Simultaneous multiple-subject analysis of respiratory gas exchange in humans

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Abstract Respiratory gas collection and analysis with indirect calorimetry allows assessment of energy and substrate use during rest and exercise and aerobic capacity. We have developed a novel system for simultaneous multiple-subject measurement of respiratory gas exchange during rest and exercise for up to five subjects, and validated the system with a series of experiments. Thirteen healthy young men (mean age 22 ± 2 years, height 172 ± 6 cm, weight 67.1 ± 11.6 kg) participated in this study. Memory problems of the mass spectrometer, which are caused by switching analyzed gases of different compositions between channels, was sufficiently eliminated with a washout of 2 s (seconds) with errors of less than 0.3% for O₂ and less than 0.4% for CO₂. The high-response mass spectrometer and specially designed mixing chamber enabled correct estimation of metabolic measurements, even for gas concentration data from a non-continuous flow of any given 1 s of every 10-s interval (spot-sampling method). Diet-induced thermogenesis (DIT) and oxygen consumption ($\dot{V}O_2$) during maximal incremental exercise were not significantly different between the novel and Douglas bag methods ($P = 0.61$ and $P = 0.56$, respectively). The limit of agreement of DIT and $\dot{V}O_2$ during maximal incremental exercise between the two methods were $2.8 \pm 43.6\%$ and $0.9 \pm 4.8\%$, respectively. We concluded that the newly developed system has reasonable validity over a wide range.

Keywords : indirect calorimetry, maximal oxygen consumption, resting metabolic rate, Douglas bag method, mixing chamber method, diet-induced thermogenesis

Introduction

Respiratory gas collection and analysis with indirect calorimetry allows us to measure the energy costs of performing a physical task. Cardiopulmonary exercise testing with respiratory gas collection and analysis can be used to develop an exercise prescription or to measure functional capacity for a given oxygen consumption level. It also has potential clinical applications for predicting energy needs for energy balance and weight management, evaluating dyspnea, risk stratification and prognosis in heart failure patients, and assessing the results of medical and surgical therapies¹.

Collection of respiratory gases during exercise or at rest has usually been performed for a single subject at a time. The primary reason for this is that the collection and anal-

ysis system itself is designed to handle only one subject at a time. Although multi-subject collection is possible using multiple systems, the duplication of hardware involves extra expense and space; and it is not always possible for a single operator to manage multiple systems without errors²). Having a single focal point for displaying the data and controlling each subject substantially improves the likelihood that problems can be identified in a timely fashion and dealt with correctly.

Thus, we have developed a novel system designed to collect respiratory gases during exercise and at rest from five subjects simultaneously. Because some procedures require significant time, this capability can result in increased testing throughput. The recording and measurement duration of an ergometric exercise load can be individually or simultaneously controlled for each channel.

Our system shows the time series plots from all channels during collection on a single display, with the user

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having to manipulate only one keyboard and mouse. In addition to saving space, we believe this arrangement reduces the chance of making errors during data collection. To validate the new system for simultaneous multiple-subject analysis of respiratory gas exchange in humans, we compared respiratory parameters of the multichannel mixing chamber (MCMC) method with the gold-standard Douglas bag (DB) method.

The aim of the study was to validate the new system for simultaneous multiple-subject analysis of respiratory gas exchange in humans.

Materials and methods

To accomplish the aims of the study, the investigation was divided into four experiments. Experiment 1 was undertaken to examine the magnitude of memory problems that occur when analyzing gases of different compositions using a mass spectrometer. Experiment 2 determined the minimum interval, as non-continuous gas concentration data, required for a representative value of gas concentration for calculation of the metabolic index. Experiment 3 was conducted to validate the novel system with DB method concerning simultaneous metabolic measurement of multiple subjects at rest and after dietary intake. The goal of Experiment 4 was to validate metabolic measurements during exercise.

Subjects

For Experiment 3, seven healthy young men underwent tests to determine resting metabolic rate (RMR) and diet-induced thermogenesis (DIT). The subjects had a mean $\pm$ SD age of 22 ± 1 year, height of 171 ± 7 cm, and weight of 66.0 ± 13.6 kg. In Experiment 4, six healthy young men (age: 23 ± 3 years, height: 174 ± 5 cm, weight: 68.4 ± 9.9 kg) were subjected to maximal exercise testing. The subjects exercised regularly at recreational levels. All of the participants provided written informed consent before the testing. The Ethics Committee of Fukuoka University approved this investigation.

Multichannel mixing chamber method

The system providing multiple-subject analysis of respiratory gas exchange can perform measurements on up to five subjects simultaneously with one source of inspired air. The newly developed system includes a fast-response mass spectrometer (ARCO-2000 MET; Arcosystem, Kashiwa, Japan), one switching system for five channels (ARCO-2000 SYSTEM-5L; Arcosystem, Kashiwa, Japan), and five mixing chambers with five pneumotachographs, all of which are controlled by a personal computer (Fig. 1). The use of this system is called the multichannel mixing chamber (MCMC) method.

For the experiment, each mixing chamber had indepen-

dent flow sensors to measure the expired gas volume. The systems were composed of a mouthpiece or mask, a corrugated tube 1.5 m in length, a pneumotachograph (0–200 l/min), and a 3-liter mixing chamber for each subject. The pneumotachographs were calibrated beforehand as follows: Each was connected by the corrugated tube to a 3-liter syringe, with five different flow rates (0.1–10 l/s) for each three strokes and no flow rate (zero). This calibration was important because in pneumotachographs, there is usually a nonlinear relationship³⁾ between the electrical signal and the flow rate. The mass spectrography apparatus took about a 1-ml sample of gas per second from the multi-sampler. The sample gas of each channel was alternately supplied to the mass spectrometer by electrically controlled solenoid valves. The mass spectrometer was calibrated using two gases: fresh room air (gas 1, defined as O₂ 20.93% and CO₂ 0.04%) and a calibration gas (gas 2, defined as O₂ 15.00%, CO₂ 4.55% and N₂ balanced). The software is able to calculate the expired minute volume ($\dot{V}_E$), oxygen consumption ($\dot{V}O_2$), carbon dioxide production ($\dot{V}CO_2$), respiratory exchange ratio (RER), and total energy expenditure (TEE) values⁴⁾.

Douglas bag method

The DB method has served as the gold standard for gas exchange measurements for more than a century^{5,6)}. Details of the DB method, as used in our laboratory, are described elsewhere^{7,8)}.

For the current study, the DBs were connected to the mixing chambers of the new system. Expired gas was collected in the DB during exercise for each minute (or for more than 1 min in the case of a low-volume sample). After gas collection, the O₂ and CO₂ gas concentrations were analyzed using the ARCO-2000 MET mass spectrometer. $\dot{V}_E$ was measured using a two-barrel, drum-type respirometer (CR-20; Fukuda Irika, Tokyo, Japan) that had been calibrated using a 3-liter syringe before testing. Just before collection in the DBs, a small sample of respiratory gas in the mixing chamber was taken to determine gas consumption for MCMC. $\dot{V}_E$ determined by the DB method was corrected for the size of the gas sample.

Experimental protocols

Experiment 1. Memory problems are a phenomenon in which a small portion of the previous sample remains in the analyzer of the mass spectrometer, which affects the measurement results of the next sample⁹⁾. The experiment included eight cycles, alternating between gas 1 and gas 2 on different channels. Each cycle lasted 10 s (5 s each for gases 1 and 2).

Experiment 2. The metabolism of one subject (age: 25 years, height: 173.7 cm, weight: 65.68 kg) was measured while he sat for 10 min, and again during the last 10 min

Fig. 1 Novel system of gas analysis. PC: personal computer, spec: spectrometer. The system can be used with up to five channels.

of maximal incremental exercise. The exercise was performed using an ergometer (Corival, Lode, Groningen, Netherlands). The workload was increased in increments of 1W every 4 s. Gas concentration was measured using the ARCO-2000 MET mass spectrometer and one mixing chamber with a pneumotachograph. This gas analyzer can measure the concentration every 0.1 s. To find the minimum interval that would represent the gas concentration at 10 s, we compared the average gas concentration for 1.0-, 2.5-, and 5.0-s intervals crossing the medium 10-s sample against the average full 10-s sample out of 10 min of continuous data, as shown in Fig. 2. $\dot{V}O_2$, $\dot{V}CO_2$, and RER calculated from the estimated gas concentrations were compared to those from the actual gas concentration to predict the percent errors of the indices from spot sampling of gas concentration.

Experiment 3. To determine RMR and DIT, subjects were brought to the laboratory by taxi between 0700 and 0730 h. The subjects were divided into groups of three and four according to their schedules. They had been instructed to avoid heavy physical exercise from the day before up to the time of the experiment. All subjects were prepared before each experiment by eating the same din-

ner the evening prior to the experiment (by 1900 h at the latest). Afterwards, they fasted and were not allowed to ingest caffeine or smoke tobacco up to the time of the experiment.

Before testing, RMR was measured for 10 min after the subject had been in a seated position for more than 30 min. Respiratory gas was collected and analyzed by the MCMC method and the DB method. Three or four subjects were simultaneously measured each day. The first 5 min of recorded data was excluded from RMR calculations. Thus, only the remaining 5 min of data was used¹⁰. At the same time, respiratory gas was collected for 5 min using the DB method. The RMR for each session was measured between 0745 and 0845 h. Each subject then had breakfast consisting of 600 kcal (15% protein, 25% fat, 60% carbohydrates) at 0900 or 0915 h. DIT was measured by having subjects put on a mask 30, 90, and 150 min following the meal. The tests were carried out in a large room (123 m³) at constant temperature ($22.8 \pm 1.3^\circ\text{C}$) and humidity ($37 \pm 11\%$).

Experiment 4. For this evaluation, subjects arrived at the laboratory after fasting at least 3 h. The test was performed using five linked mixing chambers, allowing

Fig. 2 Finding minimum interval for representative gas concentration using partially averaged continuous data for gas concentration.

measurement of five subjects simultaneously. Because of the limited number of DBs available, respiratory gas from one channel at a time was collected with DBs. During the measurement, the switching system was operated and the MCMC system continued to monitor the gases in the five mixing chambers simultaneously. An incremental exercise test was performed to a maximal level using one of three identical ergometers (Corival, Lode, Groningen, Netherlands) with each mixing chamber. The workload was increased by 1 W every 4 s to exhaustion; i.e., to the point where subjects could not continue pedaling at the given workload for 1 minute. Respiratory gas was collected in a DB five to seven times every few minutes per subject, for 1 min each time. Electrocardiography (ML-3600, Fukuda Denshi, Tokyo, Japan) using a CM-5 configuration was continuously recorded during exercise testing.

Data and statistical analysis

Data are reported as mean $\pm$ SD. For Experiment 1, memory problems at n seconds after switching from high to low gas concentration [T(n), $n = 1$ to 10 s] was calculated as follows:

$$\frac{|\text{Concentration of following gas (1 or 2)} - \text{Measured concentration at T(n)}|}{|\text{Concentration of gas 1} - \text{Concentration of gas 2}|} \times 100$$

During validation testing with MCMC and DB methods, all values were assumed to be obtained at standard temperature and pressure, dry (STPD). For Experiment 2, a one-sample t -test was used to determine overall differences among test variables at 1.0, 2.5, and 5.0 s against 10.0 s during sitting or incremental exercise. For Experiments 3 and 4, Student's paired t -test (two-tailed) was used to examine the differences between the MCMC and DB methods. When calculating DIT, if TEE after the

meal was lower than RMR, it was calculated as negative calories. Pearson's correlation coefficients were used to assess the degree of association between the two systems. Bland-Altman plots were used to show individual differences between the MCMC and DB methods¹¹⁾. This allowed calculation of the mean percent errors and the 95% limits of agreement (LOA) (mean $\pm 1.96 \times$ SD) prediction interval. The level of statistical significance was set at $P < 0.05$. PASW Statistics Version 18 software (SPSS, Inc., Chicago, IL, USA) was used for all statistical analyses.

Results

Experiment 1. The data for Experiment 1 are shown in Fig. 2. Previous gas concentration largely (~80%) affected the measurement 1 s after switching channels. Memory problems of $\dot{V}O_2$ and $\dot{V}CO_2$ decreased significantly 2 s after switching, but remained significant compared to 3 to 5 s after switching (Fig. 3).

Experiment 2. Fig. 4 shows measurement errors of $\dot{V}O_2$, $\dot{V}CO_2$, and RER determined from the average gas concentrations for durations of 10.0, 5.0, 2.5, and 1.0 s. Using spot sampling of gas concentration data, no differences among $\dot{V}O_2$, $\dot{V}CO_2$, and RER were seen at 10.0, 5.0, 2.5, or 1.0 s (Fig. 4). The standard deviation (SD) of the measurement error over 1.0 s for $\dot{V}O_2$ and $\dot{V}CO_2$ during exercise 1.2 and 1.0%, respectively, and at rest were 0.7% for both gases.

Experiment 3. Fig. 5 shows Bland-Altman plots for differences between the DB and MCMC methods during resting measurements. LOAs were as follows: $\dot{V}E_{STPD}$, $7.7 \pm 4.7\%$ (0.58 ± 0.39 l/min); $FECO_2$, $3.2 \pm 2.0\%$ ($0.09 \pm 0.06\%$); $\dot{V}O_2$, $9.3 \pm 3.9\%$ (23 ± 10 ml/min); $\dot{V}CO_2$, $10.6 \pm 4.1\%$ (23 ± 9 ml/min), and RER, $1.2 \pm 1.4\%$ ($0.010 \pm$

Fig. 3 Response time for mass spectrometry and washout of residual air caused by switching the chamber. Memory problems at n seconds after switching from high to low gas concentration [$T(n)$; $n = 1$ to 10 sec] was calculated as follows:

$$\frac{|\text{Concentration of gas (1 or 2) - Measured concentration at } T(n)|}{|\text{Concentration of gas 1 - Concentration of gas 2}|} \times 100$$

* $P < 0.05$. ** $P < 0.01$.

Fig. 4 Time measured to determine expired gas concentration calculation error. Simulated $\dot{V}O_2$, $\dot{V}CO_2$, and RER from non-continuous gas data are shown. *Left panel*: sitting at rest. *Right panel*: incremental exercise. $\dot{V}O_2$: oxygen consumption, $\dot{V}CO_2$: carbon dioxide consumption, RER: respiratory exchange ratio.

Fig. 5 Validity of the MCMC method at rest and after eating. Bland-Altman plots illustrate percent error for $\dot{V}_{E_{STPD}}$, FEO_2 , $FECO_2$, $\dot{V}O_2$, $\dot{V}CO_2$, and RER. Solid and dashed horizontal lines represent the mean percent error and 95% LOA, respectively. $FECO_2$: fraction of carbon dioxide in expired air, FEO_2 : fraction of oxygen in expired air, LOA: limit of agreement, MCMC: multichannel mixing chamber, $\dot{V}O_2$: oxygen consumption, $\dot{V}CO_2$: carbon dioxide consumption, $\dot{V}_{E_{STPD}}$: expired minute volume at standard temperature and pressure, dry, RER: respiratory exchange ratio.

0.012). LOAs measured using the MCMC method were higher than those measured using the DB method ($P < 0.001$). The LOA of fraction of O_2 in expired air (FEO_2) was $0.4 \pm 0.3\%$ ($0.07 \pm 0.06\%$), and FEO_2 measured with MCMC was lower than FEO_2 measured with DB ($P < 0.001$). In the overall analysis (using data from seven subjects to determine the RMR and DIT), a strong linear relationship was observed between the MCMC and DB methods ($R^2 = 0.9519$, $P < 0.001$) (Fig. 6A), although the TEE measured using the MCMC method was $9.5 \pm 3.9\%$ (0.11 ± 0.05 kcal/min), which was higher than that obtained with the DB method ($P < 0.001$) (Fig. 6B). For

DIT measurement, TEEs at 30 and 90 min after a meal were 0.15 and 0.19 kcal/min higher, respectively, than baseline values (Fig. 6C). There was no significant difference in DIT between the MCMC and DB methods, with an LOA of $2.8 \pm 43.6\%$. TEE at 150 minutes after meal ingestion was smaller than the RMR, as assessed by both measurement methods.

Experiment 4. Figs. 7 and 8 show correlations and Bland-Altman plots for differences between the MCMC and DB methods during the incremental exercise test. Strong linear relationships for $\dot{V}_{E_{STPD}}$, FEO_2 , $FECO_2$,

Fig. 6 Relationship and individual differences between the DB and MCMC methods at RMR and after a meal. (A) Correlation between DB and MCMC methods. (B) Bland-Altman plots for the DB and MCMC methods showing mean percent errors for the two gas analysis methods. (C) Change rate expressed in kilocalories per minute at RMR and a 600-kcal meal for the DB and MCMC methods. The subjects completed eating their meal at time zero. *Versus rest ($P < 0.05$). DB: Douglas bag method, MCMC: multichannel mixing chamber, RMR, resting metabolic rate.

$\dot{V}O_2$, $\dot{V}CO_2$, and RER were observed between the MCMC and DB methods ($R^2 = 0.984-0.995$, $P < 0.001$). LOAs for measurements using the DB method were as follows: $\dot{V}_{ESTPD}$, $2.5 \pm 4.1\%$ (1.48 ± 2.30 l/min); FEO_2 , $0.2 \pm 0.6\%$ ($0.03 \pm 0.09\%$); $FECO_2$, $1.3 \pm 2.3\%$ ($0.05 \pm 0.09\%$); $\dot{V}CO_2$, $2.5 \pm 4.6\%$ (45 ± 100 ml/min); and RER, $1.7 \pm 1.2\%$ (0.018 ± 0.012). Those measured using the MCMC method were higher than those with the DB method ($P < 0.05$). $\dot{V}O_2$ was not significantly different between the two methods, with an LOA of $0.9 \pm 4.8\%$ (9 ± 93 ml/min).

At the end of the exercise test (exhaustion), the DB-measured $\dot{V}O_2$ was $3,262 \pm 493$ ml/min (47.9 ± 5.5 ml/kg/min), $\dot{V}CO_2$ was $3,822 \pm 711$ ml/min, $\dot{V}_{ESTPD}$ was 90.38 ± 18.76 l/min, RER was 1.17 ± 0.07 , and heart rate was 189 ± 9 beats/min.

Discussion

This study confirms the validity of the new system for simultaneous multiple-subject analysis of respiratory gas exchange in humans. Although some statistically significant differences were found, the differences were so small as to be physiologically insignificant. The measurement error of oxygen uptake at rest was about 9%, and during exercise there was a very small error ($< 1\%$).

Memory problems of the mass analyzer were suffi-

ciently eliminated by a washout of ≥ 2 s. Such problems are caused by the analysis of gas samples at different concentrations when analyzing multiple mixing chambers. Therefore, we examined the switching time of the mixing chamber and a plurality of optimal washout times to determine the exact concentration of gas in a single mass spectrometer and found that memory problems could be kept very small by providing a washout of 2 + s. When using a short delay in mass spectrometer analysis, memory problems increased with increased density of the gas sample. At most concentrations, the difference seems to arise between fresh air and especially the end-tidal gas concentration, which would increase memory problems. However, memory problems for both O_2 and CO_2 were minimally affected with a washout of 2 s. For O_2 it was less than 0.3% and for CO_2 less than 0.4%. That is, the present study suggests that a washout time of 2 s negates the influence of memory problems caused by the differences between the densities of expired gas and fresh air. This small error minimally affects metabolic measurement in humans. If ventilation is constant, the error in $\dot{V}O_2$ and $\dot{V}CO_2$ for continuous gas concentration measured after sufficient washout ranged from 1.4 to 2.2% using a washout time of 1 s, and from 1.0 to 1.6% with a washout of 2 s (Table 1).

It is possible to calculate metabolic measurements correctly even for gas concentration data of non-continuous

Fig. 7 Relationship and individual differences between the MCMC and DB methods during maximal incremental exercise. Left panels show the correlations between the MCMC and DB methods for $\dot{V}E_{STPD}$, FEO_2 , and $FECO_2$. The dashed lines represent equality ($y = x$). Right panel shows mean percent errors on Bland-Altman plots. Horizontal solid and dashed lines represent the mean percent error and 95% LOA, respectively. DB: Douglas bag method, $FECO_2$: fraction of carbon dioxide in expired air, FEO_2 : fraction of oxygen in expired air, LOA: limit of agreement, $\dot{V}O_2$: oxygen consumption, $\dot{V}CO_2$: carbon dioxide consumption, $\dot{V}E_{STPD}$: expired minute volume at standard temperature and pressure, dry.

flow for any given 1 s of every 10 s (see Fig. 4). This was achieved because the respiratory gas was well mixed by the specially designed mixing chamber of the MCMC system and because of the high-response mass spectrometer. The expired gas concentration determined by the MCMC method was obtained piecemeal with more than one value at 1 s each in the mixing chamber. During a gradual increase in exercise, when multiple measurements of the expired gas concentration change during a short cycle bout, estimating the value over 1 min is particularly

important. Thus, 1-s gas samples should be obtained every 3 s per mixing chamber. We believe that this system, using only one mass spectrometer, can be further modified to measure up to 20 mixing chambers per minute.

Absolute $\dot{V}O_2$ and $\dot{V}CO_2$ measured using the MCMC method at rest and after dietary intake were slightly but significantly higher than those measured using the DB method, but relative changes were reliably measured. The intra-individual coefficients of variance in RMR determined using the DB method ($2.7 \pm 1.4\%$ [range,

Fig. 8 Relationship and individual differences between MCMC and DB methods during maximal incremental exercise. Left panels show correlations between the MCMC and DB methods for $\dot{V}O_2$, $\dot{V}CO_2$, and RER. The dashed lines represent equality ($y = x$). Right panel shows mean percent error on Bland-Altman plots for $\dot{V}O_2$, $\dot{V}CO_2$, and RER. Horizontal solid and dashed lines represent the mean percent error and 95% LOA, respectively. DB: Douglas bag method, LOA: limit of agreement, MCMC: multi-channel mixing chamber, $\dot{V}O_2$: oxygen consumption, $\dot{V}CO_2$: carbon dioxide consumption, RER, respiratory exchange ratio.

1.0–5.0%]) and the MCMC method ($3.2 \pm 2.7\%$ [range, 0.8–8.9%]) in the present study were similar to the 3–8% same-day intra-individual coefficient of variance of RMR in healthy adult humans previously reported¹²). Moreover, when comparing the DIT for a short duration (150 min), there was no difference between the 21 ± 17 kcal found with the MCMC method and the 23 ± 17 kcal obtained with the DB method. However, because there was a significant difference between the DB and MCMC methods in absolute amount of heat consumed over time, it has

been suggested that it is necessary to compensate for the difference.

The MCMC method was accurate for high-level measurements of exhaled gases during exercise. The present study found that the MCMC method is a valid system for simultaneous metabolic measurements in multiple subjects during exercise. The study also demonstrated that the MCMC method produced $\dot{V}E$, FEO_2 , $FECO_2$, $\dot{V}O_2$, $\dot{V}CO_2$, and RER values that are similar to those obtained with the DB method during sub-maximal

Table 1. Determination of time zone to decrease errors by memory problems after washout.

Time (s)	$\dot{V}O_2$	$\dot{V}CO_2$	RER
0–1	–36.6	–38.3	–3.3
1–2	–1.4	–2.2	–1.0
2–3	–1.0	–1.6	–0.7
3–4	–0.7	–1.3	–0.6
4–5	–0.5	–1.1	–0.7

(%)

$\dot{V}O_2$, $\dot{V}CO_2$, and RER were calculated when the ventilation expired volume was ideal.

and maximal exercise. The physiological parameters at the end of exercise met the criteria for determination of maximal oxygen consumption ($\dot{V}O_{2\max}$). The difference in $\dot{V}O_2$ between the DB and MCMC methods was small (0.9%), with results for each method ranging from about 500 to 3900 ml/min. There is a 2.5% error for $\dot{V}E$, which affected calculation of the respiratory index.

This study shows that despite movement of the non-steady state during exercise, high accuracy is ensured for the MCMC method. Powers et al.¹³⁾ reported $\dot{V}O_2$ measurement errors of approximately 6–63 ml/min in the non-steady state during a constant load. Foss and Hallé¹⁴⁾ examined the Oxycon Pro (Erich Jaeger GmbH, Hoechberg, Germany) mixing chamber and reported it to be only 30 ml/min lower than the same measurement obtained with the DB method. In this study, the mean error and 95% LOA of $\dot{V}O_2$ were approximately –9 ml/min and –190 to 170 ml/min, respectively, indicating no significant differences.

The measurement accuracy of the system in the present study was shown to be much better than that of some portable systems. Macfalan and Wong¹⁵⁾ reported that for the Metamax 3B (Cortex, Leipzig, Germany), the $\dot{V}O_2$ errors at rest and during exercise had a 95% LOA of –70 to 140 ml/min and –50 to 590 ml/min, respectively; and King et al.¹⁶⁾ reported that the individual $\dot{V}E$ and $\dot{V}O_2$ errors for the Aerosport KB1-C (Aeroport, Michigan, USA) had a 95% LOA of approximately –500 to 450 ml/min between rest and 250 W.

Metabolic measurement systems equipped with a ventilation meter have been reported in some studies to underestimate or overestimate ventilation. Ventilation meters with turbine and pneumotachometry expression are used in many systems. Rosdahl et al.¹⁷⁾ and Medbø et al.¹⁸⁾ reported using the Moxus Modular system (AEI Technologies, Illinois, USA) with a turbine. They noted a tendency to overestimate ventilation compared with the DB method. In addition, the same system with a pneumo-

tachometer and at high ventilation, despite the overestimation of ventilation compared with the DB method with less ventilation, was reported to have shifted to underestimation¹⁷⁾. On the other hand, measuring ventilation during submaximal exercise with the AMIS 2001 metabolic cart (Innovision, Odense, Denmark) with a differential pressure sensor was reported to cause underestimation of ventilation¹⁹⁾. The present study showed overestimation of ventilation at both rest and during exercise. Measurements in those studies were made after calibration at various flow rates. Although these studies showed significant differences, the results suggested that dispersion of small errors are scattered around the true value. However, we were unable to identify the cause of the small error that occurred between the MCMC and DB methods in our study.

Simultaneous exercise testing of several people may change the inspired gas composition of the air in the laboratory during testing to a degree that can vary depending on the number of individuals in the laboratory and their ventilatory capacity. In our study, there were up to 10 people, including examiners, subjects and spectators, in the laboratory at same time, and inspired gas concentrations were for O_2 20.90–20.92% and for CO_2 0.06–0.09%. Some previous studies calculated the air concentration in their laboratories as well^{11,14)}. It is not clear that metabolic indexes in previous studies reflect the O_2 or CO_2 concentration in the inspired air as it changes, even during the experiment itself. The expired gas of multiple subjects may affect the gas concentration of the room air (inspired air) during $\dot{V}O_{2\max}$ testing for multiple subjects if the room is small or the room is inadequately ventilated. Thus, the MCMC system was developed to enable monitoring of the inspired air concentrations continuously in real time as well as the expired gas.

The system using the MCMC method enables effective use of space in the laboratory and sufficient time for experiments. At this time, mixing chamber systems are intended for single-subject use only. The conventional system using the mixing chamber method has been developed to provide accurate measurements and the convenience of automated analysis^{20,21)}. Our new system shows time-series plots for all channels during collection on a single display. In addition, because of its ability to make simultaneous measurements, this system may reduce errors owing to biological rhythms, for example, when measuring metabolic response during ingestion of a test drug or food, as well as when measuring resting metabolism. Therefore, measuring respiratory gas in multiple subjects on the same day at the same time promotes greater experiment-related efficiency and productivity. In many cases, measurements via the DB method take a long time and must be performed by skilled technicians. There is also a period of rest, in bed or on a chair, of up to 30 minutes until the proper resting state is achieved, after which RMR is measured for about 10 min. Exercise stress

test protocols on a treadmill or cycle ergometer comprise a warm-up, main exercise, and cool-down. Our system saves space and reduces the number of individual laboratory experiments and the time required for each. It also provides a means of measuring resting metabolism and aerobic capacity in a large research study without having to duplicate expensive equipment.

In conclusion, the present study used the MCMC method to measure metabolic parameters in multiple subjects simultaneously. The study demonstrated reasonable validity over a wide measurement range when compared with the DB method. The system allowed us to measure the metabolism of several people simultaneously while conserving laboratory equipment and space.

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