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＜研究報告＞

Renal vascular responses during graded dynamic bicycling exercise in women

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Abstract

From our previous data that static exercise produced a significant decrease in the renal blood flow (RA), it was hypothesized that the blood flow in RA also decreases during dynamic exercise with graded workloads. Nine healthy female volunteers participated in the present study. After 10-min resting, the subject performed a 15-min bicycling exercise consisting of three workloads of 30%, 50%, and 70% of peak oxygen uptake ($\dot{V}O_{2peak}$) for 5 min for each workload. During dynamic exercise, the responses in oxygen uptake, minute ventilation, mean arterial blood pressure (MAP), cardiac output, and heart rate increased linearly with three workloads from rest to 30%, 50%, to 70% of $\dot{V}O_{2peak}$. However, the mean RA velocity (MVB), measured by ultrasonography, showed a linear decrease with the workloads. The index of vascular resistance in RA (RVR), calculated as MAP/RA velocity, showed a significant increase from resting level during exercise at 50% and 70% of $\dot{V}O_{2peak}$. There was a significant increase in RVR from 50% to 70% of $\dot{V}O_{2peak}$. The present data supported the hypothesis that the graded bicycling exercise produces a significant increase in the renal vascular resistance over the workload of 50% $\dot{V}O_{2peak}$.

Key words: ultrasonography, renal vascular resistance

Introduction

At rest the kidneys receive a large portion of cardiac output, over 20%, due to a lower vasoconstrictor activity in the renal vascular beds (*Rowell* 1974). However, once exercise started, it is assumed that the renal sympathetic nerve activity is increased and thereby reducing the renal blood flow. This assumption has been convinced by the observation in conscious animals showing a significant increase in renal sympathetic nervous activity during exercise (*Mastukawa et al.* 1991；*O'Hagan et al.* 1993；*Mueller et al.* 1998). In human studies, it was also suggested that exercise evoked renal vasoconstriction proportionally with the exercise intensity, but these human studies examined renal blood flow by invasive methods, such as various dye-dilution techniques based on the Fick principle (*Grimby* 1965；*Clausen et al.* 1973；*Rowell* 1973；*Pricher et al.* 2004), radionuclide

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techniques (*Flam et al.* 1990), blood sampling analysis (*Tidgren et al.* 1991 ; *Maeda et al.* 1994, 1997) and PET scanning technique (*Middlekauff et al.* 1997). Since these invasive measurements are not suitable for rapid and repeated examinations during dynamic exercise in humans, it is necessary to investigate non-invasively the renal blood flow responses during exercise in humans.

Technological developments in Doppler ultrasound have produced a noninvasive technique for measurement of flow velocity and vessel diameter. Validation of this technique has been demonstrated by the thermodilution technique (*Rådgran G* 1997), magnetic resonance imaging (*Zananiri et al.* 1993), and plethysmography (*Levy et al.* 1979 ; *Tschakovsky et al.* 1995) in human studies, and by electromagnetic flow measurements in animal studies (*Chauveau et al.* 1985 ; *Guldvog et al.* 1980 ; *Nakamura et al.* 1989). However, there have been limited studies that applied Doppler method to measure the renal blood flow during exercise (*Momen et al.* 2003, 2004 ; *Sadamoto et al.* 2004). Specifically, during dynamic exercise, there was one report (*Endo et al.* 2008). However, this study investigated only during a single workload of 40 W, and no detailed study using several exercise intensities has yet been reported. From these reasons, it was undertaken to examine the renal flow velocity non-invasively, by using Doppler ultrasound technique, during dynamic exercise with graded stepwise workloads.

Methods

Subjects

Nine normal female volunteers whose mean age was 23.0 ± 3.3 yr participated in the present study. Their average weight and height and peak oxygen uptake ($\dot{V}O_{2\,peak}$) during bicycling exercise were 58.0 ± 5.1 kg, 163.0 ± 6.0 cm, and 37.3 ± 5.4 ml/kg/min, respectively. None had any significant medical problems, and all were considered to have normal cardiovascular functions on the basis of normal medical history and physical examinations. All subjects gave informed written consent to participate in this study before the start of experiments. Volunteers abstained from caffeine for 18 hours before the study but otherwise were on an uncontrolled diet. All subjects were studied in the postabsorptive state and familiarized with the testing apparatus of experiments. The aim and protocols in the present study were approved by the Guiding Principles for Human Studies of Ethical Committee in the Japan Women's College of Physical Education.

Study protocols

The subjects reported to our laboratory on three days separated by 2-7 days. All experiments were conducted in a well-ventilated laboratory regulated at 23 ± 1℃. On the first day, the $\dot{V}O_{2\,peak}$ in each subject was determined during incremental bicycling exercise in the upright position. The workload was increased to the limit of tolerance, at a rate of 12-15 W/min on an electromagnetically braked cycle ergometer (Combi, Tokyo, Japan) at 60 rpm. The $\dot{V}O_{2peak}$ was identified by the leveling-off in heart rate (HR) responses and the level of subjective rating perceived exertion (RPE) in Borg's scale. On the second day, subjects practiced to perform bicycling exercise in the semi-recumbent position used in the following experiments on the third day. The scanning of renal blood flow was also tested using ultrasound Doppler methods. Thus, the subjects were familiarized with the experimental protocol and all measurements.

On the third day, subjects rested for 10 min while preexercise baseline levels of all variables were measured. Then, subjects performed a 15-min bicycling exercise including three workloads of 30%, 50%, and 70% of $\dot{V}O_{2\,peak}$ for 5 min for each workload and had a 3 min recovery period. The exercise was performed on the recumbent ergometer (EC-3700, Cateye Tokyo). Oxygen uptake ($\dot{V}O_2$), carbon dioxide production, minute ventilation volume ($\dot{V}_E$), heart rate (HR), arterial mean blood pressure (MAP), and cardiac output (CO) were continuously measured through the experiment. The mean blood flow velocity (MBV) in renal artery was recorded by using Doppler method for 3 min at rest condition and for last 1-min of the 5-min bicycling exercise at each workload of 30%, 50%, and 70% of $\dot{V}O_{2\,peak}$. The subject was instructed to adjust the pedaling frequency to the beep sound of 60 rpm during exercise. To obtain the highest quality of Doppler tracings possible, the Doppler transducer had to be maintained in a constant position on the subject's lower back wall. In pilot experiments, we noted that the renal artery moved with respiration phases, thus we sometimes could not maintain high-quality velocity tracings during both phases of the respiratory cycle. Nevertheless, for each subject, we obtained velocity data during the same phase of the respiratory cycle for all portions of the study protocol. No subjects performed the Valsalva's maneuver during the protocols.

Measurement of renal flow velocity.

Duplex ultrasonography (Vivid 7 Pro, GE Yokogawa medical systems, Tokyo) with a curved-array 3 C Doppler probe with a 4.0-MHz pulsed Doppler frequency was used (*Lee & Grant* 2002). The left renal artery was scanned via posterior and lateral abdominal approach from the lower back region. The measuring site of renal artery was located ~10 cm below the surface of lower back region and around 2 cm proximal to the entrance of left kidney. A real-time imaging system, without aliasing, was used to visualize the renal artery and allowed the placement of the Doppler sample volume within the lumen of the vessel to obtain the Doppler shift signal. The sample volume was kept constant at the center of the lumen and adjusted to cover the width of the vessel and the blood velocity distribution. The probe insonation angle to the skin was ~60°. Beat-to-beat Doppler signals were analyzed to determine the mean blood flow velocity (MBV) by the software built-in the equipment (Vivid 7 Pro, GE Yokogawa medical systems, Tokyo), and then the each MBV was normalized with a time constant of 5 s for the calculation of renal vascular resistance (RVR). The RVR represents the quotient of mean arterial blood pressure (MAP) and the respective MBV value. RVR is expressed in arbitrary units (a.u.) .

Cardiorespiratory measurements.

Subjects breathed through a suitable face-mask adapted for breathing through the mouth and the nose. The face-mask was connected to a Fleisch pneumotachograph (ARCO-1000, Arco system, Chiba) which gave continuous measurements of air flow. Pulmonary minute ventilation ($\dot{V}_E$), and oxygen uptake ($\dot{V}O_2$) were assessed on a breath-by-breath basis using a computerlized system with a mass spectrometer (ARCO-1000, Arco system, Chiba). Gas calibration of the mass spectrometer was performed before each experiment by utilizing a gas mixture of known composition. The data of the gas fractions and respiratory flow from the gas analysis system were continuously recorded on a computer hard disk through a 16-bit A/D board with a sampling rate of 50 Hz. Breath-by-breath analyses of $\dot{V}_E$ and $\dot{V}O_2$ were performed for all subjects in the protocol and then each value of $\dot{V}_E$ and $\dot{V}O_2$ was normalized with a time constant of 5 s. Arterial blood pressure was recorded continuously from the third finger on the left arm by photoelectric plethysmography with a Finometer (Finapres, Medical Systems BV, Amsterdam). MAP was calculated from the software of Modelflow program fixed in the Finometer. The HR, stroke volume (SV), and CO were also determined from the finger waveform using the Modelflow software. The validity of the estimated SV and CO was convinced by Sugawara et al. (2003). Similarly, beat-by-beat sequential analyses of HR, CO and MAP were performed and then normalized with a time constant of 5 s in the protocol. The RPE was monitored during exercise according to Borg's scale.

Data analysis and Statistics

The 5-s normalized data obtained for 2 min of 10-min resting period, last 1-min of 5-min exercise at each workload, and last 1-min of recovery period were respectively averaged for $\dot{V}_E$, $\dot{V}O_2$, HR, CO, MAP, MBV, RVR and RPE. These average data in each variable were used for a one-way analysis of variance (ANOVA) with repeated measures to see the effect of workload. When a significant *F*-value in the main effect of workload was observed, Dunnett's *post hoc* test or Tukey's *post hoc* test were applied to see a significant difference from the resting level in the average at three workloads and recovery period. Data are presented as means ± SD. The level of significance was set at $P < 0.05$.

Results

Fig. 1 shows the cardiorespiratory responses at rest and during exercise with three workloads of 30%, 50%, and 70% of $\dot{V}O_{2\,peak}$ and recovery period. The $\dot{V}O_2$, $\dot{V}_E$, CO, and HR during exercise increased with three workloads from rest to 30%, 50%, to 70% of $\dot{V}O_{2\,peak}$ and then gradually returned during the recovery period. There was a significant main effect of workload in one-way ANOVA (main effect, $P < 0.001$). *Post hoc* analyses demonstrated that these cardiorespiratory varibles in 30%, 50%, and 70% of $\dot{V}O_{2\,peak}$ were significantly higher than the resting level and the difference between 30%, 50%, and 70% of $\dot{V}O_{2\,peak}$ was also significant. Similar results were observed in MAP whereas the difference between 30% and 50% of $\dot{V}O_{2\,peak}$ was insignificant. The RPE increased with the workloads from 30%, 50% to 70% of $\dot{V}O_{2\,peak}$.

Fig. 2 shows the MBV and RVR responses at rest and during exercise and recovery period. The graded exercise produced a progressive decrease in MBV (one-way ANOVA main effect, $P < 0.001$). *Post hoc* analyses showed a significant decrease in the MBV from the rest to 50% and to 70% of $\dot{V}O_{2\,peak}$. The RVR increased with the increase of workloads. A significant increase from rest was found at the workloads of 50% and 70% of $\dot{V}O_{2\,peak}$. The increase in RVR between 50% and 70% of $\dot{V}O_{2\,peak}$ was also significant.

Discussion

Study findings and prior studies on the topic

The present study investigated the renal vascular responses non-invasively by using Doppler method during dynamic exercise with three workloads and found that the dynamic bicycling exercise produced a significant increase in RVR at 50% and 70% of $\dot{V}O_{2\,peak}$. This means that dynamic bicycling exercise led to the renal vasoconstriction and thereby reducing the renal blood flow over the workload of 50% $\dot{V}O_{2\,peak}$. When estimating the percent re-

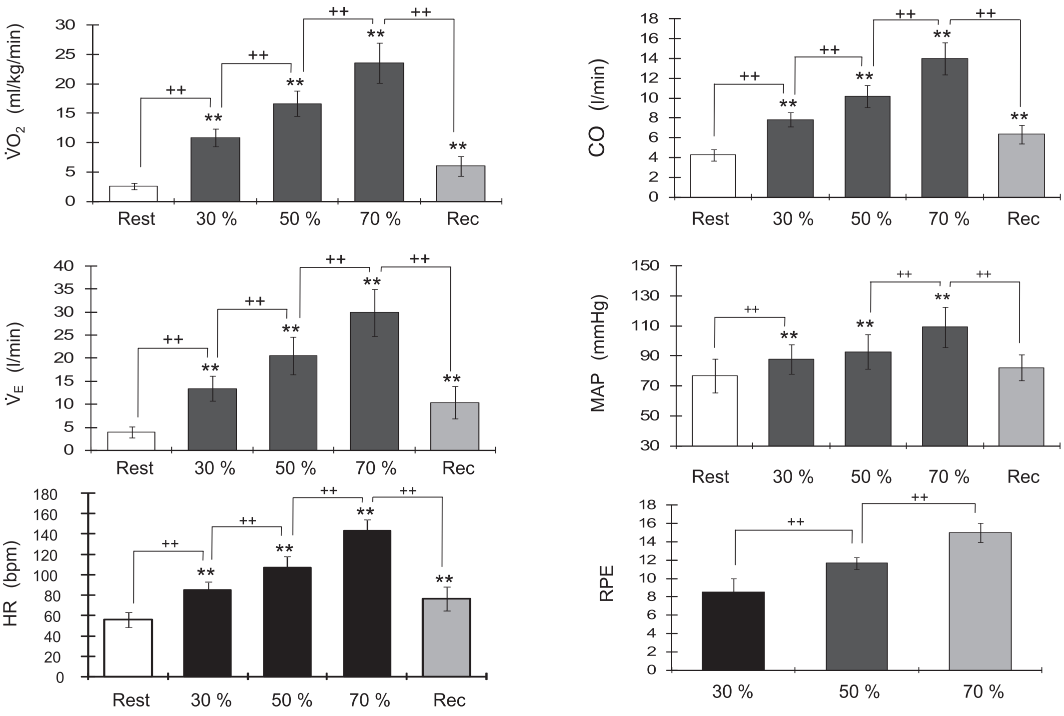

Fig. 1 Oxygen uptake ($\dot{V}O_2$), minute ventilation ($\dot{V}_E$), heart rate (HR), cardiac output (CO), mean arterial blood pressure (MAP) and rating of perceived exertion (RPE) observed at rest and during dynamic exercise with three workloads of 30%, 50% and 70% of peak oxygen uptake and during recovery period. Data expressed as means ± SD in 9 subjects. **; significant difference vs. rest, $P < 0.01$. ++; significant difference among three workloads, $P < 0.01$.

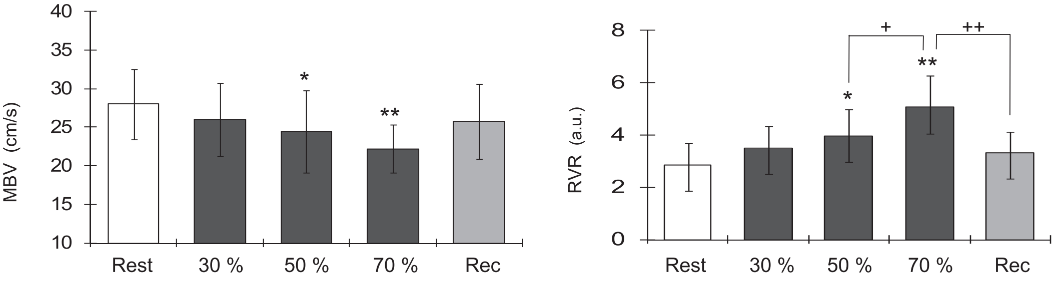

Fig. 2 Mean blood velocity (MBV) and renal vascular resistance (RVR) observed at rest and during dynamic exercise with three workloads of 30%, 50% and 70% of peak oxygen uptake and during recovery period. Data expressed as means ± SD in 9 subjects. **; significant difference vs. rest, $P < 0.01$. ++; significant difference among three workloads, $P < 0.01$.

duction in the renal blood flow from the RVR values obtained in the present results, the reduced level for 50% and 70% of $\dot{V}O_{2\,peak}$ was, respectively, about 67% and 37% of the resting blood flow. These data agree well with the previous reports, where renal flow (*Bishop et al.*

1957 ; *Grimby*, 1967), hepatic flow (*Clausen et al.* 1973) and splanchnic flow (*Rowell et al.* 1973) were shown to decrease linearly with an increase of workloads during dynamic exercise. Two studies (*Flamm et al.* 1990 ; *Osada et al.* 1999), however, reported a different reduction from the present data. In the study of Flamm et al. (1990), the blood volume of kidneys was measured by radio-labeling of red blood cells and the percent reduction from the resting level was 8 ± 2% for 50% of maximal workload (HR = 134 bpm) and 16 ± 4% for 75% of maximal workload (HR = 169 bpm). Osada et al. (1999) reported a much greater decrease in the abdominal viscera blood flow during one-legged knee extension at very-low-intensity exercise ($HR < 90$ bpm). The discrepancy between the present data and the studies in Flam et al. (1990) and Osada et al. (1999) was probably explained by different techniques for the renal flow measurements and/or different exercise modes and protocols.

Possible mechanisms responsible for the increase in RVR

The graded dynamic exercise in the present study showed a significant increase in RVR, indicating a significant increase in renal vasoconstriction over the workload of 50% of $\dot{V}O_{2\,peak}$. At first, myogenic vasoconstriction due to an elevation of MAP (*Sanchez-Ferrer et al.* 1989) was probably not responsible for the present increase in RVR over the workload of 50% of $\dot{V}O_{2\,peak}$ because the significant increase in MAP at 30% $\dot{V}O_{2\,peak}$ did not produce any corresponding changes in MBV and RVR in the present data. Alternatively, it is likely that the muscle metaboreflex played a predominant role in the increase in RVR over 50% $\dot{V}O_{2\,peak}$. Previous studies demonstrated that postexercise muscle ischemia produced a profound increase in RVR which is almost identical to RVR during static exercise (*Middlekauff et al.* 1997) or up to 60-70% of RVR during static exercise (*Momen et al.* 2003 ; *Sadamoto* 2004). It is also well-known that blood lactate accumulation during exercise, generally occurs at the intensity over 50-60% of $\dot{V}O_{2max}$ in untrained subjects (*cf. Davis,* 1979). Thus, it seems likely that the muscle metaboreflex arising from exercising muscles played an important role in the increase in RVR during dynamic exercise over 50% $\dot{V}O_{2\,peak}$ in the present study. Secondly, the muscle mechanoreflex engagement was also a possible mechanism. This explanation was convinced by animal studies (*Hill et al.* 1996 ; *Matsukawa et al.* 1990 ; *Victor et al.* 1989), where muscle mechanoreflex engagement is indicated as the primary cause for the renal vasoconstrictor during muscle contraction. Thirdly, the production of endotheline-1 (ET-1), an endothelium-derived potent vasoconstrictor peptide in human vessels, is also potent stimulus to cause vasoconstriction in nonworking muscles such as kidneys (*Maeda et al.* 1994, 1997). Further studies in humans are necessary to investigate the responsible mechanisms underlying the regulation of renal vasoconstriction during exercise.

Limitation of study

In the present study, we were not able to precisely measure renal artery diameter using ultrasound Doppler method. This is because spatial resolution decreases as the frequency of the ultrasound transducer decreases (*Kremkau* 1998). To obtain an optimal velocity signal from the renal artery, a low-frequency (4.0-MHz) transducer was employed. At this frequency level, spatial resolution of the technique is not sufficient to precisely measure diameter changes that would be seen in the relatively small renal artery. Accordingly, the RVR resistance might have some variations.

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