Recently, a change in pulmonary ventilation ($\dot{V}_E$) per unit change of carbon dioxide production ($\dot{V}CO_2$) (i.e., $\dot{V}_E$–$\dot{V}CO_2$ slope) can be measured readily from data routinely acquired during cardiopulmonary exercise testing and carries important prognostic information for chronic heart failure (CHF) patients with preserved exercise tolerance [1, 2]. According to the “cardiodynamic” hypothesis, $\dot{V}_E$ dynamics in the transient phase are mediated by the pulmonary vascular $CO_2$ flow to the lungs [3–5], and there is a strong cor-

### Abstract

We designed two experiments to investigate the relationship between ventilation ($\dot{V}_E$) and CO$_2$ output ($\dot{V}CO_2$) during exercise under the conditions of exercising different limbs, the arms as opposed to the legs (experiment 1), and of different physical training states after undergoing standard exercise training for 90 d (experiment 2). Six healthy young subjects underwent submaximal ramp exercise at an incremental work rate of 15 W/min for the arm and leg, and 11 healthy middle-aged subjects underwent an incremental exercise test at the rate of 30 W/3 min before and after exercise training. We measured pulmonary breath-by-breath $\dot{V}_E$, $\dot{V}CO_2$, oxygen uptake ($\dot{V}O_2$), tidal volume ($VT$), breathing frequency (bf), and end-tidal $O_2$ and $CO_2$ pressures ($P_{ETO_2}$, $P_{ETCO_2}$) via a computerized metabolic cart. In experiment 1, arm exercise produced significantly greater $\dot{V}_E$ than did leg exercise at the same work rates, as well as significantly higher $\dot{V}O_2$, $\dot{V}CO_2$, and bf. The slopes of the regression lines in the $\dot{V}_E$–$\dot{V}CO_2$ relationship were not significantly different: the values were 27.8±2.1 (SD) during the arm exercise, and 25.3±3.9 during the leg exercise, with no differences in their intercepts. In experiment 2, the $\dot{V}O_2$, $\dot{V}CO_2$, and $\dot{V}E$ responses at the same work rates were similar in both before and after the 90-d exercise training, whereas the heart rate (HR) and mean blood pressure (MBP) were significantly reduced after training. Exercise training did not alter the $\dot{V}_E$–$\dot{V}CO_2$ relationship, the slope of which was 31.9±4.9 before exercise training and 34.2±4.4 after exercise training. We concluded that the $\dot{V}_E$–$\dot{V}CO_2$ relationship during exercise is unaltered, independent of not only working muscle regions but also exercise training states. [Japanese Journal of Physiology, 52, 489–496, 2002]

### Key words: ventilation, CO$_2$ output, leg exercise, arm exercise, exercise training.
relation between $\dot{V}E$ and $\dot{V}CO_2$ responses during exercise [6]. Interestingly, in experiments using anesthetized animals, Cross et al. [7] and Weissman et al. [8] demonstrated similar responses in the $\dot{V}E$–$\dot{V}CO_2$ relationship to electrically induced muscle contractions in the presence and absence of intact spinal cord transmission. These results suggest the predominant contribution of humoral mechanisms to exercise hyperpnea. As far as the ventilatory response in paraplegia with spinal lesions is concerned, Adams et al. [9] and Brice et al. [10] were able to support the view of the above mentioned animal experiments. After comparing the ventilatory response between active-recovery and resting after exercise, Takahashi et al. [11] suggested that the flux of CO_2 is an important determinant of ventilatory drive.

Ramonatxo et al. [12] observed higher ventilation as a function of $\dot{V}CO_2$ during arm exercise compared with the response during leg exercise. Ishida et al. [13] also showed 20% greater ventilation during arm exercise than during leg exercise in the transient phase of exercise. Compared to leg muscles, this would probably derive from the greater content of fast-twitch (FT) fibers and fewer muscle fibers per motor unit in the arms than the legs [14], and a greater number of motor units occupying FT fibers can be recruited to maintain a given work rate or metabolic demand during arm exercises. Thus, arm exercises have been assumed to induce greater participation of the neural mechanisms in determining the ventilatory response than leg exercises [12, 15]. Our hypothesis of a different $\dot{V}E$–$\dot{V}CO_2$ relationship between arm and leg regions was that the superfluous ventilatory response at any given metabolic rate (e.g., the $\dot{V}CO_2$) could be demonstrated, because it has been assumed to be due to greater recruitments in afferent neural drive during arm exercise.

In addition, we formed an interesting hypothesis that exercise training could modify the $\dot{V}E$–$\dot{V}CO_2$ relationship during exercise, from which the CO_2 ventilatory responsiveness decreases gradually with an increased period of exercise training, whereas the ventilatory adaptations observed with training are reversible through detraining [16]. From the study of different training states, the slope in the regression line of the $\dot{V}E$ and $\dot{V}CO_2$ relationship during exercise, being very similar between trained speed swimmers and synchronized swimmers, was significantly lower than the data observed in recreational swimmers [17]; this finding proved that exercise training could induce the lower CO_2 responsiveness related to ventilation during exercise. In contrast, the higher $\dot{V}E$–$\dot{V}CO_2$ slope in severe CHF patients is associated with augmented CO_2 chemoreceptor sensitivity [1, 2, 18]. If exercise training altered the CO_2 responsiveness, it is possible that the reduction of CO_2 responsiveness can be depressed in ventilation during exercise, and that slower $\dot{V}E$ dynamics can occur at the transient phase of exercise [19]. Therefore, we can expect that the regression line in the $\dot{V}E$–$\dot{V}CO_2$ relationship will move downward and/or be sluggish after exercise training. However, the $\dot{V}E$–$\dot{V}CO_2$ relationship in these cases, such as different exercising regions and exercise training states, was very scant.

The purpose of the present study was to clarify the compelling physiological evidence that arm exercise induces a slightly greater ventilatory response, and in contrast, exercise training induces a lower ventilatory response. This clarification, in turn, would show that the characteristics of the regression line in the $\dot{V}E$–$\dot{V}CO_2$ relation could be modified by the physical training states in humans.

**METHODS**

**Subjects.** Six healthy male subjects (age 20.2±0.4 years; height 171.7±5.6 cm; weight 68.5±11.8 kg, mean±SD) participated in the experiment (experiment 1), some of which had irregular exercise activity (e.g., swimming, tennis, basketball, and badminton). The subjects performed arm exercises on one day and leg exercises on another day. Before the study, all of the subjects received a physical examination. None of the subjects were taking any medication known to affect cardiovascular function, and a 12-lead resting electrocardiogram was taken. The subjects were fully informed of any risks and discomforts associated with the experiments before they gave their written, informed consent to participate in this study, which was approved by the ethics committee of the Institutional Review Board of the School of Medicine, Kumamoto University.

In experiment 2, the study was conducted on 11 healthy, previously untrained middle-aged males (age 51.0±3.0 years; height 176.9±1.7 cm; weight 86.0±3.5 kg). The subjects were fully informed of any risks and discomforts associated with the experiments before giving their written, informed consent to participate in this study, which was approved by the ethics committee of the Institutional Review Board of Istituto di Tecnologie Biomediche Avanzate, Consiglio Nazionale delle Ricerche, Milano, Italy. The middle-aged subjects underwent a standard exercise training program (i.e., combined aerobic and strength exercises for 1.5 h every 3 d/week) for 90 d, as described in a previous report (see Method of Fukuoka et al. [20]).
Exercise protocols.

**Experiment 1.** The subjects underwent an incremental bicycle exercise (“ramp exercise” of starting from rest, 15 W every 1 min) on an electromagnetically braked cycle ergometer (75 XL, Conbi, Tokyo, Japan) until a mechanical work rate of 240 W was reached. Pedaling frequency, digitally displayed to the subjects, was kept at ~50 revolutions per minute (rpm). During the arm exercise, the center of the ergometer crankshaft was positioned at shoulder level, with slight elbow flexion in the extended position. The subjects were not restrained during the arm exercise, aside from having to sit on a high-backed chair with their feet on the floor. After 5 min of rest, the work rates were progressively augmented by a ramp of 15 W/min until a mechanical work rate of 120 W was reached. Arm cranking frequency was kept constant at ~50 rpm. All subjects performed the final work rate completely, to 120 W with the arm exercise and 240 W with the leg exercise.

**Experiment 2.** The tests were carried out under close medical supervision, and the subjects were continuously monitored by 12-lead electrocardiography (ECG). The tests were carried out in the afternoon, a few hours after a light meal. Before training (PRE) and 90 d after the beginning of the training program (POST), the subjects underwent an incremental bicycle exercise (“incremental exercise”): starting from rest, 30 W added every 3 min to voluntary exhaustion, which was defined as the inability to sustain the recommended pedaling frequency of 40–60 rpm despite vigorous encouragement by the operators. An electromagnetically braked cycle ergometer (STS 3, Cardiolime, MI, Italy) was utilized. Pedaling frequency was digitally displayed to the subjects throughout the tests.

**Measurements and data analysis.** Pulmonary $\dot{V}E$, O₂ uptake ($\dot{V}O₂$) and $\dot{V}CO₂$ were determined breath-by-breath by a computerized metabolic chart (Vmax29c, Sensor Medics, CA, USA). Expiratory flow measurement was performed by a mass flow sensor (hot-wire anemometer) calibrated before each experiment by a 3-l syringe at three different flow rates, in which its accuracy was regulated automatically within ±1.0%. Tidal volume ($\dot{V}T$), breathing frequency (bf), and $\dot{V}E$ were calculated by integrating the flow tracings recorded at the mouth of the subject. $\dot{V}O₂$ and $\dot{V}CO₂$ were determined by continuously monitoring PO₂ and PCO₂ at the mouth of the subject throughout the respiratory cycle, and from established mass balance equations, after alignment of the expiratory volume and expiratory gases tracings and A/D conversion. Calibration of the O₂ and CO₂ analyzers was performed before each experiment by utilizing gas mixtures of known composition. The digital data was transmitted to a personal computer and stored on disk. $\dot{V}O₂$ and $\dot{V}CO₂$ were expressed in STPD, and $\dot{V}E$ in BTPS. Gas exchange ratio (R) was calculated as $\dot{V}CO₂/\dot{V}O₂$. End-tidal PO₂ (PET₂) and end-tidal PCO₂ (PET₃) were determined from PO₂ and PCO₂ gas tracings. Heart rate (HR) was determined beat-by-beat from the R–R intervals by a cardiotachometer coupler (PE4000, Polar Electro, NY, USA). In experiment 2, 20 μl of earlobe capillary blood was obtained for determining lactate concentration ([La]ₐ) by an enzymatic method (ESAT 6661 Lactat, Eppendorf, MI, Italy) at rest. It was collected during the last 30 s of each step of the incremental exercise test. Mean blood pressure was calculated using the following equation: (systolic blood pressure − diastolic blood pressure)/3 + diastolic blood pressure, determined using a sphygmomanometer.

The values of $\dot{V}E$, $\dot{V}O₂$, $\dot{V}CO₂$, and HR for each work rate of both experiment 1 and experiment 2 were obtained by calculating averages of breath-by-breath or beat-by-beat values over the last 30–40 s of every 30-W work rate. Resting values were obtained by calculating averages during ~1 min of rest.

**Statistical analysis.** Data are presented as arithmetic means ± standard deviation (x ± SD). All gas exchange parameters, at the same submaximal work rate and same level of $\dot{V}CO₂$ as a function of the metabolic rate, were evaluated by two-way analysis of variance. When significant differences were found, Tukey’s post-hoc test was used to discriminate where significant differences occurred. The regression coefficients on independent variables of $\dot{V}CO₂$ were compared between the arm and leg exercises, and between the PRE and POST test periods. Significance was set at the 0.05 level.

**RESULTS**

**Experiment 1**

Figure 1 demonstrates that, as work rates increased, significantly higher $\dot{V}CO₂$, $\dot{V}O₂$, and $\dot{V}E$ were measured during the arm exercise than during the leg exercise ($p<0.05$, $p<0.01$). In addition, bf and R during the arm exercise were greater than during the leg exercise; significant differences were achieved at 90 and 120 W for bf, and 60, 90, and 120 W for R. Consequently, PET₃CO₂ and PET₃O₂ were significantly lower and higher, respectively, during the arm exercise than during the leg exercise at 120 W. However, $\dot{V}T$ showed no significant difference between the two exercise modes. Figure 2 shows the mean changes in ventila-
tory variables as a function of $V\dot{CO}_2$ during the arm and leg exercises. The rate of increase of $V\dot{E}$ with augmentation of $V\dot{CO}_2$ during the arm exercise was slightly higher than that during the leg exercise, but the difference was not significant. $V_T$ showed a similar trend with any given increase in $V\dot{CO}_2$; otherwise, the bf response was slightly higher in the arm exercise than in the leg exercise, but there were no other differences between the two exercises except for the condition of higher $V\dot{CO}_2$ of 2.0 l·min$^{-1}$. The $P_{ETCO_2}$ response was kept constant, ranging from 37 to 42 mmHg during the leg exercise. Otherwise, the response was significantly lower at $V\dot{CO}_2$ of 2.0 l·min$^{-1}$. Figure 3 presents the regression line in the relationship between $V\dot{E}$ and $V\dot{CO}_2$, obtained by averaged data from every 30 W during each exercise. The mean slope of 27.8±2.1 during the arm exercise was not significantly different from the mean slope of 25.3±3.9 during the leg exercise as shown in Table 1 ($p = 0.43$). Similarly, there was no difference in the intercepts between the two types of exercise ($p = 0.27$). Thus, the regression lines in both exercise modes are statistically the same, suggesting an absence of specificity in the regression line despite the different regions of working muscle.

**Experiment 2**

$V\dot{CO}_2$, $V\dot{O}_2$, and $V\dot{E}$ responses as a function of work rates in PRE were similar to those in POST (Fig. 4). The trend of HR in POST moved downward as com-
pared to that in PRE and became statistically significant at some work rates. Remarkably, the reduction in MBP at the same work rates also became significant in POST as compared to that in PRE. The delayed [La]b increase during incremental exercise was described in POST, but the difference in both times showed no statistical significance. These regressions between $\dot{V}E$ and $\dot{V}CO_2$, over the range of metabolic rates studied, in both PRE and POST periods, were indicated to overlap in the experiment 1 data shown in Fig. 3. The slope of the regression line in the relationship between $\dot{V}E$ and $\dot{V}CO_2$ was 31.9±4.9 in PRE and 34.2±4.4 in POST (Table 1), but was not significant between PRE and POST ($p=0.20$). In addition, the intercepts of the regression line were not statistically significant ($p=0.95$).

**DISCUSSION**

In the present study with ramp (experiment 1) and incremental (experiment 2) exercise tests, we found that, at the same mechanical rates, $\dot{V}E$ responses were significantly higher during arm exercise than during leg exercise, but similar in PRE and POST periods. However, there was no significant difference in the regression line of the $\dot{V}E$–$\dot{V}CO_2$ relationship between the two exercises, in which the slopes were 27.8±2.1 for the arms and 25.3±3.9 for the legs. In addition, exercise training did not alter the $\dot{V}E$–$\dot{V}CO_2$ relationship, in which the slopes were 31.9±4.9 in PRE and 34.2±4.4 in POST.

Wasserman et al. [21] demonstrated that there is a close relationship between the dynamic responses of $\dot{V}CO_2$ and $\dot{V}E$ to various patterns of work rate changes. The results of this study agreed with those of his previous study [22]. Based on the review of previous reports on the dynamics of $\dot{V}E$ and $\dot{V}CO_2$ at the transient phase of exercise, the “cardiodynamic” hypothesis could be supported by the evidence confirming a close correlation between the time constant $\dot{V}CO_2$ and time.

**Table 1. Summary of regression analysis for $\dot{V}E$–$\dot{V}CO_2$ relationship for each condition.**

<table>
<thead>
<tr>
<th>Condition</th>
<th>Slope</th>
<th>Intercept</th>
</tr>
</thead>
<tbody>
<tr>
<td>Arm exercise</td>
<td>27.8±2.1</td>
<td>5.1±2.4</td>
</tr>
<tr>
<td>Leg exercise</td>
<td>25.3±3.9</td>
<td>5.1±2.9</td>
</tr>
<tr>
<td>Exercise training</td>
<td></td>
<td></td>
</tr>
<tr>
<td>PRE</td>
<td>31.9±4.9</td>
<td>3.8±2.4</td>
</tr>
<tr>
<td>POST</td>
<td>34.2±4.4</td>
<td>3.7±2.6</td>
</tr>
</tbody>
</table>

Regression coefficients were calculated from a simple linear regression ($Y=AX+B$); where $Y$ is ventilation ($\dot{V}E$), $A$ is the slope of the regression line, $X$ is CO2 output ($\dot{V}CO_2$), and $B$ is the Y-intercept of the regression line. Data are shown as mean±SD.
constant $\dot{V}E$ in anesthetized dogs by bilateral peripheral nerve stimulation [23]. Lowering of the body CO$_2$ stores before the main exercise may, in part, explain the slower $\dot{V}CO_2$ dynamics, and subsequently, the slower $\dot{V}E$ dynamics [4]. In addition, acetazolamide administration, by which the CO$_2$ output is reduced via inhibition of carbonic anhydrase, has been found to result in a slowing of the $\dot{V}CO_2$ dynamics and slower $\dot{V}E$ dynamics at a moderate work rate [24]. The above evidence suggests that a strong linkage can be observed between $\dot{V}CO_2$ and $\dot{V}E$ during exercise.

As shown by the data obtained by Takano [25], arm exercise elicited higher $bf$ and lower $VT$ at a given $\dot{V}CO_2$ than did leg exercise. This was because rotational movements of the upper body during the arm exercise interfered with ventilatory movements of the thoracic muscles [26, 27]. However, there was no significant difference in the $VT$ values between the arm and leg exercises in the present study. In general, since greater FT fiber recruitment occurred in the arms during the same absolute work rate, the motor units occupying FT fiber, which can promote irradiation of the respiratory center via the afferent pathway [13], could be elicited more by greater ventilation as compared to leg exercise [14, 22]. A higher pedal rate during the leg exercise resulted in a slightly larger increase in $\dot{V}E$ and a lower $PET_{CO_2}$ at any given $\dot{V}CO_2$ as compared to the $\dot{V}E$ response at a lower pedal rate [28], indicating greater afferent neural stimulation via groups III and IV in the exercising muscles. An alternative locus of ventilatory control has been proposed to occur in or near the muscle microvasculature [29]. In animals and humans, $\dot{V}E$ has been shown to respond in a manner suggestive of an afferent drive from increased vascular conductance and/or increased tissue pressure in the exercising muscles themselves, through the involvement of Group III and IV muscle afferents. However, our results could not directly support this hypothesis regarding the neurogenic influence on exercise hyperpnea. The $\dot{V}E$–$\dot{V}CO_2$ relationship was also virtually identical during arm and leg exercises, although the responses to $\dot{V}E$ and $\dot{V}CO_2$ as a function of work rate differed between the arm and leg exercises (Fig. 1). Interestingly, with the anesthetized animal experiments, Cross et al. [7] and Weissman et al. [8] demonstrated similar responses in the $\dot{V}E$–$\dot{V}CO_2$ relationship to electrically induced muscle contractions in the presence and absence of intact spinal cord transmission, showing that this relationship is a predominant contribution to humoral mechanisms in exercise hyperpnea. Brice et al. [10] also studied the $\dot{V}E$–$\dot{V}CO_2$ relationship during electrically induced exercise in paraplegic patients, and observed a relationship similar to that in fully ambulatory human subjects. This observation suggests no role for either the peripheral neural afferents from the contacting muscles or the central command in exercise hyperpnea. Our hypothesis for neurogenical influence in arm regions was not supported, and consequently, we could not observe an alternation in the $\dot{V}E$–$\dot{V}CO_2$ relationship between arm and leg exercises. A reason for this may be that the recruitment in oxidative muscle fibers in arm regions is more developed in young subjects. Since the subjects had irregularly participated in various sports (e.g., swimming, tennis, basketball, and badminton), consequently, greater oxidative metabolism, being equivalent to leg exercise, seemed to occur in arm exercise.

In another physiological challenge, we carried out a 90-d exercise training program, and found consequently, that the slope in the regression of the $\dot{V}E$–$\dot{V}CO_2$ relationship was unaltered by exercise training. In contrast, physical training decreases the $\dot{V}E$–$\dot{V}CO_2$ slope and increases the peak oxygen uptake in patients on chronic hemodialysis [30]. Moreover, the depressed ventilatory response to $\dot{V}CO_2$ at a steady state of exercise in trained athletes was also observed as compared to subjects that engaged in recreational exercise [17]. As for the dynamics of $\dot{V}E$ and $\dot{V}CO_2$ to sinusoidal work rates in humans, the phase shift in both the $\dot{V}CO_2$ and $\dot{V}E$ responses became larger with increased periods of football training [19], providing one possibility that football training was associated with the depression of CO$_2$ ventilatory responsiveness. Actually, there was a very interesting finding that a higher $\dot{V}E$–$\dot{V}CO_2$ slope is associated with augmented chemoreceptor sensitivity in patients [1, 2].

A significantly greater $\dot{V}E$–$\dot{V}CO_2$ slope of $\sim 30$ was observed in the elderly, which was to compensate for their increased physiological dead space, as compared to that in young individuals [31, 32]. A greater value for the $\dot{V}E$–$\dot{V}CO_2$ slope, which may be due to the aging of the 50-year-old subjects, was observed in the present study. From previous reports, Miyamura and Ishida [16] reported that CO$_2$ ventilatory responsiveness decreased gradually with an increased period of exercise training, whereas the ventilatory adaptations with training observed are reversible through detraining, coincident with the data of Blum et al. [33]. Similarly, the ventilatory response to hypercapnia was significantly lower in elderly subjects as compared to the young subjects [31]. Even though we could not directly observe the CO$_2$ ventilatory responsiveness, it is likely that the lowering of CO$_2$ ventilatory responsiveness by exercise training was not remarkably observed, since aging might have already lowered CO$_2$ ventilatory responsiveness. Thus, our results for the
\( \dot{V}E-\dot{V}CO_2 \) relationship could not support the scenario for ventilatory response during exercise related to \( \dot{CO}_2 \) ventilatory responsiveness.

Although we expect that exercise training induces a significant increase in \( \dot{Q} \) at peak exercise, the time course of \( \dot{Q} \) response to submaximal absolute work rates in the PRE period would be similar to that in the POST period [34]. Similarly, since the responses to \( \dot{V}E \) and \( \dot{V}CO_2 \) as a function of work rate were also unaltered between PRE and POST periods, it will not be possible to consider the alteration in \( \dot{Q} \) after exercise training for the values of the \( \dot{V}E-\dot{V}CO_2 \) slope.

In summary, a strong relationship between \( \dot{V}E \) and \( \dot{V}CO_2 \) responses during arm exercise was recognized; the regression line during arm exercise was the same as it was during leg exercise. Even though an improvement in physiological state by exercise training was apparent in lower cardiovascular (i.e., HR and MBP) responses during exercise, the \( \dot{V}E \) and \( \dot{V}CO_2 \) responses during submaximal exercise were not altered after exercise training. The regression line in the \( \dot{V}E-\dot{V}CO_2 \) relationship during exercise did not change between PRE and POST periods. Therefore, the \( \dot{V}E-\dot{V}CO_2 \) relation during exercise was little influenced by exercising different limb regions and by the physiological training states.

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