Macrophage Polarization: Implications on Metabolic Diseases and the Role of Exercise

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ABSTRACT: Macrophages are cells of the innate immune response that trigger inflammation resolution. The phenotype of “classically activated macrophages” (M1) has anti-tumoricidal and anti-bactericidal activities. On the other hand, “alternatively activated macrophages” (M2) are involved in tissue remodeling and immunomodulatory functions. The change in the polarization of macrophages varies according to the diversity of cytokines present in the microenvironment or by the stimuli of an antigen. It involves such factors as interferon-regulatory factors, peroxisome proliferator-activated receptors (PPARs), hypoxia-inducible factors (HIFs), and signal transducers and activators of transcription (STATs). Switching the phenotype of macrophages can help attenuate the development of an inflammatory disease. Exercise can promote alterations in the number of innate immune cells and stimulates phagocytic function. Chronic exercise seems to inhibit macrophage infiltration into adipose tissue by attenuating the expression of F4/80 mRNA. Furthermore, exercise may also increase the expression of M2 markers and reduce TNF-α and TLR4 mRNA expression, which activates the inflammatory pathway of NF-κB. Chronic exercise reduces β2-adrenergic receptors in monocytes and macrophages by modulating TLR4 signaling as well as suppressing IL-12 production, a stimulator of interferon Y. In this review, we discuss macrophage polarization in metabolic diseases and how exercise can modulate macrophage plasticity.

KEY WORDS: macrophages, immune system, inflammation, exercise, transcriptional factor

I. INTRODUCTION

Despite different characterizations and features of body systems, it is clear that there is direct communication between them. For a long time, the immune system was studied by itself, unrelated to any other. Since the 1980s, however, the anatomic and functional relationships in immunity have been studied, and the role of immune cell types on metabolism has been clarified.

Central metabolic tissues, considered important for energy homeostasis, such as liver and adipose tissue, have a huge number of infiltrated cells from immune system (innate and adaptive), and their activation may vary with metabolic changes. More recent studies have included skeletal muscle in this context, especially for being capable of restoring glucose homeostasis on metabolic dysfunctions, as in type 2 diabetes. In this context, metabolic disorders affect not only the number but also the function and metabolism of immune cells. Nonetheless, despite the presence and functional contributions of many different immune cells (e.g., lymphocytes, neutrophils, eosinophils, mast cells) to metabolic tissue homeostasis, the macrophage is both numerically and functionally dominant. This cell type is one of the most studied due its sensitivity to hypertrophy of adipose tissue and subsequent considerable infiltration and polarization (covered in detail throughout the text). Furthermore, studies have shown that adipose tissue from lean individuals secretes insulin-sensitizing adipokines, influenced by alternatively
activated macrophages whereas adipose tissue from obese individuals secretes an inflammatory mediator via classically activated macrophages.\(^4\)

It is also clear that the key for this trigger in metabolic dysfunctions is the impairment of lipid and/or glycemic metabolism; the first is closely connected to atherosclerosis and the second to insulin resistance, which is related to obesity and diabetes type 2. According to Pedersen (2006),\(^5\) there is a link between inflammation, insulin resistance, and atherosclerosis, i.e., a state referred to as chronic low-grade inflammation. This state is characterized by conditions in which a 2- to 3-fold increase in the systemic concentrations of inflammatory signaling molecules (i.e., cytokines, chemokines, acute phase reactants, insulin resistance-associated adipokines, procoagulative factors, hypertensive agents) and with low serum levels of insulin sensitivity associated with adipokines such as adiponectin and visfatin.\(^6\)

Macrophages play an important role in the states or diseases mentioned previously, and their polarization to the classical phenotype is also exhibited in some diseases such as rheumatoid arthritis, inflammatory bowel disease,\(^7\) and nonalcoholic steatohepatitis.\(^8\) Non-pharmacological therapies that aim to attenuate low-grade inflammation are based on lifestyle modification that emphasize diets low in saturated fats and low in refined sugars and/or decreasing the caloric balance through regular physical exercise.\(^9\)

In this review, we summarize the polarization of macrophages on some metabolic disorders and discuss the mechanisms by which macrophages are classically activated. We also discuss the therapeutic implications of exercise on restoring the homeostasis by macrophage polarization due to exacerbated inflammatory response caused by immune cells in the development of chronic disease. The literature provides clear evidence that exercise can modulate some of the factors that influence the inflammatory response.

**II. MACROPHAGE FUNCTION, POLARIZATION, AND MECHANISMS**

Russian scientist Élie Metchnikoff first identified macrophages as cells able to phagocytize, or engulf, undesirable cells. As such, they were considered the initial protective barrier of the organism.\(^10\) These cells originate from the bone marrow and are derived from myeloid progenitor cells; subsequently, they are differentiated into monocytes, which then flow into the bloodstream. Finally, as matured cells, the monocytes can infiltrate tissues and further differentiate into macrophages, for example, in inflammation. These cells have an efficient capacity for functional specialization, as demonstrated by their different phenotypes, various locations, and responses to stimuli.

It is known that specific resident cells that are established prenatally; they originate from erythro-myeloid progenitor in the yolk sac without a monocytic intermediate.\(^11\) Resident macrophages from the liver are named Kupffer cells; in the brain they are known as microglial cells, in the bone they are known as osteoclasts, and in the peritoneum they are known as peritoneal macrophages, etc.\(^12,13\) Due to the diversity of their functions, expressed receptors, and secreted production by macrophages, they are considered an essential immune cell, targeting several regulatory events such as phagocytosis, tumoricidal and bactericidal activity, tissue repair, antigen presentation, lymphocyte regulation, and inflammation and cytokine production.\(^14\)

The functional capacity of macrophages may vary and may be influenced by a diversity of factors including some tumor products, antigens, and products of T cells (Th1 or Th2),\(^15\) resulting in alternatively activated macrophages (AAM) or M2, which are involved in tissue remodeling and immunomodulatory function. Another result may be classically activated macrophages (CAM) or M1, which have anti-tumoricidal and anti-bactericidal activity. This alternation of phenotype is associated with the cytokine that is most expressed in the microenvironment: interferon-gamma (IFN-γ), which is related to the M1 phenotype. Furthermore, bacterial lipopolysaccharide (LPS), an endotoxin present in the outer membrane of Gram-negative bacteria, may also promote the polarization to the M1 phenotype.\(^16\) On the other hand, interleukin-4 (IL-4) and -13 (IL-13) are associated with M2 macrophages, and recent studies have shown that IL-10 plays an important
role in muscle growth and regeneration, indicating that the null IL-10 mutant has impaired the capacity of M2 to proliferate myoblasts.\textsuperscript{17}

It is important to emphasize that macrophages are plastics; in other words, they can change their phenotype and their physiology and energetic metabolism depending on the environment. M1 macrophages predominate in the glycolytic metabolism, which is typically a faster means of energy production due to its importance in the first line of defense in the innate immune system. In contrast, M2 macrophages are oxidative and are associated with parasitic products, infections, and wound healing, given that a resolution phase and consequently longer-term functions are necessary.\textsuperscript{18} In aerobic glycolysis of the M1 phenotype, the activity of the respiratory chain is attenuated, resulting in the production of reactive oxygen species (ROS), which is also enhanced by elevation of the NADPH oxidase originating from the pentose phosphate pathway. Another metabolite from M1 is iNOS, which originates from the catabolism of arginine. In M2, arginase-1 is induced, producing urea, polyamines, and ornithine, which are essential for wound healing.\textsuperscript{18} Mosser and Edwards (2008)\textsuperscript{19} characterized the spectrum of macrophages as having three different features in addition to the classically activated macrophages M1 and alternatively activated M2. The first two (M1) are responses to IFNγ produced by either innate or adaptive immune cells (Th1),\textsuperscript{20} the last (M2) is an immune response reaction that originates from IL-4 produced by Th2 cells or during an innate immune response by granulocytes. Additionally, based on their gene expression, M2 macrophages can present subtypes: M2a, M2b, and M2c. Each polarized macrophage population is induced by a different chemokine exposure and drives toward a specific response. M2a and M2b are induced by exposure to IL-4/IL-13 and TLR or IL-1R agonists, respectively, and they exert immunoregulatory functions and can lead to type II responses, whereas M2c macrophages (IL-10, TGF-β, and glucocorticoid hormones) are important in suppressing immune responses and promoting tissue remodelling.\textsuperscript{21} The additional population would be regulatory macrophages, which arise from IL-10 produced by regulatory T cells, and which also secretes IL-10 to suppress immune responses and enables Th2 cell expansion.\textsuperscript{19}

As previously mentioned, the change in macrophage polarization varies according to the diversity of cytokines present in the microenvironment or stimuli by an antigen, and it involves a considerable number of factors: interferon-regulatory factors, peroxisome proliferator-activated receptors (PPARs), hypoxia-inducible-activated receptors (HIFs), and signal transducers and activators of transcription (STATs).\textsuperscript{22} In the case of M1 polarization, gene expression may be mediated by IFN-γ or LPS. IFN-γ–mediated activation is related to the JAK-mediated pathway, and it includes DNA binding sites specific to STAT1 homodimers in the promoters of the gene encoding NOS2, the MHC class II transactivator (CIITA), and IL-12. On other hand, LPS-mediated polarization requires receptor TLR4, which activates transcription factors of a pro-inflammatory response (e.g., NF-kB, IRF3 and AP1) and expresses IRF3 target genes (\textit{CCL5} and \textit{IFNβ}) and the genes downstream of IFNβ (\textit{NOS2}, \textit{MX1} and \textit{MX2}). Researchers have shown that LPS-activated macrophages, when re-stimulated (a few hours after a previous stimulation) are less responsive in the gene expression of pro-inflammatory cytokines, in an attempt to decrease the risk of exacerbating inflammation while maintaining the essential anti-microbial response.\textsuperscript{23} On the other hand, M2 macrophages are stimulated by IL-4 and IL-13 cytokines, regulated by STAT-6, and they have increased expression of such genes as arginase-1, resisting-like-α, and macrophage manose receptor (Fig. 1).\textsuperscript{22}

A question that remains open concerns tissue-resident macrophages and monocyte-derived macrophages: Is each population able to polarize in M1 or/and M2 functional phenotypes?\textsuperscript{24} Among the diversity of situations by which macrophages can be polarized, one that has been intensively investigated is the regulation of immune cell activation in adipose tissue due to its associations with the insulin resistance in this tissue.\textsuperscript{25} In a homeostatic state, adipose tissue macrophages are usually an M2 phenotype, and its inflammatory activation is associated with metabolic disease. Additionally, an important transcription factor called peroxisome proliferator activated receptor-gamma (PPAR-γ) is an important regulator
of lipid metabolism in macrophages that inhibits pro-inflammatory gene expression by transrepression of NF-κB. Furthermore, it has been hypothesized that the switch from M1 to M2 in adipose tissue may be mediated by PPAR-γ.26

The transcriptional factor PPAR, discovered in 1990, is a subfamily of the nuclear receptor superfamily of ligant-inducible transcription factors, identified by three isotypes: PPARα, PPARβ/δ, and PPARγ. PPARα is highly expressed in muscles, the liver, heart, and kidney; PPARβ/δ is abundantly expressed throughout the body but at low levels in the liver; and PPARγ is abundantly expressed in adipose tissue and macrophages. All of these iso-

FIG. 1: Macrophage polarization comparing M1 and M2 signal pathways. The different pathways underlying macrophage polarization, the receptors involved, activation via and the induced products of activation.
substances, such as adiponectin, and is able polarize M2 macrophages.

In animal models, the participation of this transcription factor on macrophage polarization and inflammatory response has been well documented. Odegaard et al. (2007) demonstrated that transgenic mice with conditional deletion of PPAR-γ in macrophages are predisposed to the development of diet-induced obesity, insulin resistance, and glucose intolerance, as well as impaired alternative macrophage activation. Furthermore, the long-term maintenance of this phenotype requires the metabolic regulators PPAR-γ and PPAR-δ, as well as the coactivator protein PGC-1β (PPARγ coactivator-1β).

III. CHRONIC INFLAMMATORY DISEASES: THE ROLE OF MACROPHAGES

Under normal conditions, a predominance of resident macrophages exists in states of low activity; the pro- and anti-inflammatory cytokines produced by the immune cells are also at equilibrium. However, the immune system responds to alterations in basal conditions, such as endocrine responses to pathologies or nutrient imbalance such as excess of body weight. When this condition of weight gain persists for a long period of time, it may trigger chronic low inflammation. A variety of metabolic disorders are related to chronic low inflammation: obesity, diabetes mellitus type 2, non-alcoholic steatohepatitis, atherosclerosis, among others. In this paper, we focus on some diseases for which treatment and/or prevention approaches include lifestyle changes.

A. Obesity

Obesity is characterized by the exacerbated accumulation of adipose tissue in the body in response to change in lifestyle and dietary habits (hypercaloric diets), and it is considered one of the higher-incidence, non-degenerative diseases of the 21st century. Adipose tissue is composed not only of adipocytes but also of a stromal vascular fraction that contains preadipocytes, endothelial cells, fibroblasts, and immune cells. With a higher caloric intake and lower expenditure of energy (i.e., a sedentary lifestyle), the hypertrophy of adipose tissue is triggered. This expansion of adipocytes also leads to a lack of oxygen in the adipose tissue (hypoxia) that stimulates chemotactic monocytes/macrophages and induces the overexpression of inflammatory cytokines, altering the homeostasis of the organism. In addition, an angiogenic event may favor the infiltration of immune cells in the tissue, an overproduction of extracellular matrix, and the exacerbated release of proinflammatory cytokines by infiltrated macrophages.

In adipose tissue hypertrophy, the recruitment of immune cells varies according to the stage and type of immune cells. In some studies, animal models fed with a high-fat diet have shown an increase in neutrophil numbers a few days after diet initiation. Subsequently, the increase in the number of macrophages and natural killers (NK) seems to occur in the weeks after a high fat diet, especially the proliferation of tissue-resident populations. In an attempt to better characterize the markers of this phenotypes, Cho et al. (2014) observed that a high-fat diet (12 weeks) not only increased the quantity of adipose-tissue macrophage content but modified the distribution of M1 (CD11c+CD301−) and M2 (CD11c−CD301+) in visceral fat.

This local accumulation of immune cells is the threshold for inflammation and activation of the immune system. Nonetheless, the most important fact in the obesity-induced inflammation and consequent insulin resistance seems to be macrophage polarization to its pro-inflammatory state (M1).

Under normal conditions, macrophages can be found in adipose tissue, and their function is to maintain the homeostasis of organic systems; however, monocytes are attracted to adipose tissue by the secretion of monocyte chemotactic protein 1 (MCP-1) in an attempt to eliminate inflammation.

Higher levels of proinflammatory cytokines released by the immune cells can lead to inflammation and further insulin resistance. In agreement with this idea, a model of deletion of MCP-1 has indicated that there is protection from the insulin resistance induced by a high-fat diet as well as a reduction of the lipid content in the liver. Indeed,
it is clear that there is cross talk between adipocytes and macrophages, starting with the exacerbated production of macrophage-derived TNF-α, which in turn acts on the TNF-α receptors of adipocytes and stimulates its lipolysis. Once the adipocytes release their contents of free fatty acids, which are potential Toll-like receptor 4 (TLR4) binders, and they consequently induce the NF-kB inflammatory pathway (Figure 1). T lymphocytes are present throughout adipose tissue, facilitating the production of pro-inflammatory cytokines, or in other words, favoring local inflammation and also spreading it systemically.

This state of low-grade inflammation involves the classical macrophage phenotype. However, the signaling pathways work as inflammatory mediators and regulators of energy metabolism. One of the first consequences of this hypertrophy and low-grade inflammation is insulin resistance in adipose tissue, caused by activation of c-Jun N-terminal kinase (JNK) and inhibitor of κ kinase (IKK), which is responsible for impairing insulin receptor phosphorylation. As a result, an increase in the lipolysis of the adipose tissue, especially the visceral fat (which also increases the efflux of fat acids in the liver), also collaborated with the higher secretion of pro-inflammatory adipokines and reduces adiponectin, an anti-inflammatory marker. The mechanisms underlying this lipolysis are not clear; however, the overexpression of proinflammatory cytokines may affect proteins related to lipid droplet stability, such as reduction of perilipin expression by TNF-α, which consequently facilitates the access of lipases to the triglyceride.

In macrophage polarization, the mechanistic target of rapamycin complex 1 (mTORC1), a nutrient/energy sensor able to regulate some metabolic processes, seems to play an important role. Moreover, elevated mTORC1 activity and consequent downregulation of Akt signaling may facilitate increased responses to LPS stimulation of macrophages, particularly adipose tissue macrophages. Furthermore, the constitutive mTORC1 activity impairs M2 polarization by attenuation of AKT activation in IL-4–induced polarization.

B. Insulin Resistance

Primarily, insulin resistance is recognized as the impairment or inability of this hormone to allow glucose uptake into peripheral tissues. Insulin action is mediated by a receptor on the membrane that triggers a cascade of interactions aimed at the translocation of the transporter to the membrane, which finally promotes the glucose uptake. The mechanisms possibly involved in the onset of insulin resistance are genetic abnormality in any proteins related to its cascade of activation, including fetal malnutrition and visceral adiposity.

The skeletal muscle is the major insulin-stimulated disposal of glucose in the body; however, the increase of fatty acid deposits in this tissue impairs the insulin sensitivity via recruitment of immune cells. In a recent study performed by Khan et al. (2015), macrophage and T-cell markers were upregulated in skeletal muscle of obese individuals compared with lean individuals. Additionally, in animal model of high fat diet, inflammatory cytokines had higher expression fate that was correlated with insulin resistance, as did macrophages and T cells localized in extramyocellular adipose tissue. This condition is also associated with MCP-1–mediated macrophage accumulation on skeletal muscle; however, insulin resistance is not an exclusive condition for obese subjects. Masharani et al. (2011) observed that insulin resistance in healthy non-obese subjects was followed by an increased in insulin receptor-1 serine phosphorylation and JNK pathway activation. Furthermore, some studies have shown that HDL plays an important role on glucose metabolism. In skeletal muscle, this lipoprotein was able to restore the impaired insulin-stimulated glucose uptake induced by lipid accumulation and macrophage recruitment.

In obesity, as mentioned above, macrophages are recruited to white adipose tissue by chemotaxis or the resident cells proliferate in the tissue. An animal study confirmed that overexpression of MCP-1 was associated with higher susceptibility to insulin resistance. The liver is another organ that can develop insulin resistance; the related mechanisms are described later in the paper.
C. Atherosclerosis

Abnormalities in the plasmatic levels of lipoproteins of the general population are not rare. Accordingly, such abnormalities are the main reason for the development of atherosclerosis, a chronic inflammatory disease characterized by the formation of lipid plaques and fibrous tissue (atheromas) in the walls of arteries. Several aspects of the initial events of the pathology must be considered: the accumulation of LDL cholesterol in the subendothelial matrix, followed by preferred areas for lesion formation (arterial branching or curvature), hemodynamic strengthening of endothelial cells, and the presence of nitric oxide synthase (NOS), which influences the permeability of the vessels. LDLs are lipoproteins that are also organized by apolipoprotein B (ApoB), a structure able to interact with matrix components and that contribute to atherogenesis. Native LDL by itself is not enough for macrophage signaling, so a modification in this lipoprotein is necessary. In the case of atherosclerosis, oxidized LDL (oxLDL) is a consequence of exposure to ROS produced by endothelial cells and macrophages. An inflammatory response is then initiated, caused by injury to the vascular endothelium, which expresses and secretes a series of molecules acting as immune system activators. Concurrently, oxLDL itself plays a role as a chemotactic factor for monocytes and adhesion molecules, increasing secretion of cytokines such as macrophage colony-stimulating factor (M-CSF). Thus begins the differentiation of monocytes into M1 macrophages that internalize oxLDL, followed by the formation of foam cells in a process mediated by a group of ‘scavenger’ receptors such as SR-A and CD36; this process seems to have primary importance and consequently initiates an immune-inflammatory response. Foam cells also produce TNF-α, inducing the formation of clots and acute coronary syndromes. NO production is inhibited by oxLDL, which enhances the vessel contraction resulting in higher blood pressure, an important risk factor for the development of atherosclerosis. On the other hand, HDL exerts a protective function by inhibiting LDL oxidation and/or removing the excess cholesterol from peripheral tissues. The formation of an atherosclerotic plaque is then initiated, which is composed of cellular elements, extracellular matrix components (fibrous cap) and a lipid core (cholesterol). Vulnerable plaques usually have a thinner fibrous cap and a necrotic core associated with hemorrhagic/thrombotic areas. Consequently, augmented biomarkers of plaque vulnerability such as pro-inflammatory cytokines (IL-12, IL-6, IL-1), smooth muscle cells (Calponin-1), extracellular matrix integrity (PCPE-1), and oxidative stress (DJ-1). Malaud et al. (2014) also used variations of plasma concentrations of local plaque biomarkers to diagnose this disease.

D. Nonalcoholic Steatohepatitis

First, it is important to clarify that nonalcoholic steatohepatitis (NASH) is a progression of the non-alcoholic fatty liver disease (NAFLD), a disease characterized by the fat accumulation on hepatocytes without a history of alcohol consumption and accompanied by metabolic disorders. Furthermore, the progression of the NAFLD can reach states of liver fibrosis or hepatocellular carcinoma. The clinical diagnosis for this complication has been studied in an attempt to develop a non-invasive diagnosis method; the liver biopsy is considered the most accurate method. Some studies have sought to elucidate biomarkers or body composition components that predict the NAFLD.

It is well known that some metabolic disorders are associated with NAFLD, such as metabolic syndrome and chronic low-grade inflammation. In addition, its pathogenesis involves two hypotheses: one regards the fat accumulation and insulin resistance on hepatocyte, the other considers oxidative stress and cytokines in the inflammation. The liver has a dual blood supply (hepatic artery and portal vein), which justifies the presence of a variety of immune innate cells in this tissue, such as Kupffer cells (resident macrophages), dendritic cells, natural killer cells, and T cells. Kupffer cells represent 80%–90% of all the macrophages in the body, and they are able to phagocytize and release proinflammatory cytokines and chemokines in the presence of pathogens. On the other hand, these cells can also
cooperate to alleviate tissue damage in some infections and/or inflammatory diseases. Additionally, the innate immune system can stimulate cells from the adaptive immune system (antigen-specific T and B lymphocytes) and can promote their differentiation and proliferation. Studies in human and animal models have shown the importance of Kupffer cells on the onset and development of NAFLD. These cells are activated by several mechanisms including pathogen-associated molecular patterns (PAMPs) and damage endogenous-associated molecular patterns (DAMPs). The first is related to dysbiosis, in which the gut microbiome is modified, and in obesity, in which gut-derived bacterial translocation is increased. The second condition is associated to the progression of disease and the presence of endogenous molecules released by adjacent damaged hepatocytes via activation of TLRs of the macrophages or Kupffer cells. Furthermore, obesity is associated with an infiltration of a population of hepatic macrophages, which is triggered by Kupffer cells (resident macrophages). Thus, infiltrated macrophages seem to be the major cause of the inflammatory state in the livers of high-fat-diet mice. Moreover, factors secreted from these cells appear to contribute to hepatic insulin resistance, whereas Kupffer cell–derived factors do not.

IV. EXERCISE AND THE IMMUNE SYSTEM

The immune system is responsible for protecting an organism from stress agents, whether by injury from invasion by pathogens (infection) or by tissue injury (inflammation), as in physical exercise, which may alter body homeostasis and immune cells composing an efficient line of defense. Exercise, both bouts and training, acts as a significant stressor during and after its performance, and exercise is able to change the behavior of immune cells as they relate to hormonal, mechanical, and metabolic alterations.

One of the earliest studies that found interaction between the immune system and exercise was conducted by Bjorn Ahlborg and Gunvor Ahlborg in 1970. They observed that during exercise there was a significant increase in the plasmatic concentration of immune cells (leucocytosis) in response to adrenergic activity. Muscle activity also leads to an immunological modulation that increases the number of immune cells, particularly neutrophils, given that these are the most abundant immune cells in the blood and have the ability to neutralize an acute inflammatory response, together with macrophages. Immune system cells, particularly lymphocytes, monocytes and macrophages, are able to produce signaling proteins or immune-modulator biomarkers, termed cytokines, with paracrine, autocrine, and endocrine functions. A unique cytokine may play different roles in various tissues, cells, and organs depending on the stimulus.

Notably, the behavior of the immune system in response to the exercise intervention depends directly on such variables of training as duration, intensity, and type of exercise. Studies dating from the end of the 1990s have reported considerable differences in response to intense stimulus, which is responsible for an increased risk of upper respiratory tract infections. Additionally, in a theory confirmed some years later by Nieman (1994), long-term exercise practiced moderately has a protective effect against the risk of infection and, therefore, is beneficial to health. In the case of long-term intense exercise, a short period of immunosuppression (3 and 72 hours) occurs mainly due to high rates of stress hormones that are circulating in the bloodstream. However, individuals who exercise regularly at moderate intensity exhibit lower risk of infection. This adaptation to training has one of its explanations in the hypothalamus-pituitary-adrenal axis and is explained by the presence of receptors for stress hormones in immune cells.

Researchers have tried to understand more deeply the mechanisms that modulate these immune responses to exercise. Thus, there has been a considerable increase in the number of studies in this field. It is currently known that both cellular and humoral components (antibodies, complement proteins, and antimicrobial peptides) are recruited in the innate and adaptive immune system in an attempt to return to the initial homeostasis. According to Costa Rosa (2004), there is an interaction between the psychoneuroimmunoendocrine axis and the skeletal muscle, and glutamine produced by skeletal muscle is essential to

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immune cells even in a quiescent state. In addition, mechanical stimulation is associated with skeletal muscle damage (fatiguing exercise protocol), as is the recruitment of immune cells via interaction of neutrophil and endothelial cells mediated by ROS.\textsuperscript{86}

Moderate exercise was able to increase the cytotoxic activity and the phagocytic capacity of peritoneal macrophage adhesion.\textsuperscript{87} This hypothesis was confirmed by Silveira et al. (2007)\textsuperscript{88} in an acute swimming session (1 h) performed with sedentary rats, which resulted in a 2.4-fold increase in macrophage phagocytic capacity as well as in an oxidative burst and in the production of nitric oxide by macrophages. In response to acute aerobic exercise, normal subjects and obese, non-diabetic subjects had an increase in skeletal muscle cytokine expression (MCP-1 and IL-6) as well as activation of the NF-κB pathway.\textsuperscript{89} In turn, the pro-inflammatory pathway results in NF-kB-activated production of cytokines such as TNF-α by macrophages, which consequently act in an autocrine manner by enhancing the activation of these immune system cells. Cortisol, which is produced by the adrenal gland within the adrenal cortex in response to stress and the low blood glucose caused by exercise, induces the secretion of IL-6 and IL-10 by macrophages, which may block NF-kB activation in an autocrine fashion.\textsuperscript{88}

Specific metabolic and hormonal alterations occur during exercises in which oxygen consumption is $\geq 60\%$,\textsuperscript{90,91} which is typical consumption of long-term aerobic exercise. There is a significant increase in plasmatic concentrations of cortisol to stimulate gluconeogenesis, by not glycidyl compounds such as amino acids. They promote protein catabolism from tissue repair and, consequently, maintain activity. Changes in this catabolic hormone are directly related to glucose concentration in the blood, verifying an inverse relationship between these variables. Glutamine is an amino acid released in catabolism from the increase in cortisol concentration. It may be used in several tissues during exercise\textsuperscript{92} and is an energy source for skeletal muscle.\textsuperscript{93} However, this amino acid is also an important substrate for maintaining immune cell activity, especially of macrophages and lymphocytes. Therefore, glutamine reduction in the bloodstream promotes a reduction in macrophage function, apoptosis, and an increase in production and release of pro-inflammatory cytokines, as TNF-α and IL-1β, which favors the installation of an immunosuppressive profile.\textsuperscript{94}

V. EXERCISE AND MACROPHAGE POLARIZATION

Two lines of studies are emerging with regard to exercise and macrophage polarization; one is related to macrophage polarization associated with adipose tissue and metabolic syndrome\textsuperscript{95} and the other regards the association with skeletal muscles damage and regenerative process.\textsuperscript{96} The anti-inflammatory and anti-atherogenic role of physical exercise, when practiced regularly, is well known to directly affect the production of anti-inflammatory cytokines, such as IL-6, IL-10, IL-18, and IL-1ra, and to yield improvements in substrate uptake and changes in amounts and phenotype of monocytes and macrophages. Below, we summarize studies that demonstrate the anti-inflammatory effects of exercise through the polarization of macrophages given different exercise protocols in human and animal models.

A. Human Model

There are some inconsistencies in data about human and rodent models with regard to recruitment of leukocytes in skeletal muscles after acute exercise; 80% of the rodent models observed some recruitment and infiltration versus 55% in humans.\textsuperscript{97} This fact becomes even more complicated when the object of the study is macrophage polarization.\textsuperscript{98} Macrophage recruitment and polarization seems to be associated to a large extent with muscle damage and appears to be limited only in subjects that perform uncustomized types of muscle contraction.\textsuperscript{98}

However, in a study conducted by Gordon et al. (2012)\textsuperscript{99} with 7 healthy subjects investigated the effect of strength training on the immune activation in skeletal muscle in response to an acute exercise session. This session comprised five upper arm exercises [biceps preacher curl, biceps concentration curl, standing biceps curl, overhead triceps extension,
triceps kickback] with three sets of six reps using the six repetition maximum (RM) weight and resting for 2 min between sets. After exercise, a reduction in monocyte chemoattraction in trained compared with untrained muscle was observed, as well as a persistent decrease in pro-inflammatory macrophage subtype M1. An increase in anti-inflammatory macrophage subtype M2 in the trained compared with untrained muscle across two exercise conditions (bilateral and unilateral arm) was also observed. Additionally, expression of the M1 gene FCGR3B (CD16b) decreased in trained muscle compared with either exercised (2.14-fold) or non-exercised (2.97-fold) untrained muscle. On the other hand, expression of the M2 genes CD163 and MRC1 (CD206) increased in the trained muscle compared with either exercised (1.61- and 1.67-fold, respectively) or non-exercised (1.22- and 1.20-fold, respectively) untrained muscle. These results suggest the potential of strength exercise training on M1 reduction and augmentation of M2 polarization of macrophages (Table 1).

A study corroborating the above findings by Fink et al. (2013) verified that the gene expression of MRC1 (mannose receptor C type 1), MGL (macrophage galactose-binding lectin), and CD163, which are anti-inflammatory M2 markers, were correlated with a high glucose disposal rate and were strongly correlated with muscle insulin sensitivity in exercised people with a BMI >25 kg/m². In this study, 15 overweight/obese subjects performed exercise interventions over 12 months. These interventions were characterized by 60 minutes of aerobic exercise (20 min warm-up and cool down, 20 min of running or cycling and 20 min of strength training) twice weekly, plus 60 min of swimming once weekly (Table 1). This training model suggests that the phenotype of strength exercise training on M1 reduction and augmentation of M2 polarization of macrophages (Table 1).

In a study conducted by Norrbom et al. (2011) to investigate the expression of PGC-1 splice variants in human skeletal muscle and the possible influence of metabolic perturbation (blood flow restriction) in response to a single one-legged knee extension exercise (the exercise session consisted of 45 min of restricted blood flow to the working leg, followed by 45 min with normal blood flow to the other leg) verified that the canonical promoter (PGC-1α-a) and the alternative promoter upstream of the canonical promoter (PGC-1α-b) were upregulated after the exercise session in the leg with blood-flow restriction. However, the fold change increase of PGC-1α-b was much greater than that of PGC-1α-a, and this condition demonstrated higher AMPK phosphorylation. In myoblasts cultured from the sample biopsies, a single bout of exercise upregulated PGC-1 in both promoter types. AMPK is a major regulator of this transcription factor from the canonical promoter and involves β-adrenergic (AICAR combined with norepinephrine) stimulation in combination with AMPK in the regulation of PGC-1α-b. Another study by Bartlett et al. (2012) was conducted with 10 active men performing acute sessions of high-intensity training on treadmills (7 min warm up at 70% VO₂max and 6 bouts of 3 min at 90% VO₂max with 3 min of active recovery at 50% VO₂max) and continuous training (50 min of continuous running at 70% VO₂max). The results demonstrated that both acute and long-term...
TABLE 1: Exercise-induced alterations on markers related to macrophage polarization

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<td>Gordon et al. (2012)</td>
<td>Young healthy individuals (N=7)</td>
<td>12-wk progressive unilateral arm resistance exercise training program. Acute bout of bilateral RE in which the trained and the untrained arm exercised at the same relative intensity</td>
<td>↓ chemoattraction, ↓M1, ↑M2</td>
</tr>
<tr>
<td>Fink et al. (2013)</td>
<td>Normal weight and overweight/ Obese individuals (N=15)</td>
<td>(20 min warm-up and cool down, 20 min running or cycling and 20 min resistance training) plus 60 min swimming once weekly</td>
<td>↑ gene expression CD206, MGL, CD163</td>
</tr>
<tr>
<td>Yakeu et al. (2010)</td>
<td>Sedentary individuals (N=17)</td>
<td>8-wk low-intensity exercise intervention (walking 10,000 steps/day; 3 times/week)</td>
<td>↑ M2 markers (AMAC1, CD14, MR and IL-4), PGC-1 (α and β)</td>
</tr>
<tr>
<td>Bartlett et al. (2012)</td>
<td>Active men (N=10)</td>
<td>Acute session - high intensity training (7 min warm-up at 70% VO₂max and six bouts of 3 min at 90% VO₂max with 3 min active recovery at 50% VO₂max) Continuous training - (50 min continuous running at 70% VO₂max)</td>
<td>↑ AMPK and p38mapk phosphorylation ↑ PGC-1α mRNA</td>
</tr>
<tr>
<td>Thomas et al. (2011)</td>
<td>Healthy, active, untrained Individuals (N=9)</td>
<td>Single 45-min bout of exercise at 70% VO₂max</td>
<td>↑ CD36 mRNA ↑ ABCA1 mRNA</td>
</tr>
<tr>
<td></td>
<td></td>
<td>8-wk training program designed to be progressive for training volume</td>
<td>↑ CD36 (protein)</td>
</tr>
<tr>
<td><strong>Mouse</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Chen et al. (2010)</td>
<td>Male C57BL/6 mice</td>
<td>8-wk training at 60% VO₂max, 5 days/week</td>
<td>↑ MKP-1</td>
</tr>
<tr>
<td>Kawanishi et al. (2010)</td>
<td>Male C57BL/6 mice</td>
<td>16-wk of 60 min/day at running speeds of 12–20 m/min, 5 times/week</td>
<td>↓ F4/80 ↑ CD163 adipose tissue ↓ CD11c, TNF-α and TLR4</td>
</tr>
</tbody>
</table>

M1, macrophages classically activated; M2, macrophages alternatively activated; CD206, cluster of differentiation 206; MGL, macrophage galactose-binding lectin; CD163, cluster of differentiation 163; MKP-1, mitogen-activated protein kinase phosphatase-1; F4/80, marker of murine macrophage populations; CD11c, cluster of differentiation 11c; TNF-α, tumor necrosis factor alpha; TLR4, Toll like receptor 4; AMAC1, alternative macrophage activation-associated CC chemokine-1; CD14, cluster of differentiation 14; MR, mannose receptor; IL-4, interleukin 4; PGC-1 (α and β), peroxisome proliferator-activated receptor gamma, coactivator 1-α and -β; AMPK, adenosine monophosphate-activated protein kinase.
exercise (when matched for average intensity, duration, and work done) induced comparable increases in AMPK and p38MAPK phosphorylation as well as PGC-1α mRNA content in human skeletal muscle (Table 1). These later studies suggest that in several models of training, and generally in the aerobic model provided, AMPK activation occurs that may act directly or indirectly as an alternative pathway for PPAR activation, given that PGC1-α is a known PPAR-γ ligant. In a recent study based on dietary antioxidant supplementation, Davies et al. (2015) suggested that, in monocytes, the effect mediated by exercise on PPARγ signaling increases the generation of PPARγ ligands via oxidation of lipoproteins rather than via ROS-mediated AMPK activation.

Finally, it is well known that, in skeletal muscles, exercise is able to upregulate genes related to PPAR-γ and results in mitochondrial biogenesis and aerobic respiration, whereas macrophage polarization of the M2 profile mediated by exercise is linked to PPAR activation in monocytes and is also associated with the anti-inflammatory effects of exercise. Recently, studies have been conducted to elucidate the mechanisms by which exercise can modulate transcriptional activity of PPAR-γ on monocytes/macrophages as well as how exercise induces alterations in its pathway. Exercise intensity and duration have been shown to provide different results in modulating transcriptional activity in humans.

B. Animal Model

In rodents, the effect of acute exercise and macrophage polarization in skeletal muscle was already evident in different models of exercise and different types of muscle fiber. In soleus muscle (predominantly oxidative fiber), Tsivite et al. (2003) observed elevation of ED1+ macrophages 24 hours after downhill exercise, but this elevation was not proceeded by ED2+macrophages. Additionally, he justified this result by absence of severe injuries induced in this muscle. In contrast, in triceps long head (predominantly glycolytic) fibers, Minari et al. (2015) using the same downhill protocol in rats, observed higher levels of mRNA gene expression CD163 after 48 hours, which indicates that macrophage polarization occurred in this muscle tissue. However, in protocols where muscle damage its deeper, macrophage polarization was more pronounced, such as in passive stretches and lengthening contractions. Unfortunately, none of these studies evaluated the influence of metabolism associated with M2 elevation or whether the macrophage polarization was influenced by differences in the type of muscle fiber.

Training protocols based on aerobic exercises are the most frequently featured in scientific studies using animal models, and this training model, acutely or chronically, is able to induce phenotypic switching from M1 macrophage to M2 macrophage in obese adipose tissue in addition to inhibiting M1 macrophage infiltration into adipose tissue. In an animal model of regular physical activity, mRNA expression of β2 and β3-adrenergic receptor on retroperitoneal adipose tissue increased compared with the sedentary group, supporting the increased sensitivity of adipose tissue to lipolysis by exercise. Paradoxically, one adaptation, as a result of physical training (18 m/min, 30 min/day, 5 days/week for 3 weeks), may be the attenuation of sympathoadrenomedullary activity, explained by the reduction in β2-adrenergic receptors in macrophages. Its receptor plays an important role in the immune system, and some studies have shown an enhancement of bactericidal activity in β2-adrenergic receptor-dependent and β2-adrenergic receptor-independent macrophages. In addition, this receptor may also influence NK-κB activation by β-arrestin-2 through desensitization of G protein-coupled receptor in macrophages. Research from 1997 suggests the inhibition of NK-κB due to cAMP and PKA activation and consequent phosphorylation of CREB (cAMP responsive element binding). Additionally, the activation of the β-adrenergic receptors can stimulate the mitogen-activated protein kinase (MAPK) cascade in a Gs-dependent or a Gs-independent way. Previous studies have shown that the mechanism whereby β-adrenergic receptor activation upregulates proinflammatory cytokines production involves neither PKA nor NF-κB. Nonetheless, this production is mediated through the stimulation of ERK1/2 and p38 MAPK.
tion of proinflammatory cytokines, whereas MAPK phosphatase-1 (MKP-1) negatively regulates macrophage MAPK activation. Chen et al. (2010)\textsuperscript{116} in his study conducted with a 7-week-old male C57BL/6 (8 weeks of training at 60% of maximum oxygen consumption, 5 days/week) observed that the mRNA level of MKP-1 in peritoneal macrophages increased in the training group, whereas stimulation with LPS (100 ng·mL\textsuperscript{-1}) elevated MKP-1 protein expressions and attenuated levels of IL-6, TNF-α, and MCP-1. It also reduced the activity of p38 MAPK, whose prolonged activation led to proinflammatory cytokines when stimulated by LPS (Table 1).\textsuperscript{117}

Furthermore, chronic exercise training (60 min/day at running speeds of 12–20 m/min 5 times/week for 16 weeks) seems to inhibit macrophage infiltration into adipose tissue, thus attenuating the expression of F4/80 mRNA in this tissue. Furthermore, exercise training may also increase expression of M2 markers (CD163), decrease CD11c (M1 marker), and reduce TNF-α and TLR4 mRNA expression, which activates the inflammatory pathway of NF-κB (Table 1).\textsuperscript{95}

Interestingly, most recent studies that demonstrate evidence of the effectiveness of physical exercise training in M1 to M2 macrophage plasticity and anti-inflammatory responses, whether in animal or human studies, are conducted mainly using aerobic exercise, whereas studies using isolated strength exercise are less abundant. However, the use of models through the training of aerobic exercise can be explained by the activation of PPAR, which may be a key protein complex.

VI. CONCLUSION

Understanding the mechanisms by which a unique cell of the immune system is able to control or change its metabolism to combat an inflammatory response may be the key for the attenuation of metabolic disorders. Prevention and treatment are always focused on the modifications of metabolic status provided by exercise, acute or long-term, moderate or vigorous. Most exercise seems to be relevant for beneficial metabolic modifications and improvements to health through the modulation of macrophage plasticity.

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