wheatley, J., Young, T., Douglas, N., Levy, P., Mcnicholas, W., Fleetham, J., White, D., Schmidt-Nowarra, W., Carley, D., Romaniuk, J. & Amer acad sleep med task, F. (1999). Sleep-related breathing disorders in adults: Recommendations for syndrome definition and measurement techniques in clinical research. *Sleep,* 22, 667-689. ISSN 0161-8105

Flint, D. H., Tuminello, J. F. & Emptage, M. H. (1993). The inactivation of fe-s cluster containing hydro-lyases by superoxide. *Journal of Biological Chemistry,* 268, 22369-22376. ISSN 0021-9258

Frazer, D. M. & Anderson, G. J. (2005). Iron Imports. I. Intestinal iron absorption and its regulation. *American Journal of Physiology-Gastrointestinal and Liver Physiology,* 289, G631-G635. ISSN 0193-1857

Gackowski, D., Kruszewski, M., Jawien, A., Ciecierski, M. & Olinski, R. (2001). Further evidence that oxidative stress may be a risk factor responsible for the development of atherosclerosis. *Free Radical Biology and Medicine,* 31, 542-547.ISSN 0891-5849

Gourdy, P., Calippe, B., Laurel, H., Tremollieres, F., Douin-Echinard, V., Lenfant, F., Bayard, F., Gueryt, J. C. & Arnal, J. F. (2008). Role of inflammatory cytokines in the effect of estradiol on atheroma. *Clinical and Experimental Pharmacology and Physiology,* 35, 396-401 ISSN 0305-1870

Guo, S., Miyake, M., Liu, K. J. & Shi, H. (2009). Specific inhibition of hypoxia inducible factor 1 exaggerates cell injury induced by in vitro ischemia through deteriorating cellular redox environment. *Journal of Neurochemistry,* 108, 1309-1321. ISSN 0022-3042

Halliwell, B. & Gutteridge, J. M. C. (2007). *Free radicals in biology and medicine,* New York, Oxford University Press. ISBN 978-0-19-856868-1

Higashi, Y., Noma, K., Yoshizumi, M. & Kihara, Y. (2009). Endothelial Function and Oxidative Stress in Cardiovascular Diseases. *Circulation Journal,* 73, 411-418. ISSN 1346-9843

Hower, V., Mendes, P., Torti, F. M., Laubenbacher, R., Akman, S., Shulaev, V. & Torti, S. V. (2009). A general map of iron metabolism and tissue-specific subnetworks. *Molecular Biosystems,* 5, 422-443. ISSN 1742-206X

Hu, F. B. (2007). The iron-heart hypothesis - search for the ironclad evidence. *Jama-Journal of the American Medical Association,* 297, 639-641. ISSN 0098-7484

Ip, M. S. M., Lam, B., Chan, L. Y., Zheng, L., Tsang, K. W. T., Fung, P. C. W. & Lam, W. K. (2000). Circulating nitric oxide is suppressed in obstructive sleep apnea and is reversed by nasal continuous positive airway pressure. *American Journal of Respiratory and Critical Care Medicine,* 162, 2166-2171. ISSN 1073-449X

Jayakumari, N. & Thejaseebai, G. (2009). High Prevalence of Low Serum Paraoxonase-1 in Subjects with Coronary Artery Disease. *Journal of Clinical Biochemistry and Nutrition,* 45, 278-284. ISSN 0912-0009

Jordan, W., Cohrs, S., Degner, D., Meier, A., Rodenbeck, A., Mayer, G., Pilz, J., Ruther, E., Kornhuber, J. & Bleich, S. (2006). Evaluation of oxidative stress measurements in obstructive sleep apnea syndrome. *Journal of Neural Transmission,* 113, 239-254. ISSN 0300-9564

Kaushik, M., Sontineni, S. P. & Hunter, C. (2010). Cardiovascular disease and androgens: a review. *International Journal of Cardiology,* 142, 8-14. ISSN

Kirbas, G., Abakay, A., Topcu, F., Kaplan, A., Unlu, M. & Peker, Y. (2007). Obstructive sleep apnoea, cigarette smoking and serum testosterone levels in a male sleep clinic cohort. *Journal of International Medical Research,* 35, 38-45. ISSN 0300-0605

Kizhakekuttu, T. J. & Widlansky, M. E. (2010). Natural Antioxidants and Hypertension: Promise and Challenges. *Cardiovascular Therapeutics,* 28, e20-e32. ISSN 1755-5914

Klein, C., Martinez, D., Hackenhaar, F. S., Medeiros, T. M., Marcolin, M. L., Silveira, F. S., Wainstein, M. V., Goncalvez, S. C. & Benfato, M. S. (2010). Carbonyl groups: bridging the gap between sleep disordered breathing and coronary artery disease. *Free Radical Research,* 44, 907-912. ISSN 1071-5762

Kokturk, O., Ulukavak, T., Mollarecep, E. & Ciftci, B. (2005). Elevated C-reactive protein levels and increased cardiovascular risk in patients with obstructive sleep apnea syndrome. *International Heart Journal,* 46, 801-809.ISSN 1349-2365

Lavie, L. (2003). Obstructive sleep apnoea syndrome - an oxidative stress disorder. *Sleep Medicine Reviews,* 7, 35-51. ISSN 1087-0792

Lefter, R., Morency, C.-E. & Joseph, V. (2007). Progesterone increases hypoxic ventilatory response and reduces apneas in newborn rats. *Respiratory Physiology & Neurobiology,* 156, 9-16. ISSN 1569-9048

Madamanchi, N. R., Vendrov, A. & Runge, M. S. (2005). Oxidative stress and vascular disease. *Arteriosclerosis Thrombosis and Vascular Biology,* 25, 29-38. ISSN 1079-5642

Mendelsohn, M. E. (2000). Nongenomic, estrogen receptor-mediated activation of endothelial nitric oxide synthase - how does it work? What does it mean? *Circulation Research,* 87, 956-960. ISSN 0009-7330

MungruE, I. N., Stewart, D. J. & HUSAIN, M. (2003). The Janus faces of iNOS. *Circulation Research,* 93, E74-E74. ISSN 0009-7330

NikI, E.(2009). Lipid peroxidation: physiological levels and dual biological effects. *Free Radical Biology and Medicine,* 47, 469-484. ISSN 0891-5849

Paravicini, T. M. & Touyz, R. M. (2006). Redox signaling in hypertension. *Cardiovascular Research,* 71, 247-258. ISSN 0008-6363

Pedram, A., Razandi, M., Wallace, D. C. & Levin, E. R. (2006). Functional estrogen receptors in the mitochondria of breast cancer cells. *Molecular Biology of the Cell,* 17, 2125-2137. ISSN 1059-1524

Pendaries, C., Darblade, B., Rochaix, P., Krust, A., Chambon, P., Korach, K. S., Bayard, F. & Arnal, J. F. (2002). The AF-1 activation-function of ER alpha may be dispensable to mediate the effect of estradiol on endothelial NO production in mice. *Proceedings of the National Academy of Sciences of the United States of America,* 99, 2205-2210. ISSN 0027-8424

Piantadosi, C. A., Carraway, M. S. & Suliman, H. B. (2006). Carbon monoxide, oxidative stress and mitochondrial permeability pore transition. *Free Radical Biology and Medicine,* 40, 1332-1339. ISSN 0891-5849

Ramara, A., Duckles, S. P., Krause, D. N. & Procaccio, V. (2007). Estrogen suppresses brain mitochondrial oxidative stress in female and male rats. *Brain Research,* 1176, 71-81. ISSN 0006-8993

Salonen, J. T., Nyyssonen, K., Korpela, H., Tuomilehto, J., Seppanen, R. & Salonen, R. (1992). High stored iron levels are associated with excess risk of myocardial-infarction in eastern Finnish men. *Circulation,* 86, 803-811. ISSN 0009-7322

Schwenke, D. C. (1998). Antioxidants and atherogenesis. *Journal of Nutritional Biochemistry,* 9, 424-445. ISSN 0955-2863

Sullivan, J. L. (1981). Iron and the sex difference in heart-disease risk. *Lancet,* 1, 1293-1294. ISSN 1293-1294

Suzuki, Y. J., Jain, V., Park, A. M. & Day, R. M. (2006). Oxidative stress and oxidant signaling in obstructive sleep apnea and associated cardiovascular diseases. *Free Radical Biology and Medicine,* 40, 1683-1692. ISSN 0891-5849

Tan, K. C. B., Chow, W. S., Lam, J. C. M., Lam, B., Wong, W. K., Tam, S. & Ip, M. S. M. (2006). HDL dysfunction in obstructive sleep apnea. *Atherosclerosis,* 184, 377-382. ISSN 0021-9150

Titorenko, V. I. & Terlecky, S. R. (2011). Peroxisome Metabolism and Cellular Aging. *Traffic,* 12, 252-259. ISSN 1398-9219

Traish, A. M., Saad, F., Feeley, R. J. & Guay, A. (2009). The Dark Side of Testosterone Deficiency: III. Cardiovascular Disease. *Journal of Andrology,* 30, 477-494.

Vanhoutte, P. M. (2009). Endothelial Dysfunction - The First Step Toward Coronary Arteriosclerosis. *Circulation Journal,* 73, 595-601. ISSN 0027-8424

Vina, J., Borras, C., Gambini, J., Sastre, J. & Pallardo, F. V. (2005). Why females live longer than males? Importance of the upregulation of longevity-associated genes by oestrogenic compounds. *Febs Letters,* 579, 2541-2545. ISSN 2541-2545

Wang, M., Crisostomo, P. R., Markel, T., Wang, Y., Lillemoe, K. D. & Meldrum, D. R. (2008). Estrogen receptor beta mediates acute myocardial protection following ischemia. *Surgery,* 144, 233-238. ISSN 0039-6060

Wong, N. D., Lopez, V., Tang, S. & Williams, G. R. (2006). Prevalence, treatment and control of combined hypertension and hypercholesterolemia in the United States. *American Journal of Cardiology,* 98, 204-208. ISSN 0002-9149

Yamauchi, M. & Kimura, H. (2008). Oxidative stress in obstructive sleep apnea: putative pathways to the cardiovascular complications. *Antioxidants & Redox Signaling,* 10, 755-768. ISSN

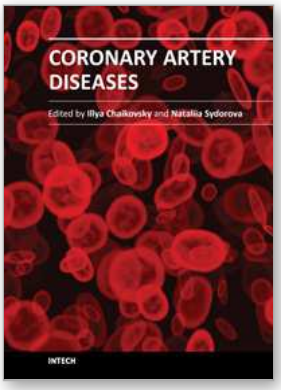
Yamauchi, M., Nakano, H., Maekawa, J., Okamoto, Y., Ohnishi, Y., Suzuki, T. & Kimura, H. (2005). Oxidative stress in obstructive sleep apnea. *Chest,* 127, 1674-1679. ISSN 0012-3692

Yamazaki, H., Haji, A., Ohi, Y. & Takeda, R.(2005). Effects of progesterone on apneic events during behaviorally defined sleep in male rats. *Life Sciences,* 78, 383-388. ISSN 0024-3205

Yoshida, H. & Kisugi, R. (2010). Mechanisms of LDL oxidation. *Clinica Chimica Acta,* 411, 1875-1882. ISSN 0009-8981

Yoshihara, D., Fujiwara, N. & Suzuki, K. (2010). Antioxidants: benefits and risks for long-term health. *Maturitas,* 67, 103-107. ISSN 0378-5122

You, S. A. & Wang, Q. (2005). Ferritin in atherosclerosis. *Clinica Chimica Acta,* 357, 1-16. ISSN 0009-8981



Coronary Artery Diseases
Edited by Dr. Illya Chaikovsky

ISBN 978-953-51-0238-0
Hard cover, 332 pages
Publisher InTech
Published online 07, March, 2012
Published in print edition March, 2012

This book has "wide geography" both literally and figuratively. First of all, this book brings together contributions from around the world, both from post-industrial countries and developing world. This is natural, because coronary artery disease is becoming pandemic worldwide. CAD is the single most frequent cause of death in developed countries, causes about 1 in every 5 deaths. Mortality from cardiovascular disease is predicted to reach 23.4 million in 2030. Moreover, in the developing world, cardiovascular disease tends to affect people at a younger age and thus could negatively affect the workforce and economic productivity. The morbidity, mortality, and socioeconomic importance of CAD make its diagnosis and management fundamental for all practicing physicians. On another hand, the book widely represents "geography" of CAD itself, i.e. many various aspects of its pathophysiology, epidemiology, diagnosis, treatment are touched in this book. This book does not pretend on complete and integral description of the Coronary artery disease. Rather, it contains selected issues on this complex multifactorial disease. Nevertheless, we hope that readers will find Coronary Artery Disease useful for clinical practice and further research.

How to reference
In order to correctly reference this scholarly work, feel free to copy and paste the following:

Mara S. Benfato, Tássia M. Medeiros and Tiago B. Salomon (2012). Coronary Artery Disease and Oxidative Stress, Coronary Artery Diseases, Dr. Illya Chaikovsky (Ed.), ISBN: 978-953-51-0238-0, InTech, Available from: http://www.intechopen.com/books/coronary-artery-diseases/coronary-artery-disease-and-oxidative-stress-

INTECH
open science | open minds

InTech Europe
University Campus STeP Ri
Slavka Krautzeka 83/A
51000 Rijeka, Croatia
Phone: +385 (51) 770 447
Fax: +385 (51) 686 166
www.intechopen.com

InTech China
Unit 405, Office Block, Hotel Equatorial Shanghai
No.65, Yan An Road (West), Shanghai, 200040, China
中国上海市延安西路65号上海国际贵都大饭店办公楼405单元
Phone: +86-21-62489820
Fax: +86-21-62489821