Hypotension induced by exercise is associated with enhanced release of adeny]l purines from aged rat artery

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EXERCISE DECREASES blood pressure in hypertensive
animals and humans (13, 28) at least partially by
inducing body weight loss and changes in prostaglan-
din metabolism (19). We showed (15) that treadmill
exercise slightly increases the endothelium-dependent
relaxation responses to ACh in thoracic aortas isolated
from young rats but does not affect those from older
rats. In addition, chronic exercise enhances endothe-

cular responses are frequently altered, with both con-
duct and resistance vessels manifesting impaired endo-

thelial cell functions (5). In addition, an association
between hypertension and hypercholesterolemia was
observed in a number of populations (34) and in aged

rats (15, 16). To clarify the beneficial effects of exercise,
we assayed the release of adenine nucleotides and
adenosine in vascular tissues, probably by ecto-

ucleotidases, is thought to be the origin of released
purines (29). On the other hand, a high rate of ATP
breakdown, such as occurs during exercise, results in
high levels of adenosine (1). Therefore, if elevated
postexercise adenine nucleotides and adenosine in ex-
tracellular spaces of vascular tissues persist as part of
the body's adaptation to exercise, the antihypertensive
effect of exercise on blood pressure may be associated
with an augmentation of ATP release from vascular
beds.

In patients with hypercholesterolemia, arterial vas-
cular responses are frequently altered, with both con-
duct and resistance vessels manifesting impaired endo-

thelial cell functions (5). In addition, an association
between hypertension and hypercholesterolemia was
observed in a number of populations (34) and in aged

rats (15, 16). To clarify the beneficial effects of exercise,
we assayed the release of adenine nucleotides and
adenosine from the caudal arteries of aged rats and
correlated the release of ATP and its metabolites with
the effect of exercise on blood pressure and serum
cholesterol.

MATERIALS AND METHODS

Animals. All animal experiments were performed in accor-
dance with the Guidelines for Animal Experimentation of
Shimane Medical University, compiled from the Guidelines
for Animal Experimentation of the Japanese Association for
Laboratory Animal Science. Female Wistar rats (100–105 wk
old) were fed a normal laboratory diet [F1 diet (in g/kg): 50
carbohydrate, 213 protein, 51 lipid, 31 fiber, 575 nitrogen-free
extract, and 80 water; total energy 42 kcal/g, Funabashi
Farm, Chiba, Japan], maintained at 23°C in relative
humidity of 50 ± 10% with automatic lighting from 0800 to
2000, and weighed. Systolic blood pressure (SBP) and
mean blood pressure were measured by the tail-cuff plethysmo-

metric method (UR-1000, Ueda, Tokyo, Japan), and the
diastolic blood pressure (DBP) was calculated as previously
described (16). The rats were then fed a high-cholesterol diet
(F1 diet containing 1% cholesterol and 1% cholic acid, Funaba-
shi Farm) and randomly divided into two groups. One group

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(11 exercised rats) was exercised 1 h/day, 5 days/wk, on a treadmill with a gradient of 10° at 5–15 m/min during the first 4 wk and at 15 m/min over the next 8 wk (15). The other group (17 sedentary rats) was only handled for 2–3 min daily, 5 days/wk for 12 wk. The rats were kept in small individual cages (20 cm long × 14 cm wide × 14 cm high).

Within at least 24 h of the last run and after an 18-h overnight fasting period, the rats were weighed and their blood pressure was measured by the plethysmographic method. The rats were anesthetized with pentobarbital sodium (65 mg/kg ip); blood was collected from the inferior vena cava into heparinized syringes, transferred to polyethylene tubes containing 1 mmol/l EDTA, and centrifuged for 20 min (3,000 rpm) at 4°C.

Plasma samples were assayed for platelet contamination with an automated hematologic analyzer (<103/µl; K-2000, Toa Medical Electronics, Kobe, J apan), and plasma levels of ATP, ADP, AMP, and adenosine were measured by HPLC with fluorescence detection.

Tissue preparation and purine release. Tissue preparation and purine release were carried out as previously described (16). After blood collection, we removed a maximal segment (∼8–13 cm, 20–30 mg wet wt) of the caudal artery, cleaned it of connective tissue while taking care not to damage the endothelium, and suspended the segment in a water-jacketed organ chamber containing 2.0 ml of modified Krebs solution (in mmol/l: 110 NaCl, 4.6 KCl, 2.5 CaCl2, 24.8 NaHCO3, 1.2 KH2PO4, 1.2 MgSO4, and 5.6 glucose, equilibrated with 95% O2-5% CO2) at 37°C for 60 min; the solution was replaced every 3 min during the last 30 min.

After the 60-min equilibration period, the bathing solution was collected by draining the organ chamber every 3 min. After the first sampling to determine spontaneous release for 3 min, the tissue was stimulated with 1 µmol/l norepinephrine for 3 min and the bathing solution (stimulation sample) was collected. The samples were processed for determination of ATP, ADP, AMP, and adenosine by HPLC fluorescence. After the release experiments, the arteries were stored in −80°C until total fatty acids were measured.

Plasma cholesterol and nitrogen oxide concentrations. Concentrations of total and free cholesterol in plasma were determined with the Cholesterol E-test and Free Cholesterol E-test kits (Wako Pure Chemical, Osaka, J apan), respectively. The plasma nitrogen oxide (NOx; nitrite/nitrate) concentration was assayed by a modification of the method of Misko et al. (24). Briefly, plasma was incubated with NADPH and ascorbic acid in the presence of nitrate reductase (Sigma Chemical, St. Louis, MO) and subsequently with 2,3-diaminonaphthalene (Dojindo Labs, Kumamoto, J apan). Fluorescence intensity was measured with a Hitachi 850 fluorescence spectrometer (Hitachi, Tokyo, J apan). Nitrite standards (>98% pure, Sigma Chemical) were freshly prepared.

Fatty acid content of plasma and tissue samples. Fatty acid levels in plasma were assayed by a modification of the one-step reaction of Lepage and Roy (20). A mixture of 100 µl of plasma, 2 ml methanol-toluene (4:1, vol/vol, containing 10 µg of tricosanoic acid as an internal standard), and 200 µl of acetyl chloride was incubated at 100°C for 60 min; 6% aqueous potassium carbonate containing 10% sodium chloride was then added, and the whole mixture was shaken for 10 min at room temperature and centrifuged at 1,800 g for 5 min. The supernatant, containing the fatty acid methyl esters, was directly subjected to gas chromatography (GC) on a model 5890 II gas chromatograph (Hewlett-Packard, Avondale, PA) equipped with a flame ionization detector and an automatic sampler (model 7673) and utilizing a 25-m × 0.25-mm ID fused-silica column (DB-WAX P/N 122-7032, J & W Scientific, Folsom, CA) programmed from 100 to 180°C at 20°C/min, 180 to 240°C at 2°C/min, 240 to 260°C at 4°C/min, and at 260°C for 5 min. The identities of the peaks were established by comparison with the peaks of reference compounds and, in part, by J MS-D 300 gas chromatography-mass spectrometry (J ed, Tokyo, J apan).

Fatty acid levels in caudal arteries were measured by a similar procedure. The stored caudal arteries (10–20 mg), transferred to a capsule precooled in liquid N2, were crushed using an amalgam mixer (UT-1600, Sharp, Osaka, J apan) and suspended in 200 µl of phosphate-buffered saline (Dulbec-co's PBS[−]) containing 0.005% butylated hydroxytoluene. The fatty acid content of 100 µl of this suspension was analyzed by GC as described above.

Fatty acid content was expressed as milligrams per deciliter of plasma or micrograms per gram of tissue wet weight. The average degree of fatty acid unsaturation (the unsaturation index) was calculated as the average number of double bonds per fatty acid residue multiplied by 100.

Statistical analysis. Results are expressed as means ± SE. Data were evaluated by regression analysis and by paired and unpaired Student's t-tests, using the computer program Stat View II (Abacus Concepts, Berkeley, CA). A level of P < 0.05 was accepted as statistically significant.

RESULTS

Body weight, food intake, and cardiovascular parameters. We observed no significant difference in body weight before and after treadmill exercise or food intake during the experimental period between exercised and sedentary rats (Table 1). The SBP and DBP of sedentary aged rats increased significantly after 12 wk; however, the blood pressure of exercised rats did not change. Exercise increased heart weight significantly (P < 0.05), and the heart rate showed a tendency to decrease (0.05 < P < 0.1) (Table 1).

During the experiments, the mortality of exercised rats was 0 of 11 and that of nonexercised rats 1 of 17. Therefore, it is unlikely that the mortality would be increased by the exercise in the present experiments.

Plasma cholesterol and NOx concentrations. Although the level of total and free cholesterol tended to be lower in the plasma of exercised rats than that of

Table 1. Body weight, food intake, systolic and diastolic blood pressure, heart weight, and heart rate in sedentary and exercised aged rats with hypercholesterolemia

<table>
<thead>
<tr>
<th></th>
<th>n</th>
<th>Body Wt, g</th>
<th>Food Intake, g rat⁻¹ day⁻¹</th>
<th>Systolic Blood Pressure, mmHg</th>
<th>Diastolic Blood Pressure, mmHg</th>
<th>Heart Wt, g</th>
<th>Heart Rate, beats/min</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Before</td>
<td>After</td>
<td>Before</td>
<td>After</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sedentary</td>
<td>16</td>
<td>333 ± 11.7</td>
<td>12.6 ± 0.33</td>
<td>146 ± 2.36</td>
<td>161 ± 3.43</td>
<td>114 ± 2.91</td>
<td>126 ± 5.30</td>
</tr>
<tr>
<td>Exercised</td>
<td>11</td>
<td>344 ± 6.36</td>
<td>12.9 ± 0.51</td>
<td>147 ± 2.7</td>
<td>149 ± 3.90*</td>
<td>111 ± 3.39</td>
<td>106 ± 6.45*</td>
</tr>
</tbody>
</table>

Values are means ± SE; n, no. of rats. Blood pressure was measured (before and after) exercise treatment. *P < 0.05; †0.05 < P < 0.1.
Table 2. Effect of exercise training on plasma concentrations of cholesterol and nitrite/nitrate

<table>
<thead>
<tr>
<th></th>
<th>n</th>
<th>T-Cho, mg/dl</th>
<th>F-Cho, mg/dl</th>
<th>NO2/NO3, µmol/l</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sedentary</td>
<td>16</td>
<td>540 ± 75.6</td>
<td>90.8 ± 17.7</td>
<td>7.18 ± 0.601</td>
</tr>
<tr>
<td>Exercise</td>
<td>11</td>
<td>459 ± 46.7</td>
<td>72.5 ± 12.9</td>
<td>7.05 ± 0.352</td>
</tr>
</tbody>
</table>

Values are means ± SE; n, no. of rats. T-Cho, total cholesterol; F-Cho, free cholesterol.

In sedentary rats, the difference was not statistically significant (Table 2). In addition, plasma NOx concentrations in aged rats were not affected by exercise (Table 2).

Fatty acid profiles in plasma and caudal arteries. In plasma, exercise did not produce a significant decrease in linoleic or linolenic acid (Table 3). Also, neither the other fatty acids (palmitic acid, oleic acid, arachidonic acid, eicosapentaenoic acid, and docosahexaenoic acid) nor the unsaturation index, a measurement of the average number of double bonds (35), altered significantly with exercise.

In the caudal arteries of these aged rats, the concentrations of oleic acid, linoleic acid, and linolenic acid decreased significantly with exercise (P < 0.05), but the other fatty acids did not alter significantly (Table 3). On the other hand, exercise significantly increased the unsaturation index (P < 0.05) of caudal arterial fatty acids (Table 3).

Release of adenine nucleotides and nucleosides from caudal artery. Measurement of the amount of adenine nucleotides and nucleosides spontaneously released from the caudal arteries over a 3-min period demonstrated a significantly higher release of total adenyl purines (ATP, ADP, AMP, and adenosine) from the arteries of exercised rats than from those of sedentary rats (Fig. 1). Treatment of these tissue samples with 1.0 µmol/l norepinephrine for 3 min increased the release of total adenyl purines; the amount released from the arteries of exercised rats was also significantly higher than that from the arteries of sedentary rats (Fig. 1).

Regression analysis of the relationship between the amount of adenyl purines released in vitro and the level of arterial fatty acids showed a significantly negative correlation between norepinephrine-induced purine release and the level of arterial oleic acid (r = -0.393, P = 0.0316). Although there was a significant positive correlation between norepinephrine-induced purine release and unsaturation index of fatty acid.

Table 3. Effect of exercise training on levels of plasma and caudal arterial fatty acids in aged rats with hyperlipidemia

<table>
<thead>
<tr>
<th></th>
<th>n</th>
<th>PA(16:0)</th>
<th>OA(18:1n-9)</th>
<th>LA(18:2n-9)</th>
<th>LNA(18:3n-3)</th>
<th>AA(20:4n-6)</th>
<th>EPA(20:5n-3)</th>
<th>DHA(22:6n-3)</th>
<th>USI</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sedentary</td>
<td>16</td>
<td>71.3 ± 7.84</td>
<td>103 ± 14.1</td>
<td>91.8 ± 9.38</td>
<td>3.58 ± 0.548</td>
<td>51.0 ± 4.94</td>
<td>2.05 ± 0.259</td>
<td>12.7 ± 1.26</td>
<td>170 ± 2.82</td>
</tr>
<tr>
<td>Exercise</td>
<td>11</td>
<td>56.8 ± 8.30</td>
<td>79.0 ± 15.0</td>
<td>67.7 ± 9.90</td>
<td>2.28 ± 0.434†</td>
<td>43.8 ± 5.02</td>
<td>1.55 ± 0.215</td>
<td>10.5 ± 0.748</td>
<td>174 ± 4.12</td>
</tr>
</tbody>
</table>

Caudal arterial fatty acids, µg/g

| Sedentary | 16 | 2.020 ± 276 | 1.622 ± 200 | 19.3 ± 4.52 | 1.855 ± 109 | 17.7 ± 2.53 | 409 ± 25.3 | 194 ± 4.03 |
| Exercise | 11 | 1.608 ± 100 | 1.040 ± 112* | 1.093 ± 95.4* | 6.82 ± 1.88* | 1.975 ± 128 | 14.5 ± 3.17 | 438 ± 69.5 | 211 ± 5.01* |

Data are expressed as means ± SE; n, no. of rats. PA(16:0), palmitic acid; OA(18:1n-9), oleic acid; LA(18:2n-9), linoleic acid; LNA(18:3n-3), linolenic acid; AA(20:4n-6), arachidonic acid; EPA(20:5n-3), eicosapentaenoic acid; DHA(22:6n-3), docosahexaenoic acid; USI, unsaturation index of fatty acid. *P < 0.05; †P < 0.1.
DISCUSSION

We have shown here that 12 wk of treadmill exercise depressed the rise in SBP and DBP normally observed in hypercholesterolemic aged rats. During exercise, one of the factors that affects arteriolar resistance is the increased blood flow to skeletal muscles to meet the increase in metabolic demand. Miller et al. (23) showed that increased blood flow enhances the endothelium-dependent relaxation induced by ACh and ADP in canine femoral arteries. In addition, the frictional force caused by an acute increase in blood flow results in shear stress, leading to relaxation of the underlying vascular smooth muscle (7) and to an increase in the production of NO, an endothelium-derived relaxing factor and a powerful vasodilator. Delp et al. (10) observed that 10 wk of treadmill exercise enhances the sensitivity and maximal endothelium-dependent relaxation of abdominal aortas of male Sprague-Dawley rats to ACh. In contrast, although we found that treadmill exercise for 12 wk produced a tendency toward increased ACh-induced endothelium-dependent relaxation of thoracic aortas from young female Wistar rats, the sensitivity to ACh was decreased by exercise in aged rats (15) and was not affected in aged hypercholesterolemic animals (data not shown).

Although other researchers have reported that NO production by coronary circulation increases with exercise (40), our study showed that plasma NOx concentration, an index of NO production, was not affected by treadmill exercise. This discrepancy may be related to the strain, sex, and/or age of the rats and/or to the relaxation response to ACh in the vascular beds. Although NO is involved in lowering vascular resistance locally in certain vascular beds (37), measuring plasma NOx concentration may not be sensitive enough to detect local changes. Further experiments are needed to clarify whether NO participates in the blood pressure-lowering effect induced by exercise training.

ATP is another endothelial cell factor released by increased blood flow and shear stress (2). We also reported (32) that a large amount of ATP is released from vascular endothelial cells by α1-adrenoceptor stimulation. ATP and ADP induce endothelium-dependent vasodilatation in precontracted arteries by binding to P2Y and/or P2U purinoceptors, (6) and adenosine induces direct vasodilatation in arterial endothelial (21) and smooth muscle cells by binding to A2 purinoceptors (4, 6). In addition, both ATP and adenosine acting via P3 purinoceptors reduce norepinephrine release from vascular sympathetic nerves (31). Adrenergic nerve stimulation induces the release of large amounts of ATP from extraneuronal sites of blood vessels via α1-adrenoceptors (29, 38), and these released purines act as autocrine and paracrine stimulators of blood vessel tone (39). The release of these endogenous adenyl purines from the arteries may produce vasodila-

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Table 4. Correlations between spontaneous and NE-induced release of 4 purine compounds from caudal arteries and blood pressure in sedentary and exercised aged rats

<table>
<thead>
<tr>
<th>Release of Purine Compounds From Caudal Arteries</th>
<th>Spontaneous</th>
<th>NE-induced</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>r</td>
<td>p</td>
</tr>
<tr>
<td>Systolic blood pressure</td>
<td>-0.411</td>
<td>0.0240</td>
</tr>
<tr>
<td>Diastolic blood pressure</td>
<td>-0.420</td>
<td>0.0207</td>
</tr>
</tbody>
</table>

Purine compounds measured were ATP, ADP, AMP, and adenosine; n = 16 (sedentary) and 11 (exercised) rats. NE, norepinephrine.
Exercise is associated with ATP release

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The effects of aging or exercise training are observed in various aspects of cell function, including ATP release from tissue. For example, Purines released from the vascular endothelium, may participate in blood pressure control in exercised rats. Our findings showed a negative correlation between plasma adenylate purine levels and blood pressure associated with aging (16) or exercise (this study). Plasma concentrations of epinephrine and norepinephrine increase during the initial stages of exercise training and are significantly lower in trained rats at rest compared with untrained rats (18). Purines released from the vascular endothelium act on purinoreceptors on adrenergic nerve terminals to reduce the release of norepinephrine (33). These findings thus suggest that sympathetic nervous activity in response to exercise may be, at least partially, negatively regulated by the ATP released from vascular endothelial cells.

A second important finding that emerges from this study is the decrease in fatty acids (i.e., oleic, linoleic, and linolenic acids) observed in caudal arteries of exercised rats, resulting in an increase in the unsaturation index. These results are consistent with those of Ohkubo et al. (26) showing that swimming exercise markedly reduces the linoleic acid content in both the iliac artery and the aorta, leading to significant increases in the unsaturation index of their vascular beds. Although daily injections of catecholamines reduce the linoleic acid content in heart muscle, these animals also exhibit reduced body weight (12). In contrast, we demonstrated that the decrease in fatty acids associated with treadmill exercise had no effect on body weight or food intake in aged rats.

Selective changes in the number and/or affinity of α1-adrenoceptors in arterial endothelial cells appear to play an important role in altering ATP release. Many researchers have demonstrated that changes in membrane unsaturated fatty acid composition correlate with changes in membrane fluidity that ultimately affect cell function. For example, after esterification and subsequent incorporation into the cell membrane, ω-3 polyunsaturated fatty acids modify the fluid mobility gradient of the phospholipid bilayer (14, 22). Moreover, supplementation with polyunsaturated fatty acids that increase “membrane fluidity” enhances the coupling between β-adrenergic receptors and adenylate cyclase (27) and membrane-associated 5'-nucleotidase and adenylate cyclase activities (3).

We recently showed (17) that administration of cis-5,8,11,14,17-eicosapentaenoic acid (EPA), an ω-3 polyunsaturated fatty acid, increases ATP release from the caudal artery and arterial EPA concentration and is associated with the repression of the blood pressure rise seen in aged hypercholesterolemic rats. These results, together with our finding that exercise caused an increase in the unsaturation index of fatty acids in the caudal arteries of aged rats, suggest that the exercise-induced enrichment of membrane lipid unsaturated fatty acids may be associated with changes in membrane fluidity, as well as the number and affinity of membrane α1-adrenoceptors, and that this may lead to enhanced ATP release from rat caudal arteries. In addition, because ATP and its metabolites can produce vasodilatation, the enhanced ATP release from vascular endothelial cells may reduce total peripheral resistance and blood pressure. Thus arterial endothelial adaptations to exercise may have important implications for the prevention and treatment of cardiovascular disease.

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