Physiological Role of Endothelin-1 in Nonworking Muscles During Exercise in Healthy Subjects

Kazuhiro Tanabe, MD; Akiko Yamamoto, MD; Noriyuki Suzuki, MD; Yasuhiro Yokoyama, MD; Naohiko Osada, MD; Masaru Nakayama, MD; Yoshihiko Akashi, MD; Atsushi Seki, MD; Hisanori Samejima, MD; Misa Oya, MD; Taizo Murahayashi, MD; Kazuto Omiya, MD; Haruki Itoh, MD; Fumihiko Miyake, MD; Masahiro Murayama, MD

Endothelin-1 (ET-1) is a potent vasoconstrictor peptide produced by vascular endothelial cells. However, the role of ET-1 in exercise-induced physiological responses is still to be investigated. The purpose of the present study was to investigate in healthy volunteers whether the ET-1 plasma concentration in nonworking muscles is changed by exercise and to investigate the physiological role of ET-1 during exercise. Bicycle ergometer cardiopulmonary exercise tests were performed in 36 healthy men (mean age, 22.5 years). Blood samples for measuring ET-1 were drawn from the cubital vein during rest and immediately after the exercise test. The ET-1 change ratio was calculated as ET-1 immediately following exercise/ET-1 during the resting state. Cardiac output (CO) was measured during the exercise test by the impendence method. Arterial venous oxygen difference (A\(\text{VO}_{2}\)/D) when CO reached 10L/min or 15L/min was calculated as A\(\text{VO}_{2}\)/D=A\(\text{VO}_{2}\)/CO. Results were as follows: (1) the ET-1 change ratio correlated inversely with exercise time at the anaerobic threshold (r=−0.37, p=0.03) and peak exercise time (r=−0.35, p=0.04); (2) the ET-1 change ratio tended toward an inverse correlation with A\(\text{VO}_{2}\)/D/work rate (r=−0.29, p=0.09); (3) the ET-1 change ratio correlated positively with A\(\text{VO}_{2}\)/D when CO reached 10L/min (r=0.42, p=0.02) and tended toward a positive correlation with A\(\text{VO}_{2}\)/D when CO reached 15L/min (r=0.32, p=0.08). These results indicate that an increase in ET-1 in nonworking muscles may participate in the exercise-induced redistribution of blood flow and in increasing the blood flow to working muscles. (Jpn Circ J 2000; 64: 27–31)

Key Words: Arterial venous oxygen difference; Endothelin-1; Exercise efficiency; Redistribution of blood flow

Vascular endothelial cells play an important role in the regulation of vascular tonus through their production of endothelin-1 (ET-1), a potent vasoconstrictor peptide. It is thought that ET-1 contributes to the regulation of vascular tonus through either a direct vasoconstrictive action or by indirect enhancement of the vasoconstrictive action of norepinephrine. However, the role of ET-1 in exercise-induced physiological responses remains to be investigated. The purpose of the present study was to investigate in healthy volunteers whether the plasma concentration of ET-1 in nonworking muscles changes with exercise and to investigate the physiological role of ET-1 in nonworking muscles during exercise.

Methods

Subjects

The subjects consisted of 36 healthy male volunteers (college students whose mean age was 22.5±1.2 years) with no history of previous serious diseases. Their present health status was checked by routine physical examination, chest X-ray, resting ECG, and routine laboratory examinations; all were within normal limits.

According to the protocol approved by the institute's Committee on Human Investigation, written informed consent for participation was obtained from all subjects before the study.

Cardiopulmonary Exercise Testing

Symptom-limited cardiopulmonary exercise tests were performed using a ramp protocol with a bicycle ergometer (1 W/3 s) to measure the anaerobic threshold (AT), the time from the start of the ramp exercise test until AT was attained (AT time), peak oxygen uptake (peak VO\(\text{2}\)), and peak exercise time. After a minute rest on the bicycle ergometer, exercise began with a 4-min warm-up (20 W) followed by an increase in load (1 W/3 s). Subjects were monitored by 12-lead ECG during exercise testing, and heart rate (HR), ST-T change, and arrhythmia were followed carefully. Blood pressure was measured at 1-min intervals by the cuff method. Carbon dioxide production (V\(\text{CO}_{2}\)), CO\(\text{2}\), and minute ventilation (VE) were measured throughout using a RM-300 respiration monitor and an MG-360 gas analyzer (Minato Ikaigaku Co, Tokyo, Japan). The measurement system for cardiopulmonary exercise testing was calibrated before starting each study. Expired gas was sampled using a breath-by-breath technique.

From these measurements, the ventilatory equivalents were calculated for O\(\text{2}\) by VE/VO\(\text{2}\) and for CO\(\text{2}\) by VE/V\(\text{CO}_{2}\), and the gas exchange ratio was determined by V\(\text{CO}_{2}/\text{VO}_{2}\). These calculations were made using a personal computer (NEC PC-9801).

AT was determined by the following conventional criteria: (1) VE/VO\(\text{2}\) increased after holding constant or decreases...
Table 1  Results of Cardiopulmonary Exercise Test

| HR at rest | 81.1±10.5 (beats/min) |
| Blood pressure at rest | 125.0±29.8/81.6±9.6 (mmHg) |
| HR at AT | 123.7±12.8 (beats/min) |
| Blood pressure at AT | 155.6±19.4/92.2±10.9 (mmHg) |
| AT | 18.6±3.0 (ml min⁻¹ kg⁻¹) |
| AT time | 236.4±61.8 (s) |
| Peak HR | 190.6±70.8 (beats/min) |
| Peak blood pressure | 204.9±18.3/91.3±11.7 (mmHg) |
| Peak VO₂ | 42.2±15.1 (ml min⁻¹ kg⁻¹) |
| Peak exercise time | 700.7±292.0 (s) |
| ΔVO₂/ΔWR | 10.7±2.9 (ml/W) |

HR, heart rate; AT, anaerobic threshold; VO₂, oxygen uptake; WR, work rate.

ing, while VE/VO₂ was constant or decreased; and (2) the gas exchange ratio started to increase steeply. The VO₂ increase rate with regard to work rate (defined as ΔVO₂/ΔWR) was calculated by linear regression from commencement of the ramp exercise until attainment of AT. Blood samples for determining the plasma concentration of ET-1 in nonworking regions were drawn from the cubital vein after 30 min of rest and immediately after the exercise test.

Measurement of Cardiac Output (CO)

Measurement of CO during the exercise test was performed by thoracic bioimpedance using an NCCOM-R7 cardiac output monitor (Biomedical Medical Instruments, Irvine, CA, USA). Thoracic bioimpedance was used to measure the pulsatile change in resistance to injected microcurrents to calculate stroke volume. Four pairs of disposable electrodes were placed on the neck and chest. The outer electrode pair injected a 70-kHz, 2.5-mA current into the thoracic tissue and the current was then sent by the inner electrode pair. Resistance to the injected current was dependent upon the fluid characteristics of the thoracic volume. CO was measured on a beat-by-beat basis, and it was represented as the average of 16 accepted beats. Arterial venous oxygen difference (AVO₂D) when CO reached 10 L/min or 15 L/min was calculated as AVO₂D = VO₂/CO.

Measurement of Plasma ET-1 Concentration

Maeda et al reported that the production of ET-1 during exercise is increased in the circulation of nonworking muscles, but not in the working muscles! In order to measure plasma ET-1 concentration during exercise that would reflect its production in nonworking regions, 12 ml of venous blood was drawn from the cubital vein and immediately aspirated into a polypropylene tube containing 4.5 mg of EDTA-2Na and 150 μl of tradirol. The sampled blood was ice-cooled immediately and centrifuged at 3000 rpm for 10 min at 4°C, thereby separating the plasma, which was maintained in frozen storage at ~70°C until analysis. Plasma ET-1 concentration was measured by radio-immunoassay.

All data are expressed as mean±SD. The paired t test was used for within-group comparisons. Regression analysis was used for correlation analysis. A p value of <0.05 was considered significant.

Results

Cardiopulmonary Exercise Test

For all subjects, the endpoint of the exercise test was leg

fatigue or shortness of breath. No subject experienced ischemic ST change or severe arrhythmia. The mean AT and peak VO₂ for the total study population were 18.6±3.0 ml min⁻¹ kg⁻¹ and 42.3±6.1 ml min⁻¹ kg⁻¹, respectively. The mean AT time and peak exercise time were 236.4±61.8 and 700.7±292.0 s, respectively. The mean ΔVO₂/ΔWR values were 10.7±2.9 ml/W (Table 1).

The mean resting HR, HR at AT, and peak HR were 81.1±10.5 beats/min, 123.7±12.8 beats/min, and 190.6±80.8 beats/min, respectively, and the corresponding blood pressures were 125.0±29.8/81.6±9.6 mmHg, 155.6±19.4/82.2±10.9 mmHg, and 204.9±18.3/91.3±11.7 mmHg respectively (Table 1).

The mean CO during the resting state, the CO at AT and the peak CO were 6.3±1.2 L/min, 12.7±2.5 L/min, and 25.4±4.6 L/min, respectively.

Exercise-Induced Change in the ET-1 Concentration

The ET-1 concentration in plasma drawn from the cubital vein did not change significantly during exercise; from 1.6±0.5 pg/ml during the resting state to 1.7±0.7 pg/ml immediately following exercise (Fig 1).

Correlation Between Plasma ET-1 Concentration and Exercise Tolerance

No correlation was observed between the resting plasma ET-1 concentration or the plasma ET-1 concentration immediately after exercise and AT, AT time, peak VO₂ or peak exercise time, and no correlation was observed between the plasma ET-1 concentration change ratio and AT or peak VO₂. The ET-1 change ratio was correlated inversely with AT time (r=−0.37, p=0.03) and peak exercise time (r=−0.35, p=0.04) (Fig 2), and displayed a tendency toward an inverse correlation with ΔVO₂/ΔWR (r=−0.29, p=0.09).

Correlation Between Plasma ET-1 Concentration and AVO₂D

No correlation was observed between the plasma ET-1 concentration during the resting state or immediately after exercise and AVO₂D when CO reached 10 L/min or 15 L/min. The ET-1 change ratio correlated positively with
Fig 2. Correlation between plasma endothelin-1 (ET-1) concentration and exercise time. The change ratio of plasma ET-1 concentration correlated inversely with anaerobic threshold (AT) time and peak exercise time.

Fig 3. Correlation between plasma endothelin-1 (ET-1) concentration and arterial venous oxygen difference (AVO2D). The change ratio of plasma ET-1 concentration positively correlated with AVO2D when cardiac output (CO) reached 10L/min and revealed a positively correlated tendency when CO reached 15L/min.

AVO2D when CO reached 10L/min $r=0.42$, $p=0.02$ and tended to correlate positively with AVO2D when CO reached 15L/min $r=0.32$, $p=0.08$ (Fig 3).

**Discussion**

In the clinical setting, Stewart et al investigated whether myocardial infarction would result in elevated plasma ET-1 levels. ET-1 levels in patients with stable coronary disease did not differ from those of normal subjects, but patients with a complicated infarction demonstrated a rapid increase in plasma ET-1. The authors concluded that the sustained increase in plasma ET-1 in patients demonstrating complications of myocardial infarction might reflect either continuing ischemia or marked depression in ventricular function. Wieczorek et al also reported that patients with either unstable angina or non-Q wave myocardial infarction had significantly higher plasma ET-1 levels than healthy controls and that ET-1 may contribute to the pathophysiology of acute coronary syndromes.

However, the role of ET-1 in healthy subjects is still to be investigated and in the present study, we focused on the physiological role of ET-1 during exercise in healthy subjects.
Maeda et al investigated whether ET-1 participates in the exercise-induced changes in blood flow distribution in muscles. In their study, the plasma ET-1 concentration in the femoral vein in the nonworking leg increased significantly after exercise, whereas that in the femoral vein in the working leg was unchanged. The arteriovenous difference in ET-1 concentration increased significantly after exercise in the nonworking leg circulation, but not in the working leg. The plasma norepinephrine concentrations in the femoral veins of both the working and nonworking leg were elevated by exercise. The authors suggested that ET-1 production was increased in the circulation of the nonworking leg by exercise, whereas the sympathetic nerve activity was augmented in both legs during exercise. The authors concluded that the increase in ET-1 production in nonworking muscles may cause vasoconstriction and hence decrease the blood flow in nonworking muscles. Nakamura et al investigated the relation between plasma ET-1 levels and peripheral blood flow. ET-1 correlated positively with diastolic blood pressure and negatively with finger skin blood flow. A negative correlation of ET-1 with oxygen saturation in the blood and local blood flow, and a positive one with the local oxygen extraction fraction, were observed in elderly subjects. In the present study, the physiological role of ET-1 in nonworking muscles during exercise was investigated from the standpoint of cardiopulmonary exercise testing and AVO2D during exercise.

During ramp exercise testing in patients with heart disease, the VO2 increase rate with regard to exercise time was lower than in normal subjects. During ramp exercise tests using a bicycle ergometer, ΔVO2/ΔWR was low in patients with heart disease and showed different values between the New York Heart Association (NYHA) functional groups. ΔVO2/ΔWR indicates the increased quantity of O2 taken up by the exercising subject, and hence by the working muscles, as the work rate is increased. If the muscle is unable to obtain O2 due to inadequate delivery, then the slope would be shallower. Although theoretically there may be several reasons why this slope is reduced, including inadequate O2 transport and limitation in O2 diffusion from capillary to mitochondria, the reduction is most likely to be occur in conditions of impaired O2 flow to the exercising extremities, such as in heart dysfunction. Then ΔVO2/ΔWR may reflect the exercise efficiency through CO redistribution. In the present study, the ET-1 change ratio tended toward an inverse correlation with ΔVO2/ΔWR even in healthy subjects, although this tendency was not statistically significant. This finding may support the hypothesis that the increase in ET-1 in nonworking muscles may participate in increasing the exercise efficiency through CO redistribution in healthy subjects. Krum and Katz reported the effects of ET-1 administration in the brachial artery on forearm blood flow at rest and during rhythmic handgrip exercise in normal subjects and patients with chronic heart failure. In their study, synthetic human ET-1 was prepared as a sterile, pyrogen-free solution in 5% dextrose in water at a concentration of 50 mg/ml and was administered as a continuous 1-ml/min infusion for 5 min through a brachial artery catheter. Administration of ET-1 decreased forearm blood flow at rest and during rhythmic handgrip exercise in normal subjects, which agreed with previous studies on the effects of ET-1 infusion on resting forearm blood flow in normal subjects that demonstrated 15-60% decrease in forearm blood flow in response to ET-1 doses ranging from 2.5 to 250 ng/min. In contrast to blood flow in normal subjects, ET-1 administration did not decrease forearm blood flow in patients with chronic heart failure. Krum and Katz concluded that the vasoconstriction response to exogenous ET-1 is attenuated at rest and during submaximal exercise in patients with chronic heart failure.

Relationship Between Plasma ET-1 Concentration and AVO2D

Arterial O2 content clearly depends on the concentration of hemoglobin and its O2 binding capacity, the alveolar PO2, pulmonary diffusion capacity, and alveolar ventilation. It is commonly assumed that arterial O2 content and hemoglobin saturation are well maintained until maximal exercise in normal individuals. However, mixed venous O2 content falls progressively with increasing VO2. AVO2D increases steadily during light and moderate exercise and reaches a maximum value of about 15 ml of O2 per 100 ml of blood in normal individuals. The first factor accounting for the widening of AVO2D is marked redistribution of CO away from inactive to active regions during exercise.13 The plasma concentration of norepinephrine is a reasonable index of this activity, and may be an index of sympathetic nervous activity, but the consistency of its close relationship to HR and to relative VO2 and its close inverse relationship with splanchic blood flow is striking.13 In the present study, the ET-1 change ratio revealed an inversely correlated tendency with ΔVO2/ΔWR, and a positively correlated tendency with AVO2D. From the physiological viewpoint, it can be surmised that in healthy subjects both O2 demand and CO are almost identical if individual work during exercise is identical regardless of individual physique. Under the same CO conditions during exercise, high AVO2D means that blood is redistributed and directed through working muscles. In applying the present results to this hypothesis, it is feasible to surmise that the change ratio of plasma ET-1 concentration is related to exercise efficiency through CO redistribution away from inactive to active regions during exercise.

In conclusion, the increase in ET-1 in nonworking muscles may participate in exercise-induced redistribution of blood flow and in increasing the blood flow to working muscles, even in healthy subjects.

Study Limitations

The present study has certain limitations. The physiological role of ET-1 in nonworking muscles during exercise in healthy subjects was examined from the standpoint of cardiopulmonary exercise testing. Further study is needed to evaluate the physiological role of ET-1 in working muscles, and in patients with heart failure to evaluate the differences in the physiological role of ET-1 between healthy subjects and cardiac patients.

References