Regular physical exercise mediates the immune response in atherosclerosis

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ABSTRACT

Atherosclerosis is a chronic inflammatory cardiovascular disease, which results from lipid accumulation in the blood vessel wall, forming a plaque, and ultimately restricting blood flow. The immune system plays a vital role in progression to plaque rupture. While recent evidence clearly indicates the anti-inflammatory function of regular exercise, the mechanisms by which regular exercise can modulate its pathophysiology is not well understood. In this review, we discuss how regular exercise can lower systemic inflammation directly via modulation of the immune system or indirectly via altered myokine concentrations and metabolites. We describe the exercise-induced responses of various myokines (such as IL-6, adiponectin, and FGF21), and how cell function in the innate immune system can be modulated via regular exercise, with the aim to modulate plaque formation in atherosclerosis.

Keywords: atherosclerosis, exercise, myokines, cytokines, IL-6, adiponectin, FGF21

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INTRODUCTION

Atherosclerosis is a chronic inflammatory disease, characterized by the formation of a plaque inside the smooth muscle wall of blood vessels [33; 141]. It is the major contributing factor to coronary heart disease [116], which is responsible for ~7 million annual deaths worldwide [139].

The inflammatory profile of atherosclerosis [137] is marked by slow, progressive structural changes within the intima, particularly in the medium and large arteries in the body [16]. If left untreated, blood vessels occlude [137], as a result of atherosclerotic plaque formation [40]. Atherosclerosis is a complex, multifactorial disease, as well as a precursor of cardiac ischemia-reperfusion, and a main risk factor for several other chronic cardiovascular diseases [48].

The response-to-retention model of atherogenesis [136] suggests that the atherogenic process initiates with subendothelial retention of lipids and lipoproteins (e.g. Apolipoprotein B, ApoB), consisting of cholesteryl fatty acyl esters and triglycerides, and low-density-lipoprotein (LDL) cholesterol. These can bind to proteoglycans in the arterial wall, particularly at arterial branch points and bifurcations [77, 128]. Increased retention rate of lipoproteins promotes fatty streaks formation inside the intima, eventually progressing into a plaque [48]. If lipids continuously accumulate in the already thickened intima [85], fibrous cap (or sclerosis) gradually forms around the lipidic core, narrowing the arterial lumen [91,48], which reduces blood flow, oxygen and nutrient supply, and, eventually, causes downstream tissue death [4, 94]. Also inside the plaque, cell apoptosis and necrotic core formation lead to thinning of the fibrous cap and plaque instability. If this plaque ruptures, it can cause cardiovascular complications such as intravascular thrombosis and myocardial infarctions.

Current therapies mainly rely on low-density-lipoprotein (LDL)- and triglyceride-lowering treatments through statins and others [10]. Other therapies aim at increasing expression of LDL receptors, in order to prevent degradation, and inhibit cholesterol absorption [15]. Bile acid sequestrants and high-density-lipoprotein (HDL)-increasing therapies have also been used. However, the side effects of the former and the lack of significant improvements of the latter have made these alternatives less popular [10; 15].

While pharmacological approaches have been widely used in a clinical context, alternative therapies, such as exercise training have clinical potential to modulate the disease progression. Regular physical exercise has been shown to improve metabolic health, cardiovascular function and improves insulin sensitivity, all factors that are known to be implicated in the onset and progression of atherosclerosis [99].

One factor that has obtained considerably less attention is the observation that regular moderate physical activity potently

lowers systemic inflammation in many cardiovascular diseases [98], and increases the concentration of circulating peripheral blood mononuclear cells (monocytes and lymphocytes). Additionally, regular exercise stimulates the release of adrenaline and cortisol, both of which with strong anti-inflammatory effects [88]. The role of small molecules secreted by skeletal muscle (myokines) and their subsequent effects on the immune response and lipid metabolism in atherosclerosis is currently unknown [36]. How exactly exercise exerts its therapeutic and anti-inflammatory effects on the prevention of plaque formation and treatment of atherosclerosis remains enigmatic [54], with just few molecular mechanisms being identified so far. Although current evidence suggests a strong anti-inflammatory effect of exercise, research on exercise-induced secretion of anti-inflammatory products is scarce [132]. In this review we provide an overview of the latest advances in exercise immunology related to atherosclerosis, and discuss whether the progression to severe atherosclerosis can be delayed by exercise, and what the underlying mechanisms are.

A literature search was performed on PubMed and Google Scholar, with search terms, such as 'exercise', 'atherosclerosis', 'immune system', 'myokines' and 'immunology' either individually or combined. Article selection was extended to cited papers considered important, as well as searches for specific myokines (i.e. FGF21), exercise and atherosclerosis.

2. IMMUNE RESPONSE AND INFLAMMATION IN ATHEROSCLEROSIS

A graphical overview of the contribution of various immune cells is provided in Figure 1. Endothelial cell injury within the blood vessels is considered to be the first step towards an atherosclerotic lesion [47]. Classic obesity markers, such as high levels of circulating lipids, particularly LDL, and metabolic dysregulation [77] are implicated in the initial stages. LDL infiltration and retention in the intima cause endothelial cells to manifest a dysfunctional phenotype [77], particularly in arterial branches and curves. These atheroprone areas are characterized by low endothelial shear stress, higher circulating levels of vasoconstrictors (e.g. endothelin-1), production of reactive oxygen species (ROS), and imbalances between nitric oxide (NO) and prostacyclin (PGI2)-mediated vasorelaxation [18]. Oxidative stress in the endothelial cells speeds LDL oxidation in the subendothelial space, and promotes a pro-atherogenic environment [147]. A lower endothelial-derived NO production reduces vasorelaxation, endothelial regeneration and integrity [86], and facilitates the accumulation and retention of atherogenic LDL in the subendothelial layer [110]. This vicious cycle ultimately activates the nuclear factor kappa B (NF-κB)-pathway that triggers the expression of pro-inflammatory cytokines in the injured cells [70], further causing endothelial dysfunction [145].

Stressed endothelial cells express higher levels of immunoglobulin-G adhesion molecules (e.g. VCAM-1, P-selectin, ICAM-1), proinflammatory receptors, and cytokines (e.g. IL-8, MCP-1 [53]). Together they are responsible for immune cell recruitment into the vascular tissue [75]. Particularly, monocytes are recruited [39] and converted into proinflammatory macrophages, which proliferate within the intima layer [106]. Figure 1 describes how the interactions between dysfunctional endothelial cells, adipocytes found at the atheroma and pro-inflammatory T-cells (Th1 and Th17) contribute towards monocyte differentiation.

2.1. Immune cells in atherosclerosis

Leukocytes are of major importance in the onset of atherosclerosis [125]. Under normal conditions, circulating monocytes are uncapable of proliferating [4, 43]. However, atherogenesis alters monocyte behaviour. Lipid accumulation inside endothelial cells increases expression levels of cellular adhesion molecules, such as E-selectin, P-selectin, VCAM-1 and ICAM-1 [24], promoting a local vascular inflammation

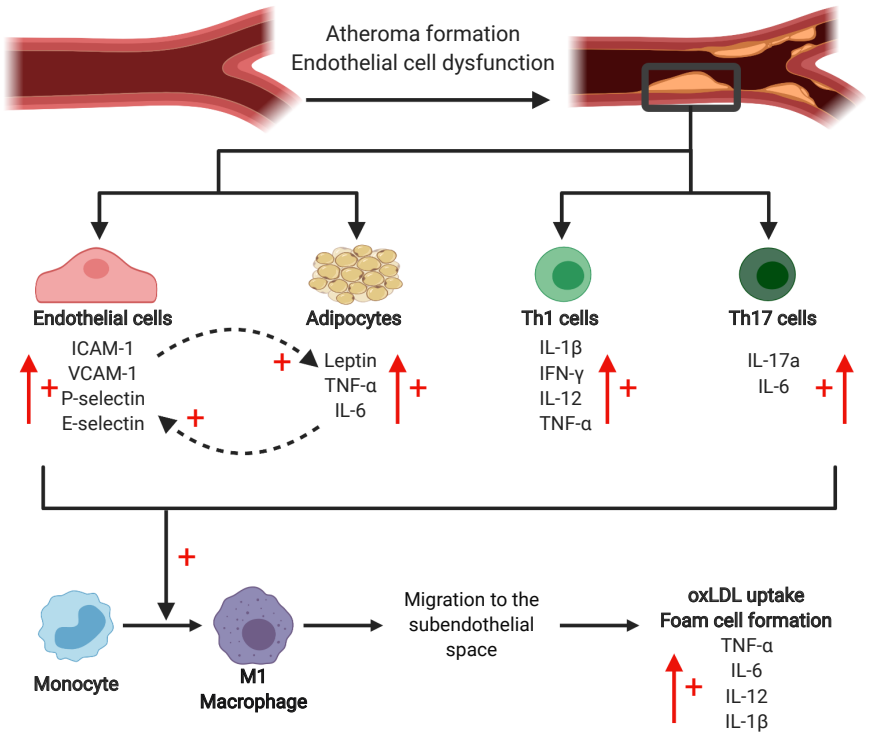

Figure 1. Interactions between endothelium, atheroma and immune system. LDL retention at the intima gives rise to atherosclerotic plaque formation. Consequently, endothelial cells become dysfunctional and express higher levels of adhesion molecules (ICAM-1, VCAM-1, P-selectin and E-selectin), contributing to monocyte recruitment towards the intima. Simultaneously, adipocytes from the accumulated LDL secrete pro-inflammatory cytokines, such as leptin, TNF-α and IL-6, whose expression stimulates, and is enhanced by endothelial cells-mediated secretion of adhesion molecules. Th1 and Th17 immune cells also found in the intima express a wide range of pro-inflammatory cytokines. Monocyte recruitment to the blood vessel is favoured by permanent expression of adhesion molecules, differentiation into pro-inflammatory M1 macrophages. Subsequent foam cells formation is facilitated by the pro-inflammatory products released by adaptive immune cells, adipocytes and endothelial cells.

response by attracting and deposition of monocytes and T-cells [42]. These cells contribute to a continuous inflammatory state via secretion of pro-inflammatory cytokines (e.g. leptin, TNF-α and IL-6) [38], causing a vicious circle by further increasing the expression of adhesion molecules and recruitment of leukocytes to the intima [55, 103].

2.1.1. Monocytes and macrophages

The interaction between monocytes and adhesion molecules, and continuous secretion of lipoprotein-binding proteoglycans [83] cause lesions to the endothelium, further facilitating the transmigration of monocytes in the intima and allowing monocytes to differentiate into macrophages [57]. Monocyte differentiation into either classically activated atherogenic M1 macrophages, or alternatively activated anti-inflammatory M2 macrophages strongly depends on the microenvironment at the artery [42]. M1 differentiation is favoured due to the exposure of monocytes TO pro-inflammatory cytokines cytokines secreted by the dysfunctional endothelium [24] and neutrophil-mediated secretion of TNF-a, IL-8 and IFN-y [60]. Pro-inflammatory macrophages regulate plasma lipoprotein metabolism and express several receptors with high-affinity to oxidized LDL (e.g. LOX-1; [25]). These will take up on oxidized LDL molecules and other lipids at the lesion site [50]. Macrophages, therefore, accumulate at the subendothelial space, due to continuous monocyte recruitment and LDL oxidation, and eventually turn into foam cells, one of the key markers in atherosclerotic plaque formation [25]. Foam cells release a series of pro-inflammatory cytokines, particularly CD40, IL-1, IL-3, IL-6, IL-8, IL-18 and TNF-α [122]. The cholesterol that has been collected and accumulated in macrophage foam cells enhances activation of inflammatory receptors [129], therefore exacerbating the inflammatory state at the atheroma. The pro-inflammatory phenotype of foam cells contributes towards plaque progression and instability, necrotic core formation, and degradation of the extracellular matrix [71].

2.1.2. T-cells

Other key players in monocyte differentiation into macrophages are T-cells. Just like monocytes, T-cells are recruited to the endothelial wall via cellular adhesion molecules (CAMs)-signalling [89], with both Th1 and Th2 cells contributing to the immune response. Th1 cells are primed by interactions between LDL molecules and oxidized-LDL-specific T-cells, found at the injured endothelium [55]. Th1 cells have an atherogenic function, as they secrete pro-inflammatory cytokines, such as IL-1β, IFN-γ, IL-2, IL-12 and TNF-α, worsening local inflammation [45]. IFN-γ, particularly, contributes to a continuous cycle of monocyte recruitment to the injured site and their subsequent differentiation into pro-inflammatory M1 macrophages [131].

Conversely, Th2 cells produce an array of anti-inflammatory cytokines, particularly IL-4, IL-5, IL-9, IL-10, IL-13 and IL-33 [60; 112]. These cytokines have a strong anti-atherogenic effect, but their exact role in the progression of atherosclerosis remains unclear, due to spatial and temporal uncertainties [79]. Early studies showed that Th2 cells prevent fatty streak formation and IL-5 lowers plasma levels of IFN-γ, possibly preventing atherosclerosis progression [79]. On the other hand, IL-4 contributes to plaque formation at later stages [79].

Th17 cells, commonly found in the atheroma, are also part of the effector T-cell subset, but with a different lineage than that of Th1 and Th2 [80]. Th17 cells secrete IL-17a, IL-17f and IL-6 [143]. Although IL-17f is known to exert an anti-atherogenic effect by increasing plaque stability [143], the overall role of Th17 cells is believed to promote disease progression due to pro-inflammatory IL-17a and IL-6 activity [2]. Their exact role is still under debate due to its ambiguous function in atherosclerosis.

Treg cells, another subset of T-cells, have a clear inhibitory effect on Th1 cells, thus reducing the pro-inflammatory milieu. Treg cells suppress the activity of CD8+ T-cells [44], which are commonly found in human atherosclerotic plaques, particularly in a more advanced stage of the disease [46, 91]. Treg cells secrete IL-10 and TGF-β, which are considered to promote plaque stability [4]. Lower Treg subpopulation are linked with atherosclerosis progression. Therefore, possible therapeutic strategies involve increasing Treg number either by cell transfer or expansion [95]. However, little is known about Treg survival upon transfer or activation/expansion, thus limiting the long-term effects on atherosclerosis progression. Additionally, these cells might behave differently on distinct stages of atherosclerosis, which makes it difficult to define a specific treatment strategy. Although promising, future studies and clinical trials should focus on the long-term contribution of the anti-inflammatory effects of Tregs [95]. Physically active individuals often have higher frequencies of circulating Treg cells, likely due to an exercise-induced increase in TGF-β, which is essential to the anti-inflammatory function of Treg cells. Current data, therefore, suggests a link between physical activity, TGF-β and optimal Treg cells function [135].

2.2. Atherosclerotic plaque

Plaque formation initiates with lipid deposition at the intima, after lipid accumulation occurs in the intima due to the injured endothelium [5], giving rise to fatty streaks. Recruited monocytes, subsequent monocyte-derived macrophages and T-lymphocytes will also accumulate at the intima [74]. As macrophages take up on oxidized LDL molecules and turn into foam cells, they secrete an array of growth factors and cytokines which stimulate migration and proliferation of vascular smooth muscle cells (VSCMs) at the intima, followed by production of extracellular matrix (ECM) and related components (e.g. collagen) to form the fibrous cap [64]. Ultimately, the atheroma is an agglomeration of leukocytes, foam cells, VSCMs, ECM and lipids, and the fibrous cap is what provides stability to the plaque.

The plaque becomes unstable once foam cells undergo apoptosis. Apoptotic cell removal is mediated by M2 macrophages [127]. However, if phagocytosis occurs at an exacerbated rate, the endoplasmic reticulum of M2 macrophages becomes stressed, culminating in cell death [134]. Combined, both apoptotic M1 and M2 macrophages make up the necrotic core. Apoptosis of M2 macrophages leads to release of lipids, inflammatory and thrombotic factors, and metalloproteinases (MMPs) [134]. Continuous recruitment of pro-inflammatory leukocytes to the atheroma causes TNF-α-induced VSMCs death [11], inhibiting VSMCs-mediated production of matrix components [26]. Particularly, CD8+ T-cells, also found at the plaque, are highly cytotoxic and promote plaque instability by inducing apoptosis of macrophages and VSMCs [67]. MMPs,

on the other hand, erode the ECM, which together with lower numbers of VSMCs contributes towards plaque susceptibility to rupture [134]. An important thrombotic mediator is tissue factor (TF), which is found. It is found in high quantities within the plaque. As its expression is enhanced in apoptotic foam cells and VSCMs. Once the plaque is ruptured, TF mediates thrombus formation [108], as well as other life-threatening ischemic cardiovascular events [77].

3. EXERCISE AS A THERAPEUTIC INTERVENTION FOR ATHEROSCLEROSIS

It is widely accepted that moderateintensity aerobic exercise improves cardiovascular, respiratory and skeletal muscle function, with little to no severe complications, and can prevent, treat and delay the development of various chronic diseases [98]. As such, regular aerobic exercise has been implicated in the prevention of the metabolic derailment, obesity, endothelial dysfunction, and insulin resistance. Generally, these effects are contributed to improved blood lipid levels, glucose signalling and skeletal muscle adaptations.

One aspect that has received little attention is that exercise also has immune-modulatory effects [88]. All skeletal muscle-derived factors that are secreted into the blood stream are called myokines [34], and more than 600 have been discovered to date. Exercise triggers the secretion of various substances including cytokines (particularly IL-6, IL-15, IL-1ra, IL-19 and sTNF-R; [121]), fostering a potent anti-inflammatory environment, and modulates immune responses [28]. Physical activity also influences leukocyte behaviour. For instance, circulating levels of non-classical, anti-inflammatory monocytes increase following exercise training [120]. High intensity exercise enhances CCR5 expression on monocytes and T-cells [6], and CCR5+ Treg cells greatly increase their expression of the anti-inflammatory cytokine IL-10, indicating potent anti-inflammatory effects of augmented CCR5 expression on the cell membrane of leukocytes [32], ameliorating inflammation in the atheroma. In addition, physical activity also alters T-cell balance by increasing circulatory levels of Treg cells [112] and inducing Th2 cell polarization, subsequently releasing higher quantities of immunosuppressive cytokines [92]. Important to note that an effective exercise protocol, capable of eliciting is optimal anti-inflammatory responses, is yet to be determined [132]. Figure 2 shows how an overview of how regular exercise affects the immune system directly, and indirectly via the release of various compounds.

3.1. Indirect effects of exercise via altered circulating metabolites

3.1.1. Endothelial shear stress-mediated substances

Physical exercise increases whole-body blood flow and endothelial shear stress [101]. Endothelial shear stress causes the endothelium to secrete vasodilators (such as NO), growth factors and others [82]. The interaction between blood flow and shear stress regulates gene expression contributing to its optimal function [148].

Angiographic studies showed a 200% higher vasodilation capacity in ultra-distance runners versus sedentary individuals [56]. This endothelium-dependent relaxation of coronary arteries and arterioles is accompanied with higher endothelial nitric oxide synthase (eNOS) expression and, subsequently, higher levels of NO [68]. Endothelial cells on the vascular lumen secrete more NO into the bloodstream [51], maintaining endothelial function and preventing local LDL accumulation. Regular exercise also improves endothelial function in ApoE-deficient mice fed on a high fat diet [37]. The areas of atherosclerotic lesions in the aortic sinus and thoracoabdominal aorta were significantly lower in the exercise group, and correlated with the amount of daily exercise. This was associated with a reduction in inflammatory cell markers in the aorta. Serum IL-6 and macrophage chemoattractant protein-1 levels were significantly lower and those of adiponectin were significantly higher in the exercise group compared to the control group [37].

While aerobic exercise increases endothelial function,

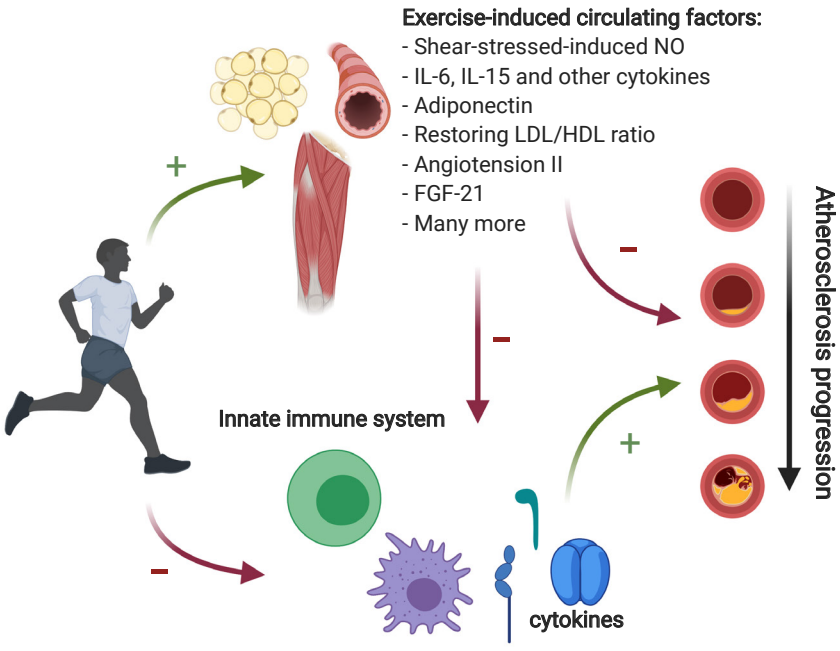

Figure 2. Schematic overview of how exercise alters the immune response in atherosclerosis. Acute exercise results altered endothelial cell function (via increased shear-stress-mediated cell signalling), and in the secretion of circulating factors, such as IL-6, IL-15, adiponectin, FGF-21 and many others. These factors, by themselves, or via the suppression of the innate immune system, are thought to alter endothelial and smooth muscle function. Exercise on the other hand also modulates macrophage and T-cell function, resulting in an additional pathway by which exercise delays the progression of plaque formation in atherosclerosis.

physical inactivity reduces overall blood flow and endothelial shear stress. Endothelial cells become dysfunctional and produce pro-inflammatory cytokines [3], changing the endothelial cells towards an atheroprone phenotype [58].

In summary, optimal endothelial function is jeopardized under conditions of inactivity or a high fat diet, which are associated with a low NO bioavailability, local attraction of inflammatory cells, causing an atheroprone environment. Regular exercise increases endothelial shear stress and endothelial-derived NO, and has the potential to revert endothelial dysfunction and reduce the local immune response.

3.1.2. Interleukin-6

Interleukin-6 (IL-6) is a key regulator of inflammation in the atherosclerotic plaque, synthesized by various cells at the vessel wall, such as macrophages, endothelial cells and smooth muscle cells [105]. IL-6 is known for exerting both anti- and pro-inflammatory properties in the course of atherosclerosis development [105]. However, when mentioned in the context of exercise training, IL-6 is a potent anti-inflammatory myokine and one of the most important biomarkers secreted by skeletal muscles released during physical activity [35]. Its inflammatory profile is more evident through chemotaxis, which brings monocytes to the vessel wall, increases chemokine production and induces higher expression levels of adhesion molecules, thus inducing an inflammatory milieu that promotes atheroma formation [140]. In contrasting, exercise-derived IL-6 triggers the production of IL-1ra and IL-10 cytokines [124], which have anti-inflammatory properties.

IL-6-mediated production of IL-10 suppresses activation and circulation levels of Th1 cells, known for exacerbation of the inflammatory state and markedly present in the atherosclerotic plaque [69]. Studies have shown that inactivation of IL-10, either genetically or via blockade of IL-6, fosters a pro-atherogenic environment, as it allows for increased transmigration of cells into the subendothelial space of the blood vessel and enhanced production of pro-inflammatory cytokines [13].

Other targets of muscle-derived IL-6 are Th1 and Th2 cells. The former is the most commonly found subset of T-cells in the atherosclerotic plaque [131]. Th1 cells have a pro-inflammatory, atherogenic profile, and worsens atherosclerosis [45 and section 2.1.2]. Opposingly, Th2 cells, which counteract inflammation via secretion of anti-inflammatory cytokines such as IL-4, are not often detected in atherosclerotic lesions [79]. However, when released during moderate endurance exercise, IL-6 acts directly on Th1 cells, via enhanced cortisol release, and suppresses its activity, whilst promoting Th2 cells differentiation [119], and higher production rates of anti-inflammatory cytokines, such as IL-4 [123].

The IL-6 signalling pathway mediates the atheroprotective effect of physical activity [126]. Exercise-induced secretion of IL-6 from skeletal muscles suppress the classical pro-inflammatory cytokines TNF-α and IL-1β that are found in high concentrations in the atheroma [93]. It is therefore likely that IL-6 can halt atherosclerosis progression by targeting pro-atherogenic markers. Although its anti- and pro-inflammatory characteristics are well documented, the exact trigger of each signaling pathway is not yet fully understood. Likely, the combination of IL-6 with other (unknown) myokines provides the key to unlock the anti-inflammatory characteristics typically seen after exercise.

3.1.3 Adiponectin

The cytokine adiponectin is mainly released by adipocytes [107], and modulates whole body lipid and glucose metabolism [107], with important anti-atherogenic and anti-inflammatory properties [90]. A study conducted by Hotta and colleagues [59] concluded that adiponectin is positively correlated with insulin sensitivity and that its serum levels decrease with obesity. Binding of adiponectin to its receptor AdipoR1 activates AMPK signalling pathway [31], increasing eNOS expression, NO synthesis and vasodilation [19], whilst its binding to AdipoR2 enhances fatty acid oxidation via PPAR-α upregulation [144].

Low circulating adiponectin levels have been implicated in the onset of type 2 diabetes mellitus and cardiovascular diseases, such as atherosclerosis [133]. The atheroprotective role of adiponectin is thought to be of indirect nature. Atherosclerotic-induced endothelial dysfunction, at its earliest stages, is mostly caused by impaired expression and activity of eNOS and poor secretion of NO, which contribute towards arterial vasoconstriction [22]. Adiponectin-knockout mice had impaired aortic vasodilation, lower NO synthesis and increased oxidative stress, accentuating even further the endothelial cell dysfunction at the intima [20]. Therefore, adiponectin might eventually emerge as a promising approach aimed at increasing NO synthesis, ameliorating endothelial dysfunction and promoting vasodilation of occluded vessels.

The atheroprotective role of adiponectin is also observed via acceleration of the reverse cholesterol transport system, a process through which high density lipoproteins (HDL) remove excess cholesterol from foam cells at the atherosclerotic plaque [107]. Adiponectin also fosters an anti-atherogenic microenvironment, via reduced expression of adhesion molecules in endothelial cells, which reduces monocyte attachment [97], and inhibits foam cell formation [96]. Altogether, these effects prevent and reduce plaque dimensions and formation, and diminish the inflammatory state in which atherosclerosis is involved.

Moderate intensity aerobic exercise increases serum adiponectin levels to the same level as several anti-diabetic drugs [1]. This would, at least in theory, improve glucose and lipid metabolism [76]. Nevertheless, exercise-induced circulating adiponectin levels are not always associated with a reduced risk of atherosclerosis and atherosclerotic burden [17]. Clearly more work needs to be performed in order to establish a causal link between increased levels of exercise-mediated adiponectin and atherosclerosis.

3.1.4 Interleukin-15

IL-15, an anti-inflammatory myokine secreted during moderate physical activity, contributes to lipid metabolism [87]. Overexpression of IL-15 is linked to a lower visceral fat mass [104]. By enhancing adipose tissue metabolism, exercise-induced induction of IL-15 possibly diminishes the concentration of circulating LDL and is a determinant factor in the treatment of atherosclerosis. More work should be performed to demonstrate a mechanistic link between exercise-induced IL-15, lipid metabolism and the onset of atherosclerosis.

3.1.5 Angiotensin II

Another key mediator of inflammation is the endothelial angiotensin II pathway, which is highly active in patients with atherosclerosis [109]. Angiotensin II promotes

vasoconstriction, fosters the progression of atherosclerosis and reduces plaque stability [100]. Angiotensin II also activates circulating immune cells and facilitates their adhesion to the endothelium and subsequent transmigration by synthesizing adhesion molecules, chemokines and cytokines [126].

Regular exercise blocks the activity of endothelial angiotensin II, and as a result inhibits these processes. Consequently, lowering angiotensin II activity reduces circulating levels of oxidized LDL and lowers macrophage recruitment to the subendothelial space. How exercise-induced alterations in angiotensin II activity ultimately (in)directly reduces atherosclerosis risk should be studied in mechanistic (animal) models.

3.1.6 Apolipoprotein E

Apolipoprotein E (ApoE) is a key, polymorphic, anti-inflammatory lipoprotein involved in plasma cholesterol homeostasis [115]. ApoE mediates the binding of VLDL to LDL- and VLDL-receptors, hence facilitating triglyceride clearance. ApoE inhibits endothelial cell activation [122], enhances HDL efflux, inhibits activation and proliferation of monocytes, and its expression on the surface of macrophages prevents foam cell formation [41]. Its interplay with the immune system is observed through suppression of NF-κB-driven inflammation [72], proliferation of CD4+ and CD8+ T-cells involved in a pro-atherogenic environment, and induction of major histocompatibility complex (MHC) II expression on macrophages, thus expanding the population of anti-inflammatory M2 macrophages [111].

Plasma triglyceride and cholesterol levels are increased in ApoE-knockout (ApoE-KO) mice, and this knock-out model is a popular model to study atherosclerosis. Shimba and colleagues [113] studied whether skeletal muscle-specific peroxisome proliferator-activated receptor γ coactivator-1α (PGC-1α) overexpression alters the progression to atherosclerosis in ApoE-KO mice. PGC-1α is known to be upregulated during endurance exercise training by approximately 3-fold [81]. Indeed, a higher expression of PGC-1α resulted in a significantly smaller atherosclerotic plaques than ApoE-KO mice with normal levels of PGC-1α. Whether this is mediated by a PGC-1α-dependent inhibition of VCAM-1 and MCP-1 mRNA expression from dysfunctional endothelial cells in the atheroma is unknown, as it is unknown whether PGC-1α can have endocrine effects. Likely, an unknown paracrine factor, released from high-oxidative muscles, contributes to the observed effect. More work should be performed to better understand the athero-protective effects overexpression of PGC-1α in skeletal muscle.

The polymorphic profile of ApoE was first described by Davignon, Gregg & Sing [29], who identified six genotypes that give rise to three isoforms. ApoE2 has an atheroprotective effect, and is linked with the highest circulating levels of HDL out of all phenotypes, as well as the lowest plasma values of total cholesterol and LDL [7,73]. ApoE3 has similar functions to ApoE2, but to a lesser extent. However, following exercise training, ApoE3 causes great reductions in circulating triglyceride and LDL/HDL levels by increasing lipid metabolism. ApoE3 also removes cholesterol from macrophages and inhibit T-cell differentiation and endothelial cell proliferation [52], through inhibition of the pro-inflammatory IL-2 signalling pathway. ApoE4, on the other hand, has a strong affinity to both VLDL and LDL, which leads to a marked competition for the LDL receptor, therefore delaying its clearance from plasma [73]. Sedentary ApoE4 carriers have a 1.4-fold greater risk of developing cardiovascular diseases, and particularly atherosclerosis [49]. Nevertheless, physically active individuals who express the ApoE4 isoform present similar lipid profiles to ApoE2- and ApoE3-carrying individuals, contributing to an enhanced atheroprotective environment [30]. Whether this exercise-mediated protection in ApoE4 carriers is due to a reduced affinity to LDL and VLDL molecules due to myokine secretion is still unknown. Similarly, what the interaction is between other ApoE isoforms and exercise training responses is currently not studied in detail.

3.1.7 Fibroblast growth factor 21 (FGF21)

Fibroblast growth factor 21 (FGF21) is a metabolic growth factor responsible for controlling glucose levels and lipid metabolism [138]. It is secreted from multiple organs across the human body in obesity-derived type 2 diabetes mellitus and cardiovascular diseases [63]. Animal studies revealed improved lipid profiles upon administration of FGF21 [21]. Muise and colleagues [84] concluded that high-fat diets induce hepatic secretion of FGF21 in mice, which resulted in an enhanced lipid metabolism. Higher serum levels of FGF21 were associated with elevated pericardial fat in people without cardiovascular disease at baseline and its reduced accumulation over time, thus highlighting its cardioprotective properties [78]. In addition to overall improvement of lipid profiles, FGF21 also has anti-inflammatory and anti-oxidant functions [65]. Therefore, FGF21 has beneficial metabolic effects which contribute towards metabolic homeostasis [63].

In humans, however, augmented serum levels of FGF21 have been linked to the development and incidence of a wide range of pathologies, including atherosclerosis [138]. Circulating levels of FGF21 increase as a response to pericardial fat accumulation, the latter being a strong determinant of atherosclerosis. Therefore, FGF21 can be used as a biomarker of cardiovascular disease, as its synthesis is markedly enhanced under conditions of adipose tissue accumulation [130]. Additionally, evidence on administration of recombinant and/or analogues of FGF21 showed several preventive effects on atherosclerosis development and progression [21]. This suggests that FGF21 in fact exerts a protective effect that contributes to reduced risk to cardiovascular diseases, likely as a compensatory cardioprotective mechanism [142].

Current research on the role of FGF21 in atherosclerosis is still scarce. The mechanisms though which FGF21 operates, likely direct or indirect via adjusting whole body metabolic homeostasis, are not fully understood yet.

4. FUTURE DIRECTIONS

Regular exercise has the potential to improve endothelial function and reduce the progression to atherosclerosis via a combination of factors. Here, we focused on the immune-modulating role of various myokines that are released during exercise. Since the field of exercise immunology are still in its relative infancy, it is important to realize that a lot of future research is needed to fully understand the cellular and molecular pathways.

One clear requirement for future understanding of the beneficial effects of exercise on atherosclerosis is the type and duration of exercise. From what is currently known, it is likely that the required exercise has to induce enough shear stress to allow endothelial function to be improved. One could argue that aerobic exercise, with a long duration and low intensity would result in more improvements compared to short-duration resistance training. However, how myokine profiles differ between various types of exercise (duration and intensities) is only marginally understood. Clearly, this is something that future research should focus on.

Regular exercise has a multitude of effects on cardiovascular function. One of them is angiogenesis which is the process of the formation of new blood vessels, via the exercise-mediated release of vascular endothelial growth factor (VEGF). It is known for being central to the pathogenesis of a wide range of diseases [14]. In atherosclerosis, however, the role of angiogenesis remains a highly unresolved issue. It has been suggested that therapeutic angiogenesis replaces dysfunctional or occluded vessels with new fully functional capillaries, and thus revascularize cardiac and peripheral tissues [146]. While atherosclerosis is mainly thought to affect larger vessels with a layer of smooth muscle, the ultimate tissue damage occurs downstream in the occlusion of capillaries by ruptured plaques. Therefore, the use of VEGF has emerged as a way to stimulate blood vessel formation in cardiovascular disease [117], but its role in atherosclerosis is highly contradictory. Whilst some studies have suggested VEGF-induced angiogenesis exerts atheroprotective effects and enhances cardiovascular health [114], others demonstrated that VEGF promotes the development of atherosclerosis [118]. As there is still no consensus on the possible benefits of angiogenesis on atherosclerosis regression and/or progression, it is highly recommended that future research needs to focus on how angiogenesis (possibly via the release of certain myokines) can alter the time course of atherosclerosis.

As atherosclerosis mainly occurs in older individuals, the development of atherosclerosis could, at least in part, overlap the process of biological ageing. Immunosenescence refers to the deterioration of the (adaptive) immune system with increasing age. Recent insights have suggested immunosenescence to play a contributing role in atherosclerosis [9]. Immunosenescence is marked by an active, pro-inflammatory subpopulation of T-cells, namely CD4+, CD8- and terminally differentiated effector memory CD45RA+ T (TEMRA) cells, which secrete high amounts of TNF-a and INF-g. TEMRA cells are highly cytotoxic and contribute to vascular inflammation, plaque disruption and worsening of atherosclerosis in older patients [12]. The risk of atherosclerosis is further enhanced by shortening of telomeres in leukocytes of elderly individuals [8] and accumulation of senescent endothelial and vascular smooth muscle cells [62]. Approximately 60% of women diagnosed with atherosclerosis, and 88% in men can be attributed to ageing-related immunosenescence [61]. Nearly all senescent cells secrete the same pro-inflammatory cytokines (IL-1, IL-1b, IL-6, IL-8, IL-18, TNF-a and IFN-γ) which have been linked with atherogenesis and its subsequent progression [23]. Physical activity is known to induce apoptosis of senescent T cells, possibly stimulating production and maintenance of naïve T-cells with ageing [66]. A 3-week endurance exercise training programme significantly reduced the number of CD8+ TEMRA cells, acting as a countermeasure to immunosenescence [102]. Therefore, as immunosenescence heavily depends on age-associated sedentarism, maintaining a physically active lifestyle throughout adulthood prevents immunosenescence. How this affects the course of atherosclerosis, independent of other lifestyle factors, remains however, an unexplored topic.

CONCLUSION

Atherosclerosis is an inflammatory chronic cardiovascular disease, caused by injury to the intimal wall within the blood vessel. Its progression is marked by a pro-inflammatory milieu induced by monocyte transmigration and differentiation into M1 macrophages, which secrete pro-inflammatory cytokines. Physical exercise is an effective non-pharmacological approach to delay and prevent atherosclerosis, likely through positive adaptations in the inflammatory pathways. These effects are mostly observed upon release of myokines IL-6 and IL-10, which block Th1 cell activity, and activate Th2 cell function. The practical link between physical exercise and atherosclerosis treatment and prevention is clear, although specific exercise prescriptions to optimally alter immune cell function are yet to be developed. Also, the direct and indirect interaction between various myokines and the immune system make future work in the field of exercise immunology exciting. We look forward to more mechanistic, therapeutic and clinical studies to better understand the beneficial effects of exercise in the development of atherosclerosis.

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