Does endothelin-1 participate in the exercise-induced changes of blood flow distribution of muscles in humans?

SEIJI MAEDA,1 TAKASHI MIYAUCHI,2 MICHIKO SAKANE,2 MAKOTO SAITO,1 SHINICHI MAKI,2 KATSUTOSHI GOTO,2 AND MITSUO MATSUDA1

1Institute of Health and Sport Sciences, 2Cardiovascular Division, Department of Internal Medicine, Institute of Clinical Medicine, and 3Department of Pharmacology, Institute of Basic Medical Sciences, University of Tsukuba, Tsukuba, Ibaraki 305, Japan

Maeda, Seiji, Takashi Miyauuchi, Michiko Sakane, Makoto Saito, Shinichi Maki, Katsutoshi Goto, and Mitsuo Matsuda. Does endothelin-1 participate in the exercise-induced changes of blood flow distribution of muscles in humans? J. Appl. Physiol. 82(4): 1107–1111, 1997.—Endothelin-1 (ET-1) is an endothelium-derived potent vasoconstrictor peptide that potentiates contractions to norepinephrine in human vessels. We previously reported that the circulating plasma concentration of ET-1 is significantly increased after exercise (S. Maeda, T. Miyauuchi, K. Goto, and M. Matsuda. J. Appl. Physiol. 77: 1399–1402, 1994). To study the roles of ET-1 during and after exercise, we investigated whether endurance exercise affects the production of ET-1 in the circulation of working muscles and nonworking muscles. Male athletes performed one-leg cycle ergometer exercise of 30-min duration at intensity of 110% of their individual ventilatory threshold. Plasma concentrations of ET-1 in both sides of femoral veins (veins in the working leg and nonworking leg) and in the femoral artery (artery in the nonworking leg) were measured before and after exercise. The plasma ET-1 concentration in the femoral vein in the nonworking leg was significantly increased after exercise, whereas that in femoral vein in the working leg was not changed. The arteriovenous difference in ET-1 concentration was significantly increased after exercise in the circulation of the nonworking leg but not of the working leg, which suggests that the production of ET-1 was increased in the circulation of the nonworking leg by exercise. The present study also demonstrated that the plasma norepinephrine concentrations were elevated by exercise in the femoral veins of both the working and nonworking legs, suggesting that the sympathetic nerve activity was augmented in both legs during exercise. Therefore, the present study demonstrates the possibility that the increase in production of ET-1 in nonworking muscles may cause vasoconstriction and hence decrease blood flow in nonworking muscles through its direct vasoconstrictive action or through an indirect effect of ET-1 to enhance vasoconstrictions to norepinephrine and that these responses may be helpful in increasing blood flow in working muscles. We propose that endogenous ET-1 contributes to the exercise-induced redistribution of blood flow in muscles.

one-leg cycle ergometer exercise; working and nonworking muscles; arteriovenous difference in endothelin-1 concentration; skeletal muscles; redistribution of blood flow to norepinephrine in human vessels (30). Thus it was thought that endogenous ET-1 contributes to the regulation of human vascular tone through its direct vasoconstrictive action or through an indirect effect of ET-1 to enhance the vasoconstrictive action of norepinephrine. Furthermore, it has been reported that systemic administration of an endothelin-receptor antagonist significantly decreased systemic blood pressure and peripheral vascular resistance in healthy humans, providing a strong suggestion that endogenously generated ET-1 contributes to basal vascular tone in humans (5). We previously reported that the circulating plasma concentration of ET-1 is significantly increased by exercise (10). However, the physiological role of ET-1 during exercise is unclear.

Endurance exercise results in a significant redistribution of tissue blood flow, in which blood flow is greatly increased in the working muscles and decreased in the splanchnic and nonworking muscle circulations (1, 4, 16–18, 28). Although it has been considered that the exercise-induced redistribution of blood flow is partly caused by the increased activity of sympathetic nerve system (2) and multiple local metabolic factors (9), the precise mechanisms are not known. It has also been demonstrated that endothelium-derived relaxing factor, which is identical with nitric oxide (NO) (6, 21), is partly involved in determining the pattern of the redistribution of tissue blood flow during exercise (25). Although this finding suggests that vascular endothelial cells may be involved in exercise-induced redistribution of blood flow, the roles of endothelin-derived vasoconstrictor substances, such as ET-1, in exercise-induced physiological responses remain to be investigated.

Although we previously reported that the circulating plasma concentration of ET-1 is significantly increased after exercise (10), the precise physiological role and origin of ET-1 during exercise are not known. To solve these questions, the present study was designed to investigate whether ET-1 production differs in the circulation of working muscles and nonworking muscles with exercise. In the present study, we used one-leg cycle ergometer exercise to address this issue. We measured plasma concentrations of ET-1 in the femoral veins of the working leg and nonworking leg and in the femoral artery in the nonworking leg in six subjects before and after one-leg cycle ergometer exercise at intensity of 110% of their individual ventilatory thresholds (VTs). We also measured plasma norepinephrine concentrations at these sampling sites.
METHODS

Subjects and protocol. Six male intercollegiate athletes (lifesavers) ranging in age from 18 to 23 yr entered the study. The study was approved by the Ethical Committee of the University of Tsukuba. This study conforms with the principles outlined in the Helsinki Declaration, and written informed consent was obtained from all the athletes. All exercise was performed by the subjects in the seated position on a cycle ergometer (model 232C50, Combi). The cycle ergometer exercise was performed by the subjects using only the right leg. The subjects did not participate in any intensive or long-lasting training 1 day before the test. To determine individual VT, the subjects performed the one-leg ergometer test with stepwise increases in intensity (15 W for 3 min, followed by 6-W increases every minute until the subjects felt exhausted). Both O2 uptake (VO2) and minute ventilation were measured by using the breath-by-breath method (K2, Cosmed). Individual VTs were calculated by using regression analysis of the slope of the VO2 and minute ventilation plot (20).

All of the subjects performed the one-leg cycle ergometer exercise of 30-min duration at 110% of their individual VTs. Our previous study showed that the exercise at 90% of VT for 30-min duration caused a significant increase in circulating plasma ET-1 concentration (10). This is the reason why we chose the load of exercise that we did (110% VT for 30-min duration) in the present study. The exercise intensity was 65.5 ± 0.9 (SE) W. The one-leg exercise tests were performed within 12 days after the day of determination of individual VTs. Heart rate was continuously measured by a pulse watch (model MRC-1200, Nihon Kohden). All of the exercise protocols were performed at a constant room temperature (25°C). Each athlete stopped oral intake of food and liquid, including water, 1 h before exercise. Before and after the exercise tests, the plasma ET-1 concentration in the femoral vein of the working leg and the nonworking leg and in the femoral artery of the nonworking leg was measured with the subjects in the supine position. Before and after the cycle exercise tests, plasma ET-1 and norepinephrine concentrations in both sides of the femoral veins (i.e., working leg and nonworking leg) and in the femoral artery in the nonworking leg were measured. Because it has been reported that plasma concentration of various substances in the artery is not different among various portions of the body, plasma ET-1 and norepinephrine concentrations in femoral artery were measured in only one side of nonworking leg in this study. Each athlete lay down in a supine position within 15 s after the stopping the exercise, and the femoral venous blood of the working leg was sampled within 60 s (i.e., the first sampling was performed within 75 s). The blood samples were taken by puncture. Blood sampling was done in the following order: the femoral vein in the working leg, the femoral vein in the nonworking leg, and the femoral artery in the nonworking leg. The blood samples were taken within 1 min of each other; i.e., the blood samples of all the sites (3 sites) were taken within 3 min. We previously confirmed that the exercise-induced increase in plasma ET-1 lasts for a relatively long time (at least 30 min) (10).

Measurement of plasma ET-1 concentration by sandwich-enzyme immunoassay. Each blood sample was placed in chilled tubes containing aprotinin (300 kallikrein-inactivating units/ml) and EDTA (2 mg/ml) and was then centrifuged at 2,000 g for 15 min at 4°C. The plasma was stored at −30°C until use. Plasma (1 ml) was acidified with 3 ml of 4% acetic acid, and immunoreactive ET-1 was extracted with a Sep-Pak C18 cartridge (Waters, Milford, MA) as previously described in papers from our laboratory (10, 14, 15, 19, 26). The elutes were reconstituted with 0.25 ml of assay buffer and were subjected to sandwich-enzyme immunoassay. Sandwich-enzyme immunoassay for ET-1 was carried out as previously described by using immobilized mouse monoclonal antibody AwETN40, which recognizes the NH2-terminal portion of ET-1, and peroxidase-labeled rabbit anti-ET-1 COOH-terminal peptide (15–25) Fab’ (10, 14, 15, 26). The Fab’ fragment of this rabbit antibody was used as an enzyme-labeled detector antibody after being coupled with horseradish peroxidase. The coefficient of variation (CV) of the ET-1 assay for the intra-assay variation was 11%, and the CV for the interassay variation was 13% (12).

Measurement of plasma norepinephrine concentration. Plasma norepinephrine concentration was measured by using a radioenzymatic assay based on the method of Peuler and Johnson (22). Plasma samples from each subject were assayed in the same assay run and were determined in duplicate or triplicate.

Statistics. Values are expressed as means ± SE. Statistical analysis was carried out by analysis of variance followed by Scheffé’s F-test for multiple comparisons. P < 0.05 was accepted as significant.

RESULTS

At the end of one-leg exercise, heart rate and systolic blood pressure increased significantly (Table 1). Hematocrit increased significantly and body weight decreased significantly after exercise (Table 1). Thirty minutes after exercise, heart rate, systolic blood pressure, and hematocrit returned to the preexercise level.

Immediately and 30 min after exercise, the plasma ET-1 concentration in the femoral vein was significantly increased in the nonworking leg (Fig. 1). However, in the working leg, the plasma ET-1 concentration in the femoral vein was not increased either immediately or 30 min after exercise (Fig. 1). There were no significant differences in the plasma ET-1 concentration in the femoral artery before, immediately after, and 30 min after exercise (Fig. 1). Immediately and 30 min after exercise, the arteriovenous difference in ET-1 concentration was significantly increased in the circulation of the nonworking leg but not in that of the working leg (Fig. 2). Therefore, it was suggested that the production of ET-1 was increased in the circulation of nonworking muscles by exercise.

In all sampling sites (femoral veins of both the working leg and nonworking leg and the femoral artery

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<th>Table 1. Body weight, heart rate, systolic blood pressure, and hematocrit before and after 30 min of one-leg exercise</th>
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<td>Before Exercise</td>
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<tr>
<td>Body weight, kg</td>
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<td>Heart rate, beats/min</td>
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<td>Systolic blood pressure, mmHg</td>
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Values are means ± SE of 6 subjects. Subjects performed a one-leg cycle exercise test of 30-min duration at 110% of their individual ventilatory thresholds. Significantly different vs. before exercise, *P < 0.05; †P < 0.01.
of the nonworking leg), the plasma concentrations of norepinephrine were significantly increased immediately after exercise (Fig. 3), and they returned to the basal level 30 min after exercise (Fig. 3). Immediately after exercise, the increase in plasma norepinephrine concentration in the vein of the working leg (2.5-fold) was almost comparable to that of nonworking leg (2.2-fold) (Fig. 3). Immediately after exercise, the increase in plasma norepinephrine concentration in the artery of nonworking leg was 1.6-fold (Fig. 3).

**DISCUSSION**

In the present study, we measured plasma concentrations of both ET-1 and norepinephrine in femoral veins in both the working leg and nonworking leg and in the femoral artery before and after one-leg exercise of 30-min duration. The arteriovenous difference in ET-1 concentration of the nonworking leg was significantly increased after exercise, whereas that in the working leg was not significantly different before and after exercise. These findings suggested that the production of ET-1 was increased in the circulation of nonworking muscles by exercise. Because it has been reported that ET-1 is produced by the vascular endothelial cells, but not by the skeletal muscles in the limbs (11, 24), the possibility was suggested that the production by vascular endothelial cells was increased during exercise in inactive skeletal muscle. It is thought that endogenously generated ET-1 contributes to basal vascular tone in healthy humans; it has been reported that systemic administration of the endothelin-receptor antagonist TAK-044 significantly decreased systemic blood pressure and peripheral vascular resistance in healthy humans (5). Therefore, it was considered that the increased ET-1 production in nonworking leg may cause the increase in vascular tone, thereby contributing to the redistribution of blood flow during exercise. Such a decrease in flow in nonexercising muscles would maximize the blood flow available to the active muscles.

The present study showed that the plasma norepinephrine concentrations were significantly elevated immediately after exercise in the femoral veins of both the working and nonworking legs, suggesting that the sympathetic nerve activity was augmented in both legs during exercise. In the present study, plasma levels of both ET-1 and norepinephrine were shown to rise significantly during exercise in the nonworking leg. In addition to the vasoconstrictor effect of ET-1, low concentrations of ET-1, which do not produce vasoconstriction, potentiate contractions in response to norepinephrine in human arteries (30). In experimental animals, it has also been demonstrated that a low dose of
ET-1 enhances adrenergic vasoconstriction in perfused rat mesenteric arteries (27). Therefore, in vessels in the nonworking leg, it was possible that ET-1 potentiated norepinephrine-induced vasoconstriction. Therefore, the increase in ET-1 in nonworking muscles may cause vasoconstriction through a direct action or by enhancing the vasoconstriction in response to norepinephrine. These mechanisms may contribute to the exercise-induced redistribution of blood flow in muscles and augment blood flow to the working muscles. This finding could have implications for conditions in which there is augmented adrenergic vasoconstriction within nonactive vascular beds during exercise. Norepinephrine levels in both legs returned to baseline at the 30-min time point despite the elevated ET-1 level in the nonworking leg. It was unclear why venous ET-1 levels were elevated at 30 min postexercise when norepinephrine levels had returned to baseline.

Because ET-1 is produced by vascular endothelial cells in the limb muscle, but is not produced by skeletal muscle cells or skin tissues (fibrous tissues, keratinous tissues, etc.) (11, 24), our results suggest that vascular endothelial cells increase ET-1 production during and/or after exercise in the nonworking leg but not in the working leg. However, the following hypothesis is also possible. During and immediately after exercise, tissue blood flow in nonworking leg may be decreased by vasoconstriction (2, 9). When local circulating blood flow is decreased, the level of local venous plasma ET-1 concentration could be elevated without an increase in ET-1 production by vascular endothelial cells. Therefore, there is the possibility that ET-1 was accumulated during exercise in the tissue of nonworking leg because of a decreased blood flow and that the venous plasma concentration of ET-1 in the nonworking leg was elevated after exercise by a washout effect. However, we believe that this possibility is unlikely because we have previously demonstrated that, although the forearm blood flow decreased following surgical stress in humans, the ET-1 output from the forearm, calculated by the forearm blood flow and the arteriovenous difference of ET-1 concentrations, significantly increased after the surgery (19).

The mechanism for the difference in the production of ET-1 between working muscles and nonworking muscles by exercise remains to be elucidated. It has been shown that both mechanical factors (such as hemodynamic shear stress) and neurohumoral factors (such as angiotensin II, arginine vasopressin, etc.) affect the production of ET-1 in cultured vascular endothelial cells (3, 8, 24, 31). It has been reported that low levels of shear stress stimulate and higher levels of shear stress depress the release of ET-1 in the cultured vascular endothelial cells (8). The difference in blood flow between the working and nonworking legs (increase and decrease, respectively, in blood flow by exercise) might cause the difference in the levels of shear stress on vascular endothelial cells of the legs. Therefore, it is possible that differences in the levels of shear stress on vascular endothelial cells between working and nonworking legs is one of the causal factors for difference in ET-1 production seen in the legs. Alternatively, the following hypothesis may be also possible. NO or other vasodilating factors (such as prostacyclin) have been reported to be released into the exercising muscles from the vascular endothelium (7, 25). Because it also has been reported that these vasodilating factors (NO and prostacyclin) inhibit the production of ET-1 in vascular endothelium (23, 24), these factors might suppress ET-1 release during exercise. It is also reasonable to speculate that within the working leg, release of metabolic vasodilating factors is interfering with this process. Because it has been reported that a low dose of ET-1 potentiates vascular contractions to norepinephrine (30), there may be interactions among the blood flow, the sympathetic nervous system, and the release of various endothelium-derived vasoconstricting and vasodilating factors in the regulation of blood flow in exercising and nonexercising muscles. Therefore, it is possible that there may be neuronal-endothelial interactions in working and nonworking muscles that affect the release of ET-1.

The circulating plasma ET-1 levels in healthy humans (1.0–1.5 pg/ml) (11, 14, 15, 19, 24) are considered to be below a level that produces contractions in human vessels (11, 13, 24, 30). However, local interstitial concentrations of ET-1 around vessels in vivo in humans are not known. The report of Haynes et al. (5) that systemic administration of the endothelin-receptor antagonist TAK-044 significantly decreased systemic blood pressure and peripheral vascular resistance in healthy humans strongly suggests that endogenously generated ET-1 contributes to basal vascular tone in humans. In the present study, the levels of the circulating plasma ET-1 (~1.2 pg/ml) in the healthy subjects were in accordance with the previous reports (11, 14, 15, 19, 24). In the present study, the exercise increased plasma ET-1 level in the femoral vein of the nonworking leg by 46% (see Fig. 1). However, it is possible that an increase in local ET-1 levels around the vascular endothelium (especially around vascular smooth muscles) in the nonworking leg by the exercise is far greater than that in the plasma.

The present study has the following study limitations. First, the blood was sampled after exercise when blood pressure was declining, and we obtained the blood by the puncture of vessels and not by indwelling catheters. Thus the study of more frequent measurement of plasma ET-1 levels by using indwelling catheters is needed. Second, there was no evidence that blood flow to the nonworking leg of the present subjects actually decreased during exercise. Third, it was also unclear why venous ET-1 levels were elevated at 30 min postexercise when norepinephrine levels had returned to baseline.

In summary, we have demonstrated that the arteriovenous difference in ET-1 concentration was significantly increased in the circulation of the nonworking muscles but not in that of working muscles after exercise. These findings suggested that the production of ET-1 was increased in the vascular endothelial cells of the nonworking muscles by exercise. The present
study also demonstrated that the plasma norepinephrine concentrations were elevated by exercise in the femoral veins of both the working and nonworking legs and, therefore, that the sympathetic nerve activity was augmented in both legs during exercise. Therefore, the present study provides a new hypothesis that the increase in production of ET-1 in nonworking muscles may cause vasoconstriction and hence decrease blood flow in nonworking muscles through its direct vasoconstrictive action or through an indirect effect of ET-1 to enhance vasoconstriction to norepinephrine. We propose that endogenous ET-1 participates in the exercise-induced changes in distribution of blood flow in skeletal muscles and may be helpful in increasing the blood flow to working muscles.

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Address for reprint requests: M. Matsuda, Dept. of Sports Medicine, Institute of Health and Sport Sciences, Univ. of Tsukuba, Tsukuba, Ibaraki 305, Japan.

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