REVIEW

Is the anti-inflammatory effect of regular exercise responsible for reduced cardiovascular disease?

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ABSTRACT

Engaging in regular physical activity reduces the risk of developing CVD (cardiovascular disease), but it is not certain to what degree this may be due to the anti-inflammatory effects of exercise. Following acute exercise, there is a transient increase in circulating levels of anti-inflammatory cytokines, whereas chronic exercise reduces basal levels of pro-inflammatory cytokines. Exercise training also induces the expression of antioxidant and anti-inflammatory mediators in the vascular wall that may directly inhibit the development of atherosclerosis. Limited studies in humans and more comprehensive assessments in animal models have confirmed that exercise is atheroprotective and helped identify a number of the mechanisms to explain these effects. This review explores the relationship between systemic and vascular wall inflammation and the role that the anti-inflammatory effects of exercise have on the development and progression of CVD.

INTRODUCTION

It is well established that physically fit individuals have a reduced risk of developing CVD (cardiovascular disease) and other age-related chronic disorders [1]. However, the mechanisms responsible for the protective effects of exercise are not completely clear. CVD is associated with chronic low-level inflammation indicated by modest (2–4-fold) elevations in circulating pro-inflammatory cytokines and the acute-phase reactant CRP (C-reactive protein) [2]. It is difficult to determine if these inflammatory variables are a cause or a consequence of particular disorders, but recent evidence suggests they may play a key role in the pathogenesis of CVD and other chronic diseases [3]. One way that exercise may modify CVD risk is by reducing inflammation. The purpose of this review is to examine the effects of regular exercise on established markers and mediators of inflammation and discuss the impact that changes in these variables may have on the development and progression of CVD.

INFLAMMATION AND CVD

Atherosclerotic CVD is the leading cause of death in developed countries [4]. Atherosclerosis was formerly characterized as an accumulation of lipids in the artery...
wall, but today is recognized as a chronic inflammatory disorder that results from the interactions between modified lipoproteins and various components of the immune system, including monocyte-derived macrophages, T-lymphocytes and a variety of cytokines secreted by these and other cells in the artery wall [5].

Many factors contribute to the development of atherosclerosis, but the oxidation of the protein and lipid components of LDL (low-density lipoprotein) particles is believed to be among the most important initiating events [5]. Elevated LDL is a well-known CVD risk factor and can drive the development of atherosclerosis even in the absence of other known risk factors. LDL is normally permeant to the arterial endothelium [6], but when levels are elevated it can accumulate in the subendothelial (intimal) space, where it becomes oxidized by enzymes such as myeloperoxidase, 15-LO (lipoxygenase) and iNOS (inducible nitric oxide synthase) [5]. According to the ‘Response-to-Retention Hypothesis’, ox-LDL (oxidized LDL) is retained in the intima and associates with extracellular matrix proteins where it initiates many of the pro-inflammatory effects associated with the development of atherosclerotic plaques [7]. The inflammation initiated by ox-LDL is mediated largely by the local production of pro-inflammatory cytokines in the artery wall. These cytokines promote endothelial cell dysfunction, regulate macrophage and T-cell activation and proliferation, alter SMC (smooth muscle cell) phenotype, induce apoptosis, and promote plaque rupture and thrombosis [8–10].

Chronic systemic inflammation is also believed to contribute to the development of atherosclerosis. Circulating levels of numerous inflammatory variables have been shown to modify CVD risk, including CRP, TNF-α (tumour necrosis factor-α), IL (interleukin)-6, IL-2, IL-7, IL-8, IL-18, sCD40L (soluble CD 40 ligand) and MCSF (macrophage colony-stimulating factor) (reviewed in [11]). Several of these markers are increasingly being used for CVD risk stratification [12], but determining whether they are a cause or a consequence of atherosclerosis remains a challenge. Identifying the contribution that individual cytokines make to the development of atherosclerosis is difficult due to the interactions and redundancy between the numerous pro- and anti-inflammatory cytokines that define chronic inflammation. Furthermore, most secreted cytokines are rapidly cleared by surrounding cells or inactivated by soluble inhibitors, thus measuring their circulating levels does not necessarily reflect their biological activity in tissues. A thorough examination of the role of each of these cytokines in atherosclerosis is beyond the scope of this review, but serum IL-6, TNF-α and CRP have emerged as particularly strong independent risk factors for CVD [13], thus a brief overview of the mechanisms believed to be responsible for the relationship between these markers and atherosclerosis is outlined below.

IL-6 is found primarily in the circulation, secreted by numerous cell types, including activated macrophages and lymphocytes, in response to IL-1 and TNF-α. IL-6 is traditionally described as a pro-inflammatory cytokine based on the fact that serum levels increase in response to sepsis [14], it stimulates the production of pro-inflammatory cytokines from monocytes and macrophages [15], and up-regulates CRP production by the liver [16]. Elevated serum IL-6 is associated with unstable angina [17], increases the risk of future MI (myocardial infarction) [18] and has been linked to all-cause and CVD mortality [19]. IL-6 also has been hypothesized to promote atherosclerosis directly by enhancing endothelial expression of chemokines and adhesion molecules, increasing endothelial dysfunction and promoting a procoagulant state [20].

IL-6 also has several anti-inflammatory actions. These include inhibiting the production of TNF-α in response to pro-inflammatory stimuli, such as endotoxin [21], and stimulating the production of the anti-inflammatory cytokines IL-1ra (IL-1 receptor antagonist), IL-10 and sTNFRs (soluble TNF-α receptors) [22]. In vivo studies appear to support an anti-inflammatory role for IL-6; deficiency in IL-6 has been shown to promote atherosclerosis in both C57/Bl6 [23] and ApoE (apolipoprotein E)-deficient (ApoE-/-) mice [24], and had no effect on atherosclerosis in LDLr (LDL-receptor)-deficient (LDLr-/-) mice [25]. Perhaps due to these apparent pro- and anti-inflammatory activities, the role of IL-6 in atherosclerosis and other inflammatory disorders, such as Type 2 diabetes [22], is controversial. Because TNF-α stimulates the production of IL-6 in cells and tissues, it has been hypothesized that chronically elevated IL-6 is simply a marker for ongoing TNF-α production during low-grade inflammation and may not contribute directly to the pathogenesis of inflammatory diseases [22].

In contrast with IL-6, TNF-α is clearly a pro-inflammatory cytokine. TNF-α mediates local inflammatory responses, the systemic acute-phase response and is thought to play a central role in the development of CVD, Type 2 diabetes and the metabolic syndrome [26]. In terms of CVD, elevated circulating levels of TNF-α predict risk of MI [18], the severity of PAD (peripheral arterial disease) [27] and the extent of carotid artery atherosclerosis in healthy middle-aged men [28]. TNF-α is prevalent in atherosclerotic lesions [29] and induces the expression of adhesion molecules such as ICAM-1 (intracellular adhesion molecule-1) [30] and E-selectin [31]. TNF-α as well as IL-1 also promote low shear-stress-induced neointimal hyperplasia [32], a hallmark feature of atherosclerosis. In addition, TNF-α deficiency inhibits aortic atherosclerosis in ApoE-/- mice, despite these mice having slightly elevated cholesterol levels [33]. This evidence strongly suggests that TNF-α has a direct effect on the development and progression of atherosclerosis.
CRP, an acute-phase protein secreted from the liver in response to IL-6, has emerged as a strong predictor of CVD risk in some studies [34], prompting some to suggest it should be measured as a routine clinical risk assessment test [35]. Indeed, evidence from primary prevention trials indicates that elevated CRP levels predict future CVD events [36]; however, there is little evidence to date that demonstrates lowering CRP levels reduces CVD risk [12]. Furthermore, recent reviews have cast doubt on the efficacy of CRP in risk prediction for CVD, suggesting that it does not significantly improve risk discrimination enough to recommend widespread screening [37].

In addition to being a marker of inflammation associated with atherosclerosis, some evidence suggests that CRP may also be a causal risk factor. CRP has been implicated in all phases of atherosclerosis, from the initial recruitment of inflammatory cells into the arterial wall to plaque rupture [3]. It exerts particularly important effects on endothelial function by inducing endothelial cell expression of adhesion molecules, such as ICAM-1, VCAM-1 (vascular cell adhesion molecule-1) and P-selectin [38], inhibiting the expression and activity of eNOS (endothelial nitric oxide synthase) [34] and reducing prostacyclin release [39]. CRP also induces monocyte recruitment by up-regulating endothelial cell expression of MCP-1 (monocyte chemotactic protein-1) [40] and induces monocyte chemotaxis by up-regulating monocyte expression of the MCP-1 receptor CCR-2 (CC chemokine receptor-2) [41]. Together, these factors promote the recruitment, binding and uptake of inflammatory cells, such as macrophages and T-cells, into the artery wall.

CRP also is found in atherosclerotic lesions co-localized with foam cells [42]. It has been shown to bind and opsonize LDLs in the artery wall, enhancing their uptake by macrophages [43], and activates the complement pathway [44], leading to the production of pro-inflammatory cytokines by vascular cells. CRP also promotes plaque instability by inducing endothelial expression of MMP (matrix metalloproteinase)-1 and MMP-10 [45], and promotes thrombosis by inducing the expression of PAI-1 (plasminogen activator inhibitor-1) [46].

Once considered just a marker of inflammation, this evidence indicates that CRP may be directly involved in the development and progression of atherosclerosis. However, some recent reports suggest that many of the inflammatory in vitro effects attributed to CRP may have been artifacts caused by the presence of contaminants in the CRP preparation used in the experiments [11]. Furthermore, the overexpression of CRP in ApoE−/− mice had either no effect [47] or produced only a modest increase [48] in atherosclerosis, and one report even indicates some protective effects of CRP on atherogenesis in mice [49]. As a result, there is considerable controversy in the literature as to whether CRP is directly involved in the development of atherosclerosis and its complications. More research will be needed clarify these issues.

EXERCISE AND MARKERS/MEDIATORS OF INFLAMMATION

Exercise/oxidation paradox

Atherosclerosis has been described as an inflammatory response to ox-LDL in the artery wall [5]. Although physical fitness and regular exercise are associated with reductions in CVD risk, acute exercise paradoxically increases oxidative stress. During exercise, whole-body $\dot{V}$O$_2$ (oxygen consumption) increases dramatically and is associated with increases in the production of ROS (reactive oxygen species) by skeletal muscle and other tissues, although the primary sources of this exercise-induced oxidative stress is controversial [50]. The increased oxygen flux through the mitochondrial electron transport chain is believed to be the main source of free radical generation during exercise, but many metabolic pathways in various tissues contribute to the generation of free radicals by reactions during exercise through the induction of enzymes such as xanthine oxidase, NADPH oxidase and myeloperoxidase [51]. The induction of these pro-oxidant enzymes by skeletal muscle and other tissues leads to an increase in plasma markers of oxidative stress, such as F2-isoprostanes and myeloperoxidase. Circulating levels of these markers are independently associated with CVD risk [52,53], but it is unclear if oxidative stress in the circulation directly promotes the oxidation of LDL in the vascular wall. Wetzstein et al. [54] have shown that acute exercise increases the susceptibility of LDL to undergo in vitro oxidation, implying that this may also happen in the circulation. However, ox-LDL in the plasma is cleared rapidly by the liver [55], so probably does not promote inflammation like LDL that is oxidized and retained in the artery wall. There is even evidence that plasma oxidative stress may be atheroprotective. Meilhac et al. [56] have shown that acute exercise in mice increases antibodies to oxidatively modified lipoproteins in the plasma, but this was associated with the induction of the antioxidant enzyme catalase in the vascular wall after just 1 week of exercise. This indicates that an exercise-induced plasma oxidative stress may stimulate an arterial antioxidant response, which should inhibit LDL oxidation, inflammation and ultimately atherosclerosis [56]. Additional studies are needed to confirm this hypothesis.

In contrast with acute exercise, chronic exercise appears to enhance antioxidant defences in skeletal muscle, the circulation and the vasculature by a variety of mechanisms. In skeletal muscle, exercise training increases the activity of the antioxidant enzymes glutathione peroxidase and SOD (superoxide dismutase) [50,51]. These
Atherosclerosis is an inflammatory disorder that can be initiated by the oxidation of LDL in the sub-endothelial space via enzymes such as 15-LO and iNOS. Ox-LDL induces the expression of pro-inflammatory cytokines (not shown) that promote vascular dysfunction, macrophage and T-cell activation, proliferation and foam cell formation, and alter SMC phenotype. Inflammation also promotes VC, a regulated process similar to bone formation that is mediated by resident SMCs, CVCs and COPCs that can differentiate into cells with an osteoblast-like phenotype and promote mineral (hydroxyapatite) deposition. Chronic exercise has a number of anti-inflammatory effects that may inhibit both atherosclerosis and VC. These include altering circulating levels of lipids and inflammatory mediators and inducing endothelial cell expression of eNOS and antioxidant enzymes, such as catalase and ecSOD. See text for more details. Solid arrows indicate movement of cells, and broken arrows represent regulatory actions; (+) and (−), induction and inhibition respectively; Ex+ and Ex−, chronic exercise increases or decreases respectively, circulating levels or tissue expression of the indicated protein. Abbreviations: ALP, alkaline phosphatase; BMP, bone-matrix protein; EC, endothelial cell; FC, foam cell; mΦ, macrophage; OC, osteocalcin; OP, osteopontin; Th, T-helper cell.

Adaptations may dampen the metabolic perturbations in skeletal muscle induced by bouts of acute exercise. Chronic exercise also reduces markers of oxidative stress in the plasma, including F2-isoprostanes [57] and myeloperoxidase [58]. In the vasculature, exercise training-induced increases in eNOS activity have many direct and indirect effects on oxidative stress and inflammation. For example, Fukai et al. [59] demonstrated that 3 weeks of treadmill running in C57/Bl6 mice produced approx. 3-fold increases in the aortic expression of eNOS and ecSOD (endothelial cell SOD), a potent antioxidant in the vasculature. However, ecSOD expression was not increased by exercise in eNOS−/− mice, indicating that the changes in ecSOD were dependent on the exercise-induced increases in eNOS expression. NO also has been shown to induce HO-1 (haem oxygenase-1) gene expression by VSMCs (vascular SMCs) [60]. HO-1 has potent antioxidant and anti-inflammatory functions via reducing tissue levels of the pro-oxidant haem and inhibiting monocyte recruitment into the vascular wall [61]. Lastly, endothelium-derived NO may reduce inflammation in the artery wall by inhibiting activation of NF-κB (nuclear factor-κB). NF-κB is a transcription factor that is involved in the regulation of many of the pro-inflammatory genes linked to atherosclerosis, and probably mediates many of the anti-atherogenic effects ascribed to NO, including inhibiting leucocyte binding and chemotaxis, the aggregation of platelets and the proliferation of SMCs (reviewed in [62]). As a result, the exercise-induced induction of eNOS may be responsible for a significant part of the anti-inflammatory effects of exercise training in the vascular wall (Figure 1).

**EXERCISE AND CIRCULATING CYTOKINES**

Because systemic markers of inflammation are elevated in CVD and other diseases, modifying their levels through exercise or other means may have therapeutic potential. The cytokine response to exercise has been the subject of...
several recent reviews [22,26], so will only be discussed briefly here. During acute exercise, working muscles release a number of cytokines into the circulation, including IL-6, that may modulate systemic low-level inflammation [63]. The exercise-induced increase in IL-6 stimulates the appearance of other anti-inflammatory cytokines, including IL-1ra, sTNFR and IL-10, whereas circulating levels of pro-inflammatory cytokines, such as TNF-α and IL-1, are generally not increased by acute exercise [22].

Studies examining the effects of chronic exercise on cytokine production and circulating cytokine levels have been somewhat conflicting. Cross-sectional studies indicate that increasing levels of physical activity and/or fitness are generally associated with modest reductions in circulating levels of TNF-α and IL-6, and increased levels of IL-10 [26]. Consistent with this, Smith et al. [64] observed a 58% reduction in the production of the pro-inflammatory cytokines IFN-γ (interferon-γ), TNF-α and IL-1β, and a 36% increase in the production of the anti-inflammatory cytokines TGF-β (transforming growth factor-β), IL-4 and IL-10, by cultured mononuclear cells from ischemia patients following 6 months of exercise training compared with baseline levels. In addition, some studies demonstrate reduced circulating levels of TNF-α, IL-6, IFN-γ and other markers of systemic inflammation following exercise training, although several others do not (reviewed in [26]). These discrepancies may be due to a number of factors, including differences in subject characteristics, the type and intensity of the exercise intervention, the timing of the blood samples taken and inherent variability in the assays used to measure many of these cytokines. Nevertheless, the preponderance of evidence suggests that both acute and chronic exercise is associated with modest improvements in markers of systemic low-grade inflammation. However, the clinical significance of these improvements is uncertain because they may not reflect changes occurring at the tissue level.

EXERCISE AND CRP

A number of studies have been published regarding the effects of both acute and chronic exercise on serum CRP levels. Studies in marathon runners have found that strenuous acute exercise produces large transient increases in CRP [65,66]. This finding is consistent across most studies and is probably due to the exercise-induced release of IL-6 and other pro-inflammatory cytokines from skeletal muscle that regulate CRP production in the liver [22].

Although high-intensity or prolonged acute exercise increases CRP, chronic exercise has been hypothesized to reduce circulating levels of CRP and other inflammatory markers. Cross-sectional studies consistently demonstrate an inverse relationship between serum CRP and both physical activity level and cardiorespiratory fitness [67]. These associations remain after controlling for potential confounders in some studies [68], although others suggest they may be influenced by factors, such as gender, body composition and the type of physical activity. For example, in NHANES III (Third National Health and Nutrition Examination Survey [69]), jogging and aerobic dancing were associated with lower CRP levels when compared with other forms of activity, including swimming, cycling and weightlifting. Several observational studies indicate that chronic physical activity reduces CRP levels more in males than females [70–72], and no relationship between physical activity levels and CRP is found in some studies after adjusting for BMI (body mass index) and other risk factors [73,74].

Data from prospective studies examining the effect of exercise training on CRP levels have been inconclusive. Exercise training appears to blunt the acute exercise-induced increase in CRP [75]; however, a meta-analysis of RCTs (randomized controlled clinical trials) published recently concluded that aerobic exercise training does not reduce basal CRP levels [76]. This meta-analysis included data from 323 subjects in five separate trials in which subjects were randomized to either a control group or an aerobic exercise intervention lasting at least 8 weeks. Overall, there was a 3% reduction in CRP levels in the exercise groups, but this change was not statistically significant. Of note, one RCT that was published after this meta-analysis found that 10 months of aerobic exercise, but not flexibility and resistance exercise, significantly reduced serum CRP by 10–15% [77].

In a recent paper by Plaisance and Grandjean [78], the authors reviewed data from a number of longitudinal studies and concluded that long-term exercise training significantly reduces CRP levels, and that this effect may be independent of baseline CRP levels, body composition and weight loss. However, none of the studies reviewed were RCTs. In the only study with a control group, CRP was reduced from 1.19 to 0.82 mg/l (31%) in highly trained runners after 9 months of increased training in preparation for a marathon, but did not change significantly in non-training control subjects [79]. Although the reduction in CRP in the training group in this study was statistically significant, the physiological relevance of such a small absolute change in CRP levels is unknown. Furthermore, because the subjects in the ‘training’ group were already highly fit runners, the significance of these findings to the population at large is also uncertain.

Additional RCTs assessing the effects of exercise training on CRP levels are clearly needed. Most of the studies conducted to date have included interventions of 4 months or less, many have included subjects with relatively low baseline CRP levels and almost none have not controlled for statin use. In addition, it is uncertain
whether exercise has a direct effect on CRP levels independent of weight loss. Weight loss has consistently been shown to reduce CRP levels [80]. Most exercise training interventions have shown modest or no effects on CRP after statistically correcting for weight loss, but few studies have assessed the effect of exercise on CRP in the absence of weight loss [80]. Longer-term studies controlling for these factors are needed before any significant conclusions can be drawn regarding the impact of regular exercise on serum CRP levels.

**EXERCISE AND ATHEROSCLEROSIS**

Although observational studies suggest a protective effect of exercise on CVD risk, few studies have attempted to examine directly the effect of exercise, either alone or in combination with other therapies, on the development or progression of atherosclerosis in humans (Table 1). In one study, Hambrecht et al. [81] found that LTPA (leisure time physical activity) in excess of 1500 kcal/week (where 1 kcal ≈ 4.184 kJ) inhibited the progression of angiographically defined coronary atherosclerotic lesions in patients with CAD (coronary artery disease), whereas regression of lesions was observed in patients expending greater than 2200 kcal/week. Likewise, Schuler et al. [82] found a significant regression of coronary atherosclerotic lesions in seven out of 18 patients with angina following a 1 year diet and exercise intervention compared with regression of lesions in just one out of 18 patients receiving ‘usual care’. To test the effects of a longer-term intervention on inflammation and atherosclerosis, Rauramaa et al. [83] conducted a 6-year RCT in a population-based sample of 140 middle-aged men. Changes in chronic inflammation were assessed by measuring serum CRP, and the progression of atherosclerosis was assessed by measuring carotid artery IMT (intima-media thickness). In the full cohort, neither CRP levels nor the progression of carotid artery IMT differed between the intervention and control groups. However, in a subgroup of patients not taking statins, there was a 40 % decrease in the progression of IMT in the exercise group compared with the controls.

Table 1  Prospective studies of exercise and atherosclerosis/CAD in humans

<table>
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<th>Reference</th>
<th>Subjects/intervention</th>
<th>Method</th>
<th>Results</th>
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<tr>
<td>[81]</td>
<td>CAD patients randomized to 1 year of unsupervised aerobic exercise</td>
<td>Coronary artery atherosclerosis assessed by angiography</td>
<td>No change or regression of lesions in 90 % of subjects in the intervention group, but only 55 % of subjects in the control group.</td>
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<td>[82]</td>
<td>Angina patients selected for 1 year of supervised and unsupervised exercise training ( n = 18 ) or ‘usual care’ ( n = 18 )</td>
<td>Coronary artery atherosclerosis assessed by angiography</td>
<td>Regression of lesions in seven out of 18 subjects in the intervention group, but only one out of 18 subjects in the control group.</td>
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<td>[83]</td>
<td>Population sample of middle-aged men randomized to 6 years of unsupervised low-/moderate-intensity exercise ( n = 70 ) or ‘usual activity’ ( n = 70 )</td>
<td>Carotid artery IMT assessed by ultrasonography</td>
<td>Regression of lesions in seven out of 18 subjects in the intervention group, but only one out of 18 subjects in the control group.</td>
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<td>[85]</td>
<td>CAD patients randomized to a 1 year multiple risk factor intervention programme (diet, exercise, weight loss, smoking cessation and lipid medication) ( n = 145 ) or ‘usual care’ ( n = 155 )</td>
<td>Coronary artery atherosclerosis assessed by angiography</td>
<td>Narrowing of diseased coronary artery segments was reduced 47 % in the treatment group compared with controls.</td>
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<tr>
<td>[86]</td>
<td>CAD patients randomized to a 1 year multiple risk factor intervention programme (vegetarian diet, smoking cessation, stress management and moderate exercise) ( n = 28 ) or ‘usual care’ ( n = 20 )</td>
<td>Coronary artery atherosclerosis assessed by angiography</td>
<td>Average percentage diameter stenosis decreased 2.2 % in the treatment group and increased 3.4 % in the control group.</td>
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<td>[87]</td>
<td>A total of ten CAD patients assigned to 1 year of supervised intense exercise training (no control group)</td>
<td>Extent of ischaemic ST-segment depression during exercise measured by ECG</td>
<td>Maximum degree of ST-segment depression during exercise was reduced by 20 %, despite a 20 % increase in heart rate/systolic blood pressure product.</td>
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An important aspect of these mouse studies is that they identified numerous mechanisms through which exercise exerts its protective effects. One important finding involves the relationship between exercise, cholesterol metabolism and atherosclerosis. In the study by Meilhac et al. [56], cholesterol levels were reduced by 15% and aortic atherosclerosis by 40% in exercise-trained mice compared with sedentary controls. However, the animals in this study were LDLr−/− mice, indicating that the LDLr is not responsible for exercise-induced reductions in plasma cholesterol levels. Furthermore, Pynn et al. [97] have shown that exercise-induced reductions in atherosclerosis were independent of changes in cholesterol levels, providing evidence that the protective effects of exercise extend beyond its well-documented benefits in lipid metabolism [103].

Several studies have examined the combined effects of exercise training and antioxidant supplementation on atherosclerosis in mice. Napoli et al. have found that antioxidant supplementation to exercising mice synergistically reduced atherosclerosis [96], prevented plaque rupture [102] and prolonged survival [102]. In contrast, Meilhac et al. [56] have found that vitamin E supplementation attenuated exercise-induced increases in aortic catalase expression and offset the exercise-induced reduction in atherosclerosis. One primary difference between these studies was the mode of exercise used: Meilhac et al. used treadmill running, whereas Napoli et al. used swimming. ‘Swim training’ in rodents appears to impose an enhanced stress response compared with treadmill running [104], although it is not evident why this would account for the different effects of antioxidant supplementation on atherosclerosis between these studies.

In agreement with the findings of Meilhac et al. [56], there is some evidence that antioxidant supplementation during exercise may have deleterious effects on the anti-inflammatory response to exercise in humans. In a study by Fischer et al. [105], antioxidant supplementation inhibited the expression and release of IL-6 from skeletal muscle during exercise, and this subsequently blunted the release of the anti-inflammatory mediators IL-1ra and cortisol. This indicates that oxidative stress from acute bouts of exercise induces beneficial adaptations; if blocked by antioxidant supplementation, the adaptive benefits of exercise may not be realized. On the basis of these discrepant findings, more work is needed to clarify if antioxidant supplementation during exercise is beneficial or should be contraindicated in certain circumstances.

**INFLAMMATION AND VC (VASCULAR CALCIFICATION)**

A component of CVD that is often underappreciated is VC. VC has been recognized for over 150 years [106], but has typically been considered a manifestation of normal
aging and a frequent complication of the late stages of atherosclerosis [107,108]. However, several heritable diseases are characterized by extensive VC early in life and in the absence of atherosclerotic lesions, indicating that atherosclerosis and VC are pathologically distinct and controlled by different genetic pathways [109]. VC was considered previously to be relatively benign, but has been independently associated with a variety of clinical end points, including amputation [110,111], stroke [112], MI [113–115], poor surgical outcomes [116] and CVD mortality [113–115,117,118].

VC can occur in either the intimal or medial layer of the vascular wall. Intimal calcification is most often associated with advanced atherosclerotic plaques and occlusive lesions [119] and has been implicated in plaque disruption and thrombosis [120]. Calcification of the medial layer, which is especially prevalent in diabetics and patients with ESRD (end-stage renal disease) [121–123], can develop in conjunction with or in the absence of intimal calcification and has different pathological consequences. Medial calcification is associated with increased artery wall IMT [124–130], increased arterial stiffness and ventricular afterload [123,124] and is a strong predictor of future CVD mortality in both diabetes [110] and ESRD [131–133].

Previously thought to be a passive precipitation of calcium and phosphate, more recent research has shown that VC occurs via a highly regulated process similar to bone formation involving OLCs (osteoblast-like cells) in the artery wall [134–136]. OLCs express proteins normally found in osteoblasts, such as alkaline phosphatase, bone matrix proteins, osteocalcin and osteopontin [136,137], and are capable of producing a bone-like matrix and mineralizing in the presence of calcium and phosphorus [119]. The origin of these OLCs is controversial, but evidence suggests that they may be derived from either COPCs (circulating osteoprogenitor cells), or the differentiation of resident stem cells, VSMCs, pericytes or a subpopulation of cells in the vascular wall known as CVCs (calcifying vascular cells) [138].

Similar to many other chronic diseases, inflammation-related variables, such as CRP, pro-inflammatory cytokines and markers of oxidative stress, are believed to play a key role in the development and progression of VC. Cultured SMCs and CVCs begin to express bone-related proteins and mineralize if treated with ox-LDL [139,140], H2O2 or xanthine oxidase [141], homocysteine [142], AGEs (advanced glycation end products) [143] or leptin [144]. TNF-α promotes in vitro calcification of vascular cells [145], as does co-culture of CVCs with monocytes/macrophages [146]. Elevated circulating levels of CRP is also associated with increased VC in renal patients [126], but it is not known whether it directly contributes to mineralization.

VC may also be enhanced by low serum levels of calcification inhibitors. Calcium and phosphate are tightly regulated near their saturation levels in the circulation and it has been hypothesized that circulating inhibitors of calcification are necessary to prevent vascular and other soft tissue calcification under normal conditions. Several inhibitors function in the arterial wall, including osteopontin, osteoprotegerin and matrix GlA protein, whereas fetuin-A, also known as AHSG (a2-Heremans–Schmid glycoprotein), has been shown to inhibit the formation of hydroxyapatite crystals in the circulation [147]. Indeed, fetuin-A deficiency in mice promotes organ and soft tissue calcification [148], and low circulating levels of fetuin-A in ESRD patients may contribute to the excessive VC in this population [149–151].

Fetuin-A has also been associated with other inflammatory conditions, including Type 2 diabetes and the metabolic syndrome [152]. Fetuin-A has been classified as a reverse acute-phase reactant, because serum levels fall significantly during injury and infection [153]. It is secreted primarily by the liver, but is also expressed by macrophages [154]. The role of fetuin-A in inflammation is still being elucidated, but its promoter has several potential IL-6-responsive elements [153], it has been shown to inhibit TNF-α signalling [155], and has been shown to have a role as a counter-regulator of macrophage deactivation [153]. It currently is not known if modifying fetuin-A levels either pharmacologically or by other means would help protect against the development of VC or other inflammatory disorders.

EXERCISE AND VC

Interventions such as exercise that modify inflammation could in theory influence the extent of VC or its rate of progression. However, few studies have attempted to address the effectiveness of exercise in preventing VC, and the data from these studies are rather equivocal [156,157]. Taylor et al. [156] found no relationship between physical activity levels and coronary artery calcium scores in a group of healthy middle-aged subjects, whereas Desai et al. [157] found a modest inverse relationship between calcification and physical activity level in asymptomatic individuals with multiple CVD risk factors. However, these were both observational studies that utilized questionnaires to estimate physical activity levels. Objective measures of cardiorespiratory fitness [e.g. VO2max (maximum VO2)] were not obtained in either study, so the relationship between fitness and calcification was not determined.

Exercise may inhibit VC by many mechanisms. Exercise training in mice induces the expression of antioxidant enzymes in the vascular wall, including catalase [56] and SOD [59], both of which inhibit the oxidation of LDL in the vascular wall. Because ox-LDL promotes osteoblastic differentiation of vascular cells in vitro
The exercise-induced increase in antioxidant defences in the vascular wall may inhibit osteogenesis. In addition, endurance exercise training has beneficial effects on many other putative risk factors for VC, including elevated circulating glucose, CRP and low HDL-cholesterol (high-density lipoprotein-cholesterol), so it is reasonable to assume that exercise may inhibit VC via risk factor reduction.

There are no published prospective studies that have examined the effect of exercise training on VC or risk factors associated with its development in humans or animal models. We are currently conducting an exercise training intervention in an inbred strain of mice to examine the effect of physical activity on markers and mediators of atherosclerosis and VC. We have used microarray analysis and a cytokine antibody array to compare aortic gene and protein expression in sedentary mice and mice that exercised on a treadmill for 3 months. Our preliminary data (E. J. Tomayko, L. Feeney and K. R. Wilund, unpublished work) indicate that exercise training has a significant effect on the aortic expression of a number of genes and proteins that have been implicated in VC. For example, we found approx. 2.5–3-fold increases in expression of several inhibitors of VC, including fetuin-A, fetuin B [158] and sclerostin, an antagonist of bone morphogenetic proteins [159,160]. By contrast, the expression of several proteins believed to promote the differentiation of vascular cells into OLCs, including Shox2 [161] and sonic hedgehog protein (Shh-n) [162], were reduced 2–3-fold in the exercise-trained mice. These preliminary data indicate that exercise may induce changes in aortic gene and protein expression consistent with protection against VC. More work is being planned to validate and extend these findings.

SUMMARY/FUTURE DIRECTIONS

Both cross-sectional and longitudinal studies indicate that regular exercise reduces the risk of developing atherosclerotic CVD and other chronic diseases associated with low-grade inflammation. Recent research has begun to elucidate the mechanisms responsible for the protective effects of exercise, but many questions remain. Regular exercise has been shown to reduce markers of oxidative stress and inflammation in both the plasma and the vasculature and these changes may contribute significantly to the inverse relationship between physical activity and CVD risk observed in epidemiological studies. Additional research is needed to clarify the role that systemic markers of inflammation play in the pathogenesis of CVD as well as the role that exercise has in modulating these effects.

In addition to modifying inflammation-related risk factors, exercise training also has been shown to directly inhibit atherosclerosis in a variety of animal models. Studies assessing the effect of exercise on atherosclerosis in humans are difficult to interpret due to methodological concerns associated with current imaging techniques. Future studies using enhanced imaging technologies may allow for better assessments of the effect of exercise on plaque development, progression and lesion characteristics.

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