

Arterial Thickness and Immunometabolism: The Mediating role of Chronic Exercise

B.M.M. Antunes^{1,*}, S.U. Cayres², F.S. Lira¹ and R.A. Fernandes²

¹Exercise and Immunometabolism Research Group, Department of Physical Education, Universidade Estadual Paulista, UNESP, São Paulo-Brazil; ²Research Group Related to Physical Activity, Department of Physical Education, Universidade Estadual Paulista, UNESP, São Paulo-Brazil



Abstract: Metabolic alterations and cardiovascular diseases, such as atherosclerosis, are associated with lifestyle modifications, particularly the increase of physical inactivity and poor eating habits, which contribute to one of the main causes of death in modern times. Cardiovascular diseases are positively correlated with several illnesses, such as obesity, hypertension and dyslipidemia, and these disorders are known to contribute to changes in immune cells, cytokines and metabolism. Atherosclerosis is a chronic inflammatory disease characterized by the formation of lipid plaques and fibrous tissue (atheroma) in the artery walls and this process is related to the oxidation of LDL-c (low density lipoprotein) and the formation of a particle, termed LDL_{ox}, which can generate toxic injury to the vessel wall. In this atherogenic process there is an inflammatory response generated by the injury in the vascular endothelium, which in itself is able to express and secrete a variety of molecules, such as myeloid colony-stimulating factors (M-CSF), monocyte chemoattractant protein-1 (MCP-1) and tumor necrosis factor alpha (TNF- α), that act as activators of the immune system. Therefore, the main purpose of this review is to highlight the immuno-metabolic alterations involving the thickening and stiffness of arteries observed in atherosclerosis, and how chronic exercise can act as an anti-inflammatory and anti-atherogenic approach.

Keywords: Atherosclerosis, chronic exercise, inflammation, lipid profile, metabolism.

EPIDEMIOLOGICAL AND GENETIC ASPECTS OF THE ATHEROGENIC PROCESS

Current literature has identified that heart diseases are the main cause of death in the world [1], and this fact is worrying, given that behavioral habits, such as inadequate exercise and poor food intake are associated with cardiovascular risk factors from childhood [2, 3]. In this way, excess of body fat has an important role, primarily related to the atherogenic process [4, 5]. Indeed, in accordance with Grimaldi *et al.* [6], the atherogenic process consists of the deposition and formation of atheroma plaques in blood vessels; given that higher carotid intima-media thickness (CIMT) is a powerful indicator of cardiovascular risk among adults [7] and is related to myocardial infarctions, strokes and atherosclerosis [8-10].

Research of the epidemiological and genetic aspects of this condition has investigated the interaction of these processes. Indeed, epidemiology is an area that studies the interaction between the environment and the risk factors that may affect a population. Current results [5] indicate that cardiovascular risk factors, such as body fat, elevated blood pressure and dyslipidemia are essential components related to cardiovascular outcomes and they have direct interactions with the elevation of low lipoprotein density-cholesterol (LDL-c) in the blood stream.

*Address correspondence to this author at the Exercise and Immunometabolism Research Group, Department of Physical Education, Universidade Estadual Paulista, UNESP, Rua Roberto Simonsen, 305, 19060-900, Presidente Prudente, São Paulo, Brazil; Tel: +55 18 3229-5906; E-mail: bah_tunes@hotmail.com

In parallel, interesting results have been found in children. Cayres *et al.* [2] showed that regular breakfast intake is inversely related to blood pressure and triacylglycerol in apparently healthy adolescents. Another study analyzed obese adolescents [11], and found that skipping meals, mainly breakfast, is associated with a concentration of lipids and glucose. However, it remains unclear how this process is related to intima-media thickness and eating behaviors in this population. On the other hand, the atherogenic process has been strongly investigated in its genetic aspects. Recently, the epigenetic process, mainly methylation DNA, is associated with the regulating of genes and can be associated with cardiovascular outcomes in adulthood [6].

ARTERIAL INTIMA-MEDIA THICKNESS: PROCESSES AND MECHANISMS

According to Jourdan *et al.* [12], the intima-media thickness (IMT) is understood as the distance between the two echogenic lines that show the lumen/intima interface and media/adventitia of the arterial wall. In general, this process is silent and is associated with inflammatory response, such as interleukin-6, C-reactive protein (CRP) and reactive oxygen species [13, 14].

The literature has demonstrated that higher carotid intima-media thickness (CIMT) is a powerful indicator of cardiovascular risk factors, mainly in adults [7], but this is not clear in the pediatric population [12] especially when the cut-off aspects are analyzed. This increased arterial IMT is related to higher permeability of the intima wall in response to

CRP [13, 15]. CRP is an acute-phase protein produced by the liver in response to interleukin-6 [14]. Taken together, these components are essential to vascular inflammation [14, 16]. Besides this, there is an infiltration of LDL-c into the endothelial space (between the intima and media layer), which undergoes the oxidation process (LDL-ox), and which is a powerful toxic agent through the atherogenic process [15].

The interaction of this pathway results in the formation and deposit of 'foam cells', higher arterial thickness related to higher monocyte adhesion, cytotoxicity and vascular dysfunction [15, 17]. Taken together, there is a reduction in availability of nitric oxide, and consequently, alterations in the intima-media layer structure (vasoconstriction) [17].

IMMUNO-METABOLIC ALTERATIONS IN ATHEROGENIC PROCESS

Major chronic diseases acknowledged in the 21st century, such as obesity, diabetes *mellitus* type 2, dyslipidemia, cancer and atherosclerosis showed an inflammatory response to several metabolic alterations mainly verifying modifications in the biomarkers production, as pro and anti-inflammatory cytokines, as well as an immunological pattern [18]. The worsening in the immuno-metabolic alterations and in the advancement of the disease is positively associated with poor eating behavior and a sedentary lifestyle being that this last is a risk factor for metabolic diseases which is termed by some authors as the disease of physical inactivity [19].

In the atherogenic process, as well as in several illnesses, metabolic and inflammatory alterations concurrently occur because, in the face of a metabolic disorder, the immune system is recruited in order to reestablish the body's homeostasis, just as immunological cells are mobilized when some organ or tissue does not act in an efficient and regular form [20]. One of the first metabolic abnormalities verified in the atherogenic process is related to the plasmatic concentration of lipids, primarily by cholesterol fraction imbalance. In other words, increases of very low density lipoprotein (VLDL-c), low density lipoprotein (LDL-c), and hypertriglyceridemia (excess of triacylglycerol (TAG) plasmatic) associated with a decrease in high density lipoprotein (HDL-c) are a significant risk factor for the development of cardiovascular disease. In addition, TG/HDL-c and LDL-

c/HDL-c ratios are well established predictors of cardiovascular diseases such as atherosclerosis [21-23].

Morphologically, LDL-c is a lipoprotein that delivers cholesterol to different body tissues, and among lipoproteins shows small size and density. Given this characteristic, its infiltration is easier and more harmful [24, 25]. Because of this lipid impairment, and particularly because of the increase in LDL-c plasmatic concentration, this lipoprotein particle is oxidized by oxidative stress as mediated by reactive oxygen species (ROS). This process is associated with shear stress in response to high concentrations of LDL-c [26]. LDL oxidation originates in the molecules of oxidized LDL, termed LDL_{ox}, which can generate toxic injury to the vessel wall through atheroma plaque formation and adhesion [27, 28].

Modifications in lipoprotein profiles and LDL_{ox} sedimentation are damaging factors to organisms. An inflammatory process may occur with significant activation of both innate and adaptive immunity. In order to reestablish homeostasis, there is a significant increase in the amount of immune cells, mainly neutrophils and monocytes, recruited by cytokines, lipid mediators and chemotactic proteins such as monocyte chemoattractant protein-1 (MCP-1) [29, 30]. Neutrophils are polymorphonuclear cells in elevated plasmatic amounts and compose the front line of the immune system. These neutrophils have the ability of repairing local injuries by recognizing, adhering in the region and phagocytosing, because these cells are an active phagocytes of innate immune response, while on the other hand monocytes are recruited and differentiated into macrophages after tissue infiltration and are able to perform phagocytosis, have a rapid response capacity and stay longer in the injured tissue. Thus they are considered effector cells of the final stages of the natural immune response. Simultaneously, several inflammatory biomarkers such as interleukins (IL-1 β and IL-6) and tumor necrosis factor (TNF- α) are produced and released into the bloodstream in response to the inflammatory process and may act as pro inflammatory cytokines with the ability of giving feedback to the inflammatory cycle and may lead to chronic inflammation and disease aggravation. In Fig. (1) the atherogenic process is illustrated by showing the contributions to lesions in the vessel wall of excess LDL-c in the blood.

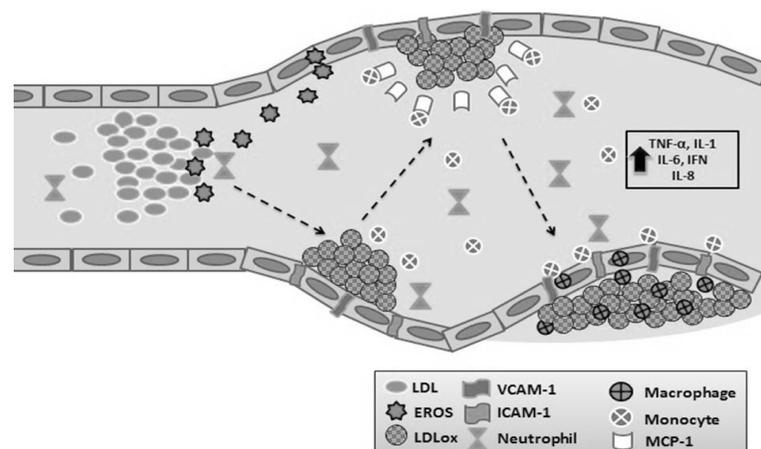


Fig. (1). Atherogenic process by LDL_{ox}.

INTERACTION MECHANISMS BETWEEN ARTERIAL THICKNESS AND IMMUNOMETABOLISM

Different mechanisms are involved in arterial thickness and stiffness. Recruitment of immune cells and biomarker production is needed in order to recover metabolic homeostasis, and is highly relevant to understand the metabolic and molecular adjustments that surround these significant interactions.

A key molecule in arterial thickness, as well as primarily in the stiffening process that is directly affected by immunometabolic responses is nitric oxide (NO). Nitrous oxide is a vasodilator hormone synthesized from L-arginine amino acid in endothelium, via nitric oxide synthase (NOS), which is essential in the maintenance of vascular homeostasis and is responsible for distensibility in muscular arteries and large vessels *in vivo* [31]. It occurs partly in response to flow-mediated dilation in the conduit arteries in humans [32], besides protecting the vessel by attenuating proliferation, adhesion and cellular migration, as well as molecule adhesion expression, ROS and platelet aggregation [33].

The reduction of NO bioavailability, with consequent arterial stiffness, is significantly high in virtue of endothelial injuries that are followed by the inflammatory response of pro inflammatory cytokines (TNF- α and IL-6) and C-

reactive protein (CRP) synthesis, release of adhesion molecules and immune cells mobilization, and it may be that all of these inflammatory mechanisms are mediated by transcriptional factors such as nuclear factor *kappa* B (NF- κ B) [34, 35]. Cytokines follow the signs of inflammatory response together with adhesion molecules that are in endothelial cells. They are not active in a normal state, but when the endothelium is injured, these protein molecules are expressed and release intercellular adhesion molecules (ICAM-1) and vascular cell adhesion molecules (VCAM-1) in order to mobilize the immune system, mainly neutrophils and monocytes, by chemotaxis [36].

Generally, the endothelium regulates substrate production, such as adhesion molecules, by NO, which acts directly on NF- κ B inhibition. When there are injuries in the vessel wall, mediated by LDL-c excess, there is NADPH oxidase activation with superoxide anions production (O₂⁻), or ROS, leading to a reduced production of endothelial nitric oxide synthase (eNOS), resulting in NO availability, and a significant increase in NF- κ B activation by *toll like receptor 2* (TLR2) activation via LDL-c. Consequently, TNF- α , IL-6, chemokine and adhesion molecules are released as an inflammatory response [37]. These alterations, illustrated in Fig. (2), constitute the immune-metabolic profile in arterial stiffness and thickness that is a risk factor for the installation

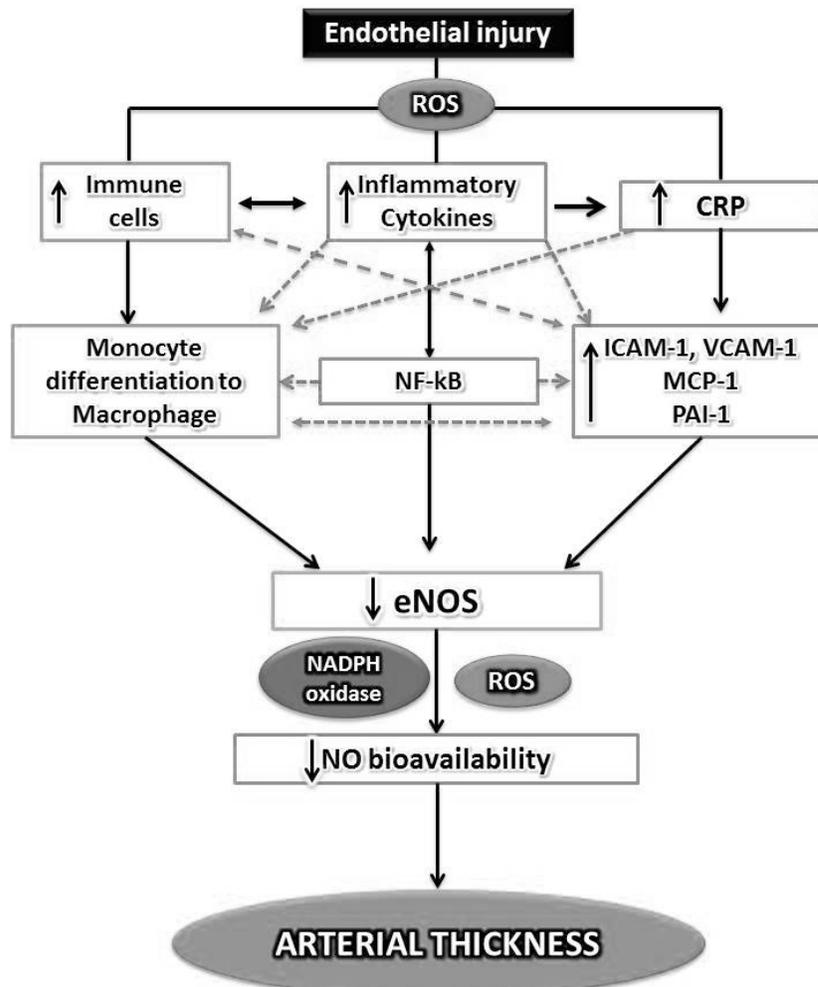


Fig. (2). Mechanism of interaction between Immunometabolism and Arterial thickness.

and development of several cardiovascular diseases such as atherosclerosis, myocardial dystrophy and coronary artery disease.

THE MEDIATOR AND ANTI-ATHEROGENIC ROLE OF CHRONIC EXERCISE

It is well documented that physical inactivity associated with poor eating behavior contributes significantly to the installation and development of chronic illness, as well as modifications in inflammatory biomarkers in the blood in response to inflammation [18]. On the other hand, regular chronic exercise combined with healthy dietary habits is recommended as a powerful strategy for the treatment and prevention of these diseases by virtue of their anti-inflammatory and anti-atherogenic effects [38]. Chronic exercise (which means structured physical activity) can positively influence risk factors that are associated with cardiovascular diseases such as hypertension, diabetes mellitus type 2, obesity, dyslipidemia and endothelial dysfunction [39, 40].

As previously mentioned, the oxidative stress acting on LDL oxidation and the formation of LDLox, is a determinant alteration that injures the vessel wall, but paradoxically, exercise also induces an oxidative stress which would appear associated with anti-atherogenic effects and that functions by inducing arterial antioxidant enzymes. The creation of these antioxidant enzymes not only reduces oxidative damage, but also decreases the generation of oxidants *in situ* [41, 42]. In a study conducted by Meilhac *et al.* [43], with C57BL/6 and LDLr^{-/-} male mice trained on a treadmill with and without vitamin-E supplementation (synthetic antioxidant) throughout 12 weeks, they observed that acute exercise provides antioxidant protection in the arterial wall by increasing antibodies for oxidatively modified proteins, catalase and eNOS, and in addition, they verified that supplementation with vitamin-E could be deleterious to exercisers by inhibiting antioxidant enzymes in the arterial wall. In an 8-week study conducted by Vezzoli *et al.* [44] with healthy master runners stratified into two groups (moderate-intensity continuous and high-intensity discontinuous training), they observed that regardless of the training model, both induced similar beneficial effects of reducing the resting levels of oxidative stress biomarkers in plasma and urine (thiobarbituric acid reactive substances (TBARS), 8-hydroxy-2-deoxy-guanosine (8-OH-dG) and total antioxidant capacity (TAC)), and in addition, the authors suggested that both might cause a higher level of exercise-induced oxidative stress when compared to a workload-matched session.

In general, exercise creates beneficial oxidative stress, by increasing eNOS activity and other antioxidant enzymes, and consequently significantly altering the NO plasmatic concentrations. According to the metabolic aspects, studies suggest that the beneficial effects on coronary arterial disease are mediated through an increase in inducible nitric oxide synthase (iNOS) phosphorylation by protein kinase B (Akt) [45]. This protein, together with AMP-activated protein kinase (AMPK), is responsible for the phosphorylation of arterial eNOS during running exercise [46, 47] and represents an important molecular mechanism for adaptation to chronic exercise. AKT and AMPK are complex proteins

responsive to physical stress by increasing ATP consumption. Additionally, by being phosphorylated and activated, they may activate gene transcription factors such as proliferator-activated receptors (PPAR) that regulate the gene expression of several proteins, especially proteins related to lipids and lipoprotein metabolism, glucose homeostasis, cell proliferation and inflammation.

According to a paper by Yakeu *et al.* (2010), 17 sedentary individuals involved in 8 weeks of low-intensity exercise intervention, chronic exercise was observed to be associated with a upregulation of M2 markers (CD14 and MR (mannose receptor)) and downregulation of M1 markers (MCP-1) as well as to increase plasmatic concentration of anti-inflammatory cytokines such as IL-4 and IL-10 and decrease IL-6 and TNF- α levels after exercise. These modifications are mediated by differentiation towards an M2 macrophage phenotype via an increase in PPAR activation. The important anti-inflammatory and anti-atherogenic role awarded to chronic exercise may be mediated by M2 polarization due to PPAR activation.

On the other hand, it is not clear in the literature what the most effective exercise intensity is in the prevention/control of pro-inflammation agents [48], but chronic exercise at moderate intensity has been evocated as effective in the promotion of an anti-inflammatory background and prevention of atherosclerosis. In fact, in rodent models, chronic exercise is related to the increased release of superoxide dismutase (a recognized anti-inflammatory agent) by shear stress [49]. Moreover, insulin resistance affects the ROS production in sub-endothelial space, as well as reduces NO availability [50]. In human models, prolonged chronic exercise programs reduce insulin resistance and inflammatory status in diabetic patients even without significant weight loss [48].

Thus, we conclude that chronic exercise, when performed systematically, is a potent strategy for the prevention and treatment of the systemic inflammation of several diseases, such as atherosclerosis, by increasing anti-inflammatory cytokine production, antioxidant enzyme activation, immune cell mobilization and alterations in gene transcription patterns.

CONFLICT OF INTEREST

Barbara de Moura Mello Antunes, Fábio Santos Lira, Rômulo Araújo Fernandes, and Suziane Ungari Cayres declare that they have no conflicts of interest that are directly relevant to the content of this article.

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