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ORIGINAL PAPER

Effect of ramp bicycle exercise on exhaled carbon monoxide in humans

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Abstract The effect of exercise on the increase of exhaled CO in smokers compared to non-smokers has not been clarified yet. In this study we compared the dynamics of exhaled CO before, during and after exercise between smokers and non-smokers. A group of 8 smokers and a group of 8 non-smokers underwent a bicycle exercise in a ramp fashion to near maximum intensity. Ventilation and gas exchange, and CO exhalation were continuously measured every 30-s before, during and after the exercise. The fraction of CO (F_{CO}) in the exhaled air decreased gradually, but the total amount of exhaled CO ($\dot{V}_{CO}$) increased in a linear manner during the ramp exercise, and F_{CO} and $\dot{V}_{CO}$ returned to the pre-exercise level within several minutes after exercise in all subjects. A linear relationship was observed between $\dot{V}_{O_2}$ and $\dot{V}_{CO}$, and between $\dot{V}_E$ and $\dot{V}_{CO}$ in both the whole period of measurement and during the ramp exercise period in all subjects. However, the $\dot{V}_{CO}$ at 0 W, the peak $\dot{V}_{CO}$ and the slope coefficients in the regression equation between $\dot{V}_{CO}$ and $\dot{V}_{O_2}$, and between $\dot{V}_{CO}$ and $\dot{V}_E$ in the ramp exercise as well as the entire periods of measurement were significantly higher in smokers compared with those in non-smokers, and these were correlated with the number of cigarettes smoked per day. It was concluded that CO exhalation increases linearly with the increase of $\dot{V}_{O_2}$ and $\dot{V}_E$ during exercise, and habitual smoking shifts these relationships upward depending on the number of cigarettes smoked daily.

Keywords Exhaled CO · Ramp exercise · Smoking · Oxygen uptake · Minute ventilation

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Introduction

It has been demonstrated that carbon monoxide (CO) is produced endogenously from various types of cells and has many pathophysiological roles, including neurotransmission, vascular regulation, anti-inflammatory, anti-proliferative, and anti-apoptotic responses, similar to those of other gaseous transmitters such as nitric oxide and hydrogen sulfide [1–3]. It has also been shown that the concentration of exhaled CO is affected by normal and the pathophysiological conditions, including airway and lung inflammation [4–7], cigarette smoking [8–11], exercise [5, 12] and aging [13].

However, little has been published about the effect of exercise on exhaled CO. Horváth et al. [5] reported that the fraction of CO in the exhaled air (F_{CO}) decreased slightly, but the total amount of exhaled CO ($\dot{V}_{CO}$) increased significantly during 60 W and peak bicycle exercises in eight young healthy subjects. They also showed that $\dot{V}_{CO}$ returned to baseline 5 min after exercise. If the increase of CO exhalation during exercise is a reflection of CO production from the active tissues, including muscle, vasculature and the heart, measurements of exhaled CO could be a useful tool in understanding the regulatory and protective function of CO during exercise. However, little has been

published concerning the exhaled CO during exercise and recovery.

It is well known that cigarette smoke can increase exhaled CO concentrations and carboxyhemoglobin (COHb) in the blood [8–11]. In an attempt to assess the effect of cigarette smoking, this paper aimed at analyzing the dynamic changes in exhaled CO against exercise workload in smokers by comparing them with those of non-smokers. Specifically we measured the dynamic response of exhaled CO against exercise workload, minute ventilation and oxygen uptake in healthy non-smoking subjects using a metabolic measurement system and ramp exercise protocol, which have been used previously to evaluate the dynamic response of cardiorespiratory indices in a wide range of exercise workloads. Subsequently we measured the dynamic response in smokers by using the same measurement system and protocol.

Materials and methods

Subjects

Sixteen healthy males (15 students and 1 university staff member), 8 of whom were cigarette smokers, volunteered for this study. The physical characteristics and smoking history of the subjects are shown in Tables 1 and 2. They were all in good health and were not treated with any medications relating to cardiovascular, metabolic or inflammatory diseases. Written informed consent was obtained after full explanation of the risk and scientific significance of the study. This study was approved by the ethics committee for human studies of Toyohashi University of Technology.

Protocols

Subjects exercised on an electrically braked bicycle ergometer, which was designed in our laboratory [14]. The experiments were carried out in a ramp manner to near maximum intensity followed by a rest in a seated position for 2 min and unloaded cycling for 3 min. The ramp exercise terminated when the heart rate of the subject exceeded 170 beats/min (end point of ramp exercise). The rate of increase of workload was set at 15 W/min. Ventilation and gas exchange variables were measured every 30 s using the mixing chamber method reported in [15]. The metabolic measurement system consists of a face-mask, a pneumotachograph, a mass-spectrometer, a personal computer and a chamber with a total volume of 4 l. Minute ventilation ($\dot{V}_E$), oxygen uptake ($\dot{V}_{CO}$), carbon dioxide output and fractions of oxygen, carbon dioxide and nitrogen were calculated online every 30 s. All respiratory

Table 1 Physical characteristics and experimental results of 8 non-smokers

Subjects	Age (years)	Height (cm)	Weight (kg)	Ex-duration (min′s″)	$\dot{V}_{CO}$(0W) (μl/min)	Peak $\dot{V}_{CO}$ (μl/min)	Correlation (r) ($T\dot{V}_{O_2} - T\dot{V}_{CO}$)	Correlation (r) ($T\dot{V}_E - T\dot{V}_{CO}$)	Slope coeff. ($E\dot{V}_{O_2} - E\dot{V}_{CO}$)	Slope coeff. ($E\dot{V}_E - E\dot{V}_{CO}$)
N1	46	177	80	13′00″	13.3	38.4	0.88	0.95	0.013	0.420
N2	22	166	55	10′30″	16.1	38.6	0.93	0.96	0.019	0.399
N3	21	173	70	11′00″	12.1	34.9	0.88	0.96	0.017	0.545
N4	21	174	60	12′30″	18.1	26.7	0.79	0.92	0.006	0.317
N5	23	168	59	14′30″	13.7	28.9	0.92	0.94	0.010	0.305
N6	21	170	58	15′30″	12.4	35.2	0.95	0.90	0.008	0.193
N7	22	184	61	14′30″	14.5	37.7	0.93	0.97	0.013	0.546
N8	22	174	73	13′00″	10.3	26.0	0.94	0.96	0.010	0.298
Mean (±SD)	24.8 (8.6)	173.3 (5.6)	64.5 (8.8)	13′04″ (1′42″)	13.8 (2.4)	33.3 (5.3)	0.90 (0.05)	0.95 (0.02)	0.012 (0.004)	0.378 (0.124)

"Ex-duration" indicates the duration of ramp exercise. "Correlation (r)" indicates the correlation coefficient between $\dot{V}_{O_2}$ and $\dot{V}_{CO}$, and between $\dot{V}_E$ and $\dot{V}_{CO}$ in the whole period of measurements from rest to recovery as titled $T\dot{V}_{O_2} - T\dot{V}_{CO}$ and $T\dot{V}_E - T\dot{V}_{CO}$. "Slope coeff" indicates the slope coefficient in the regression equation between $\dot{V}_{O_2}$ and $\dot{V}_{CO}$, and between $\dot{V}_E$ and $\dot{V}_{CO}$ for the data set obtained during ramp exercise period as shown $E\dot{V}_{O_2} - E\dot{V}_{CO}$ and $E\dot{V}_E - E\dot{V}_{CO}$

Table 2 Physical characteristics, smoking history and experimental results on 8 smokers

Subjects	Age (years)	Height (cm)	Weight (kg)	No. of Cig./day	Smoking years (years)	Ex-duration (min′sec″)	$\dot{V}_{CO}$(0W) (μl/min)	Peak $\dot{V}_{CO}$ (μl/min)	Correlation (r) ($T\dot{V}_{O_2} - T\dot{V}_{CO}$)	Correlation (r) ($T\dot{V}_E - T\dot{V}_{CO}$)	Slope Coeff. ($E\dot{V}_{O_2} - E\dot{V}_{CO}$)	Slope Coeff. ($E\dot{V}_E - E\dot{V}_{CO}$)
S1-1	21	180	59	20	6	12′00″	76.7	227.6	0.93	0.96	0.091	1.909
S1-2						(11′00″)	(116.1)	(353.0)	(0.95)	(0.96)	(0.180)	(4.526)
S2-1	20	171	72	15	5	9′30″	59.4	134.8	0.86	0.96	0.062	2.379
S2-2						(10′00″)	(191.2)	(313.4)	(0.81)	(0.93)	(0.095)	(4.602)
S3	21	169	60	15	7	11′30″	12.5	37.1	0.88	0.93	0.016	0.300
S4	21	173	62	5	1	11′00″	22.9	70.8	0.96	0.98	0.036	1.212
S5	22	176	80	20	7	12′30″	159.8	298.1	0.93	0.94	0.072	3.137
S6	19	175	65	10	6	9′30″	57.8	131.0	0.97	0.97	0.063	2.376
S7	20	182	60	20	1	13′30″	99.9	153.2	0.73	0.90	0.023	1.234
S8	20	175	61	30	7	9′00″	153.2	262.8	0.87	0.93	0.095	5.375
Mean (±SD)	20.5 (0.5)	175.1 (4.3)	64.9 (7.4)	17 (7.5)	5 (2.6)	11′04″ (1′36″)	80.3 (54.6)	164.4 (91.5)	0.89 (0.08)	0.94 (0.03)	0.057 (0.030)	2.240 (1.541)
G-Diff.	$p < 0.05$	N.S.	N.S.			$p < 0.05$	$p < 0.01$	$p < 0.01$	N.S.	N.S.	$p < 0.01$	$p < 0.01$

"No. of Cig." and "Smoking years" mean the average number of cigarettes smoked per day and the length of habitual smoking in each subject. Other parameters are the same as in Table 1. Two subjects (S-1 and S-2) repeated the same measurement on different days, and the second data (-2) were shown below the first measurement (-1). The second data were excluded from the data calculation. G-Diff" indicates the statistical significance of the difference in the group mean values between smokers and non-smokers

variables were stored on a magnetic disk and simultaneously printed out on paper every 30 s at the end of the experiment. The concentration of CO in the exhaled breath drawn from the mixing chamber was analyzed using the CO analyzer (mBA-2000, TAIYO, Japan), which provided CO concentrations every 1 s, with a resolution of 0.1 ppm. The delay time of the measurements between the flow and gas signals analyzed by the mass spectrometer and the CO analyzer was 5 and 10 s, respectively. Two-point calibration was performed using ambient air and a standard gas, mixed with O_2, CO_2 and N_2 for the mass-spectrometer, and a CO absorber and standard gas, mixed with CO, O_2, CO_2 and N_2 for the CO analyzer, respectively. Heart rate was monitored simultaneously with an ECG monitor (AC-611G, Nihonkohden, Japan), using a standard chest lead throughout the measurement, to determine the end point of ramp exercise. The experimental room was controlled to maintain the temperature and air ventilation, and the expired air from the subjects was pulled out of the room. F_ICOs measured as the CO concentration in the room ranged from 0.32 to 0.54 ppm throughout the experiments.

All experiments were undertaken from 1:30 p.m. to 5:30 p.m. The subjects had lunch or smoked a cigarette at least 2 h before the experiment. Two smokers (S1 and S2) repeated the same measurement on different days, in which the subjects smoked just before the experiment.

Data processing and analysis

The fraction of CO in the exhaled air (F_ECO) was averaged every 30 s to estimate the mean F_ECO, taking into account the time delay. The amount of carbon monoxide exhaled per minute ($\dot{V}_{CO}$) was estimated as follows:

$$\dot{V}_{CO(STPD)} = \dot{V}_{E(STPD)} \times (F_{ECO} - F_{ICO}) \quad (1)$$

where F_{ECO} and F_{ICO} represent the CO concentrations in the expired and inspired gases, respectively. In this study, the value of ($F_{ECO} - F_{ICO}$) was defined as the net fraction of CO and was indicated as ΔF_{CO}. Thus, the former equation could be rewritten as follows:

$$\dot{V}_{CO(STPD)} = \dot{V}_{E(STPD)} \times \Delta F_{CO}. \quad (2)$$

Results obtained from both groups are expressed as mean ± SD. Linear regression and Pearson's correlation analyses were used to elucidate the linear relationship between $\dot{V}_{O_2}$ and $\dot{V}_{CO}$, and between $\dot{V}_{E(BTPS)}$ and $\dot{V}_{CO}$ in both the whole period of measurement and the ramp exercise period. Mann–Whitney U test was applied to examine the significance of the difference between the groups, and statistical significance was accepted at $p < 0.05$.

Results

There was no statistical difference in the subjects' height and weight between groups, but the age was higher in non-smokers. As seen in the 5th column of Table 1 and in the 7th column of Table 2, the exercise duration from the beginning to the end point of ramp exercise was significantly ($p < 0.05$) longer in non-smokers. $\dot{V}_{CO}$ at 0 W ($\dot{V}_{CO}(0\,W)$) and Peak $\dot{V}_{CO}$ were significantly ($p < 0.01$) higher in smokers compared with those in non-smokers. A positive correlation was found between the numbers of cigarette smoked per day and $\dot{V}_{CO}(0\,W)$ ($r = 0.78$), and peak $\dot{V}_{CO}$ ($r = 0.73$) (Tables 1, 2). Also, positive correlations were seen between the Brinkman Index and $\dot{V}_{CO}(0\,W)$, and Peak $\dot{V}_{CO}$ ($r = 0.63$ and 0.67, respectively).

Typical examples of the ΔF_{CO}, $\dot{V}_{CO}$, $\dot{V}_{O_2}$, and $\dot{V}_{E(BTPS)}(\dot{V}_E)$ against time, and the relationships between $\dot{V}_{CO}$ and $\dot{V}_{CO}$, and between $\dot{V}_E$ and $\dot{V}_{CO}$ are shown in Figs. 1 (a non-smoker) and 2 (a smoker). These figures showed that the ΔF_{CO} decreased gradually during the ramp exercise but rapidly returned to pre-exercise levels after the cessation of exercise. $\dot{V}_{CO}$, $\dot{V}_{O_2}$ and $\dot{V}_E$ appeared to increase linearly or curvilinearly during the ramp exercise period. As a consequence, high and significant correlations were found between $\dot{V}_{O_2}$ and $\dot{V}_{CO}$, and between $\dot{V}_E$ and $\dot{V}_{CO}$ in the whole data set throughout rest, exercise and recovery in all subjects (Tables 1, 2). But a small dissociation of the linearity in the relationship between $\dot{V}_{O_2}$ and $\dot{V}_{CO}$ was also observed, especially in the recovery phase in both subjects. This tendency was also observed in many other subjects. Furthermore, some differences were also observed between smokers and non-smokers. Note that the absolute values of F_{CO} and $\dot{V}_{CO}$ were higher in smokers, and the slope coefficients of the regression equation between $\dot{V}_{O_2}$ and $\dot{V}_{CO}$,

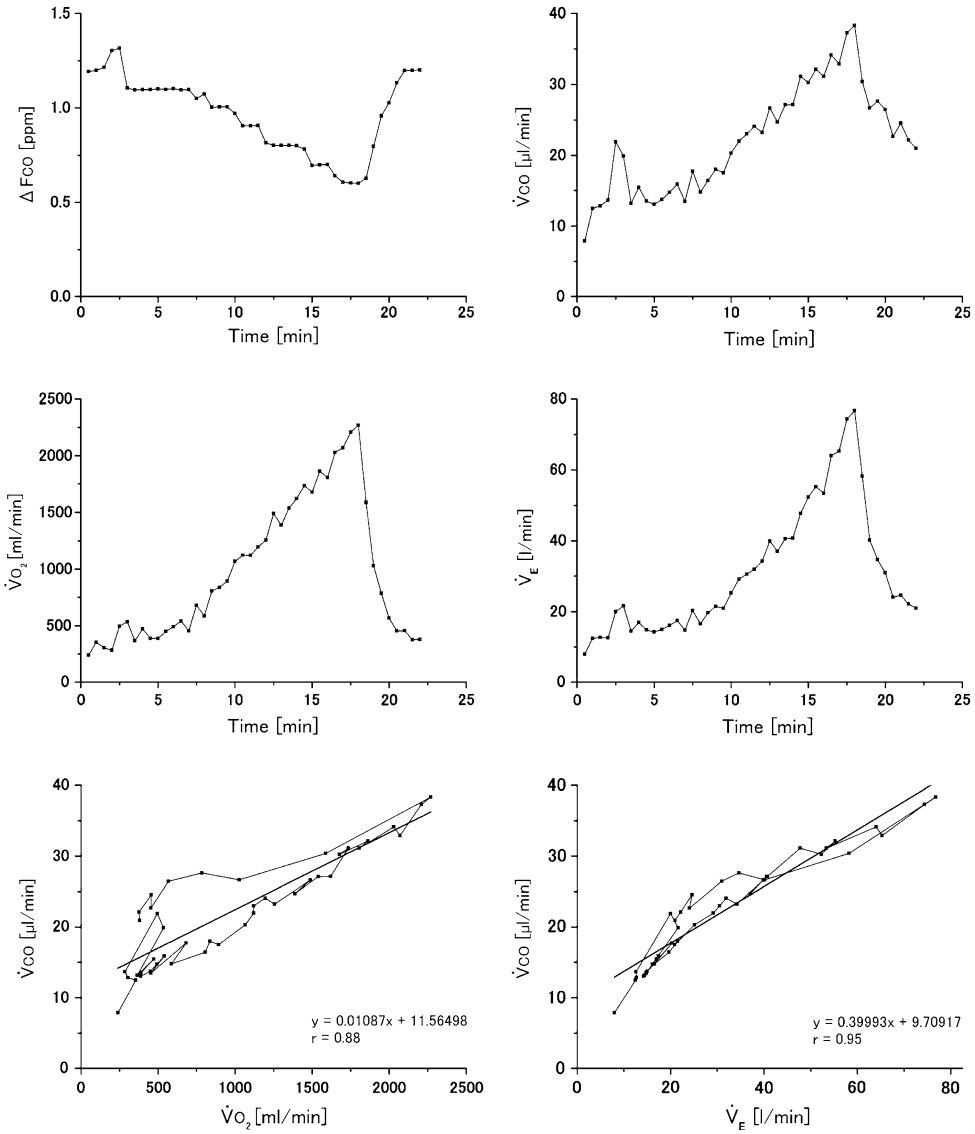

Fig. 1 Changes in ΔF_{CO}, $\dot{V}_{CO}$, $\dot{V}_{O_2}$, $\dot{V}_E$ against time and the relationship between $\dot{V}_{O_2}$ and $\dot{V}_{CO}$, and between $\dot{V}_E$ and $\dot{V}_{CO}$ in one non-smoker (N-1). Note that ΔF_{CO} gradually decreased, but $\dot{V}_{CO}$ increased linearly during ramp exercise, similar to the changes of $\dot{V}_{O_2}$ and $\dot{V}_E$. A significant correlation was observed between $\dot{V}_{O_2}$ and $\dot{V}_{CO}$, and between $\dot{V}_E$ and $\dot{V}_{CO}$ (*lower panel*)

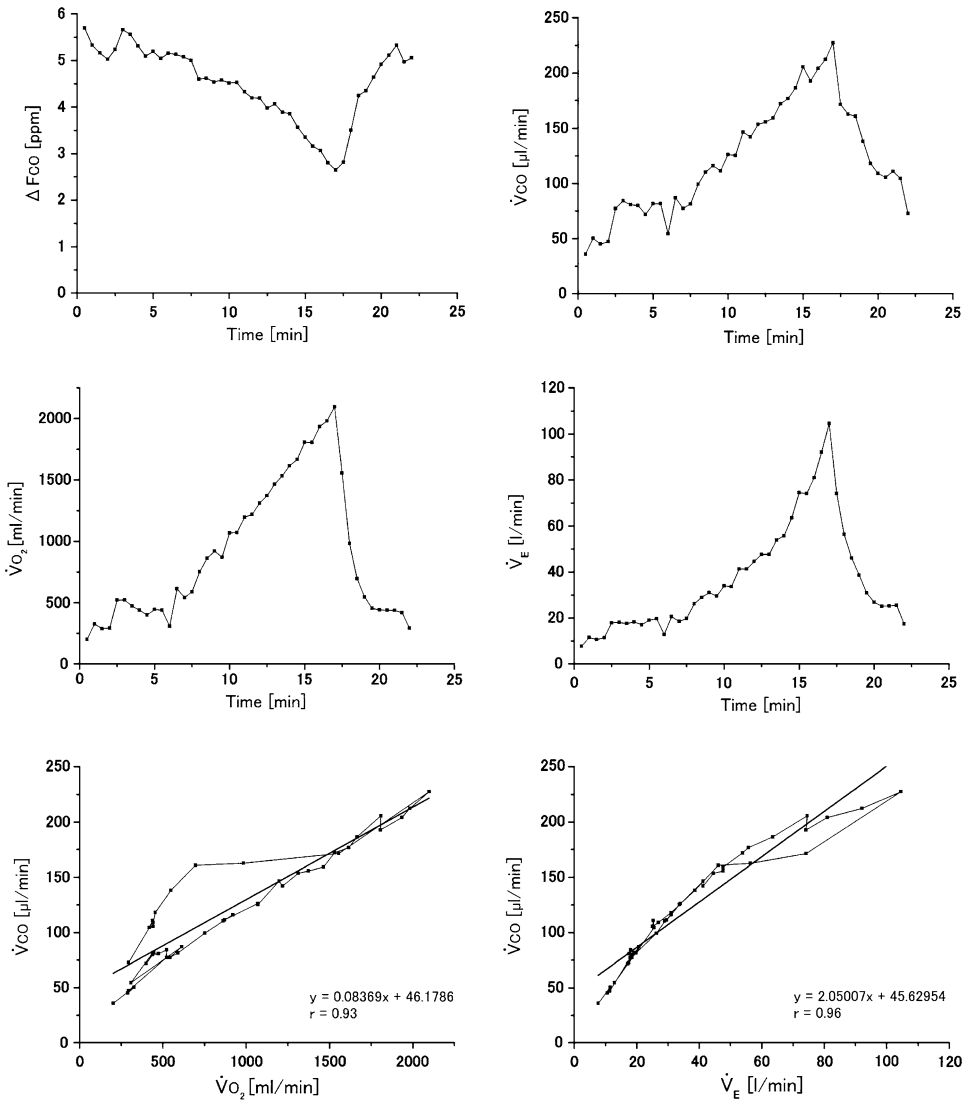

Fig. 2 Changes in ΔF_{CO}, $\dot{V}_{CO}$, $\dot{V}_{O_2}$, $\dot{V}_E$ against time and the relationship between $\dot{V}_{O_2}$ and $\dot{V}_{CO}$, and between $\dot{V}_E$ and $\dot{V}_{CO}$ in one smoker (S-1). Note that ΔF_{CO}, $\dot{V}_{CO}$, and the slope coefficients and intercepts of the regression equation between $\dot{V}_{O_2}$ and $\dot{V}_{CO}$, and between $\dot{V}_E$ and $\dot{V}_{CO}$ are higher compared with those in non-smokers

and between $\dot{V}_E$ and $\dot{V}_{CO}$ were significantly ($p < 0.01$) higher in smokers compared with those of non-smokers (the slope coefficients: 0.011 ± 0.003 vs. 0.073 ± 0.047 in the $\dot{V}_{O_2}$ and $\dot{V}_{CO}$ relationship; 0.385 ± 0.016 vs. 2.872 ± 1.860 in the $\dot{V}_E$ and $\dot{V}_{CO}$ relationship).

The rates of increase in $\dot{V}_{CO}$ against $\dot{V}_{O_2}$ and $\dot{V}_E$ during the ramp exercise period are shown in Figs. 3 (non-smokers) and 4 (smokers), respectively. It would appear that the relationship between $\dot{V}_E$ and $\dot{V}_{CO}$ was curvilinear rather than linear, but the relationship between $\dot{V}_{O_2}$ and $\dot{V}_{CO}$ was linear in both groups. Moreover, it was found that the inter-individual difference in $\dot{V}_{CO}$ against $\dot{V}_{O_2}$ and $\dot{V}_E$ was much larger in smokers. The mean values of $\dot{V}_{CO}$ at 0 W exercise ($\dot{V}_{CO}(0\ W)$) and at the end of ramp exercise (Peak $\dot{V}_{CO}$) in non-smokers were 13.8 ± 2.5 and 33.5 ± 5.3 μl/min, respectively, and these were significantly ($p < 0.01$) lower than those of smokers (80.2 ± 54.8 and 166.4 ± 91.5 μl/min). The slope coefficients of the regression equation between $\dot{V}_{O_2}$ and $\dot{V}_{CO}$, and between $\dot{V}_E$ and $\dot{V}_{CO}$ in smokers were significantly ($p < 0.01$) greater than those of non-smokers (Tables 1, 2).

Figure 5 shows the effect of smoking just prior to the experiment on exhaled CO in two smokers (S1 and S2). It was clearly demonstrated that a cigarette smoked immediately before the trial remarkably enhanced $\dot{V}_{CO}$ against $\dot{V}_{O_2}$ and $\dot{V}_E$ compared with the first trial.

Discussion

In this study we evaluated the dynamic response of the exhaled CO to exercise in relation to $\dot{V}_{O_2}$ and $\dot{V}_E$ and compared these relationships between smokers and non-smokers. Results showed that that F_{CO} decreased gradually, but $\dot{V}_{CO}$ increased in a linear fashion during a ramp

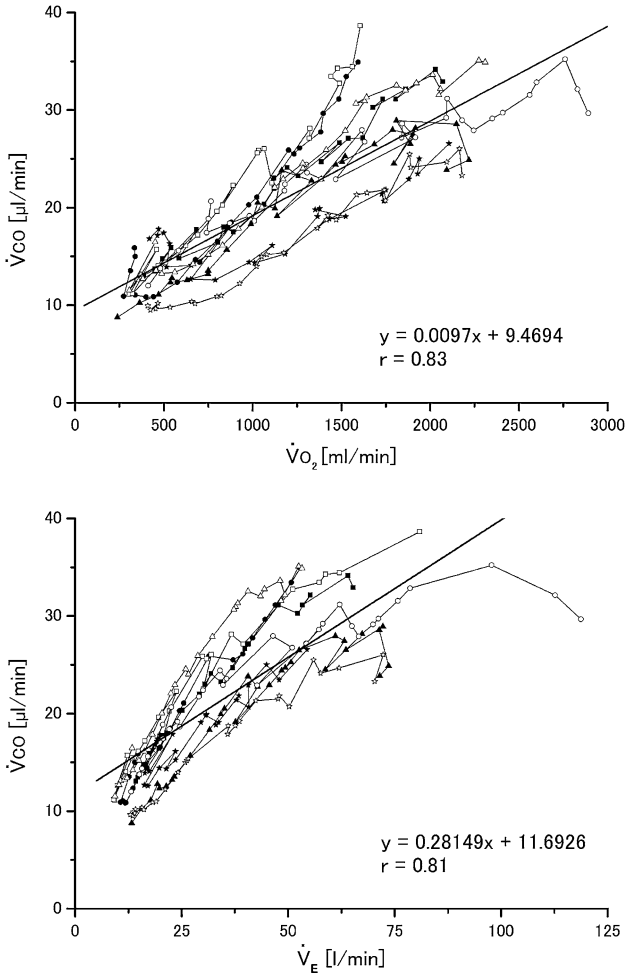

Fig. 3 Relationship between $\dot{V}_{O_2}$ and $\dot{V}_{CO}$ (*upper panel*) and between $\dot{V}_E$ and $\dot{V}_{CO}$ (*lower panel*) obtained during the ramp exercise period on 8 non-smokers. Note that the relationship between $\dot{V}_{O_2}$ and $\dot{V}_{CO}$ appears to be linear, but the relationship between $\dot{V}_E$ and $\dot{V}_{CO}$ appears to be curvilinear. The pattern of the changes among subjects was very similar

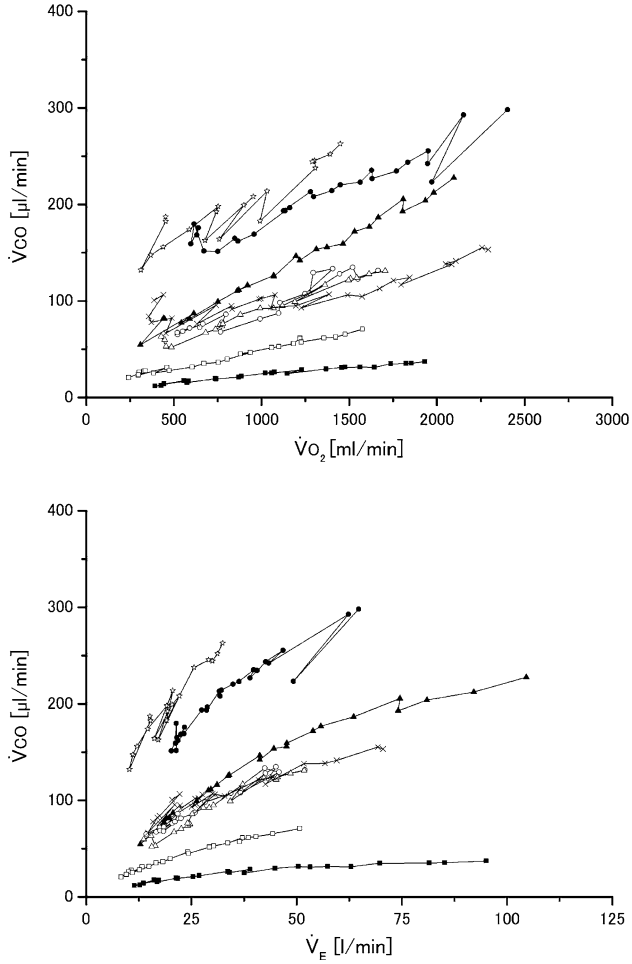

Fig. 4 Relationship between $\dot{V}_{O_2}$ and $\dot{V}_{CO}$ (*upper panel*) and between $\dot{V}_E$ and $\dot{V}_{CO}$ (*lower panel*) obtained during ramp exercise period on 8 smokers. Note that high correlations were observed between $\dot{V}_{O_2}$ and $\dot{V}_{CO}$ ($r = 0.89$–0.99), and between $\dot{V}_E$ and $\dot{V}_{CO}$ ($r = 0.98$–0.99), respectively. Also note that the scale of $\dot{V}_{CO}$ was ten times larger than that of in Fig. 3

exercise, and that after the cessation of exercise, F_{CO} and $\dot{V}_{CO}$ returned to the pre-exercise level within several minutes, concomitant with the changes of $\dot{V}_{O_2}$ and $\dot{V}_E$. Furthermore, a linear relationship was detected between $\dot{V}_{O_2}$ and $\dot{V}_{CO}$, and between $\dot{V}_E$ and $\dot{V}_{CO}$, not only throughout the whole period of measurement, from rest to exercise and recovery, but also during the ramp exercise period in both smokers and non-smokers. Values of F_{CO} and $\dot{V}_{CO}$, and the rates of increase of $\dot{V}_{CO}$ against $\dot{V}_{O_2}$ and $\dot{V}_E$, not only during ramp exercise, but also for the whole period of measurement from rest, exercise and recovery, were significantly higher in smokers compared with those in non-smokers.

Horváth et al. [5] observed that VCOs at rest, at 60 W and at the peak of intensity exercise were 27.4, 72.1 and 98.2 µl/min, respectively, and returned to baseline 5 min after the cessation of exercise in 8 healthy young subjects with a mean age of 14.2 ± 1.2 years. They also noted that the F_{CO} in the exhaled air decreased gradually depending on the exercise intensity. The present results were generally consistent with those observations in which F_{CO} decreased and $\dot{V}_{CO}$ increased depending on the intensity of exercise; however, $\dot{V}_{COs}$ in this study were considerably lower than those reported by Horvath et al. [5]. The reason for the discrepancy in $\dot{V}_{CO}$ between Horvath et al. [5] and the present findings in non-smokers cannot be explained here. Differences could be due to the subjects used and/or experimental protocols. Additionally, a previous study evaluated the F_{CO} and/or $\dot{V}_{CO}$ under only a few constant-load exercises [5]. In the present study, F_{CO}, $\dot{V}_{CO}$, $\dot{V}_{O_2}$ and $\dot{V}_E$ were measured continuously at rest, during ramp exercise to near maximal intensity and recovery every 30 s

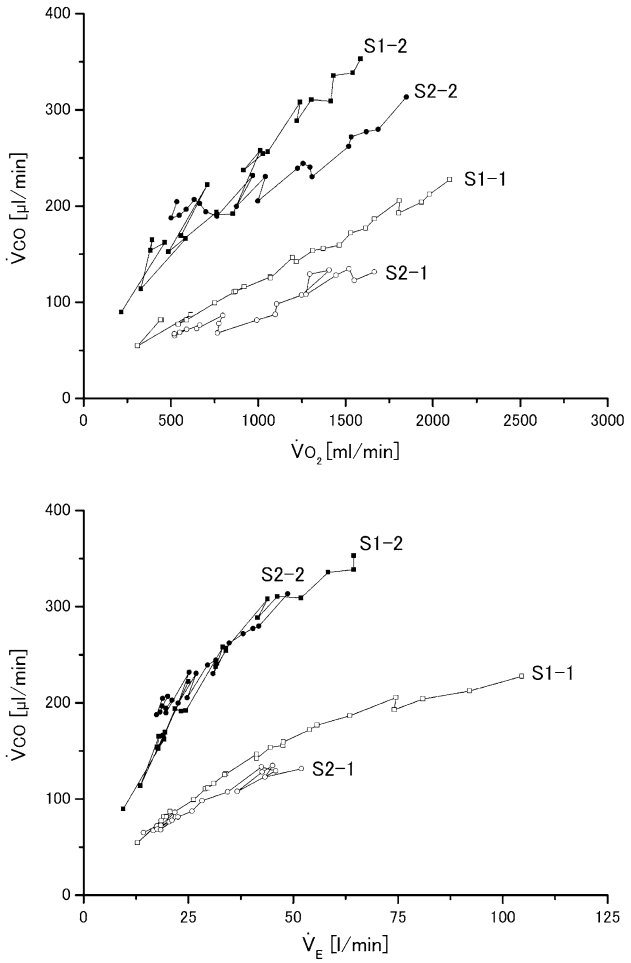

Fig. 5 The effect of cessation time of smoking on $\dot{V}_{CO}$ in two subjects. In the first measurement, the exercise testing started at least 2 h from the last smoking (S1-1 and S2-1). However, in the second measurement on different days, the exercise started immediately after smoking (S1-2 and S2-2), respectively. Note that $\dot{V}_{CO}$ increased remarkably compared with the first measurement

using a mixing chamber method. The method used in the present study could provide information about the dynamic responses of F_{CO} and $\dot{V}_{CO}$ to a wide range of exercise and the recovery period accompanied with other respiratory variables. A linear relationship was observed between the exercise intensity and F_{CO} or $\dot{V}_{CO}$, between $\dot{V}_{CO}$ and $\dot{V}_{O_2}$, and between $\dot{V}_E$ and $\dot{V}_{CO}$ in both groups, smokers and non-smokers, in a wide range of exercises from unloaded to near maximal. However, this does not necessarily indicate that CO production in the body increases with the increase of exercise intensity or oxygen utilization, because the origin and the roles of the exhaled CO remain uncertain [1, 7, 16, 17].

Coburn [18] reported that the total amount of CO stored in the human body could be estimated as 10 ml and that more than 80% of CO in the body appeared in the blood, bound chemically to hemoglobin. In the present study the total amount of $\dot{V}_{CO}$ during the ramp exercise period for 13 min in one non-smoker (N-1), shown in Fig. 1, can be roughly estimated as 320 μl. This may correspond to only 4% of the CO stored in the blood and may reduce the COHb only from 1 to 0.96% [18]. By contrast, $\dot{V}_{CO}$ during ramp exercise in one smoker (S-1), shown in Fig. 2, can be roughly estimated as 1800 μl, which is more than 5 times larger than that of the non-smoker (N-1) shown in Fig. 1. It has been reported that the CO concentration in the exhaled air and COHb in the blood were higher in smokers compared with nonsmokers [8–11]. In other words, smokers have a high capacity to provide CO in exhaled air compared with non-smokers. However it is still unclear whether blood CO content can be provided into the exhaled gas. Vogel and Gleser [19] had reported that the COHb concentration (%) gradually decreased according to the exercise intensity from 1.7% at rest to 1.0% at maximal exercise. This suggests indirectly that COHb may be the major source of the exhaled CO. A higher exhalation of CO during exercise in smokers and an enhancement of CO output just after smoking, compared with the long-term cessation of smoking as shown in Figs. 4 and 5, indicate that blood CO content may contribute to CO exhalation, especially during exercise.

However, other possibilities regarding the origin of exhaled CO during exercise cannot be excluded. Some studies suggest that the gene expression of heme oxygenase-1 (HO-1), which is an inducible isoform of CO synthase in the active muscles, is derived from long-term exercise [20, 21]. Steensberg et al. [20] demonstrated enhancement of IL-6 and HO-1 mRNA expression after knee extensor exercise for 2 h in humans. Essig et al. [21] also reported that in rat limb muscle, HO-1 mRNA increased 7-fold after 1 h of exhaustive running. Although they did not measure CO production directly, these results suggest that CO can be produced in the active muscles during exercise. Doubt still remains about whether these gene expressions occur simultaneously with exercise and whether the CO produced in the active muscle rapidly diffuses to the blood.

However, a contribution of the upper and lower airways to the exhaled CO has also been suggested. Many studies have reported the effect of airway and lung injuries on F_{CO}, including asthma, chronic obstructive pulmonary disease, bronchiectasis, cystic fibrosis and viral infection of the airway [4–7]. These studies, using a single breath hold technique to measure F_{CO}, indicated that inflammation of the lower airway can enhance CO production in the epithelium, endothelium and vascular smooth muscles through an increase in the enzymatic activity of HO-1. Andersson et al. [16] also reported that the nasal and paranasal sinuses

contributed to the production of CO in human airways, and suggested that the epithelial cells in the nasal and paranasal airways are the major source of CO in the upper airway. In the present study, subjects breathed freely with a face mask, so the nasal and oral airways might have contributed to the exhaled CO. In addition, recent studies suggest that cigarette smoke enhances HO-1 expression in the cells located in the airway and the lungs [22, 23]. These observations confirm that the upper and lower airways are the sources of exhaled CO. However, it is still unknown whether the CO production in these sites is enhanced by a short-term exercise like in the present study. Further studies will be required to resolve these problems.

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