Chapter 1

Exercise and Atherogenesis

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Prevalence of Arteriosclerotic Cardiovascular Disease

According to the World Health Organization, ischemic heart disease and stroke are the top two causes of mortality worldwide, accounting for a total of 14.1 million deaths in 2012 [1]. In the United States, diseases of the heart remain the leading cause of death in both men and women, accounting for 614,348 deaths in 2013; cerebrovascular diseases rank fourth, accounting for an additional 133,103 deaths [2]. The disturbing worldwide increase in the prevalence of type 2 diabetes [3], whose co-morbidities include micro- and macrovascular disease does not bode well for future improvements in the incidence of deaths due to cardiovascular disease (CVD) unless lifestyle changes and therapeutic advances successfully address this issue [4].

Exercise and All-cause Mortality

A number of studies have shown that regular, moderate-intensity, physical exercise reduces mortality due to all causes, particularly deaths due to CVD [5-8]. Regular physical exercise has also been shown help prevent the onset of type 2 diabetes mellitus and to favorably impact on the prevalence of other risk factors for CVD including hypertension, obesity, hyperlipidemia, insulin resistance, and the metabolic syndrome [9-16]. Involvement in moderate to vigorous physical activity in later life (55-73 years of age) has been shown to decrease the incidence of major chronic diseases, depression and physical or cognitive...
impairment even in persons who have been inactive most of their lives [17]. Persons with established CVD, including those with heart failure, ischemic heart disease, and peripheral vascular disease have also been shown to benefit by regular involvement in moderate-intensity exercise programs [18-20].

Insufficient physical activity is one of the 10 leading risk factors for global mortality, contributing to some 3.2 million deaths per year. Globally, in 2010, 20% of men, 27% of women, and 78% of boys and 84% of girls aged 11-17 years did not meet WHO recommendations for exercise health. The statistics were similar for citizens of the United States, with 25% of men, 39% of women, 78% of boys and 84% of girls failing to meet WHO recommendations. During this period, it is estimated that insufficient physical activity caused 69.3 million disability-adjusted life years–2.8% of the total–globally [21].

Recommended Levels of Exercise

The evidence supporting the beneficial effects of physical exercise is sufficient to have prompted the National Institutes of Health in 1999 to recommend that “Children and adults alike should set a goal of accumulating at least 10 min of moderate-intensity physical activity on most, and preferably all, days of the week” [22]. This amount of exercise has subsequently been determined to be suboptimal and in a 2014 report on global health, the World Health Organization recommended that adult men and women should accumulate at least 150 minutes of moderate intensity physical activity per week and that young people aged 5-17 years should accumulate at least 60 minutes of physical activity of moderate to vigorous intensity daily [21]. A recent policy statement issued by the American Heart Association on the value of primordial and primary prevention of CVD included physical exercise in their recommendation of lifestyle modifications [23].

Exercise and Atherogenesis

This chapter will review the role that myokines, adipokines, endothelial cells, macrophages, telomeres and interleukins play in atherogenesis and how their activities can be modified by regularly performed exercise. No attempt is made to review the effect of exercise on risk factors for atherosclerotic cardiovascular disease (ASCVD) such as hypertension, obesity, hyperlipidemia and type 2 diabetes mellitus. I will start with a brief review of what is currently understood about the immunopathology of atherogenesis.

Atherogenesis

The earliest manifestation of atherosclerosis is the development of subendothelial fatty streaks in large and medium sized arteries. The streaks consist primarily of oxidized or glycated low density lipoprotein (LDL), monocytes, macrophages and foam cells – macrophages bloated by their ingestion of modified lipid. The deposits have
been identified in young adults and can fluctuate in extent depending on a number of variables, including plasma lipid levels. These early manifestations of atherosclerosis appear to represent an innate immune response in which monocytes respond to endothelial cell (EC) and vascular smooth muscle cell (VSMC) chemotactic signals, bind to EC adhesins, migrate into the intima, evolve into macrophages and under the auspices of a mixture of surface scavenger and toll-like receptors (TLRs), ingest modified lipoproteins [24]. Both VSMCs and ECs can differentiate into macrophages thereby contributing to the inflammatory response and foam cell pool.

With time, fatty streaks evolve into atherosclerotic plaques containing elements of both the innate and adaptive immune systems. Proinflammatory cellular elements include Th1 cells, M1-polarized macrophages, IL-12+ dendritic cells, B2 cells, NK cells and NKT cells. Anti-inflammatory elements include Th2 cells, Th3 cells, regulatory T cells, M2-polarized macrophages and regulatory (B1) B cells. Mast cells and a variety of TCD3+ subpopulations have also been identified in atherosclerotic plaques. There is a mixture of proinflammatory cytokines (TNF-α, IFN-γ, IL-1, IL-12, IL-18) and anti-inflammatory cytokines (IL-4, IL-6, IL-10, TGF-β). Antibodies secreted under the direction of Th1 and Th2 cells, Type V collagen produced by infiltrating mast cells, and complement components add to the mixture. Resident macrophages can evolve into dendritic-like cells with advanced capabilities of binding to and activating Th1 cells, thus enhancing the atherosclerotic process. Ultimately, the plaque assumes its most advanced form, with a fibrous cap produced by VSMCs, and a necrotic core containing foam cells, cellular debris, cholesterol crystals, and a mixture of partially digested lipids. Calcium deposits produced by VSMC-derived osteoblasts are also present. Rupture of the cap exposes platelets to the underlying thrombogenic constituents resulting in clot formation and occlusion of an already narrowed lumen. It is this event that precipitates most myocardial infarctions [24-26].

There is a Yin-Yang relationship between the atherogenic and atheroprotective elements, with the former winning out in the process of plaque formation. There is now evidence that moderate levels of physical activity done on a regular basis can arrest or even reverse this relationship [27,28].

Physical Activity and Atherogenesis

Exercise and Myokines

Myokines are contraction-related proteins secreted from skeletal muscle that act on signaling pathways within myocytes and/or other tissues. The first identified and most studied myokine is IL-6, which activates 5’ adenosine monophosphate activated protein kinase (AMPK) and/or phosphatidylinositol-3-kinase (PI3K) to increase
fat oxidation and glucose uptake within myocytes. The level of circulating IL-6 increases in an exponential fashion (up to 100 fold) in response to exercise and declines in the post-exercise period. The increase is related to exercise intensity, duration, the mass of muscle recruited and one’s endurance capacity. Released into the circulation, IL-6 induces lipolysis and fat oxidation in other tissues, including adipocytes, while exerting both local and systemic anti-inflammatory effects by suppressing the production of TNF-α and IL-1 and by stimulating the production of the anti-inflammatory cytokines IL-10 and IL-1 receptor antagonist (IL-1Ra) in immune cells. Although IL-6 is produced by most nucleated cells, including hepatocytes, endothelial cells, T cells and macrophages, it is likely that a significant proportion of exercise-related increases in circulating IL-6 is from contracting skeletal muscle, and that IL-6 contributes to the anti-inflammatory effects of exercise by its effects on lipid metabolism and cytokine production [29].

Exercise also increases the expression of peroxisome proliferator-activated receptor γ coactivator 1α (PGC-1α) in muscle. PGC-1α, a transcription coactivator, stimulates the expression of the membrane protein fibronectin type III domain containing 5 (FNDC5), which is prototypically cleaved to form the myokine irisin. Irisin drives the transformation of white fat cells into brown-in-white or brite, cells with a phenotype similar to brown fat cells. Brown adipose tissue serves as a thermogenic organ in which mitochondrial respiration is uncoupled from ATP production to dissipate energy [30]. In obese mice elevated levels of plasma irisin is followed by a reduction in body weight and an improvement in metabolic homeostasis. Administration of irisin to diabetic mice has been shown to improved fatty acid oxidation and glucose utilization by regulating the AMPK signaling pathway; treated mice experienced reductions in fat weight and serum total cholesterol and triglycerides, attesting to the importance of this myokine as a regulator of glucose and lipid metabolism [31].

A study involving 102 healthy participants doing aerobic and strength endurance training over a period of 26 weeks found no difference between pre- and post-exercise levels of serum irisin; the authors brought into question the validity of several previous studies reporting post-exercise rises in serum irisin levels [32]. In a more recent study involving 28 overweight or obese subjects, 8 weeks of resistance training increased circulating levels of irisin in proportion to muscle mass [33]. Acute strenuous resistance exercises have consistently shown a transient elevation of irisin in the immediate post-exercise period [34-36]; this is very similar to what is seen with IL-6.

Adipokines

It is only recently that we have recognized the critical role of adipose tissue in maintaining metabolic homeostasis. Adipocytes are not simply storage depots for free
fatty acids (FFA); they are critical endocrine organs, that, by the production of adipokines, serve to regulate a variety of metabolic processes and to modulate total body mass. Although over 50 adipokines have been identified with diverse functional roles, adiponectin and leptin have been most closely studied.

Adiponectin enhances insulin sensitivity in muscle and liver and increases FFA oxidation in several tissues, including myocytes. It also decreases serum levels of FFA, glucose, and triacylglycerol. This hormone is a potent inhibitor of TNF-α-induced monocyte expression of adhesion molecules; it also inhibits the transformation of macrophages into foam cells, and thus displays several atheroprotective properties. In mice deficient in apolipoprotein E, adiponectin reduces plaque formation in aortic sinuses by 30% as compared to controls. Plasma concentrations of adiponectin fall with increasing obesity, contributing to insulin resistance and hyperinsulinemia in overweight individuals and, possibly, enhancing the atherogenic process [37].

Leptin is produced in adipocytes and serves to downregulate appetite by binding to leptin receptors in the hypothalamus. Animals and humans deficient in the leptin gene develop morbid obesity and diabetes, both risk factors for atherosclerosis. Unfortunately, although plasma levels of leptin increase with increasing body weight, the rise is accompanied by an increased expression of suppressor-of-cytokine-signaling (SOCS-3) which blocks the central effects of leptin, nullifying the intended feedback control of obesity [37].

In a well-designed study, levels of the adipokine C1q/TNF-related protein 1 (CTRP1) have been found to be elevated in the sera and peripheral blood mononuclear cells (PBMCs) of coronary artery disease patients, coronary endarterectomy specimens, and aortic atherosclerotic plaques. CTRP1 caused a concentration-dependent expression of adhesion molecules and inflammatory markers in human endothelial cells and PBMCs and induced p-38 dependent monocyte-endothelium adhesion in vitro and the recruitment of leukocytes to mesenteric venules in C57BL/6 mice. Intraperitoneal injection of recombinant CTRP1 protein promoted atherogenesis in apoE (-/-) mice which was attenuated in CTRP1 (-/-)/apoE (-/-) double knockout mice. Thus CTRP1 joins other proinflammatory proteins that function as both a marker and promoter of atherogenesis [38].

Exercise and Adipokines

Lee and associates found that ten weeks of forced exercise restored acetylcholine-induced endothelial-dependent vasodilatation in the aortas of type 2 diabetic mice. This effect was blunted in adiponectin knockout mice, suggesting that some of the beneficial effects of exercise on EC function may have been mediated by adiponectin [39]. Another study demonstrated that two years of weekly T’ai Chi exercises performed by individu-
als with cardiovascular risk factors resulted in significant increases in circulating levels of adiponectin [40]. Nascimento and associates found that 8 months of moderate intensity exercise done by 80 overweight and obese children increased adiponectin levels in proportion to reductions in their body mass index; leptin levels and serum inflammatory markers (TNF-α, CRP) fell in response to exercise [41]. A study involving 92 premenopausal women found a similar dose-response effect of exercise-induced weight loss on circulating levels of adiponectin and leptin [42]. In contrast, Sjogren and associates found that 6 months of prescribed physical exercise in 30 sedentary, overweight and abdominally obese subjects failed to alter circulating levels of adiponectin; in addition, pre- and post-exercise biopsies of subcutaneous fat failed to show changes in the expression of adiponectin and a cadre of inflammatory cytokine mRNAs when compared to controls [43]. However, these findings can be explained by the fact that the exercising group only lost 1.5 kg (1.0 kg more that the control group). In a study involving 59 overweight and 52 obese adult subjects, 12 months of moderate intensity exercise resulted in lowering of serum levels of adiponectin; once again, however, subjects did not lose weight as a result of the exercise program [44]. Thus it appears that post-exercise increases in adiponectin can only be anticipated if subjects lose (fat) weight.

Table 1 contains a list of some of the better studied myokines, myo-adipokines, and adipokines.

<table>
<thead>
<tr>
<th>Myokines</th>
<th>Adipo-myokines</th>
<th>Adipokines</th>
</tr>
</thead>
<tbody>
<tr>
<td>BDNF</td>
<td>ANGPTL4</td>
<td>Adiponectin</td>
</tr>
<tr>
<td>IL-7</td>
<td>FGF21</td>
<td>Leptin</td>
</tr>
<tr>
<td>IL-15</td>
<td>FSTL1</td>
<td>Resistin</td>
</tr>
<tr>
<td>Irisin</td>
<td>IL-6</td>
<td></td>
</tr>
<tr>
<td>LIF</td>
<td>MCP-1</td>
<td></td>
</tr>
<tr>
<td>Myonectin</td>
<td>PEDF</td>
<td></td>
</tr>
<tr>
<td></td>
<td>VEGF</td>
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</tbody>
</table>

ANGPTL4-angiopoietin like 4; BDNF-brain-derived neurotrophic factor; FGF-fibroblast growth factor; FSTL1-follistatin-like 1; IL-interleukin; LIF-leukemia inhibitory factor; MCP-1-monocyte chemoattractant protein 1; PEDF-pigment endothelial derived factor; VEGF-vascular endothelial growth factor. *After Raschke and Eckel [27].

Vascular Endothelial Cells

An early event in atherogenesis is the activation of vascular endothelial cells (ECs) in response to the injurious effects of glycated or oxidized LDL, oscillatory or diminished levels of shear stress, infection with agents such as CMV, HIV-1, or Chlamydia pneumoniae, free radicals arising during oxidative stress, heat shock protein 60, hypertension, cigarette smoking and/or inactivity [10-16,45-51]. Activated ECs upregulate their production of chemokines, adhesins, VSMC growth and activation factors and downregulate their production of TGF-β, vasodilators, antioxidants, and anticoagulants. The endothelium is dysfunctional, particularly with regard to its ability to maintain normal vascular tone, and is distinctly proatherogenic [25,52-56] (Table 2).
Table 2: Functional Characteristics of Proatherogenic Vascular Endothelial cells.

<table>
<thead>
<tr>
<th>Monocyte &amp; T cell recruitment</th>
<th>VCAM-1, RANTES, MCP-1</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vascular smooth muscle cell growth</td>
<td>PDGF-A, PDGF-B, TGF-β</td>
</tr>
<tr>
<td>Vasoconstriction</td>
<td>ET-1, ECE, ACE, NO, PGI₂, CNP, AM</td>
</tr>
<tr>
<td>Clotting</td>
<td>tPA, TM, NO</td>
</tr>
<tr>
<td>Oxidation</td>
<td>COX-1, COX-2, SODs</td>
</tr>
</tbody>
</table>

VCAM-1-vascular cell adhesion molecule 1; RANTES-regulated on activation normal T expressed and secreted chemokine; MCP 1-mono-ocyte chemoattractant protein 1; PDGF-platelet-derived growth factor; TGF-β, transforming growth factor β; ET-1- endothelin 1; ECE-endothelin-converting enzyme; ACE-angiotensin-converting enzyme; NO-nitric oxide; PGI₂-prostacyclin; CNP-C-type natriuretic peptide; AM-adrenomedullin; TM-thrombomodulin; tPA-tissue-type plasminogen activator; COX-cyclooxygenase; SODs-superoxide dismutases.

Exercise and Endothelial Cells

Exercise can normalize EC function in persons prone to atherogenesis [57]. The manner in which this occurs is incompletely understood, but hemodynamic forces, particularly shear stress, are known to exert a powerful influence on EC phenotype and function, and likely play a major role in the atheroprotective effects of exercise. In addition, aerobic exercise has been shown to protect against endothelial dysfunction by increasing nitric oxide and hydrogen peroxide production in LDL receptor-deficient mice [58].

Shear stress mechanosensors include integrins, platelet EC adhesion molecules (PECAM)-1, cadherins, glyco-calyx (whose intraluminal projections uncoil in the direction of flow) and primary cilia. Shear stress is also sensed by caveolae (small eNOS-binding membrane invaginations), G-protein-coupled receptors, and tyrosine kinase receptors. These events initiate a number of signal transduction pathways that regulate gene expression of focal adhesion kinase (FAK), Rho family GTPases, PI3 kinase, mitogen-activated protein kinases (MAPKs), protein kinase C (PKC) and nuclear factor–kB (NF-kB). The resultant change in endothelial phenotype is dependent on the type of shear stress. Laminar blood flow produces predominantly antegrade shear stress along the EC surface which favors an atheroprotective EC phenotype, whereas retrograde, oscillatory and slow shear stress of the type that occurs at bifurcations in the arterial tree predispose to an atherogenic EC phenotype.TWIST1, a transcription factor activated by a number of signal transduction pathways (STAT3, Wnt, Ras, MAPK) has been shown to be expressed preferentially at low shear stress regions of adult arteries where it promotes atherogenesis by inducing EC proliferation and inflammation [59].

Evidence supporting the role of shear stress in maintaining a healthy endothelial cell phenotype is found in a study done by Restaino and associates demonstrating that endothelial dysfunction in the popliteal arteries of 10 young healthy men following prolonged sitting was medi-
ated by a reduction in shear stress [60]. In addition, Seawright and associates found that short-term increases in pressure and shear stress attenuated age-related declines in endothelial function in skeletal feed muscle feed arteries of experimental rats [61]. Shear stress levels in the arteries of humans during exercise have been shown to be in the range that produces an anti-atherogenic EC phenotype, and, in some areas, reduce oscillatory flow [62]. In mice, 5 weeks of voluntary wheel running was found enhance mitochondrial biogenesis in vascular endothelium through a shear stress-dependent mechanism [63].

**Monocytes and Macrophages**

Monocytes and macrophages are the dominant cell type in the early stages of atherogenesis and continue to represent a key effector cell population in the later stages when cell-mediated immune responses gain a progressively important foothold. In atherogenesis, recruited monocytes can evolve into a variety of phenotypes with different functional characteristics. On the atherogenic side, this includes M1-polarized macrophages and IL-12+DCs and on the atheroprotective side M2-polarized macrophages and CD11c+DC-like cells.

TLRs play a central role in macrophage activation and provide a strong link between local innate and adaptive immune responses in atherosclerosis. Activation of these receptors triggers an intracellular signaling cascade mediated through myeloid differentiation factor 88 or toll/interleukin-1 receptor-domain-containing adapter-inducing interferon-β, leading to the secretion of pro- and anti-inflammatory cytokines [64]. LDL-R permits ingestion of native LDL and scavenger receptors SR-A, CD36, CD68 permit ingestion of lipids that have been oxidized, glycated, or acetylated with consequent enhancement of foam cell formation. These modified lipids (most notably ox-LDL) are potent stimulators of proinflammatory cytokine and chemokine secretion, including TNF-α, IL-1β and monocyte chemotactic protein 1 (MCP-1) [65].

**Exercise and Monocytes and Macrophages**

A study in which 17 sedentary subjects undertook an 8-week low-intensity exercise program (walking 10,000 steps/day, three times a week) documented an upregulation of M2 phenotype markers (AMAC1, CD14, MR, IL-4) and a downregulation of M1 phenotype markers (MCP-1, TNF-α, IL-6) in circulating monocytes. This was associated with a corresponding increase in plasma levels of Th2 cytokines and a decrease in plasma levels of Th1 cytokines [66].

A study done in C57BL/6J mice found that exercise enhanced skeletal muscle insulin sensitivity by promoting influx of M2 macrophages [67]. Several studies in diet-induced obese mice have demonstrated that exercise inhibits inflammation in adipose tissue by suppressing macrophage infiltration and by accelerating phenotypic switching from M1 to M2 macrophages [68-70].
Telomeres

Telomeres are specialized nucleoprotein structures that protect chromosomal ends from degradation. These structures progressively shorten during cellular division and can signal replicative senescence when shortened below a critical level.

The premature aging syndromes Progeria (Hutchinson-Guilford-Progeria syndrome, HGPS) and Werner’s syndrome (WRNS) have in common early onset atherosclerosis and ASCVD-related death. Although HGPS is due to a gain-of-function mutation in the LMNA gene and WRNS is due to a loss-of-function mutation in Werner syndrome ATP-dependent helicase, both conditions are characterized by premature senescence and exhaustion of mesenchymal stem cells (MSCs), the source of VSMCs in arterial walls; in both conditions, there is also premature shortening of telomeres. Interestingly, MSC exhaustion and telomere shortening are also characteristic of the normal aging process [71] with telomere shortening predisposing to the development of metabolic disorders and ASCVD [72].

Effect of Exercise on Telomere Length

In a meta-analysis of 37 clinical studies, Mundstock and associates found no clear association between exercise and telomere length; however, as they noted, there was an important methodological diversity in these studies and only a few were done prospectively [73].

In a recently published clinical study on exercise and telomere length, Denham and associates have resolved this uncertainty. They found that endurance athletes (n = 61) had significantly longer leukocyte telomeres and expression of telomere regulating genes (TERT and TPPI) than healthy controls (n = 61). On dividing the results based on distance covered per week bicycling or running, they found that subjects in the top two quartiles had the longest telomeres. Importantly, they also noted that the resting heart rate, a measure of cardiorespiratory fitness, was an independent predictor of leukocyte telomere length and TERT and TPPI mRNA expression [74]. Thus, their study provides one explanation for the well documented association between lifespan and cardiorespiratory fitness.

Interleukins

Interleukin-1

Histopathologic data indicate that IL-1 is abundant in atherosclerotic tissue compared with healthy controls and that levels are particularly increased within the vessel walls of subjects with the acute coronary syndrome (ACS). The naturally occurring antagonist of IL-1, IL-1 receptor antagonist (IL-1Ra) is also found in atherosclerotic tissue, and the balance between these 2 cytokines may determine a number of cellular responses [75]. IL-1 is secreted by a variety of cells, including ECs, macrophages, monocytes, lymphocytes, neutrophils and fibroblasts. Cells displaying IL1R1 include, most prominently, T cells, epithelial cells, ECs and fibroblasts [76].

IL-1α remains mostly in the cytosol, where it func-
tions as an autocrine messenger. It is secreted by senescent VSMCs in atherosclerotic lesions and can, as an autocrine, induce a proinflammatory senescence-associated VSMC phenotype, prompting the release of high levels of multiple chemokines and proinflammatory cytokines. These senescent VSMCs also release active matrix metalloproteinase-9, secrete less collagen, upregulate multiple inflammasome components, and prime adjacent epithelial cells and VSMCs to a proadhesive and proinflammatory state [77].

IL-1β is cleaved for biological activity by the inflammasome protease, caspase c [75]. It is a potent proinflammatory cytokine, capable of activating ECs and VSMCs in atherosclerotic lesions. It enhances VSMC proliferation and migration through the upregulation of their P2Y2 receptors and consequent RAGE expression and HMGB1 release [78]. IL-1β can also trigger the transdifferentiation of VSMCs into the stellate VSMC phenotype via the secretion of PGE2 and cAMP-dependent protein kinase activation [79]. This cytokine promotes the differentiation of naïve CD4+ T cells into the proinflammatory Th17 phenotype [76].

Persons genotypically predisposed to generating large amounts of IL-1β are at high risk of developing ACS if they have elevated levels of apoB-associated oxidized phospholipids (OxPL/apoB+), emphasizing the critical role plasma lipids play in potentiating the inflammatory effects of IL-1β [80]. There is also an association between IL1Ra gene polymorphisms and the severity of coronary artery disease. In this regard, minimally modified LDL induces gene expression of IL-1β in human PBMCs in a dose-dependent manner and gene expression of IL-β1 has been shown to be elevated in PBMCs of patients with coronary artery disease [81]. Studies on the role of IL-1 in atherogenesis have shown that IL-1 knockout mice develop less atherosclerosis than control mice.

**Tumor necrosis factor-β**

There is comparatively little literature on the specific role of TNF-α in atherogenesis. In blood cellular sources of TNF-α include Th1 cells, some Th2 cells, some cytotoxic T cells and activated M1-polarized macrophages [76]. Like the other proinflammatory cytokines it is proficient in inducing a proadhesive and proinflammatory state in ECs. It also increases vascular permeability, facilitating the influx of cellular and plasma elements into subendothelial areas and enhances the calcification of VSMCs in atherosclerotic lesions [82]. Importantly, TNF-α inhibits the anti-inflammatory cytokine, IL-10. It can also deliver activating signals to M1 macrophages. In experimental apolipoprotein E knockout mice with hyperlipidemia and coronary artery EC dysfunction, neutralization of TNF-α or deletion of the TNF-α gene, has been shown to reestablished normal EC function [83].

**Interferon-γ**

IFN-γ is often referred to as a master regulator of atherogenesis. It is produced by Th1 cells (main source),
cytotoxic T cells, NK cells, NK T cells, B2 B cells and activated M1-polarized macrophages [76]. In one study 70% of atherosclerotic plaques contained IFN-γ mRNA [26] and in another study, the majority (81%) of T cell clones isolated from atherosclerotic plaques were shown to produce IFN-γ [84].

IFN-γ upregulates the expression of adhesion molecules (ICAM-1, VCAM-1) in ECs, prompting the influx of monocytes and macrophages during the early stages of atherogenesis. It promotes the differentiation and subsequent migration of VSMCs into plaque regions, and can destabilize fibrous caps by inhibiting collagen synthesis in VSMCs. IFN-γ is responsible for activating M1-polarized macrophages, NK cells and NKT cells. It also induces macrophage expression of a number of genes involved in cholesterol metabolism, including ApoE, ATP-binding cassette transporter A1 (ABCA1), and Acetyl-CoA acetyltransferase 1 (ACAT1). By increasing ACAT1 expression and attenuating ABCA1 expression, IFN-γ has been shown to reduce cholesterol efflux in foam cells, thereby contributing to the accumulation of cholesterol esters in these cells. It further contributes to foam cell development by upregulating receptors involved in the binding of phosphatidylerine and oxidized lipids, and can initiate foam cell apoptosis, causing them to expel their contents into the intima, contributing to the lipid-rich necrotic core and extracellular matrix degradation [85].

The role of IFN-γ in atherogenesis has been demonstrated in transgenic mice with targeted disruption of apoE and IFN-γ receptor genes (aopE0/IFN-γR0 mice). These mice demonstrate a reduction in atherosclerotic lesion size, cellularity and lipid content [86].

**Anti-Atherogenic Interleukins**

**Interleukin-4**

IL-4 has been shown to be present in 10% of atherosclerotic plaques [26] and to be secreted by 81% of T cell clones isolated from atherosclerotic plaques [84]. It is produced in Th2 cells (main source), γ/δ T cells, NKT cells, mast cells, eosinophils and basophils. IL-4 exerts its anti-inflammatory effects by inhibiting the differentiation of IFN-γ-producing Th1 cells and, secondarily, the transition of monocytes into M1-polarized macrophages. IL-4 also promotes the differentiation of antigen-stimulated naïve T cells into Th2 cells. It has been shown to directly promote M2-polarized macrophage differentiation via the MAPK signaling pathway [87].

**Interleukin-10**

IL-10 is a key regulator of the inflammatory response. It is mainly produced by Th2 cells, Tregs, Tc2 cells, M2 macrophages, B1 B cells, monocytes and dendritic cells. Its primary function is to limit the production of TLR agonist-induced production of chemokines and cytokines (including IL-1α, IL-1β, and TNF-α) in macrophages and dendritic cells. IL-10 directly suppresses monocyte and
macrophage functions by downregulating their surface expression of class II MHC molecules and costimulatory molecules CD80/CD86. It also inhibits cytokine production and proliferation of CD4+T cells (both Th1 and Th2 phenotypes) through its suppressive effects on antigen presentation and expression of co-stimulatory molecules [76]. It causes “massive” downregulation of TNF mRNA in macrophages [88].

**Transforming Growth Factor -β**

TGF-β1 has been shown to be present in large amounts in atherosclerotic plaques [26]. It is mainly produced in Th2 cells, Th3 cells, Tregs, Tr1 cells, and M2-polarized macrophages. TGF-β1 is secreted in latent form and must bind to α2β2 integrins on epithelial cells or αvβ8integrins on immune cells to become active. The anti-inflammatory effects of this interleukin are evidenced by the fact that TGF-β1-deficient mice die within 4 weeks of massive infiltrations of lymphocytes and macrophages in multiple organ systems in what appears to be a systemic autoimmune response. In this regard, TGF-β1 activates CD4+CD25+Foxp3+ regulatory T cells whose anti-inflammatory effects are mediated by both TGF-β1 and IL-10. TGF-β1 inhibits TNF-α at the translation level in macrophages [89].

It should be noted that, in conjunction with other cytokines, TGF-β1 can promote the differentiation of Th22 cells (TGF-β1 + IL-6) and Th17 cells (TGF-β1 + IL-6, IL-21 and IL-23); both these phenotypes are pro-inflammatory, emphasized the contextual nature of TGF-β1 actions.

**Exercise and Interleukins**

In a study involving 43 adult subjects at risk of developing ASCVD, we found that 6 months of moderate-intensity exercise attenuated PBMC production of IFN-γ, TNF-α and IL-1α and augmented their production of IL-2, IL-4, IL-6, IL-10 and TGF-β1 [79]. Study participants averaged two and one-half hours of moderate intensity exercise per week – the same amount of exercise recommended for adult men and women by the World Health Organization for maintenance of health. Participants divided their time between aerobic exercises (57%), resistance exercises (35%) and flexibility exercises (8%) and spent an average of 71 minutes in each training session which occurred on average three times a week. Extended over a six-month period, this amount of exercise was sufficient to reduce the spontaneous and PHA-induced production of the atherogenic cytokines by 24% and 59% respectively, and to increase the spontaneous and PHA-induced production of the anti-atherogenic cytokines by 89% and 50%, respectively. Importantly, in PHA-stimulated cultures, proinflammatory cytokine production fell in proportion to the time subjects spent in each training session doing aerobic exercises [90].

A subsequent study involving 28 subjects with documented coronary artery disease found that 12 weeks of aerobic exercise training resulted in a reduction in plasma
levels of IL-1, IFN-γ, and CRP, and an increase in IL-10 levels [19]. Recent research involving type 2 diabetics showed that a combination of aerobic and resistance exercise was particularly effective in reducing blood levels of IL-1β and TNF-α and increasing levels of IL-4, IL-10 and adiponectin [91]. Tartibian and associated found that 16 weeks of low to moderate levels of aerobic exercise decreased serum levels of IL-6, TNF-α, and IL-1β in 15 post-menopausal women [92]. Other studies, including one on the effects of exercise on inflammatory markers in HIV+ subjects, gave similar results [93-95] (Table 3).

### Summary

Long-term moderate intensity exercise exerts its beneficial effects by changing the balance between immune cells producing atherogenic cytokines and those producing atheroprotective cytokines. An example of this change is seen in the shift from M1-polarized to M2 polarized macrophages in blood and adipose tissue following exercise training. There are likely multiple contributors to this change, including shear stress-related normalization of endothelial cell function and the production of anti-inflammatory myokines, most notably, IL-6. By increasing telomere length, exercise may also exert a favorable effect on age-related contributions to atherogenesis.

<table>
<thead>
<tr>
<th>Subjects</th>
<th>Duration</th>
<th>Intensity</th>
<th>Resistance</th>
<th>Aerobic</th>
<th>Flexible</th>
<th>Time/session</th>
<th>Result</th>
<th>Ref</th>
</tr>
</thead>
<tbody>
<tr>
<td>51 women, 15 men, aged 60-40</td>
<td>26 w</td>
<td>66% MHR</td>
<td>SET grp (40)</td>
<td>No</td>
<td>Tread- mill grp (20)</td>
<td>40</td>
<td>No change in atherogenic cytokines</td>
<td>32</td>
</tr>
<tr>
<td>11 women, 17 men, overweight or obese, mean age 26</td>
<td>6 w</td>
<td>65-85% MHR</td>
<td>SET grp (33)</td>
<td>SET grp (10)</td>
<td>No</td>
<td>40</td>
<td>No change in atherogenic cytokines</td>
<td>33</td>
</tr>
<tr>
<td>5 girls, 62 boys, overweight or obese</td>
<td>8 m</td>
<td>Not defined</td>
<td>Not defined</td>
<td>Not defined</td>
<td>Not defined</td>
<td>10</td>
<td>No change in atherogenic cytokines</td>
<td>41</td>
</tr>
<tr>
<td>157 pre-menopausal women</td>
<td>5 m</td>
<td>30-80% MHR</td>
<td>No Tread- mill, cycling</td>
<td>No</td>
<td>Stretch (8%)</td>
<td>40</td>
<td>↑irisin levels in resistance grp</td>
<td>33</td>
</tr>
<tr>
<td>14 women, 29 men, age 36 ± 10</td>
<td>12 m</td>
<td>56 ≥ 80%</td>
<td>MHR</td>
<td>No</td>
<td>Tread- mill, cycling</td>
<td>50</td>
<td>↑irisin levels, ↓TNF-α, BMI</td>
<td>42</td>
</tr>
<tr>
<td>57 girls, 62 boys, overweight or obese</td>
<td>8 m</td>
<td>Not defined</td>
<td>Not defined</td>
<td>Not defined</td>
<td>Not defined</td>
<td>5</td>
<td>↑irisin levels, ↓TNF-α, BMI</td>
<td>44</td>
</tr>
<tr>
<td>10 women, 18 men, CAD, age 46±12</td>
<td>12 w</td>
<td>60±80% MHR</td>
<td>No</td>
<td>Various</td>
<td>No</td>
<td>40</td>
<td>↑irisin levels, ↓TNF-α, BMI</td>
<td>45</td>
</tr>
<tr>
<td>15 post-menopausal women</td>
<td>16 w</td>
<td>≥ 55% MHR</td>
<td>No</td>
<td>Various</td>
<td>No</td>
<td>25-30 m</td>
<td>↑irisin levels, ↓TNF-α, BMI</td>
<td>46</td>
</tr>
<tr>
<td>27 women, 15 men, mean age 46</td>
<td>8 m</td>
<td>60-80%</td>
<td>MHR</td>
<td>Weight loss (10%)</td>
<td>Various (50%)</td>
<td>Stretch (6%)</td>
<td>30-120 min</td>
<td>↑irisin levels, ↓TNF-α, BMI</td>
</tr>
<tr>
<td>11 women, 28 men, CAD age 59 ± 2</td>
<td>6 w hospital, 6 w home</td>
<td>50-80% VO2 max</td>
<td>No</td>
<td>Tread- mill, cycling</td>
<td>No</td>
<td>30-40 m</td>
<td>↑irisin levels, ↓TNF-α, BMI</td>
<td>48</td>
</tr>
<tr>
<td>13 women, 17 men, overweight or obese age 42 ± 11</td>
<td>12 w</td>
<td>High</td>
<td>NRT</td>
<td>No</td>
<td>Stretch (30-40% body fat)</td>
<td>30</td>
<td>↑irisin levels, ↓TNF-α, BMI</td>
<td>49</td>
</tr>
<tr>
<td>15 men, age 58 ± 7</td>
<td>14 w</td>
<td>High</td>
<td>NRT</td>
<td>No</td>
<td>Stretch (40-70% body fat)</td>
<td>30</td>
<td>↑irisin levels, ↓TNF-α, BMI</td>
<td>50</td>
</tr>
</tbody>
</table>

MHR—maximum heart rate; SET—strength endurance training; NLRT—non-linear resistance training; CAD—coronary artery disease; HIV—human immunodeficiency virus; m—minutes; w—weeks; av—average; grp—group; hosp-hospital training facility. Note that although serum levels of IL-6 increase briefly following acute exercise, they fall in the setting of long-term exercise; in contrast, PBMC production of this pleiotropic cytokine increases following long-term exercise.
Recommendations

Exercise is the single most cost-effective way to preserve life and well-being, yet it is seldom prescribed by health care professionals.

The author recommends that health care givers prescribe exercise to all eligible adults in the following manner: 1. Calculate the patient’s maximum heart rate (MHR) using the formula MHR = 220-age; 2. Have the patient start exercising at 40-50% of MHR and then gradually increase the level to 70-80% of MHR; 3. Have the patient exercise at 70-80% of MHR at least 150 minutes per week in accordance with WHO recommendations.

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