Hypotension induced by exercise is associated with enhanced release of adeny1 purines from aged rat artery

M. HASHIMOTO, K. SHINOZUKA, Y. TANABE, S. GAMOH, T. HARA, M. S. HOSSAIN, Y. M. Kwon, M. KUNITOMO and S. MASUMURA. Hypotension induced by exercise is associated with enhanced release of adeny1 purines from aged rat artery. Am J. Physiol. 276 (Heart Circ. Physiol. 45): H970–H975, 1999.—To determine whether the antihypertensive effects of exercise are associated with release of ATP and its metabolites from arteries, we assayed blood pressure and the release of adeny1 nucleotides and nucleosides from the caudal arteries of exercised and sedentary aged hypercholesterolemic rats. Exercise on a treadmill for 12 wk significantly decreased the rise in systolic blood pressure and the release of adeny1 nucleotides and nucleosides from the caudal arteries of exercised rats compared with those of sedentary rats. Oxygen consumption and heart rate were increased significantly by exercise in aged sedentary rats. The exercise-induced decrease in blood pressure was significantly greater in aged exercised rats than in sedentary rats. Release of adenine nucleotides and adenosine in aged exercised rats, both spontaneously and in response to norepinephrine, significantly decreased with age. These results suggest that exercise alters the membrane fatty acid composition in aged rats as well as the release of adeny1 from vascular endothelium and that these factors are associated with the regression of the rise in blood pressure normally observed with advanced age.

EXERCISE DECREASES blood pressure in hypertensive animals (13, 28) at least partially by inducing body weight loss and changes in prostaglandin metabolism (19). We showed (15) that treadmill exercise significantly 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 endothelium-dependent vasodilatation (8, 36) and increases the expression of endothelial nitric oxide (NO) synthase (9, 30). Taken together, these findings suggest that exercise may affect vascular mechanical responses by altering endothelial cell function.

Using endothelial cells isolated from rat caudal arteries, we (16) showed that the amount of ATP released in response to α1-adrenoceptor stimulation decreases with advancing age and that this extracellular ATP, together with its metabolites, participates in blood pressure changes associated with aging. ATP causes vasodilatation by stimulating the release of NO/endothelium-derived relaxing factor from endothelial cells (11) and by hyperpolarizing smooth muscle cells (25). Adenosine enhances vasodilatation by direct action on vascular endothelial (21) and smooth muscle (4) cells. These purines derive primarily from endothelial cells and, to a lesser extent, from smooth muscle cells (32). In addition, ATP, which rapidly degrades to ADP, AMP, and adenosine in vascular tissues, probably by ectonucleotidases, 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 adenyl nucleotides and adenosine in extracellular 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 vascular responses are frequently altered, with both conduit and resistance vessels manifesting impaired endothelial 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 nucleosides 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 accordance 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 30 water; total energy 42 kcal/g, Funabashi Farm, Chiba, J. p.] maintained at 23 ± 2°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 plethysmographic method (UR-1000, Ueda, Tokyo, J. p.), 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, Funabashi 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 mM EDTA, and centrifuged for 20 min (3,000 rpm) at 4°C.

Plasma samples were assayed for platelet contamination with an automated hematocrit analyzer (<10% µl; K-2000, Toa Medical Electronics, Kobe, Japan), 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 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 µM 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, Japan), respectively. The plasma nitrogen oxide (NOx; nitrite/nitrate) concentration was assayed by a modification of the method of Aspergillus niger nitrate reductase (Sigma Chemical, St. Louis, MO) and subsequently with 2,3-diaminonaphthalene fluorescence 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, Japan), 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 Aspergillus niger nitrate reductase (Sigma Chemical, St. Louis, MO) and subsequently with 2,3-diaminonaphthalene (Dojindo Labs, Kumamoto, Japan). Fluorescence intensity was measured with a Hitachi 850 fluorescence spectrometer (Hitachi, Tokyo, Japan). Nitrite standards (>98% pure, Sigma Chemical) were freshly prepared.

Fatty acid content of plasma and tissuesamples. 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 tolune phase, 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°C to 200°C at 2°C/min, 200°C to 240°C at 4°C/min, 240°C to 260°C at 4°C/min, and 260°C to 300°C. 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, Japan).

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, Japan) and suspended in 200 µl of phosphate-buffered saline (Dulbecco's PBS–I) 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 in 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 |
|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|
|                 | n               | Body Wt, g      | Food Intake, g rat⁻¹ day⁻¹ | Systolic Blood Pressure, mmHg | Diastolic Blood Pressure, mmHg | Heart Wt, g | Heart Rate, beats/min |
| Sedentary       | 16              | 333 ± 11.7      | 12.6 ± 0.332                | 146 ± 2.36                  | 161 ± 3.43                  | 114 ± 2.91  | 126 ± 5.30                  | 429 ± 10.3                  |
| Exercised       | 11              | 344 ± 6.36      | 12.9 ± 0.510                | 147 ± 2.7                   | 149 ± 3.90*                  | 111 ± 3.39  | 106 ± 6.45                  | 399 ± 11.7                  |

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.

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 purine release in response to norepinephrine and fatty acid unsaturation index (Fig. 2), the correlation between spontaneous purine release and unsaturation index, although positive, was not statistically significant.

Plasma levels of adenine nucleotides and adenosine. The levels of adenine nucleotides and nucleosides in plasma revealed that in aged rats exercise did not produce a statistically significant increase (0.05 < P < 0.1) in total plasma concentrations of these purines (Fig. 3). Regression analysis revealed a significant positive relationship between plasma purine levels and the norepinephrine-induced release of adenyl purines from rat caudal arteries (r = 0.636, P = 0.0005).

Relationship between blood pressure and adenyl purines. Regression analysis of the relationship between blood pressure and the amount of plasma adenyl purines demonstrated a significantly negative correlation between SBP and plasma purine concentration (r = −0.473, P = 0.0147) and also a significantly negative correlation between purine release (spontaneous or norepinephrine-induced) and blood pressure (Table 4).

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) mg/dl</th>
<th>OA(18:1n-9) mg/dl</th>
<th>LA(18:2n-9) mg/dl</th>
<th>LNA(18:3n-3) mg/dl</th>
<th>AA(20:4n-6) mg/dl</th>
<th>EPA(20:5n-3) mg/dl</th>
<th>DHA(22:6n-3) mg/dl</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>5.58 ± 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>

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; †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 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 $\alpha_1$-adrenoceptor stimulation. ATP and ADP induce endothelium-dependent vasodilation in precontracted arteries by binding to $P_2\gamma$ and/or $P_2\zeta$ purinoceptors, (6) and adenosine induces direct vasodilation in arterial endothelial (21) and smooth muscle cells by binding to $A_2\zeta$ purinoceptors (4, 6). In addition, both ATP and adenosine acting via $P_3$ 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 $\alpha_1$-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-

![Fig. 2. Relationship between unsaturation index of caudal arterial fatty acids and NE-induced release of total of 4 purine compounds (ATP, ADP, AMP, and adenosine) from caudal arteries of aged rats. Purine release was measured over 3 min. ○, ○, sedentary and exercised aged rats, respectively.](https://www.ajpheart.org/doi/10.1210/ajpheart.2017-0109)

![Fig. 3. Plasma concentrations of adenyl purines in sedentary and exercised aged rats; n = 16 (sedentary rats) and 11 (exercised rats).](https://www.ajpheart.org/doi/10.1210/ajpheart.2017-0109)

<table>
<thead>
<tr>
<th>Release of Purine Compounds</th>
<th>Spontaneous</th>
<th>NE-induced</th>
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<tbody>
<tr>
<td>Purine compounds measured were ATP, ADP, AMP, and adenosine; n = 16 (sedentary) and 11 (exercised) rats. NE, norepinephrine.</td>
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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](https://www.ajpheart.org/doi/10.1210/ajpheart.2017-0109)
ntation via P2y, A2, and P3 purinoceptor stimulation and thus decrease blood pressure. Our findings of increased adeny purine release from the arteries of exercised rats and of an inverse relationship between purine release and the increases in SBP and DBP normally observed in aging rats suggest that ATP and its metabo-
ites ADP and adenosine, which are released from vascular endothelium, may participate in blood pres-
sure control in exercised rats.

Our findings showed a negative correlation between plasma adeny purine levels and blood pressure associ-
ated with aging (16) or exercise (this study). Plasma concentrations of epinephrine and norepinephrine in-
crease 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 purinoceptors on adrenergic nerve terminals to reduce the release of norepineph-
rine (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 aged rats, and of an inverse relationship between purine release and the increases in SBP and DBP normally observed in aging rats suggest that ATP and its metabo-
ites ADP and adenosine, which are released from vascular endothelium, may participate in blood pres-
sure control in exercised rats.


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