Effects of Exercise on Cardiovascular Outcomes in Monkeys With Risk Factors for Coronary Heart Disease

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Objective—Exercise reduces the risk for coronary heart disease. However, the mechanisms mediating the beneficial effects of exercise remain ambiguous. In particular, it is uncertain whether exercise inhibits the development of atherosclerosis, a major pathobiologic process underlying heart disease.

Methods and Results—To address this question, adult male monkeys were fed an atherogenic diet while assigned to one of four experimental conditions for 34 months: 1) runner/no group disruption, ie, “stable” (n=19); 2) runner plus frequent social group disruption, ie, “unstable” (n=19); 3) sedentary/stable (n=15); or 4) sedentary/unstable (n=18). Neither exposure to exercise nor social group disruption significantly affected the resulting coronary artery atherosclerosis extent or lumen areas (all ANOVA values, P>0.05). When compared with sedentary individuals, exercise animals had lower resting heart rates (119.0±3 vs 132.0±3 bpm, P=0.002), greater echocardiographically measured left ventricular ejection fractions (77.2±0.01% vs 73.8±0.01%, P=0.02), greater quantitative angiographically measured dilation of coronary arteries to phenylephrine (2.6±1% vs −3.7±1% change from baseline diameter, P=0.003), and a reduced cortisol response to an adrenocorticotropin challenge. These measures were not significantly affected by social condition.

Conclusions—Thus, exercise improved some measures of cardiovascular health and reduced stress responsivity but did not inhibit progression of coronary artery atherosclerosis or promote positive artery remodeling. It is concluded that exercise may exert cardioprotective effects without influencing atherosclerosis extent. (Arterioscler Thromb Vasc Biol. 2003;23:864-871.)

Key Words: stress ■ atherosclerosis ■ heart rate ■ ejection fraction ■ vascular reactivity

Exercise training has assumed an important role in both primary and secondary prevention of coronary heart disease. Moderate to vigorous exercise is often prescribed for the prevention of ischemic heart disease, as well as for treatment after myocardial infarction, angioplasty, coronary bypass surgery, and heart transplantation. Exercise is also suggested for individuals diagnosed with congenital heart disease and stable congestive heart failure.1 The reported physiological benefits of exercise include increased physical performance and angina threshold in patients, as well as improved myocardial perfusion.2,3 Additionally, exercise has been shown to lower heart rate (HR) and improve coronary artery reactivity.1 Exercise, in combination with other interventions (lipid-lowering diet or drugs) has been shown to reduce lumen stenosis (measured as a change in lumen diameter).3-5

Conversely, dyslipoproteinemia and psychosocial stress are risk factors for the development of coronary heart disease. Elevated plasma concentrations of total plasma cholesterol (TPC), LDL cholesterol (LDL-C), and reduced concentrations of HDL cholesterol (HDL-C) promote the formation of coronary artery atherosclerosis and impair dilation of coronary arteries.6,7 Psychosocial stress exacerbates progression of coronary artery atherosclerosis in nonhuman primates.8-11 Additionally, both chronic and acute stressors impair endothelium-mediated dilation of coronary arteries.7,12

It is clear that exercise has beneficial effects on general cardiovascular health. However, it remains uncertain whether exercise independently of other interventions can inhibit the progression of coronary artery atherosclerosis, particularly in individuals otherwise at risk because of exposure to a high-fat/cholesterol diet or psychosocial stress. A single previous study using monkeys suggested that exercise is anti-atherogenic; however, this investigation was conducted on relatively young individuals with a small number of animals in each group.13 Here, we report the results of a larger investigation, involving adult animals and incorporating a stress manipulation as well as exposure to an atherogenic diet. Consistent with studies on human patients, exercise had beneficial effects on indices of cardiovascular function. However, we observed no additional benefit with respect to inhibition of atherosclerosis.
Methods

Subjects
Original study animals were 95 adult male cynomolgus monkeys (Macaca fascicularis), imported as adults (average age 7.0 years, estimated by dentition) from Indonesia (Primate Research Center, Bogor Indonesia). After a 1-month quarantine, animals were placed in social groups containing 5 animals each. Groups were housed in pens measuring 2 m × 3 m × 2.5 m, which allowed sufficient space for social interaction but not for substantial locomotor activity. Mortality associated with chronic diarrhea (approximately 20%) was unusually high during this pre-experimental period. This necessitated the importation of 16 additional monkeys. The new monkeys arrived within 8 months of the original group and before any animals were assigned to experimental conditions. Seventy-one monkeys underwent all experimental procedures and comprised the final subject population. All procedures involving animals were conducted in compliance with state and federal regulations and standards and with the approval of the Wake Forest University Institutional Animal Care and Use Committee. The animal facilities and procedures of Wake Forest University Medical School are AAALAC approved.

Experimental Design

Overview
The study was a 2 × 2 factorial design in which the treatments were exercise (runner, sedentary) and social condition (stable, unstable social groups). As part of this design, all animals consumed a diet relatively high in fat and cholesterol, designed to model that typically consumed by North Americans.

The experiment was conducted in four phases. Phase 1 consisted of a 5-month period during which baseline measures were collected. Phase 2 consisted of a 10-month period during which all animals were trained to run on a motorized exercise wheel. During phases 1 and 2, the monkeys ate a chow (-) diet (Ralston Purina Company). At the beginning of phase 3, all social groups were reorganized across groups to insure approximate equivalence in willingness and ability to running. Half of the groups were then randomly assigned to the running condition, with the remainder sedentary. Phase 3 continued for 8 months, during which the running animals became fully conditioned, and the sedentary animals were allowed to decondition. All animals began consuming the atherogenic diet during this phase.

Phase 4 (34 months in duration), began with the assignment of half of the running and sedentary monkeys to the unstable social condition. The social groups of the “unstable” monkeys were reorganized at monthly intervals thereafter. The social groups of the “stable” monkeys remained constant for the remainder of the experiment. The four final treatment conditions were: 1) runner/stable (n = 19); 2) runner/unstable (n = 19); 3) sedentary/stable (n = 15); and 4) sedentary/unstable (n = 18). At the beginning of phase 4, the four treatment conditions were equivalent in plasma lipids.

Exercise
The monkeys exercised in a motor-driven wheel that had a diameter of 107 cm and an adjustable speed control that ranged from 0 to 6 km/h. At the beginning of phase 2, monkeys were familiarized with the wheel by spending equal amounts of time sitting and running slowly (1.5 km/h). The speed of the wheel and the amount of time spent running was increased gradually. By the end of phase 2, monkeys were running consistently for 30 minutes at 3.5 km/h, 3 days per week. At every running session, each monkey was rated on a 5-point scale according to predetermined criteria. The determin-ation of running ability was used as a randomization factor in the assignment of animals to the running or sedentary conditions which were done at the beginning of phase 3. Following assignment to final experimental groups (phase 4), monkeys in exercise groups received additional conditioning until they ran 40 minutes per day, 3 days per week, at 3.5 km/h. This level of training has been shown to result in aerobic conditioning in monkeys of this species. While monkeys were exercising, their sedentary counterparts were removed from their social groups and placed in single cages for an equal amount of time as a sham procedure. All monkeys were observed continuously while running on the exercise wheel and were not made to run to exhaustion or if they showed any evidence of injury or distress.

Social Condition
We have observed previously that coronary artery atherogenesis in male monkeys is exacerbated when animals are exposed to a disrupted social environment. In these studies, social disruption or instability was achieved by periodic reorganization of monkeys among social groups. The same manipulation was used in the current experiment. Starting with phase 4, the reorganization of group memberships occurred on a monthly basis and followed a schedule that permitted each monkey to be housed with 4 new animals at the start of each reorganization.

Diet
The experimental diet was formulated at our facility to model typical North American consumption. The diet had a wheat flour base and derived 43% of calories from fat, 18% from protein, and 39% from carbohydrates; lard, beef, butter, soft margarine, and egg yolk provided the majority of the fat, while protein was derived from casein and lactalbumin as well as the wheat flour. The diet was fed to each social group in two daily portions, morning and late afternoon.

In Vivo Measurements

Restraint
Animals were tranquilized with ketamine HCl (10 to 15 mg/kg) for all physical handling and sampling.

HR and Blood Pressure Measurements
The methods used for HR evaluations have been described previously. Briefly, HR was measured by using portable ECG telemetry units (Kuettelf and Esser, Model TM-7 patient monitors) secured beneath nylon mesh jackets and maintained in place for several days. To assess the effects of the experimental manipulations, HR was monitored during undisturbed overnight conditions (the 18-hour period from 15:00 until 09:00, recorded on 3 consecutive nights) at baseline and 4 times during the experiment. We report here determinations made near the end of the study. During the final phase of the study, HR was also recorded at midday (~12:00) and in relation to an exercise session (before, during, and 10 minutes after); all animals (runner and sedentary) were exposed to the exercise wheel for this determination. Systolic and diastolic blood pressures were measured once every 3 months during phase 4 of the experiment by using methods described previously.

Myocardial and Coronary Artery Function Tests
Myocardial function was evaluated via echocardiography on two occasions during phase 4 and by using methods published previously. Briefly, left ventricular thickness and ventricular chamber size were measured with B- and M-mode technology. Ejection fractions (at rest) were calculated as the difference between ventricular chamber size in systole and diastole. Coronary artery function was assessed on one occasion, immediately before necropsy. In this procedure, animals were first anesthetized and then a 3F custom-designed catheter was advanced from the left femoral artery to the left main coronary artery. Quantitative coronary angiography (QCA) was used to assess the change in coronary artery diameter (from control diameter) in response to intracoronary infusion of: 1) acetylcholine (10⁻⁶ M, final concentration in the coronary artery); 2) nitroglycerin (50 μg); and 3) phenylephrine (1 μg). Control diameters were measured before and between infusions of the agonists. There was approximately 20 minutes between infusions of agonists. The QCA methods, sensitivity, and reproducibility have been published previously.

Adrenocorticotropicin (ACTH) Challenge
To assess reactivity of the pituitary adrenocortical (“stress”) system, an ACTH challenge was done once in phase 4, toward the end of the
study and by using techniques previously described.18 The challenge was conducted in two parts. At approximately 08:00, animals were briefly captured and given dexamethasone intramuscularly (0.125 mL/kg) to suppress endogenous adrenocortical activation. Four hours later, animals were captured, anesthetized, and given a bolus of ACTH (Cortrosyn: 10 ng/kg) injected into the saphenous vein. Venous blood samples from the contralateral femoral vein were taken at the time of injection, and at 15 and 30 minutes after injection. Cortisol was extracted from the plasma with diethyl ether and measured by radioimmunoassay.

**Body Weight**

Body weight was determined whenever animals were anesthetized, but no less than once per month. Analyses are based on the mean of measures taken during the last 12 months of the experiment.

**Plasma Lipids and Lipoproteins**

TPC and HDL-C were measured at 3-month intervals by using methods described previously.19 20 As stated above, TPC and HDL-C values were used as a randomization variable to assign monkeys to the four experimental conditions. Plasma concentrations of LDL+very LDL concentrations (LDL+VLDL-C) were calculated from the TPC and HDL-C concentrations. Analyses are based on the mean of measures taken during phase 4 of the experiment.

**Behavioral Observations**

To document the effects of exercise and social condition on behavior, 30-minute observations were made of each social group twice per week using techniques described previously.21 Each animal was thus observed approximately 250 times during Phase 4. During these observations, technicians used a computer (Tandy TRS model 100) to record all aggressive, submissive, and affiliative interactions. Observations were made between 09:00 and 16:00, with times of day balanced across groups. After collection, the data were used in the calculation of 5 behavioral indices encompassing the range of social behavior typically observed in this species: 1) the rate of total aggression (fights initiated per monkey per hour); 2) the rate of intense aggression (fights with biting or grabbing initiated per monkey per hour); and 3) the percentage of time spent in passive body contact, grooming, or sitting alone at a distance.9 21 For each behavior, the mean score for each animal across phase 4 was used in all subsequent analyses.

**Post-Necropsy Experimental Endpoints**

**Organ Measurements**

The heart was removed, trimmed and weighed at necropsy (before removal of the coronary arteries (see below). The atria and right ventricle were removed from the left ventricle and the left ventricle weighed separately. The adrenal glands were removed, trimmed, and weighed at necropsy. The ratio of total adrenal weight (right plus left) to body weight was calculated.

**Assessment of Coronary Artery Atherosclerosis**

Monkeys were euthanized, and the cardiovascular system was then perfusion-fixed with 10% neutral buffered formalin at physiological pressure (100 mm Hg) for 1 hour, as described previously.8 11 Morphometric assessment of the atherosclerosis extent, arterial size, and lumen size was accomplished by using a computer-assisted stylus, as described in detail elsewhere.8 11 As in previous studies, the mean of 15 sections of coronary artery (5 sections each of the left circumflex, left anterior descending, and right coronary arteries) was used as the atherosclerosis index for each animal.

**Statistical Analysis**

Values shown are mean±SEM. Data were tested for assumptions of parametric analysis and were subjected to linear transformation where necessary for all surviving animals (n=71), a series of two-factor analyses of variance (Exercise runner, sedentary × Social Condition stable, unstable) were used to determine the effect of the experimental manipulations on the dependent measures. For measures obtained both at baseline and during the experiment—HR, blood pressure, and body weight—two-factor (Exercise runner, sedentary × Social Condition stable, unstable) analyses of covariance (covarying the baseline values) were used. For the atherosclerosis outcome only, a separate (Exercise runner, sedentary × Social Condition stable, unstable) ANOVA was conducted by using only the animals from the original cohort surviving to the end of the study (n=58). This analysis did not differ in outcome from the analyses based on all 71 monkeys. Tests of significance were two-tailed at P<0.05.

**Results**

**In Vivo Determinations**

**Heart Rate and Blood Pressure**

Analysis of covariance revealed that undisturbed (overnight) HRs were significantly lower in runners than sedentary monkeys (Table 1). HRs measured at midday were higher across all monkeys than overnight measures (P<0.001). However, the significant difference between runners and sedentary animals persisted (Table 1). HRs were also measured in all monkeys while in an exercise wheel, before, during, and 10 minutes after an exercise challenge session conducted within the last 4 months of the study. On placement in the wheel, HR was equivalently elevated in all groups (168±3 bpm). Furthermore, HR was similarly increased in all

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**TABLE 1. HR and Blood Pressure**

<table>
<thead>
<tr>
<th></th>
<th>Runner/Stable (n=19)</th>
<th>Runner/Unstable (n=19)</th>
<th>Sedentary/Stable (n=15)</th>
<th>Sedentary/Unstable (n=18)</th>
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<tbody>
<tr>
<td><strong>HR, bpm</strong></td>
<td></td>
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<tr>
<td>Undisturbed (overnight)</td>
<td>118.06±4.24</td>
<td>117.50±3.95</td>
<td>132.00±4.28</td>
<td>134.51±4.72</td>
</tr>
<tr>
<td>Midday</td>
<td>124.20±5.70</td>
<td>128.50±5.21</td>
<td>142.70±5.64</td>
<td>141.60±5.93</td>
</tr>
<tr>
<td>After exercise</td>
<td>177.60±4.59</td>
<td>177.99±4.72</td>
<td>188.36±5.17</td>
<td>189.09±4.73</td>
</tr>
<tr>
<td><strong>Blood pressure, mm Hg</strong></td>
<td></td>
<td></td>
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<tr>
<td>Systolic</td>
<td>95.47±5.27</td>
<td>99.05±4.23</td>
<td>95.27±5.10</td>
<td>101.28±3.20</td>
</tr>
<tr>
<td>Diastolic</td>
<td>50.42±3.33</td>
<td>54.05±2.41</td>
<td>51.80±3.55</td>
<td>59.5±3.31</td>
</tr>
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</table>

Main Effect: Exercise × Social Condition

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<thead>
<tr>
<th></th>
<th>Exercise</th>
<th>Social Condition</th>
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<tbody>
<tr>
<td>F=10.37</td>
<td>NS</td>
<td>NS</td>
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<tr>
<td>P=0.002</td>
<td></td>
<td></td>
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<tr>
<td>F=7.32</td>
<td>NS</td>
<td>NS</td>
</tr>
<tr>
<td>P=0.01</td>
<td></td>
<td></td>
</tr>
<tr>
<td>F=5.18</td>
<td>NS</td>
<td>NS</td>
</tr>
<tr>
<td>P=0.03</td>
<td></td>
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</table>

Interaction: Exercise × Social Condition

<table>
<thead>
<tr>
<th></th>
<th>Exercise</th>
<th>Social Condition</th>
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<tbody>
<tr>
<td>NS</td>
<td></td>
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</table>

Undisturbed HR means are adjusted for baseline measure±SEM. Other values are mean±SEM. n is total number of animals evaluated.
animals during the exercise challenge (228±3 bpm). After the challenge, however, HR was significantly lower in the exercise monkeys than in their sedentary counterparts, with no significant differences related to Social Condition or the Exercise × Social Condition interaction. Finally, the experimental manipulation did not significantly affect blood pressure (Table 1).

**Echocardiographic Evaluation of Myocardial Function**
Monkeys in the Exercise condition had significantly greater ejection fractions than those in the sedentary condition (Table 2); there were no significant effects associated with Social Condition or the interaction between Exercise and Social Condition.

**Coronary Artery Reactivity**
There was an effect of experimental condition of coronary artery response to phenylephrine. Coronary arteries of runners dilated in response to intracoronary infusion of phenylephrine (2.6±1.5%), whereas those of sedentary monkeys constricted (3.7±1.1%) (P=0.002). There was no effect of experimental condition on coronary artery response to acetylcholine or nitroglycerin, (all P>0.1, Table 2).

**ACTH Challenge**
To assess the effects of Exercise and Social Condition on adrenal responsivity, the cortisol responses to the ACTH challenge were subjected to a repeated measures analysis of covariance; the 15- and 30-minute cortisol values were the repeated measures and the cortisol value at time 0 was used as the covariate. The analysis revealed significant effects for Exercise (runners lower than sedentary) and the repeated measure (higher at 15 minutes than 30, Figure 1), with no significant effects associated with Social Condition or the interaction of Social Condition and Exercise (data not shown).

**Plasma Lipids and Lipoprotein Measurements**
There was no significant difference in TPC concentrations among the runner/stable, runner/unstable, sedentary/stable, and sedentary/unstable monkeys (11.97±0.62 mmol/L, 11.63±0.54 mmol/L, 11.37±0.78 mmol/L, and 11.60±0.74 mmol/L, respectively). Additionally, there were no significant effects of the exercise manipulation or social condition on plasma measures of HDL-C, LDL+VLDL-C, or triglycerides (Table 3).

### TABLE 2. Myocardial Function and Coronary Artery Responses

<table>
<thead>
<tr>
<th></th>
<th>Runner/Stable</th>
<th>Runner/Unstable</th>
<th>Sedentary/Stable</th>
<th>Sedentary/Unstable</th>
<th>Main Effect</th>
<th>Interaction</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Exercise</td>
<td>Social Condition</td>
</tr>
<tr>
<td>Ejection fraction, %</td>
<td>75.9±1.5</td>
<td>78.5±0.9</td>
<td>74.8±1.3</td>
<td>72.8±1.6</td>
<td>F=6.03</td>
<td>NS</td>
</tr>
<tr>
<td></td>
<td>(n=19)</td>
<td>(n=18)</td>
<td>(n=14)</td>
<td>(n=16)</td>
<td></td>
<td>NS</td>
</tr>
<tr>
<td>Acetylcholine, %</td>
<td>0.36±1.53</td>
<td>0.35±1.41</td>
<td>2.65±1.63</td>
<td>0.72±1.10</td>
<td>NS</td>
<td>NS</td>
</tr>
<tr>
<td></td>
<td>(n=14)</td>
<td>(n=12)</td>
<td>(n=12)</td>
<td>(n=10)</td>
<td></td>
<td>NS</td>
</tr>
<tr>
<td>Nitroglycerin, %</td>
<td>1.06±1.17</td>
<td>1.40±1.67</td>
<td>2.84±2.12</td>
<td>−1.95±0.97</td>
<td>NS</td>
<td>NS</td>
</tr>
<tr>
<td></td>
<td>(n=16)</td>
<td>(n=13)</td>
<td>(n=10)</td>
<td>(n=10)</td>
<td></td>
<td>NS</td>
</tr>
<tr>
<td>Phenylephrine, %</td>
<td>2.30±2.48</td>
<td>2.94±1.92</td>
<td>−3.60±1.62</td>
<td>−3.84±1.84</td>
<td>F=9.84</td>
<td>NS</td>
</tr>
<tr>
<td></td>
<td>(n=11)</td>
<td>(n=12)</td>
<td>(n=10)</td>
<td>(n=10)</td>
<td></td>
<td>NS</td>
</tr>
</tbody>
</table>

*Values are mean±SEM. n is total number of animals evaluated.*
that the experimental manipulations did not affect either or their interaction on intimal area or lumen area, suggesting there was no significant effect of Exercise, Social Condition, bodyweight, or the ratio of adrenal weight to body weight.

Social Behavior
Behavioral responses are contained in Table 4. It can be seen that running monkeys spent more time in body contact with each other than did their sedentary counterparts. There were no significant effects of exercise on any other behavior. The social manipulation did not influence the rate at which animals initiated fights or the percentage of time that they spent in various behavioral states.

Postmortem Determinations

Body and Organ Weight
As shown in Table 5, the left ventricle weight of the runners was greater than those of the sedentary monkeys. There were no significant differences associated with Social Condition or the Exercise × Social Condition interaction. Furthermore, there were no significant differences among treatment groups in body weight, heart weight, the ratio of left ventricular weight to heart weight, or the ratio of adrenal weight to body weight.

Atherosclerosis Extent
There was no significant effect of Exercise, Social Condition, or their interaction on intimal area or lumen area, suggesting that the experimental manipulations did not affect either atherosclerosis or artery size (Figure 2).

Discussion
The hypothesis of this study was that chronic exercise would induce beneficial effects on cardiovascular outcomes, even in animals subjected to periodic social disruption (ie, stress). The study showed that, while exercise produced a significant conditioning effect on the cardiovascular system, it did not inhibit the progression of coronary artery atherosclerosis, lead to arterial remodeling (ie, luminal enlargement), or result in changes to plasma lipids or lipoproteins. Furthermore, the absence of an exercise effect on atherosclerosis was not influenced by exposure to the social disruption manipulation, which itself did not significantly affect atherosclerosis extent. Finally, animals that exercised displayed diminished cortisol responsivity to an ACTH challenge, suggesting that exercise attenuated the classic stress response of the pituitary adrenocortical axis; the social manipulation was not associated with any alterations in stress response. Taken together, the data presented here indicate that chronic exercise improves cardiovascular function and reduces stress responsivity without concomitant changes in plasma lipids or atherosclerosis progression.

Exercise Effects on Cardiovascular Conditioning
The exercise regimen used in the present experiment produced many of the beneficial effects on cardiovascular conditioning that have been reported previously. Exercise reduced resting HRs (compared with sedentary animals) and HR after acute exercise, although it did not reduce maximal HR during exercise. The exercise regimen also increased myocardial contractility (increased ejection fraction of the left ventricle) and increased the size of the left ventricle. While the exercise regimen did not promote endothelium-mediated dilation (the response to acetylcholine), it did reduce constriction to phenylephrine.

Documented beneficial effects of exercise on the cardiovascular conditioning include a reduction in resting HRs, an increase in left ventricular size, an increase in myocardial contractility, a reduction in blood pressure of hypertensive individuals, and increased arterial distensibility. Thus, it would appear that, for the most part, the exercise regimen used in the current experiment produced conditioning effects that were not influenced by exposure to social disruption.

TABLE 3. Plasma Lipid and Lipoproteins Concentrations (mmol/L)

<table>
<thead>
<tr>
<th></th>
<th>Main Effect</th>
<th>Interaction</th>
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<tbody>
<tr>
<td></td>
<td>Exercise</td>
<td>Social Condition</td>
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<tr>
<td></td>
<td></td>
<td>Exercise × Social Condition</td>
</tr>
<tr>
<td>TPC</td>
<td>NS</td>
<td>NS</td>
</tr>
<tr>
<td>HDLC</td>
<td>NS</td>
<td>NS</td>
</tr>
<tr>
<td>LDL+VLDLC</td>
<td>NS</td>
<td>NS</td>
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<tr>
<td>TPC/HDLc</td>
<td>NS</td>
<td>NS</td>
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</table>

Values are mean ± SEM.

TABLE 4. Social Behaviors*

<table>
<thead>
<tr>
<th></th>
<th>Main Effect</th>
<th>Interaction</th>
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<tbody>
<tr>
<td></td>
<td>Exercise</td>
<td>Social Condition</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Exercise × Social Condition</td>
</tr>
<tr>
<td>Total fights initiated</td>
<td>NS</td>
<td>NS</td>
</tr>
<tr>
<td>Intense fights initiated</td>
<td>NS</td>
<td>NS</td>
</tr>
<tr>
<td>Time spent grooming, %</td>
<td>NS</td>
<td>NS</td>
</tr>
<tr>
<td>Time spent in contact, %</td>
<td>NS</td>
<td>NS</td>
</tr>
<tr>
<td>Time spent alone, %</td>
<td>NS</td>
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Values are mean ± SEM. n is total number of animals evaluated.
*Rates calculated per hour per monkey (for behavioral actions).
consistent with previously reported cardiovascular conditioning effects of exercise.

One apparent discrepancy between previously published data and the result reported in this study is that exercise (in the present study) did not promote endothelium-mediated dilation of atherosclerotic arteries. Exercise has been shown to promote endothelium-mediated dilation by upregulating production of NO.26–31 The lack of effect of exercise on endothelium indicated dilation may be due to a lack of effect on atherosclerosis extent which has been shown to modulate vascular reactivity.6 We did, however, observe a modest effect of exercise on constrictor responses to phenylephrine, which is consistent with previous studies showing a beneficial effect of exercise on adrenergically mediated constriction.32

Exercise Effects on Intermediate Endpoints of Coronary Heart Disease

Exercise was not associated with significant changes in plasma lipids or lipoproteins. As reviewed by Shepard and Balady,1 exercise has modest effects on lowering plasma TPC and LDL-C concentrations. It is unclear why a similar effect was not observed in the present study. One possibility is that TPC concentrations in the monkeys were higher than those in people considered to have elevated TPC concentrations. Since the effects of exercise on TPC concentrations among people are modest at best, it seems more likely that any TPC-lowering properties of exercise may not have been sufficient to overcome the relatively high TPC concentrations in monkeys. Another possibility is that the stress associated with forced exercise might have prevented beneficial changes. However, the results of the ACTH challenge test suggest that stress was reduced, not increased, by the exercise manipulation.

Although there are reports in the literature that exercise tends to increase HDL-C concentrations (especially the HDL2 subfraction), no such effect was observed in the current study. As with the TPC concentrations, it is possible that the relatively suppressed HDL-C levels of the cholesterol-fed monkeys in this experiment were not amenable to the modest effect usually observed in association with an exercise manipulation, etc. Similarly, Couillard et al33 reported that men with isolated low HDL-C did not show HDL-C changes after exposure to an exercise regimen.

Exercise did not inhibit progression of coronary artery atherosclerosis or promote lumen enlargement (positive coronary artery remodeling) in monkeys housed in stable or unstable social groups. These data may be interpreted in one of two ways. Either a moderate exercise regimen is not anti-atherogenic, or the anti-atherogenic effects of exercise were suppressed in the present study by the diet-induced dyslipoproteinemia used as part of the experimental paradigm. Unfortunately, there are no human trials relating direct

<table>
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<th>Table 5. Body and Organ Weights</th>
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<tr>
<td></td>
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<tr>
<td>Body weight, kg</td>
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<tr>
<td>Left ventricle weight, g</td>
</tr>
<tr>
<td>Heart weight, g</td>
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<tr>
<td>Left ventricle weight/heart weight ratio</td>
</tr>
<tr>
<td>Total adrenal weight/body weight ratio</td>
</tr>
</tbody>
</table>

Body weight means are adjusted for baseline measure±SEM. Other values are mean±SEM. n is total number of animals evaluated.

Figure 2. Atherosclerosis extent in the coronary artery (mean intimal area and lumen area in mm²) for all animals in the four groups: runner/stable, runner/unstable, sedentary/stable, and sedentary/unstable (P>0.05).
measures of atherosclerosis to exercise. Exercise has been associated with a reduction in lumen stenosis\textsuperscript{1} and ischemic ST-segment depression, but neither is a direct measure of atherosclerosis. Furthermore, in contrast to the present experiment, the studies reporting a beneficial effect of exercise on lumen stenosis generally include lipid-lowering interventions. Notably, observational investigations following large cohorts of people frequently show reductions in cardiovascular disease associated with increasing amounts of exercise.\textsuperscript{1–5,27}

Such studies are, however, inherently limited by the high degree of self selection exhibited by exercise participants. Nonetheless, the results of these studies can be reconciled with the major outcome of the current investigation if the general health benefits of exercise conditioning prolong life even in the face of advancing atherosclerosis. Of relevance in this context is the recent report by Ford\textsuperscript{34} relating exercise to decreased C-reactive protein, suggesting that exercise may be cardioprotective in part because of anti-inflammatory effects. Although no inflammation markers were measured in the present study, the reduction in adrenocortical responsivity in the exercised monkeys is perhaps indicative of reduced inflammatory potential.

The effects of exercise on development of atherosclerosis in nonprimate animal models are varied. The effects of exercise on atherosclerosis in pigs are reviewed by Carey.\textsuperscript{35} While both negative and positive studies are cited, the effectiveness of exercise to inhibit atherogenesis appears to depend on the duration and intensity of the exercise regimen.\textsuperscript{35} Hasler et al\textsuperscript{36} report that exercise retards the development of atherosclerosis in rats fed a hyperlipemic diet. Furthermore, exercise is reported to inhibit development of atherosclerosis in LDL-receptor knockout mice.\textsuperscript{37} Because of the genetic similarities between nonhuman and human primates and the similarities in atherosclerotic lesion development, we believe that findings reported in this study represent a more translatable effect of exercise on atherogenesis than those findings in nonprimate models. However, we cannot rule out the possibility that exercise might have had positive effect on atherosclerosis progression with a more extensive and intense exercise regimen as has been noted in pigs.\textsuperscript{35}

We note that one previous study using cynomolgus monkeys suggested that exercise inhibits progression of atherosclerosis.\textsuperscript{13} It is not entirely clear why results of the present study are not consistent with the earlier outcome. The study by Kramsch et al\textsuperscript{12} used younger animals than those in the present study, as judged by their body weights. Furthermore, there were significant methodological differences in the two studies. First, the Kramsch study used nonmotorized exercise wheels in contrast to the motorized wheels used here. Animals in the Kramsch study could thus meter their own exercise. Furthermore, the current study randomly assigned animals to the exercise and sedentary conditions only after all animals had been initially conditioned; this was not done in the Kramsch study, raising the possibility that animals may have been assigned to the exercise group based on their willingness to run. Two other factors could have specifically biased the Kramsch study in favor of the exercised group: 1) the exercised—but not the sedentary control—monkeys had free access to monkey chow, a diet with known anti-atherogenic effects (all animals in the current study consumed only the atherogenic diet following assignment to their final experimental conditions); and 2) the sedentary animals in the Kramsch study were housed in single cages to prevent physical activity; single-cage housing accelerates atherogenesis in this species.\textsuperscript{38}

Considerations of the Psychosocial Stress Paradigm

The present study was designed to determine whether exercise could inhibit the atherogenic effects of the stress associated with repeated reorganization of social group membership. However, the results revealed no exacerbation of atherosclerosis in the reorganized groups and thus did not provide an adequate basis for testing the hypothesis that exercise protects against the atherogenic effects of stress. Although it is not known for certain why the stress manipulation in the current study did not accelerate atherogenesis, the behavioral data offer a clue. In previous experiments groups that were similarly manipulated by group disruption differed from controls in the pattern of fighting (greater numbers of severe fights initiated) and grooming (more grooming).\textsuperscript{21} No such effects were observed in the present study, suggesting that the manipulation was not particularly stressful. Further evidence that the manipulation was not particularly stressful is the lack of effect of social condition on cortisol response. Unlike previous studies, an estrogen-implanted female was not used as a provocation in the reorganized groups. Perhaps such additional stimulation is necessary to produce a stress response. Another possibility is that the monkeys used in this experiment were, by chance, unusually pacific.

Study Limitations

As stated in Methods, all monkeys were trained to run and were then graded on their ability/willingness to do so. To avoid the bias related to self-selection, monkeys were then divided into runners and nonrunners, equalizing groups for initial running grade. Studies involving exercise in people are usually biased by self selection because willing runners are included in the exercise groups and the less willing are in the nonexercise group. Thus, the procedure of equalizing the groups of monkeys for running grade may have diluted the treatment condition, making it harder to detect the beneficial effects of exercise on atherosclerosis as sometimes seen in human studies. Unfortunately, the numbers of monkeys were not sufficiently high to do subgroup analysis based on running grade.

There is current interest in the effects of treatment/prevention on atherosclerotic plaque inflammation. It is believed that atherosclerotic inflammation may be more predictive of coronary heart disease risk than plaque size, per se, and that exercise may specifically target such inflammation.\textsuperscript{34,35} The study reported here was designed to examine the effects of exercise on plaque size. We did not examine the effects of exercise on plaque inflammation. Any effects of exercise on inflammation would thus have gone undetected.

In conclusion, the current study suggests that a moderate exercise regimen produces favorable cardiovascular conditioning effects and reduces stress responsivity, but does not inhibit progression of coronary artery atherosclerosis or promote artery remodeling in monkeys with marked, diet-
induced dyslipoproteinemia. It is concluded that exercise serves an important role in reducing the risk of heart disease. However, other appropriate therapies/alterations in lifestyle need to be considered for reducing the risk of coronary heart disease in individuals at high risk.

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