Long-term Exercise and Atherogenic Activity of Blood Mononuclear Cells in Persons at Risk of Developing Ischemic Heart Disease

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ALTHOUGH THE AGE-ADJUSTED death rate due to cardiovascular disease (CVD) has declined in the past 25 years, heart disease remains the leading cause of death in the United States, accounting for 733,834 deaths, or 31.6% of total mortality, in 1996.1

A number of studies have shown that moderate-intensity physical activity reduces the incidence of all-cause mortality, particularly deaths due to CVD.2-9 The accumulated evidence on the health benefits of physical activity prompted participants in a National Institutes of Health Consensus Conference to recommend that “children and adults alike should set a goal of accumulating at least 30 minutes of moderate-intensity physical activity on most, and preferably all, days of the week.”10

Despite the documented health benefits, the mechanism whereby physical activity prevents CVD is incompletely understood, although it is probably multifactorial. Risk factors such as hypertension,11 obesity,12 hyperlipidemia,13 and insulin resistance14 may respond favorably to moderate levels of physical activity, thereby protecting against CVD. However, because exercise also protects against CVD in smokers and in persons

Context Increasing evidence demonstrates that atherosclerosis is an immunologically mediated disease in which the secretion of atherogenic and atheroprotective cytokines, by infiltrating blood mononuclear cells, plays an important role. It is not known whether long-term exercise alters this atherogenic and atheroprotective activity directly.

Objective To determine the effect of long-term exercise on the atherogenic activity of blood mononuclear cells in persons at risk of developing ischemic heart disease.

Design Before-after trial using a 6-month individualized, supervised exercise program, with an enrollment period from December 1996 to October 1997.

Setting Hospital-based community wellness center.

Participants Of 110 persons who responded to a public request for volunteers, 52 met the inclusion criteria (risk ratio for myocardial infarction ≥ 1.7 based on serum complement and/or C-reactive protein levels, and normal exercise treadmill test results). Forty-three of the 52 enrollees (25 women [mean age, 49.7 years] and 18 men [mean age, 48.1 years]) completed the study; 9 withdrew for personal reasons. Additional risk factors for ischemic heart disease included hypercholesterolemia (65.1%), a family history of coronary heart disease (62.8%), inactivity (60.5%), hypertension (32.6%), obesity (25.6%), smoking (11.6%), and diabetes mellitus (4.7%).

Main Outcome Measures Blood levels were compared at baseline and after the exercise program had been completed for the following: spontaneous and phytohemagglutinin-induced production of interleukin 1α, tumor necrosis factor α, and interferon gamma (atherogenic cytokines), and interleukin 4, interleukin 10, and transforming growth factor beta 1 (atheroprotective cytokines) by blood mononuclear cells; lymphocyte phenotypes and mitogenic responses to phytohemagglutinin; and serum C-reactive protein levels.

Results Subjects exercised for a mean of 2.5 (range, 0.3-7.4) hours per week. Mononuclear cell production of atherogenic cytokines fell by 58.3% (P < .001) following the exercise program, whereas the production of atheroprotective cytokines rose by 35.9% (P < .001). Changes in transforming growth factor beta 1 and in phytohemagglutinin-induced atherogenic cytokine production after the exercise program were proportionate to the time subjects spent performing repetitive lower-body motion exercises (P < .02), indicating a dose-response relationship. After the exercise program, changes in cellular function were reflected systemically by a 35% decrease in serum levels of C-reactive protein (P = .12).

Conclusions Our data suggest that long-term exercise decreases the atherogenic activity of blood mononuclear cells in persons at risk of developing ischemic heart disease. This may be a mechanism whereby physical activity protects against ischemic heart disease.
without evident risk factors,\textsuperscript{9} it appears to favorably influence the course of atherosclerosis in ways yet to be discovered. Whatever the reasons, reports have documented a strong and independent association of low cardiorespiratory fitness and low levels of physical activity and the risk of death due to CVD.\textsuperscript{2-10}

There is increasing evidence that atherosclerosis may be an immunologically mediated disease.\textsuperscript{15-24} Early atherosclerotic lesions contain activated immune cells, including CD4\textsuperscript{+} and CD8\textsuperscript{+} T cells, monocytes, macrophages, and endothelial cells. These cells are responsible for the local production of a variety of cytokines that have been identified in atherosclerotic lesions, including interleukin (IL) 1, IL-2, IL-4, IL-6, IL-10, tumor necrosis factor alpha (TNF-\(\alpha\)), interferon gamma (IFN-\(\gamma\)), and transforming growth factor beta (TGF-\(\beta\)). There is provocative but, as yet, inconclusive evidence that this immune reaction may be in response to an infectious agent.\textsuperscript{25-27} Heat shock protein (HSP) 60,\textsuperscript{19,23} or oxidized low-density lipoprotein (LDL),\textsuperscript{28} Whatever the inciting events, in transplanted human hearts, activation of coronary endothelial cells is predictive of the subsequent development of coronary atherosclerosis,\textsuperscript{29} lending credence to the importance of the immune response in the early stages of CVD. Indirect evidence is also found in reports documenting an association between blood levels of acute-phase reactants, notably C-reactive protein (CRP),\textsuperscript{30,31} fibrinogen,\textsuperscript{32} and C3\textsuperscript{33} and the future risk of heart attack. Acute phase reactants are produced by hepatocytes in response to several cytokines, notably IL-1, IL-6, and TNF-\(\alpha\), and often serve to minimize tissue damage that follows an inflammatory response.

In this study, we investigated the possibility that long-term exercise benefits persons at risk of developing ischemic heart disease by favorably altering the production of cytokines with atherogenic and atheroprotective properties. According to current evidence, the cytokines with atherogenic properties include IL-1, TNF-\(\alpha\), and IFN-\(\gamma\).\textsuperscript{16-21} The cytokines with atheroprotective properties include IL-4, IL-10, and TGF-\(\beta\).\textsuperscript{33-30} Because blood leukocytes provide the major source of immune cells in atherosclerotic lesions\textsuperscript{21} and because it is not feasible to obtain these cells from arterial biopsy specimens, we have chosen to measure the effect of exercise on the production of these cytokines by blood mononuclear cells. We have also determined the effect of exercise on serum levels of CRP, an acute-phase protein that has been speculated to serve as a marker of the severity of the immune-mediated inflammatory response in atherosclerosis.\textsuperscript{30}

### METHODS

#### Subjects

This study was approved by the institutional review board of East Tennessee State University, Johnson City, Tenn. Each participant read and signed the informed consent in the presence of an investigator.

Subjects were recruited from the general public by placing an outline of the study and a request for volunteers in 3 local newspapers. A total of 110 persons responded and were screened for participation in the study. Of these, 54 had serum C3 and/or CRP levels that placed them at greater risk of future heart attack (risk ratio = 1.7).\textsuperscript{30,32} and they underwent exercise treadmill testing (ETT) using a modified Bruce protocol. Fifty-two subjects had normal ETT results and qualified for final inclusion in the study. These subjects were enrolled in a hospital-based wellness center where, after analysis of their medical history and previous levels of activity, they were assigned to supervised and individually tailored exercise programs. To minimize seasonal influences on the results, 4 to 5 subjects were enrolled each month over an 11-month period extending from December 1996 to October 1997. Subjects were not paid for participating in the study. Forty-three subjects (18 men, 25 women) successfully completed the study. Nine subjects withdrew for personal reasons. Summaries of subject demographics and risk factors for CVD are shown in [Table 1](#).

#### Medications taken daily during the study included aspirin (30.2%), antihypertensive agents (25.6%), thyroid hormones (16.3%), lipid-lowering agents (14%), antidepressants (11.6%), insulin (2.3%), and an oral hypoglycemic (2.3%). Nineteen subjects (44.2%) consumed an average of 2.3 alcoholic beverages per week. Medications taken daily only by women included estrogen (64%), progestins (28%), biophosphonates (8%), and oral contraceptives (4%).

Immunologic studies were performed at baseline and after completion of the 6-month exercise program. All blood samples were drawn in the morning without fasting. Plasma samples were immediately frozen and stored at \(-70^\circ\text{C}\) until assayed.

#### TABLE 1. Risk Factors for Cardiovascular Disease*  

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Men (n = 18)</th>
<th>Women† (n = 25)</th>
<th>Overall (n = 43)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, mean (SD), y</td>
<td>48.1 (8.0)</td>
<td>49.7 (7.2)</td>
<td>49.0 (7.5)</td>
</tr>
<tr>
<td>Hypertension‡</td>
<td>61.1</td>
<td>12</td>
<td>32.6</td>
</tr>
<tr>
<td>Elevated total cholesterol-HDL ratio§</td>
<td>83.3</td>
<td>52</td>
<td>65.1</td>
</tr>
<tr>
<td>Family history of coronary heart disease</td>
<td>66.7</td>
<td>60</td>
<td>62.8</td>
</tr>
<tr>
<td>Diabetes mellitus</td>
<td>5.6</td>
<td>4</td>
<td>4.7</td>
</tr>
<tr>
<td>Smoker</td>
<td>5.6</td>
<td>16</td>
<td>11.6</td>
</tr>
<tr>
<td>Obesity§</td>
<td>27.8</td>
<td>24</td>
<td>25.6</td>
</tr>
<tr>
<td>Inactivity‡</td>
<td>55.6</td>
<td>64</td>
<td>60.5</td>
</tr>
</tbody>
</table>

*All data are presented as percentages except for age. All subjects had blood levels of C3 and/or C-reactive protein that placed them at greater risk of future heart attack (risk ratio = 1.7); 6.9% of subjects had no other risk factors; 18.6% had 1 other risk factor; 18.6% had 2 other risk factors; 23.3% had 3 other risk factors; 27.9% had 4 other risk factors; and 4.7% had 5 other risk factors.

†Of the women, 68% were amenorrheic (44%, postmenopausal; 24%, posthysterectomy and oophorectomy). Of the amenorrheic women, 71% were taking estrogen replacement medication.

‡Subjects younger than 50 years of age were considered to have hypertension if they had a systolic blood pressure of at least 140 mm Hg, those older than 50 years if their systolic blood pressure was at least 150 mm Hg, or if at any age they had a diastolic blood pressure of at least 90 mm Hg on 3 or more occasions.

§The total cholesterol-high-density lipoprotein (HDL) ratio was greater than 4.96.

¶Body mass index was greater than 30 kg/m\(^2\).

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morning at approximately the same time of day. Subjects were instructed not to exercise for at least 24 hours prior to blood drawing.

**Blood CRP**
C-reactive protein serum levels were assayed before and after the exercise program by radial immunodiffusion using CRP LL and CRP EL Narorid kits (The Binding Site Ltd, Birmingham, England).

**Lymphocyte Phenotyping**
Immunophenotyping of blood lymphocytes was performed with lysed whole blood and a FACSscan flow cytometer (Becton Dickinson, San Jose, Calif) and fluorescein- and phycoerythrin-labeled murine monoclonal IgG antibodies to measure levels of T lymphocytes (CD3+); T helper lymphocytes (CD4+); T cytotoxic lymphocytes (CD8+); T lymphocytes displaying major histocompatibility complex II antigen (DR+); vascular adhesion molecule 4 (VLA-4) (CD49d); Fas antigen (CD95+); or gamma-delta antigen T-cell receptor (TCRgd+); B lymphocytes (CD3−CD19+); and natural killer (NK) cells (CD3−CD19+CD56+).

**Table 2. Effect of Exercise on Cytokine Production by Blood Mononuclear Cells**

<table>
<thead>
<tr>
<th></th>
<th>Before Exercise, pg/mL</th>
<th>After Exercise, pg/mL</th>
<th>% Change Value</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Phytohemagglutinin Negative Cultures</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>IL-1α</td>
<td>119.0 (101)</td>
<td>104.1 (87)</td>
<td>−13</td>
<td>.047</td>
</tr>
<tr>
<td>TNF-α</td>
<td>3340 (203)</td>
<td>1621 (180)</td>
<td>−51</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>IFN-γ</td>
<td>7270 (1087)</td>
<td>2101 (495)</td>
<td>−71</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>IL-4</td>
<td>123 (27)</td>
<td>239 (31)</td>
<td>+94</td>
<td>.001</td>
</tr>
<tr>
<td>IL-10</td>
<td>1136 (70)</td>
<td>1242 (70)</td>
<td>+9</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>TGF-β1</td>
<td>2347 (168)</td>
<td>3350 (139)</td>
<td>+43</td>
<td>&lt;.001</td>
</tr>
</tbody>
</table>

**Phytohemagglutinin Positive Cultures**

<table>
<thead>
<tr>
<th></th>
<th>Before Exercise, pg/mL</th>
<th>After Exercise, pg/mL</th>
<th>% Change Value</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>IL-1α</td>
<td>96 (15)</td>
<td>92 (16)</td>
<td>−3</td>
<td>&gt;.05</td>
</tr>
<tr>
<td>TNF-α</td>
<td>364 (60)</td>
<td>262 (43)</td>
<td>−26</td>
<td>.003</td>
</tr>
<tr>
<td>IFN-γ</td>
<td>23 (4)</td>
<td>13 (1)</td>
<td>−44</td>
<td>.007</td>
</tr>
<tr>
<td>IL-4</td>
<td>12 (2)</td>
<td>12 (2)</td>
<td>0</td>
<td>&gt;.05</td>
</tr>
<tr>
<td>IL-10</td>
<td>5 (4)</td>
<td>52 (14)</td>
<td>+940</td>
<td>.003</td>
</tr>
<tr>
<td>TGF-β1</td>
<td>3350 (139)</td>
<td>3350 (139)</td>
<td>+43</td>
<td>&lt;.001</td>
</tr>
</tbody>
</table>

Sources of cytokines in blood mononuclear cell preparations include monococytes (interleukin [IL] 1, tumor necrosis factor [TNF]-α, IL-10, and transforming growth factor [TGF]-β1); T lymphocytes (IL-1, TNF-α, interferon [IFN]-γ, IL-4, and IL-10); natural killer (NK) cells (IFN-γ and TNF-α); and B cells (IL-4 and IL-10). Mononuclear cells sources in atherosclerotic lesions are similar except that NK and B cells are usually rare or absent. Data are presented as mean (SEM).

**Cytokine Production**

Blood mononuclear cell preparations containing 15% to 20% monocytes and 80% to 85% lymphocytes were isolated from venous blood using AccuPrep (Accurate Chemical & Scientific Corp, Westbury, NY), washed 3 times at 10°C with sterile phosphate-buffered saline solution (pH, 7.4; 0.1 mol/L), and suspended at a concentration of 2 × 10⁶ cells/mL in Rosewell Park Memorial Institute Growth Medium, formula 1640 (Mediatech, Herndon, Va) containing 5% heat inactivated human AB serum (vol/vol), 1-glutamine (2 mmol/L), penicillin (50 U/mL), and gentamicin and streptomycin (50 µg/mL each). Preparations were incubated under 5% carbon dioxide at 37°C for 48 hours with and without the T-cell mitogen phytohemagglutinin (PHA) (5 µg/mL). Culture supernatants were rendered cell free by centrifuging at 1000g for 10 minutes at 12°C, and stored in 1- to 2-mL aliquots at −120°C for later use. Supernatants were subsequently assayed for IL-1α, IL-4, IL-10, IFN-γ, TNF-α, and TGF-β1 using solid-phase enzyme-linked immunosassay kits (Quantikine, R & D Systems, Inc, Minneapolis, Minn, for IL-1α and TGF-β1; Predicta, Genzyme Corp, Cambridge, Mass, for TNF-α; CytoScreen, BioSource International, Inc, Camarillo, Calif, for IFN-γ; and Immuno-Notech, Inc, Westbrook, Me, for IL-4 and IL-10).

**Mitogenic Assays**
Mitogenic responses were measured by adding methyl-[3H]-thymidine (0.74 MBq, 50 µL) to culture samples removed 6 hours prior to supernatant harvesting. Labeled samples were incubated under 5% carbon dioxide at 37°C for a period of 6 hours and the cells collected on glass fiber filter paper using a Mash II Cell Harvester (Microbiology Associates, Walkersville, Md). Samples were air dried, placed in vials containing Scinti-Verse II, and assayed with a Beckman LS9800 liquid scintillation counter (Beckman, Fullerton, Calif). Proliferative responses are expressed as net counts per minute (Δcpm), calculated as cpm in PHA-stimulated cultures minus cpm in unstimulated cultures. The mitogenic assay used in this study had been optimized in accordance with standard procedures using varying concentrations of PHA, cells per well, and incubation times.

**Statistical Analysis**
Statistical analysis was performed using STATISTICA 5.1 F (Statsoft, Inc, Tulsa, Okla). The 2-sided t test for paired samples was used to determine the significance of differences between cytokine, lymphocyte phenotype, and CRP measurements in blood samples taken before and after the exercise program. A 2-sided t test for independent samples was used to determine within-group differences. A χ² analysis was used to determine the significance of differences between expected and observed frequencies. Unless stated otherwise, results are expressed as the mean (SEM).

## RESULTS

### Production of Atherogenic Cytokines

The production of IFN-γ and TNF-α by blood mononuclear cells was significantly attenuated in both PHA-negative and PHA-positive cultures by exercise (TABLE 2). Compared with baseline values, IFN-γ levels fell by 44% and 71% and TNF-α values by 28% and 51% in cultures with and without added PHA, respectively (P = .007). In addition, IL-1α secretion fell by 13% in PHA-stimulated cultures (P = .047). Overall the production of atherogenic cytokines fell by 58.3% after the exercise program (P < .001).

### Production of Atheroprotective Cytokines

In contrast to the effects of exercise on the production of atherogenic cytokines, the production of IL-4, IL-10, and TGF-β1 by blood mononuclear cells was significantly augmented by exercise (Table 2). In PHA-negative cultures, IL-10 levels increased by 94% and TGF-β1 by 43% (P = .003). In PHA-positive cultures, significant changes were seen in IL-4 (94% increase) and...
TGF-β1 (37% increase) \( (P \leq .001) \). Overall, the production of atheroprotective cytokines increased by 35.9\% after the exercise program \( (P < .001) \).

### Lymphocyte Phenotypes
There were no significant changes in the numbers or percentages of lymphocyte phenotypes following exercise (TABLE 3).

### Mitogenic Responses
Proliferative responses of lymphocytes to PHA decreased following exercise, from a mean (SEM) value of 9142 (760) Δcpm to 3155 (584) Δcpm \( (P < .001) \).

### CRP Levels
C-reactive protein levels measured before the exercise program ranged from 0 to 0.9 mg/dL in the lower quartile to 5.8 to 37.5 mg/dL in the upper quartile, with a mean (SEM) value of 4.81 (1.09) mg/dL. Values taken after the exercise program decreased by 35\% to 3.13 (0.64) mg/dL \( (P = .12) \) (2-sided t test). The frequency of values in the upper quartile dropped by 50\% following exercise \( (P = .01) \) (χ² analysis).

### Exercise Parameters
The mean number of hours per week that subjects underwent supervised exercise was 2.5 (range, 0.3–7.4 hours per week). The mean duration of each exercise session was 70 minutes (range, 36–123 minutes), and the mean number of visits per week was 2 (range, 0.3–5.3 visits per week). The mean percentage of time spent doing different exercises was as follows: weight lifting (35\%), walking or running on a treadmill (32\%), cycling (16\%), stretching (8\%), aerobics (3\%), rowing (3\%), climbing (2\%), and skiing (1\%).

Four subjects (9.3\%) changed their diet to one that was lower in energy intake and animal fat, and 2 of the 5 tobacco users discontinued smoking during the study. By completion of the study, 15 subjects (34.9\%) had lost weight, with the mean weight for all subjects falling from 80.1 to 77.4 kg \( (P = .02) \). There were no changes in medications or alcohol consumption during the study.

### Relationship of Exercise Parameters to Cytokine Production
The percentage decrease in atherogenic cytokine production by PHA-stimulated mononuclear cells was proportionate to the time subjects spent performing repetitive lower-body motion exercises (ie, walking, running, cycling, rowing, climbing, aerobics, and skiing) \( (r = –0.267, \ P = .002) \). The correlation was most significant for IFN-γ \( (P = .03) \) (FIGURE), followed by IL-1α \( (P = .05) \) and TNF-α \( (P = .09) \).

In contrast, both the spontaneous and PHA-induced production of TGF-β1 increased in proportion to the time spent doing these exercises \( (r = 0.354, \ P = .02) \). No other correlations were found between exercise parameters and main outcome measures.

### Within-Group Variations
Blood mononuclear cells taken from men prior to exercise produced more IFN-γ and TNF-α in cultures without added PHA than did mononuclear cells taken from women \( (P = .04) \). Following exercise, men spontaneously produced more IL-1α and TNF-α than women \( (P = .004) \). In contrast, men had

<table>
<thead>
<tr>
<th>TABLE 3. Effect of Exercise on Blood Lymphocyte Levels*</th>
</tr>
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<tbody>
<tr>
<td></td>
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<tr>
<td></td>
</tr>
<tr>
<td>CD3&lt;sup&gt;+&lt;/sup&gt;</td>
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<td>CD3&lt;sup&gt;+&lt;/sup&gt;CD4&lt;sup&gt;+&lt;/sup&gt;</td>
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<tr>
<td>CD3&lt;sup&gt;+&lt;/sup&gt;CD16&lt;sup&gt;+&lt;/sup&gt;CD56&lt;sup&gt;+&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

*There are no significant differences between before and after exercise values \( (P > .05) \). Data are presented as mean (SEM). TCR indicates T-cell receptor.

### Figure: Effect of Exercise Duration on the Production of Interferon Gamma (IFN-γ)
by Phytohemagglutinin-Stimulated Mononuclear Cells

The average number of hours per week each subject spent doing repetitive lower-body motion exercises (walking, running, cycling, rowing, climbing, aerobics, and skiing) is plotted against the change in IFN-γ production that occurred following these exercises \( (r = 0.3, \ P = .03) \). The production of IFN-γ decreased in proportion to the duration of exercise.
lower circulating levels of CD4+ and VLA-4+ T cells prior to and after exercise (P≤.049), and lower levels of CD95+ T cells following exercise (P = .03). Mitogenic responses before the exercise program were also lower in men (P = .03), whereas there was no difference in proliferative responses to PHA in samples between men and women taken after the exercise program.

Mononuclear cells of hypertensive subjects produced significantly more IL-1α and TNF-α than those of normotensive subjects following exercise (P≤.03). The production of IL-1α and TNF-α was also higher in hypertensive subjects before the exercise program, with differences approaching statistical significance (P≤.13). This finding may explain in part the higher levels of IL-1α and TNF-α in men, since they had a higher prevalence of hypertension than did women.

No within-group differences could be demonstrated as a result of menopause, estrogen therapy, use of aspirin or other medications, alcohol consumption, obesity, hypercholesterolemia, smoking, diabetes mellitus, dieting, weight loss, or smoking cessation during the study.

**COMMENT**

In our study, 6 months of moderate-intensity exercise attenuated blood mononuclear cell production of cytokines with predominantly atherogenic properties, while simultaneously augmenting the production of cytokines with predominantly atheroprotective properties.

The decrease in atherogenic cytokine production by PHA-stimulated mononuclear cells and the increase in mononuclear cell production of the atheroprotective cytokine TGF-β1 after the exercise program were proportionate to the time subjects spent in performing repetitive lower-body motion exercises, indicating the existence of a dose-response relationship between exercise and mononuclear cell function in our subjects. The changes in immune cell function were accompanied by a reduction in serum CRP, a possible systemic reflection of decreased IL-1α and TNF-α production and a favorable sign in persons at risk of developing CVD. Importantly, the changes were unrelated to the ingestion of aspirin or other medications, or to dieting, weight loss, or smoking cessation during the study. Subjects included men and women who had been selected from the general population and who had well-defined risk factors for ischemic heart disease.

We found that exercise had a particularly significant attenuating effect on the production of IFN-γ and TNF-α. Both of these cytokines have been identified in early and advanced atherosclerotic lesions and are postulated to play preeminent roles in atherogenesis. Tumor necrosis factor alpha is produced primarily by monocytes and macrophages and is a potent proinflammatory cytokine. Interferon gamma is produced in T-helper type 1 (Th1) lymphocytes, NK cells, and cytotoxic T lymphocytes and is the most important cytokine regulating the activities of mononuclear phagocytes and is postulated to play preeminent roles in atherogenesis. Both cytokines can activate endothelial cells, monocytes, macrophages, and smooth muscle cells, thereby contributing to leukocyte recruitment, endothelial cell procoagulant activity, LDL oxidation, and foam cell formation in atherosclerotic lesions.

The importance of IFN-γ in atherogenesis has recently been demonstrated in transgenic mice with targeted disruptions of the APOE gene and the IFN-γ receptor gene (apoE0/IFN-γR0 mice). These mice demonstrate a substantial reduction in atherosclerotic lesion size, cellularity, and lipid accumulation, and an increase in plasma concentrations of potentially atheroprotective phospholipid/apolipoprotein A-IV rich particles when compared with APOE 0 mice, suggesting that IFN-γ promotes and modifies atherosclerosis through its local effects in the arterial wall as well as by its effects on plasma lipoproteins.

In contrast to the atherogenic cytokines, the production of cytokines that possess predominantly atheroprotective properties was augmented by exercise. In unstimulated blood mononuclear cell cultures, the cytokine most affected was IL-10, followed by TGF-β1. The secretory responses of PHA-stimulated cultures were generally similar, except that IL-4 production was also up-regulated by exercise. These cytokines effectively down-regulate delayed-type hypersensitivity reactions of the type seen in atherosclerotic lesions, primarily by suppressing mononuclear phagocyte and Th1 lymphocyte functions. Although TGF-β is thought to be primarily responsible for stimulating collagen and proteoglycan synthesis by vascular smooth muscle cells in atherosclerotic plaques, it inhibits the proliferation and migration of these cells in atherosclerotic lesions and is thought to function primarily as an atheroprotective cytokine. In this regard, low plasma levels of TGF-β have been documented in patients with ischemic heart disease, possibly due to its sequestration into an inactive pool by lipoproteins.

It is of note that, in our study, mononuclear cell production of TGF-β1 increased in proportion to time spent doing exercises traditionally considered to be cardioprotective.

It is probable that the changes in blood mononuclear cell function among the subjects after undergoing the exercise program resulted, at least in part, from an augmented suppression of TNF-α and IFN-γ production by the atheroprotective cytokines. Furthermore, because blood leukocytes are thought to provide the main source of immune cells in atherosclerosis, similar functional changes may have occurred in mononuclear cells present in atherosclerotic lesions. This possibility is supported indirectly by the decrease in serum levels of CRP in our subjects after the exercise program. It remains uncertain, however, what initiated these changes. Since herpes viruses and chlamydia have been isolated from atherosclerotic plaques...
it is possible that physical activity stimulated the immune system to eradicate these agents. Alternatively, it may be that exercise reduced levels of oxidized LDL or HSP 60 in fatty streaks and atherosclerotic plaques in our subjects. Both of these molecules have been postulated to be a target of the autoinmune attack in atherosclerosis and are capable of independently activating endothelial cells.19,23,24 Whatever the explanation, our finding that moderate long-term exercise reduces blood mononuclear cell production of cytokines with predominantly atheroprotective properties, while simultaneously increasing their production of cytokines with predominately atheroprotective properties, provides an insight as to how physical activity helps protect against CVD.

REFERENCES