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ORIGINAL ARTICLE

The Moxus Modular metabolic system evaluated with two sensors for ventilation against the Douglas bag method

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Abstract This study evaluated the Moxus metabolic system with the Douglas bag method (DBM) as criterion. Reliability and validity were investigated in a wide range of ventilation and oxygen uptake and two sensors for determining ventilation were included. Thirteen well-trained athletes participated in one pre-test and four tests for data collection, exercising on a cycle ergometer at five submaximal powers (50–263 W) and at VO_{2max}. Gas exchange variables were measured simultaneously using a serial setup with data collected on different days in an order randomized between Moxus with pneumotachometer (MP) and turbine flowmeter (MT) sensors for ventilation. Reliability with both sensors was comparable to the DBM. Average CV (%) of all exercise intensities were with MP: 3.0 ± 1.3 for VO_2, 3.8 ± 1.5 for VCO_2, 3.1 ± 1.2 for the respiratory exchange ratio (RER) and 4.2 ± 0.8 for V_E. The corresponding values with MT were: 2.7 ± 0.3 for VO_2, 4.7 ± 0.4 for VCO_2, 3.3 ± 0.9 for RER and 4.8 ± 1.4 for V_E. Validity was acceptable except for small differences related to the determination of ventilation. The relative differences in relation to DBM at the powers including VO_{2max} were similar for both sensors with the ranges being: +4 to −2 % for V_E, +5 to −3 % for VO_2 and +5 to −4 % for VCO_2 while RER did not differ at any power. The Moxus metabolic system shows high and adequate reliability and reasonable validity over a wide measurement range. At a few exercise levels, V_E differed slightly from DBM, resulting in concomitant changes in VO_2 and VCO_2.

Keywords Moxus Modular metabolic system · Douglas bag method · Validity · Reliability · Turbine flowmeter · Pneumotachometer · Oxygen uptake · Carbon dioxide production · Ventilation · Respiratory exchange ratio

Introduction

Determination of oxygen uptake in humans is a long-standing key method traceable back to 1790 (Hollman and Prinz 1997). The historical development of indirect calorimetry using closed and open-circuit respirometry has recently been reviewed thoroughly and inexpensive equipment for determination of oxygen uptake was suggested as the most suitable future method for estimations of energy expenditure during field conditions (Shephard and Aoyagi 2012). Historically, since the early nineteenth century, the classical Douglas bag technique (Douglas 1911) has been extensively used to measure oxygen consumption and energy expenditure and has over the years proved to afford the investigator high validity and reliability (Åstrand 1952; Taylor et al. 1955; Mitchell et al. 1958; Åstrand and Saltin 1961; Saltin and Åstrand 1967; Åstrand and Rodahl 1986). Consequently, numerous of authors recommend the Douglas bag technique as the criterion method for these determinations (Casaburi et al. 2003; Macfarlane 2001; Hodges et al. 2005).

Over the past 30 years, however, the Douglas bag technique has been successively replaced by automated systems and today most measurements are performed with automated and computerized metabolic systems in a less time-consuming manner and with more variables being

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easily attainable. Alongside this expansion an increased number of different brands of equipment have become commercially available for the investigator. Equally, it has become much more difficult for the individual investigator to keep the automated metabolic systems under good control. The need for further analysis of new automated metabolic systems has been emphasized in the literature (Macfarlane 2001; Hodges et al. 2005) and guiding principles for these investigations have been reported (Atkinson et al. 2005; Hodges et al. 2005).

The use of a mixing chamber is generally considered to be a more reliable setup than breath-by-breath analysis. However, over the past 10 years few evaluations of stationary metabolic systems with mixing chambers against the Douglas bag method (DBM) have been reported (Bassett et al. 2001; Jensen et al. 2002; Foss and Hallén 2005; Crouter et al. 2006). For determining ventilation, Foss and Hallèn used a turbine flowmeter on the expiratory side, Jensen et al. and Crouter et al., used a pneumotachometer on the inspiratory side, whereas Bassett and coworkers (2001) used a pneumotachometer on both sides to compare these setups. One previous study has found larger than acceptable errors with an early model of a turbine flowmeter when compared to a pneumotachometer in the same investigation (Yeh et al. 1987).

The Moxus metabolic system is a commercially available, automated, stationary metabolic system for laboratory use. It is designed for measuring with high demands on reliability and accuracy (AEI Technologies Inc., Naperville, IL, USA). It is equipped with a mixing chamber and with gas analyzers that are stable, fast-responding and precise (Macfarlane 2001). Ventilation can be determined on the inspiratory side either with a turbine flowmeter as on earlier models, or with a pneumotachometer as on the model being commercially available at present. This system has been used in a number of scientific investigations (Burgomaster et al. 2005; Devries et al. 2005; Moore et al. 2005; Liu et al. 2003) and has not been evaluated against a criterion method until recently (Medbø et al. 2012). However, in that study, the Moxus system was evaluated in a smaller VO_2 range (1.2–3.75 L min^{-1}) and with the turbine flowmeter merely which is no longer commercially available.

Methods

Approach for the investigation

The objective of the present study was to study reliability and validity of the Moxus metabolic system in a wide measurement range and to include a comparison of two sensors for ventilation, i.e., a pneumotachometer and a turbine flowmeter. For this purpose, we used the DBM as the reference method and simultaneous measurements of the gas exchange variables by a serial coupling setup between the Moxus system and the DBM in each experiment. The reliability of each ventilation sensor was investigated by testing them in separate experiments twice on different days and, from these data, the coefficient of variation and typical error were calculated. Furthermore, data collection with the pneumotachometer and turbine flowmeter was randomized to avoid potential bias from order effects. The validity of the Moxus system with each ventilation sensor was evaluated by analysis of the data collected with these setups and the concomitantly collected data with the DBM. Statistical evaluations were performed at each power to detect differences in relation to DBM.

To this end well-trained athletes were asked to participate in one pre-test and four tests for data collection, exercising on a cycle ergometer at five submaximal powers (50–263 W) and at VO_{2max}.

Participants

Thirteen well-trained endurance athletes participated. Their physical characteristics were: age 29 ± 4.3 years, height 182 ± 6.1 cm, body mass 75 ± 8.7 kg, VO_{2max} 4.8 ± 0.4 L min^{-1} or 64 ± 5.2 mL kg min^{-1} (mean ± SD). They were all well familiar with cycling as a work mode: 12 undertook regular training and competition in road cycling and 1 was a former elite rower who used cycling as a regular form of endurance training. All were still competing but at various levels and rates. Two belonged to the Swedish national team, six were active at national elite level and five were active but not at elite level. All were volunteers and prior to their participation they were informed about the purpose, procedure and possible risks related to the tests. They were also informed about their rights to cease participation at any time without explanation, in accordance with the Helsinki Declaration, and the study was approved by the Karolinska Institute Ethics Committee. The participants were asked to avoid strenuous or prolonged exercise and to keep to their normal diet 24 h preceding the tests. Each person participated in one pre-test for familiarization and in four tests for data collection.

Experimental design

A serial coupling setup was used to collect the gas exchange variables simultaneously with Moxus metabolic system and the DBM (see Fig. 1). Data were collected in randomized order between the Moxus pneumotachometer (MP) and Moxus turbine (MT) setups. In half the group, the order of the tests was MT-MP-MT-MP; in the other half MP-MT-MP-MT. For the collection of expired air, a suitably sized Hans Rudolph face mask was used (model: 8940

small, 8930 medium or 8920 large) together with a Hans Rudolph two-way non-rebreathing valve (model 2730 Large 2-way NRVB, Y-shape, Hans Rudolph Inc., Kansas City, MO, USA). Prior to and during the entire exercise tests, leaks between the face and the face mask were carefully checked for.

When the Moxus metabolic system was used with the turbine flowmeter, this was attached directly on the inspiratory side of the breathing valve, and a corrugated plastic hose (length 2.5 m inner diameter 35 mm, Hans Rudolph Inc., Kansas City, MO, USA) was attached between the expiratory side of the breathing valve and the mixing chamber. When a pneumotachometer (PNT) was used with this system, an additional 2.5 m plastic hose was attached between the inspiratory side of the breathing valve and the PNT since the PNT was attached to the stand of the metabolic cart and not directly on the breathing valve. The serial coupling between the Moxus metabolic system and the DBM was completed by attaching a hose (length 55 cm and inner diameter 35 mm) between the outlet of the mixing chamber and the inlet of the three-way valve for Douglas bag air collection.

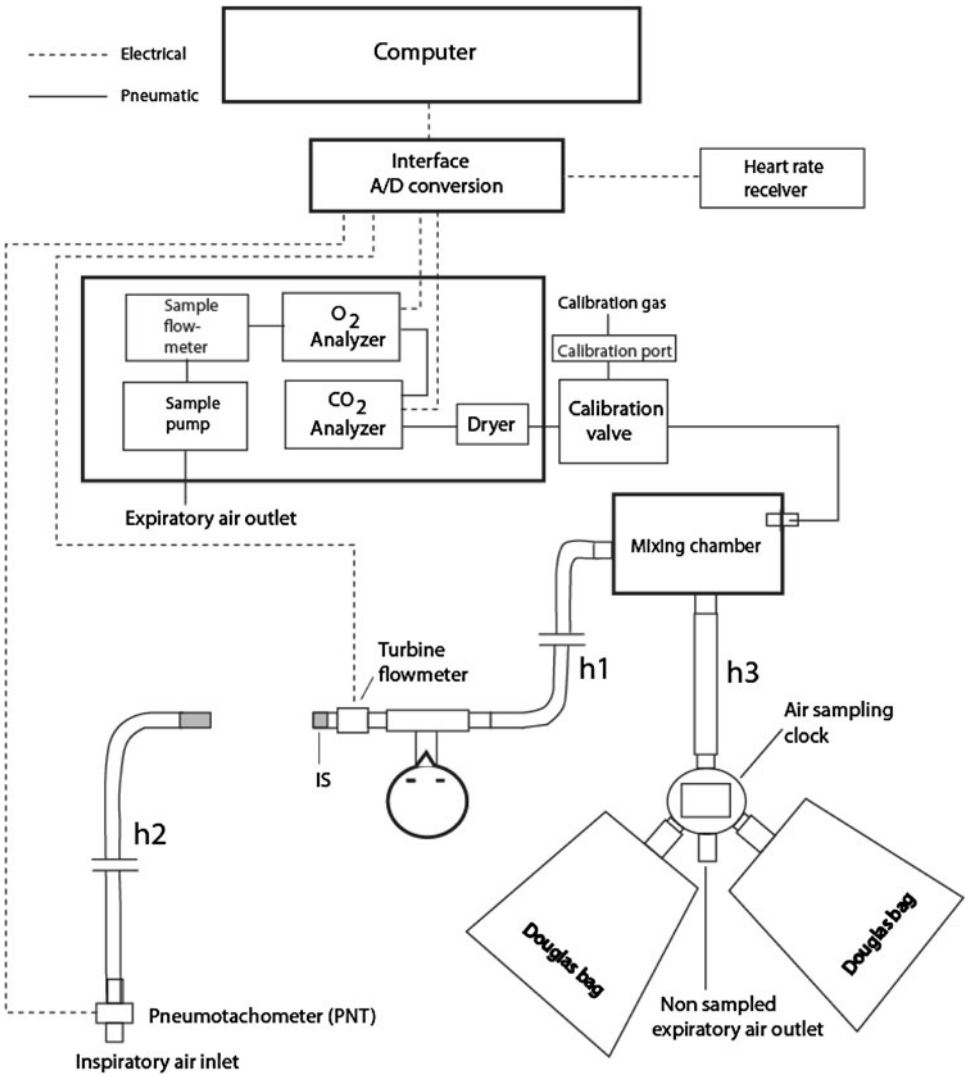


Fig. 1 Illustration of the experimental setup for simultaneous measurements with the Moxus metabolic system and the Douglas bag method using a serial coupling setup. When the turbine flowmeter was used it was attached directly on the inspiratory side of the breathing valve (*IS*) and a hose (*h1*) was attached between the expiratory side of the breathing valve and the mixing chamber. When the pneumotachometer (PNT) was used, an additional hose (*h2*) was attached between the inspiratory side of the breathing valve and the PNT. Another hose (*h3*) was attached between the outlet of the mixing chamber and the inlet of the three-way valve for Douglas bag air collection, for the serial coupling between the Moxus metabolic system and the DBM. Before being measured in the gas analyzers, expired air is sampled at the outlet of the mixing chamber, transported through the calibration valve and dehydrated in the desiccant box. The vacuum flow of the sample pump is measured in a flowmeter connected to the outlet of the gas analyzers. An analog-to-digital conversion is performed in the interface box and the output is transferred to the computer for further processing

The manual data entry function in the Moxus software was used during data collection at start and stop of each Douglas bag collection to obtain a time marker and thereby synchronization between the data collected by the Moxus Douglas bag systems over the particular time frame. With this command, markers were introduced in the tabular results generated by the Moxus system with a 10-s resolution, from which the nearest data were selected to match those from the DBM. The Douglas bag volumes were corrected for the removal of the expiring air volumes by the O_2 and CO_2 analyzers both within the continuous data collection during the test (250 mL min^{-1} by the Moxus metabolic system) and during the subsequent analysis of the gas fractions in the Douglas bags (250 mL min^{-1}).

Graded exercise test

A Monark pendulum ergometer cycle (manually adjusted 828E, Monark Exercise AB, Vansbro, Sweden) was used in all the tests. Prior to the study, the ergometer cycle was calibrated using high-accuracy weights and, before each test, the braking-force scale was checked and zero adjusted if needed. All subjects were instructed to keep the cadence within ±1 rpm of the target value. The cadence was constantly shown on a display visible to both the subject and the investigator. Every minute the position of the pendulum was checked with respect to a scale and the braking force adjusted if necessary. The graded exercise test consisted of a submaximal exercise for 9 min at 50 W, 6 min at 100 W and 5 min at 150 W at a cadence of 50 rpm followed by work for 4.5 min at 210 W and for 4 min at 263 W at a cadence of 70 rpm. From then on, the VO_{2max} test began at a cadence of 90 rpm and 2 min exercise at 180 W for all. This was followed by the next power (for 1 min), individually based on the subject's physical capacity. Power was increased for all by 22.5 W every min until fatigue. As a result of the differences in maximal aerobic power of the participating athletes, the last power 30 s prior to termination of VO_{2max} ranged between 394 and 529 W (average 448 ± 34 W). The metabolic variables were measured and averaged for 240 s at 50 W, 180 s at 100 W, 120 s at 150 W, 90 s at 210 W and for 60 s at 263 W. VO_{2max} was calculated by averaging the highest consecutive values over 60 s at maximal exercise. For the Douglas bags, this was based on sampling periods of 30 s and for the Moxus system three consecutive 10-s periods were used. The highest VO_2 attained by the DBM was taken as VO_{2max} and compared with the values from the Moxus metabolic system in the same sampling period.

A 20-μL blood sample was taken from the fingertip for lactate analysis at the end of the 210 and 263 W exercise periods to make sure that these powers were low enough to avoid lactate accumulation. Blood was also sampled at 1 and 3 min after VO_{2max}. Immediately after each test, all samples were analyzed on a BIOSEN C-line analyzer (EKF-Diagnostik, Barleben/Magdeburg, Germany). The average blood lactate values were: 1.0 ± 0.18 mmol L^{-1} at 210 W, 1.5 ± 0.72 mmol L^{-1} at 263 W, 12.4 ± 1.32 mmol L^{-1} and 12.7 ± 1.4 mmol L^{-1} 1 and 3 min after the test, respectively.

Equipment for the metabolic measurements

The Douglas bag method

Specifications and quality control of the specific Douglas bag system have previously been described in detail (Rosdahl et al. 2010). In the present study, this system was used with other gas analyzers and other stopcocks on the Douglas bags, and re-checked. The fractional concentrations of oxygen were determined with the S-3A Oxygen analyzer, those of carbon dioxide were determined with the CD 3-A Carbon dioxide analyzer, with a P-61B infrared sensor (AEI Technologies Inc., Naperville, IL, USA). These analyzers were included in the Moxus metabolic system and thus also used for determination of the O_2 and CO_2 fractions when the system was used with either a pneumotachometer or a turbine flowmeter. The gas analyzers were carefully checked beforehand for accuracy and linearity using high-precision calibration gases. The Douglas bags were fitted with new gastight stopcocks (type 546 d40DN32, PVCU, EPDM PN16, Georg Fischer Piping Systems Ltd., Shaffhausen, Switzerland) with an inner diameter of 32 mm and fittings with an outer diameter of 40 mm to which the breathing tubing was connected. The Douglas bags were custom made in gastight polyurethane coated with polyamide fabric in sizes to hold 120 and 160 L volumes (C Fritze Consulting, Svedala, Sweden). Prior to the study, all were checked for leaks with 0.1 kg weight load on top of the air-filled bags (no leaking after 2 h with the detection limit being 135 mL) and gas diffusion (no change in O_2 % or CO_2 % after 2 h at room temperature). All Douglas bags were flushed with expired air before they were used for the first time each day. During the collection of expired air, the Douglas bags were placed in a bag stand with a three-way valve and a timer (Fabri AB, Spånga, Sweden). Volume was determined with a custom made and balanced spirometer tank (an enlarged copy of a Collins Tissot tank with an adjusted balance, Fabri AB, Spånga, Sweden) with a fast-responding temperature sensor attached on top of the inner cylinder (certified accuracy ±0.5 °C, GMH 3230, Greisinger electronic GmbH, Regenstauf, Germany). Ordinary algorithms were used (in which the spirometer temperature was included) to transform the volume to STPD and to BTPS conditions

(Carpenter 1964). Volume determination accuracy with the spirometer has been verified previously (Rosdahl et al. 2010) and was checked again prior to the present study.

Prior to each sequence of analysis, the gas analyzers were calibrated with a high-precision gas mixture from one gas cylinder of 15.03 % O_2 and 6.01 % CO_2 and another gas cylinder with 21.00 % O_2 and 0.03 % CO_2 (accuracy: O_2 15.024–15.036 % and CO_2 6.004–6.016 %, Air Liquide AB, Kungsängen, Sweden). Ambient conditions were measured with accurate and certified equipment (atmospheric pressure 0.2 % rel. full scale, GMH 3160; room temperature ±0.5 °C and relative humidity ±2 % abs., GMH 3330, Griesinger electronic GmbH, Regenstauf, Germany).

The Moxus metabolic system

The overall description of the Moxus metabolic system is illustrated in Fig. 1. The system is designed for use in five different modes depending on the requirements of the investigation. The "Moxus or Max II system mode" is illustrated since this should be used when the expired air from human subjects is sampled from the mixing chamber (or directly from the breathing valve if the data were to be acquired breath by breath). Ventilation is measured on the inspiratory side of the breathing valve and the system used in the present study was set up to allow recordings either with a pneumotachometer (PNT) or with a turbine flowmeter. Expired air is sampled at the outlet of the mixing chamber, transported through the calibration valve and dehydrated in the desiccant box with molecular sieve and silica gel indicator before being measured in the gas analyzers. The vacuum flow is determined by the setting of the sample pump (250 mL min^{-1}) and is measured in a flowmeter connected to the outlet of the gas analyzers. All analog input signals are transferred to the interface box and an analog-to-digital conversion is performed at 250 Hz with 12-bit resolution. The output from the interface is transferred to the computer and the Max II software as single data points either breath-by-breath or on a one-data-point-per-second basis. This "raw data" are then saved (every 30 s) before any calculations are performed and can be re-opened and viewed later. The software allows the investigator to select different time intervals for different types of data and to change the way the data are viewed on the screen during the test. Prior to each test, the necessary parameters to convert the ATP flow data (inspired air at Ambient Temperature, Pressure and humidity) to BTPS and STPD units must be entered manually in the subject data screen. Atmospheric pressure, ambient temperature, and relative humidity should be entered with an accuracy of ±2 mmHg, ±1 °C and 5 % (abs.), respectively.

The following algorithms are used by the software to transform from ATP to BTPS and from BTPS to STPD: ATP to BTPS = (310.16/(273.16 + Temperature)) × (Pressure − P)/(Pressure − 47.04), where P = Humidity × (31.82 − C × ((0.078 × C + 1.152) × C + 9.133))/100 C = (0.2 × Temperature) − 6. BTPS to STPD = 0.001159 × (Pressure − 47.07). The temperature is given °C and the unit for water saturation pressure is given in mmHg.

The software version used in the present study was Windows Version 2.4.01. Theory of operation for the system and formulas for calculation of the metabolic variables are described in detail in Appendix A in the Moxus and Max II Instruction Manual, (AEI Technologies Inc., Naperville, IL, USA).

Mixing chamber: The total mixing volume is 6.9 L, i.e., the sum of the chamber volume (4.2 L) and the volume of the hose and breathing valve (2.7 L). The mixing chamber is active, i.e., has a fan placed inside to smooth out variations from the exhaled air breath by breath.

Gas analyzers: The fractional concentration of oxygen is determined with the S-3A Oxygen analyzer; model N-22M with a stabilized zirconia cell (AEI Technologies Inc. Naperville, IL, USA). According to the Instruction Manual, the accuracy is ±0.01 % O_2 on the percent oxygen scale within the working range, which is calibration gas value ±5 % of full-scale value. Response speed is 90 % of final value in less than 100 ms. The carbon dioxide fractional concentration is determined with a CD 3-A Carbon dioxide analyzer, with a P-61B infrared-based sensor (AEI Technologies Inc., Naperville, IL, USA). According to the instruction manual, the accuracy is ±0.02 % CO_2 on the percent oxygen scale or 1 % of the reading, whichever is larger. The speed of response is user selectable to 24 or 400 ms to 90 % of final value for a step change in carbon dioxide concentration.

The actual response speed of both analyzers depends on the pump flow rate and the time needed for the sample to travel from the mixing chamber to the sensor, i.e., the delay time. Recommended sample flow rate is 250 mL min^{-1}, for the present application. The delay time can be determined by the investigator with a procedure described in the Instruction Manual.

Turbine flowmeter measurement system: The manufacturer's specifications of the VMM-400 turbine flowmeter system are as follows: linear flow range 0.1–12 L s^{-1} (6.0–720 L min^{-1}), accuracy 1.5 %, dead space 38 mL, and a pressure drop of 0.45 Pa at 120 L min^{-1} (or 2 L s^{-1}) due to the flowmeter's resistance. The pickup method of the system is infrared based and optically focused.

Pneumotachometer: The pneumotachometer (PNT) was a Hans Rudolph series 4813 designed for athlete spirometry applications (Hans Rudolph Inc., Kansas City, MO, USA). As stated in the documentation, the calibrated flow range

was 80–800 L min^{-1} and the accuracy ±2 % of this specific model. In the Moxus interface, the flow signal from the PNT is converted to a linearized signal using a calibration look-up table which corrects non-linearity in the PNT, transducer and electronics. The actual volume (inspiratory ATPS) is determined through a flow-triggered comparator circuit/integrator.

Calibration: Prior to the entire study, the delay time, i.e., the time needed for the sample to travel from the mixing chamber to the gas analyzer sensors was carefully determined following the procedure described in the instruction manual. The present values achieved and used were: mixed volume delay 6.9 L, O_2 delay 6.9 s and CO_2 delay 5.6 s. Values entered and used for the inspired fractional concentrations of O_2 and CO_2 were 20.93 and 0.03 %, respectively. Prior to each test, the gas analyzers, the PNT and turbine flowmeter were calibrated carefully following the instruction manual. In brief, the gas analyzers were first calibrated alone and then through software to calibrate their analog outputs and compensate for analog-to-digital conversion errors (overriding the analyzer calibration). In both steps, two calibration levels were used from one gas cylinder with 15.03 % O_2 and 6.01 % CO_2 and from another with 21.00 % O_2 and 0.03 % CO_2 (accuracy: O_2 15.024–15.036 % and CO_2 6.004–6.016 %, Air Liquide AB, Kungsängen, Sweden). The PNT and turbine flowmeter was calibrated with a 3 L (2,995 mL) certified calibration syringe (Hans Rudolph Inc., Kansas City, MO, USA) connected to the mouth port of the breathing valve and pumped five times. Additionally, verification was performed by pumping the syringe five times at varying speeds while verifying that the average percentage error was below ±2 %. The calibration was repeated if the percentage error was higher than 2 %, although this was rare. Ambient conditions were measured with accurate and certified equipment (atmospheric pressure 0.2 % rel. full scale, GMH 3160; room temperature ±0.5 °C and relative humidity ±2 % abs., GMH 3330, Griesinger electronic GmbH, Regenstauf, Germany).

Calculations and statistics

Reliability

Student's paired *t* tests were used to detect significant differences between the first and second tests with each system, i.e., whether any order effects were present. The typical error (TE) and coefficient of variation (CV) were used to express the reliability of the measured metabolic variables. TE was calculated by dividing the standard deviation of the difference between the values from the first and the second test with $\sqrt{2}$ and CV was calculated by dividing TE with the average values of both tests multiplied by 100.

Validity

To compare the data from the two setups using the Moxus metabolic system (MP and MT) with the concomitantly measured data with the DBM, all values for each participant from the first and the second tests with each method were averaged prior to further use. Statistical evaluations were thereafter performed to detect differences between MP versus DBM and between MT versus DBM at each submaximal power and at VO_{2max}. Absolute and relative values were evaluated separately. Following previous recommendations (Hodges et al. 2005), Student's paired *t* tests were used to compare whether results of the two methods differed significantly. In addition, agreement between the methods was graphically displayed with Bland–Altman plots (Bland and Altman 1999). The 95 % limits of agreement were not included in the B–A plots since the degree of systematic error between DBM and MP displayed a clear relationship to the size of the measured value. The Graph-Pad Prism 4 software package was used to create the Bland–Altman plots (Graph-Pad software Inc., San Diego, CA, USA).

Results

Reliability

The reliability of all recorded metabolic variables as measured with the DBM, the Moxus turbine flowmeter (MT) and the Moxus pneumotachometer (MP) setup are presented in Table 1 as the coefficient of variation (CV) and typical error (TE) calculated for the duplicate tests performed at five submaximal and at VO_{2max}. Generally, the CV and TE were well acceptable for the variables measured with the DBM, MP and MT as indicated by CV ranges of 1.2–5.3 % for VO_2, 2.0–6.0 % for VCO_2, 1.8–5.1 % for the respiratory exchange ratio (RER) and 2.8–7.2 % for V_E at the various powers. No significant differences were detected between the test–retests in any of the variables at any of the powers. Further, no systematic change was apparent in CV with increased work rate.

Validity

Overall was the validity of the Moxus metabolic system with both sensors for ventilation acceptable. RER did not differ at any power while small differences in VO_2 and VCO_2 were noted. These were primarily related to the

Table 1 Day-to-day reliability of metabolic variables measured with the Douglas bag method and the Moxus metabolic system

	Power	Douglas bag				Moxus pneumotachometer				Douglas bag				Moxus turbine flowmeter			
		CV (%)	TE	$T_1 - T_2$	P	CV (%)	TE	$T_1 - T_2$	P	CV (%)	TE	$T_1 - T_2$	P	CV (%)	TE	$T_1 - T_2$	P
VO_2 (mL min^{-1})	50 W	4.5	44	3	NS	5.3	54	7	NS	1.7	17	5	NS	3.1	33	9	NS
	100 W	2.8	43	−5	NS	3.6	56	−5	NS	1.2	19	12	NS	3.0	48	10	NS
	150 W	2.3	46	−20	NS	2.6	54	−20	NS	1.3	28	5	NS	2.4	52	−12	NS
	210 W	2.6	70	18	NS	2.2	59	18	NS	2.5	68	−5	NS	2.9	80	−14	NS
	263 W	2.2	72	−29	NS	1.7	57	−29	NS	1.4	46	13	NS	2.4	83	12	NS
	VO_2max	1.8	87	37	NS	2.3	108	37	NS	2.5	120	−12	NS	2.5	117	−64	NS
VCO_2 (mL min^{-1})	50 W	6.0	51	−7	NS	5.7	51	−17	NS	5.1	45	−4	NS	5.3	49	3	NS
	100 W	3.8	51	−28	NS	4.1	57	−30	NS	4.2	57	12	NS	4.9	70	15	NS
	150 W	4.0	73	−37	NS	3.7	69	−34	NS	4.9	88	44	NS	4.5	85	2	NS
	210 W	2.7	66	8	NS	2.0	49	−17	NS	3.8	94	17	NS	4.4	111	11	NS
	263 W	2.8	85	−11	NS	2.0	61	−15	NS	3.4	106	33	NS	4.3	137	39	NS
	VO_2max	2.8	156	8	NS	5.0	270	48	NS	3.9	217	−95	NS	4.6	250	−132	NS
RER	50 W	5.0	0.04	−0.01	NS	5.1	0.04	−0.01	NS	5.0	0.04	−0.03	NS	4.8	0.04	−0.02	NS
	100 W	3.6	0.03	−0.01	NS	3.5	0.03	−0.01	NS	3.1	0.03	−0.02	NS	3.5	0.03	−0.01	NS
	150 W	3.4	0.03	−0.01	NS	3.5	0.03	0.00	NS	5.0	0.04	0.00	NS	3.6	0.03	−0.01	NS
	210 W	2.4	0.02	0.00	NS	2.2	0.02	0.00	NS	2.9	0.03	−0.01	NS	2.9	0.03	0.00	NS
	263 W	2.3	0.02	0.00	NS	2.3	0.02	0.00	NS	2.6	0.02	0.00	NS	2.7	0.03	0.00	NS
	VO_2max	1.8	0.02	0.00	NS	1.9	0.02	0.00	NS	2.2	0.03	−0.01	NS	2.3	0.03	0.00	NS
V_E (L min^{-1})	50 W	5.2	1.3	−0.1	NS	4.8	1.2	0.0	NS	4.7	1.1	−0.5	NS	5.6	1.4	−0.2	NS
	100 W	4.5	3.1	0.0	NS	4.0	2.7	0.5	NS	3.6	2.5	0.6	NS	3.5	1.3	−0.1	NS
	150 W	4.7	2.1	−1.0	NS	4.2	1.9	−0.8	NS	2.8	1.3	0.0	NS	3.4	1.5	−0.5	NS
	210 W	4.3	2.6	0.4	NS	3.2	1.9	0.2	NS	3.6	2.1	0.0	NS	4.8	2.9	−0.2	NS
	263 W	4.0	3.0	1.1	NS	3.6	2.6	1.1	NS	3.2	2.4	1.0	NS	4.5	3.4	1.2	NS
	VO_2max	5.1	8.7	0.3	NS	5.5	−0.7	9.0	NS	6.9	11.7	−0.8	NS	7.2	11.9	−1.1	NS

The test–retest Douglas bag data collected in serial connection with the Moxus pneumotachometer setup is given in the column to the left of the test–retest Moxus pneumotachometer data, and accordingly, the test–retest Douglas bag data collected in serial connection with the Moxus turbine flowmeter setup are given in the column to the left of the test–retest Moxus turbine flowmeter data

CV, Coefficient of variation is the TE expressed as a percentage of the mean value of the test–retest values; *TE*, typical error of measurement expressed in mL min^{-1} for VO_2 and VCO_2 and L min^{-1} for V_E; $T_1 - T_2$, the absolute difference between the test (T_1) and retest (T_2); *P*, the *P* values from the Student's paired *t* tests performed with the test and retest values

Table 2 Validity of Moxus metabolic system with the pneumotachometer setup

	Power	DBM	M-pneumotach	Absolute diff.		Relative diff. (%)	
		Mean ± SD	Mean ± SD	Mean ± SD	*P*	Mean ± SD	*P*
VO_2 (mL min^{-1})	50 W	979 ± 48	1026 ± 40	47 ± 21	***	4.6 ± 2.1	***
	100 W	1520 ± 146	1557 ± 151	37 ± 21	***	2.4 ± 1.3	***
	150 W	2030 ± 51	2051 ± 58	21 ± 38	NS	1.0 ± 1.8	NS
	210 W	2688 ± 53	2684 ± 57	−5 ± 51	NS	−0.2 ± 1.9	NS
	263 W	3304 ± 80	3251 ± 87	−52 ± 46	**	−1.6 ± 1.4	**
	VO_{2max}	4749 ± 428	4599 ± 387	−150 ± 97	***	−3.2 ± 2.1	***
VCO_2 (mL min^{-1})	50 W	854 ± 61	900 ± 62	45 ± 20	***	5.0 ± 2.1	***
	100 W	1329 ± 152	1371 ± 152	42 ± 18	***	3.1 ± 1.3	***
	150 W	1813 ± 74	1839 ± 85	26 ± 28	**	1.4 ± 1.5	**
	210 W	2458 ± 87	2475 ± 91	17 ± 36	NS	0.7 ± 1.4	NS
	263 W	3090 ± 86	3043 ± 97	−47 ± 34	***	−1.6 ± 1.1	***
	VO_{2max}	5581 ± 491	5369 ± 411	−213 ± 216	**	−3.9 ± 4.0	**
RER	50 W	0.872 ± 0.03	0.873 ± 0.03	0.001 ± 0.00	NS	0.06 ± 0.56	NS
	100 W	0.874 ± 0.03	0.876 ± 0.03	0.003 ± 0.01	NS	0.32 ± 1.05	NS
	150 W	0.894 ± 0.03	0.894 ± 0.03	0.000 ± 0.01	NS	−0.06 ± 1.07	NS
	210 W	0.916 ± 0.02	0.921 ± 0.03	0.005 ± 0.01	NS	0.50 ± 1.31	NS
	263 W	0.936 ± 0.03	0.936 ± 0.03	0.000 ± 0.01	NS	0.02 ± 1.25	NS
	VO_{2max}	1.178 ± 0.03	1.176 ± 0.03	−0.002 ± 0.01	NS	−0.15 ± 1.00	NS
V_E (L min^{-1})	50 W	24.1 ± 2.0	24.9 ± 1.8	0.83 ± 0.41	***	3.4 ± 1.8	***
	100 W	34.5 ± 3.6	35.0 ± 3.5	0.47 ± 0.40	**	1.4 ± 1.2	**
	150 W	45.7 ± 3.6	45.5 ± 3.5	−0.17 ± 0.62	NS	−0.4 ± 1.4	NS
	210 W	60.3 ± 4.1	59.4 ± 3.8	−0.96 ± 0.72	***	−1.6 ± 1.2	***
	263 W	75.6 ± 5.2	73.4 ± 5.1	−2.25 ± 1.06	***	−3.1 ± 1.5	***
	VO_{2max}	169.7 ± 22.3	165.4 ± 20.7	−4.27 ± 3.14	***	−2.5 ± 1.8	***
F_EO_2 (%)	50 W	16.12 ± 0.29	16.04 ± 0.30	−0.08 ± 0.04	***	−0.48 ± 0.2	***
	100 W	15.73 ± 0.35	15.65 ± 0.36	−0.08 ± 0.05	***	−0.49 ± 0.3	***
	150 W	15.65 ± 0.38	15.55 ± 0.39	−0.10 ± 0.06	***	−0.63 ± 0.4	***
	210 W	15.62 ± 0.34	15.51 ± 0.33	−0.10 ± 0.05	***	−0.68 ± 0.3	***
	263 W	15.69 ± 0.35	15.60 ± 0.35	−0.09 ± 0.05	***	−0.58 ± 0.3	***
	VO_{2max}	17.39 ± 0.33	17.40 ± 0.33	0.01 ± 0.05	NS	0.03 ± 0.3	NS
F_ECO_2 (%)	50 W	4.34 ± 0.27	4.40 ± 0.29	0.06 ± 0.03	***	1.32 ± 0.6	***
	100 W	4.70 ± 0.32	4.77 ± 0.34	0.07 ± 0.03	***	1.44 ± 0.5	***
	150 W	4.86 ± 0.34	4.94 ± 0.35	0.08 ± 0.02	***	1.52 ± 0.4	***
	210 W	4.99 ± 0.34	5.10 ± 0.33	0.11 ± 0.04	***	2.13 ± 0.9	***
	263 W	5.00 ± 0.34	5.08 ± 0.34	0.07 ± 0.05	***	1.46 ± 1.0	***
	VO_{2max}	4.06 ± 0.39	4.04 ± 0.39	−0.02 ± 0.07	NS	−0.58 ± 1.6	NS

Absolute and relative differences between the Douglas bag method (DBM) and the Moxus metabolic system with the pneumotachometer setup (M-pneumotach). V_E is given as BTPS, while VO_2 and VCO_2 are given as STPD. The levels of significance are based on Student's paired *t* tests in which the absolute and the relative differences, respectively, between the Douglas bag method (DBM) and the Moxus metabolic system were used

* $P < 0.05$. ** $P < 0.01$, *** $P < 0.001$

determination of ventilation and the relative differences in relation to DBM at all powers including VO_{2max} were overall similar for both sensors. The validity of metabolic variables as measured with the MT and MP setup is expressed in more detail as the differences against the DBM criterion in Tables 2 and 3, and Figs. 2 and 3.

The Moxus pneumotachometer setup

As evident in Fig. 2 and Table 2, the trend of an increasing deviation range between the MP system and the DBM appears related to size of the measured values. At the lowest powers of 50 and 100 W, the MP setup measured

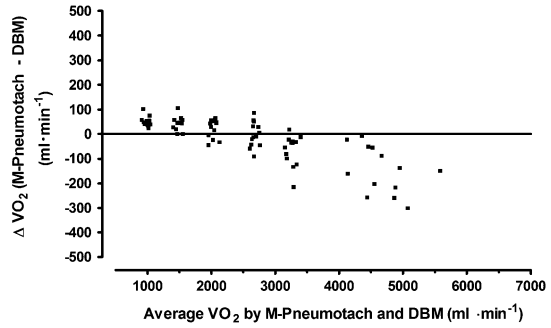
Table 3 Validity of Moxus metabolic system with the turbine flowmeter setup

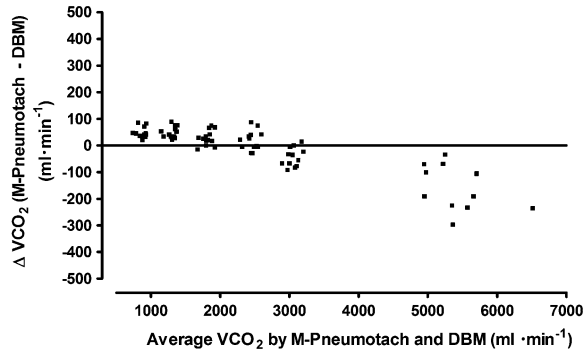
	Power	DBM	M-turbine	Absolute diff.		Relative diff. (%)	
		Mean ± SD	Mean ± SD	Mean ± SD	*P*	Mean ± SD	*P*
VO_2 (mL min^{-1})	50 W	1007 ± 54	1061 ± 62	53 ± 24	***	5.0 ± 2.2	***
	100 W	1558 ± 141	1626 ± 154	67 ± 30	***	4.1 ± 1.7	***
	150 W	2058 ± 51	2151 ± 64	92 ± 41	***	4.3 ± 1.8	***
	210 W	2700 ± 62	2794 ± 65	94 ± 67	***	3.3 ± 2.4	***
	263 W	3317 ± 83	3399 ± 96	83 ± 92	**	2.4 ± 2.7	**
	VO_2max	4777 ± 460	4681 ± 432	−96 ± 160	NS	−2.1 ± 3.4	NS
VCO_2 (mL min^{-1})	50 W	875 ± 50	923 ± 55	49 ± 21	***	5.2 ± 2.1	***
	100 W	1350 ± 138	1414 ± 146	64 ± 28	***	4.5 ± 1.9	***
	150 W	1799 ± 72	1889 ± 59	90 ± 40	***	4.8 ± 2.1	***
	210 W	2452 ± 99	2540 ± 85	88 ± 54	**	3.5 ± 2.1	**
	263 W	3105 ± 142	3180 ± 125	75 ± 78	**	2.3 ± 2.5	**
	VO_2max	5582 ± 550	5425 ± 518	−158 ± 167	**	−2.9 ± 3.1	**
RER	50 W	0.859 ± 0.03	0.861 ± 0.03	0.002 ± 0.01	NS	0.24 ± 0.6	NS
	100 W	0.857 ± 0.03	0.861 ± 0.03	0.004 ± 0.01	NS	0.47 ± 1.2	NS
	150 W	0.865 ± 0.03	0.871 ± 0.03	0.006 ± 0.02	NS	0.66 ± 2.3	NS
	210 W	0.901 ± 0.03	0.904 ± 0.02	0.003 ± 0.01	NS	0.34 ± 1.4	NS
	263 W	0.932 ± 0.04	0.931 ± 0.04	−0.001 ± 0.01	NS	−0.14 ± 1.4	NS
	VO_2max	1.172 ± 0.03	1.164 ± 0.03	−0.008 ± 0.01	*	−0.71 ± 1.1	*
V_E (L min^{-1})	50 W	24.4 ± 2.3	25.5 ± 2.4	1.1 ± 0.57	***	4.2 ± 2.1	***
	100 W	34.6 ± 3.4	35.7 ± 3.5	1.1 ± 0.65	***	3.2 ± 1.8	***
	150 W	44.4 ± 3.1	45.6 ± 3.2	1.2 ± 0.61	***	2.7 ± 1.3	***
	210 W	59.1 ± 4.0	60.0 ± 4.2	1.0 ± 0.93	**	1.6 ± 1.5	**
	263 W	74.6 ± 4.9	75.1 ± 5.3	0.6 ± 1.31	NS	0.7 ± 1.7	NS
	VO_2max	169.9 ± 22.8	165.9 ± 22.0	−3.9 ± 3.61	**	−2.4 ± 2.2	**
F_EO_2 (%)	50 W	16.04 ± 0.31	15.98 ± 0.31	−0.06 ± 0.02	***	−0.39 ± 0.1	***
	100 W	15.61 ± 0.42	15.54 ± 0.41	−0.07 ± 0.04	***	−0.44 ± 0.3	***
	150 W	15.44 ± 0.39	15.33 ± 0.40	−0.10 ± 0.03	***	−0.68 ± 0.2	***
	210 W	15.48 ± 0.36	15.37 ± 0.35	−0.11 ± 0.05	***	−0.71 ± 0.3	***
	263 W	15.59 ± 0.40	15.49 ± 0.39	−0.11 ± 0.08	***	−0.69 ± 0.5	***
	VO_2max	17.35 ± 0.37	17.34 ± 0.35	−0.01 ± 0.06	NS	−0.06 ± 0.3	NS
F_ECO_2 (%)	50 W	4.36 ± 0.28	4.41 ± 0.28	0.05 ± 0.02	***	1.2 ± 0.6	***
	100 W	4.74 ± 0.38	4.81 ± 0.40	0.07 ± 0.03	***	1.4 ± 0.7	***
	150 W	4.93 ± 0.40	5.04 ± 0.38	0.11 ± 0.10	**	2.2 ± 2.0	**
	210 W	5.05 ± 0.36	5.16 ± 0.34	0.10 ± 0.05	***	2.1 ± 1.1	***
	263 W	5.08 ± 0.38	5.16 ± 0.35	0.09 ± 0.08	**	1.7 ± 1.5	**
	VO_2max	4.08 ± 0.39	4.06 ± 0.39	−0.02 ± 0.06	NS	−0.5 ± 1.6	NS

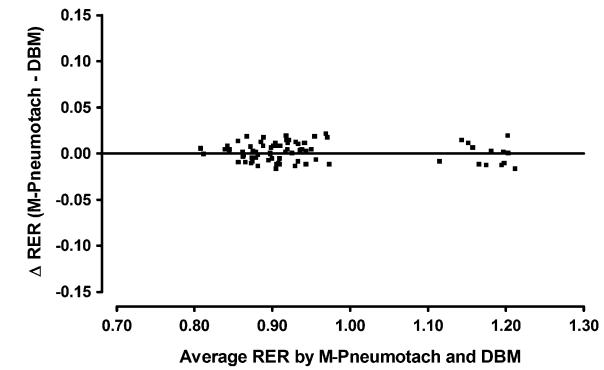
Absolute and relative differences between the Douglas bag method (DBM) and the Moxus metabolic system with the turbine flowmeter setup (M-turbine). V_E is given as BTPS, while VO_2 and VCO_2 are given as STPD. The levels of significance are based on Student's paired *t* tests in which the absolute and the relative differences, respectively, between the Douglas bag method (DBM) and the Moxus metabolic system were used

* $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$

VO_2 higher than the DBM (4.6 and 2.4 %, respectively), while no difference occurred at the medium power ranges of 150 and 210 W. Conversely, at 263 W and at VO_2max, the MP setup measured VO_2 lower than the DBM (−1.6 and −3.2 %, respectively). Similar to the VO_2 results, the MP setup measured VCO_2 higher than the DBM at 50 and 100 W (5.0 and 3.1 %, respectively), but with no difference at 210 W, and lower values at 263 W and at VO_2max. In accordance with the results for VO_2 and VCO_2, V_E was measured higher at 50 and 100 W (3.4 and 1.4 %,







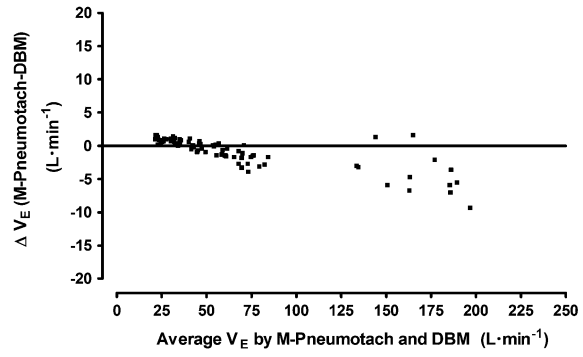
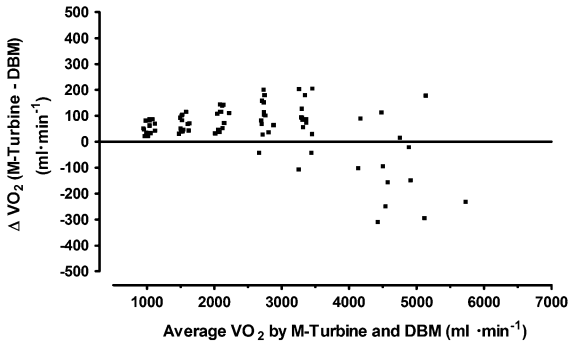
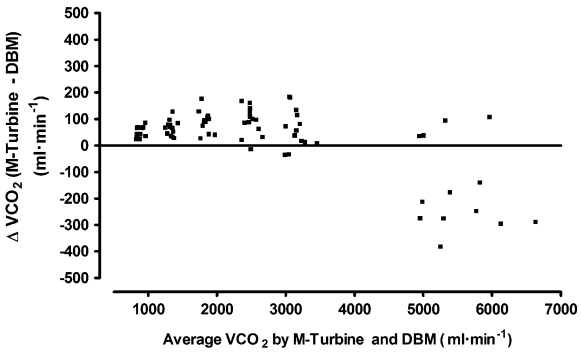
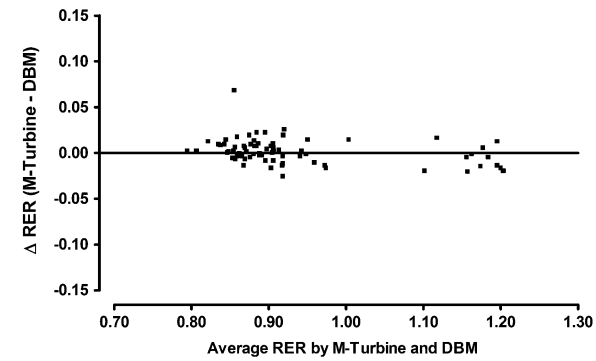


Fig. 2 Bland–Altman plots showing the agreement between measurements with the Moxus metabolic system with the pneumotachometer setup and the Douglas bag method (DBM). *Y*-axes show absolute differences in VO_2 (STPD), VCO_2 (STPD), RER and V_E (BTPS). *X*-axes show the mean values for both methods. All data points represent the average of duplicate measurements with both methods collected during cycle ergometer exercise at 50, 100, 150, 210, 263 W and at VO_{2max}. Validity of the Moxus metabolic system with the pneumotachometer setup







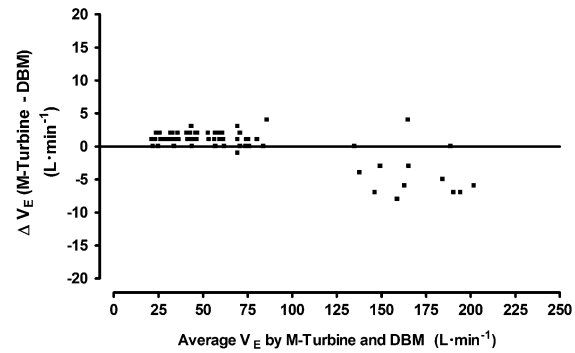


Fig. 3 Bland–Altman plots showing the agreement between measurements with the Moxus metabolic system with the turbine flowmeter setup and the Douglas bag method (DBM). *Y*-axes show absolute differences in VO_2 (STPD), VCO_2 (STPD), RER and V_E (BTPS). *X*-axes show the mean values for both methods. All *data points* represent the average of duplicate measurements with both methods collected during cycle ergometer exercise at 50, 100, 150, 210, 263 W and at VO_{2max}. Validity of the Moxus metabolic system with the turbine flowmeter setup

respectively), with no or a very small difference at 150 and 210 W, and slightly lower at 263 W and at VO_{2max}. At all submaximal powers, the MP setup measured F_EO_2 lower and F_ECO_2 higher than the DBM, while no difference was found at VO_{2max}. The RER did not differ between the MP setup and the DBM at any power (Table 2).

The Moxus turbine flowmeter setup

As evident in Fig. 3 and Table 3, VO_2 measured with the MT setup was higher than that of the DBM at all submaximal powers the (1.7 and 2.7 %, respectively), while no difference was found at VO_{2max}. Similarly, VCO_2 was measured higher at all submaximal powers (2.3–5.2 %) while values at VO_{2max} were significantly lower (−2.9 %). In accordance with the results for VO_2 and VCO_2, V_E was high at submaximal powers of 50–210 W (range 1.6–4.2 %), with no difference at 263 W, and low (−2.4 %) at VO_{2max}. At all submaximal powers, the MT setup measured F_EO_2 slightly lower and F_ECO_2 slightly higher than the DBM, while no difference was found at VO_{2max} (Table 3). For RER, a small difference reaches statistical significance at VO_{2max} but no differences were seen at any of the other work rates.

Discussion

In the present study, the reliability and validity of the Moxus metabolic system were for the first time evaluated with two different measuring devices for ventilation using the DBM as reference system. A wide range in ventilation and oxygen uptake, including VO_{2max}, was measured.

Reliability

The *day-to-day* reliability of all the metabolic variables as measured with the Moxus metabolic system, with both types of ventilation sensors, was found to be acceptable and agreed closely with that of the DBM within a wide measurement range. To the best of our knowledge, there are no expert agreed statements regarding acceptable limits for reliability and naturally the limits may vary depending on the study purpose. However, since we consider the quality assurance guidelines for exercise physiology laboratories from the Australian Institute of Sport (Tanner and Gore 2012) being well elaborated and useful for most measurement conditions, these limits (for VO_2, a typical error <0.15 L min^{-1} or CV < 3 % at VO_{2max}) has been taken as our criterion for acceptable reliability. The results for reliability expressed as the coefficient of variation (CV) for VO_2 and V_E are discussed below in relation to results in the literature and the criterion method.

Surprisingly few previous studies have reported the *day-to-day* reliability of automated systems with a mixing chamber. Useful reference values can be found in two comprehensive investigations (Wilmore et al. 1998; Skinner et al. 1999) who studied the reproducibility of maximal exercise test data in the HERITAGE family study in a group of 390 sedentary subjects, using the Sensor Medics 2900 metabolic system with a mixing chamber. Wilmore et al. (1998) reported a *day-to-day* CV of 4.7 and 3.6 % for VO_2 and 6.7 and 5.3 % for V_E at 50 W and 60 % of VO_{2max}, respectively, while Skinner et al. (1999) reported a *day-to-day* CV of 5.1 % for VO_{2max} and 9.5 % for V_{Emax}. More results with the DBM are available. With sedentary subjects, Carter and Jeukendrup (2002) found the *day-to-day* CV to be 3–5 % for VO_2 and 5–5.7 % for V_E at 100 and 150 W submaximal exercise. In the same investigation, the *within-test* CV was slightly lower than the *day-to-day* CV at the same powers, around 2.5 % for VO_2 and 2.5–4 % for V_E. Further, a very low *day-to-day* CV of 1.9 % has been reported by Jensen and Johansen (1998) for VO_{2max} in athletes investigated with the DBM, the attendant CV for V_{Emax} being 11.8 %. At submaximal powers, the CV for VO_2 was 7.4–7.5 % with no values reported for V_E. In addition, a later study on athletes by Jensen et al. (2002) reported a low *within-test* CV of 1.8 % for VO_2 and 4.8 % for V_E at submaximal powers with the DBM.

Judging the CV results from different studies is intricate as the influences of several methodological aspects need to be considered. Among these are the training status of the subjects, the power, the type of exercise and whether the *day-to-day or within-test* CV being studied. To the best of our knowledge, the literature of the past 10 years offers only four investigations of automated metabolic systems with mixing chambers that were relevant for comparison with our results (Crouter et al. 2006; Jensen et al. 2002; Foss and Hallén 2005; Medbø et al. 2012). With five submaximal powers at 50–250 W, Crouter and co-workers reported a CV of 4.7 % for VO_2 with the automated metabolic system and 5.3 % with the DBM for all powers combined. In our study, we obtained two CV values for VO_2 of approximately 5 % at 50 W, whereas the other CV values at submaximal powers ranged between 1.2 and 3.6 %. Concerning the *day-to-day* CV for V_E, our values ranged between 3.2 and 5.6 % for the Moxus system and 2.8–5.2 % for the DBM. Crouter et al. (2006) reported 7.3 % with the automated metabolic system and 8.5 % with the DBM. However, since the participants in those authors' study were healthy males but not athletes, this may be a factor to consider in the assessment of the results. Higher reproducibility is easier to obtain with well-trained participants than with untrained. Hence, the higher CV values seen in Crouter et al. may be related to differences

in metabolic measurement systems or to differences in the training status of the participants, a combination of both factors. In a comprehensive evaluation study of the Oxycon Pro system with a mixing chamber, Foss and Hallén (2005) reported a very low overall CV of 1.2 and 1.0 % for VO_2 and V_E, respectively, when this was calculated and expressed as *within-test* CV between the automated system and the DBM. Interestingly, in this study, an electromagnetically braked cycle ergometer was used which likely will reduce the variation attributable to fluctuations in workload.

The *day-to-day* CV values obtained at VO_{2max} with the Moxus metabolic system were low at around 2.5 % with close agreement with the DBM values in the present study and similar to the values of ≤3 % as reported by Medbø et al. (2012) for the Moxus system with a turbine flowmeter. These values are also similar to or slightly higher than the *within-test* CV values at VO_{2max} of 1.9 % for the Amis metabolic cart and 1.8 % for the DBM as reported by Jensen et al. (2002). Equally, the *day-to-day* CV at V_{Emax} in our study ranged between 5.5 and 7.2 % for the Moxus system and 5.1 and 6.9 % for the DBM, i.e., slightly higher than the values of 4.8 % reported by Jensen et al. (2002) for both DBM and the Amis metabolic cart.

Overall, the present results strongly indicate that the *day-to-day* reliability of the Moxus metabolic system used with two different measuring devices for ventilation, i.e., the pneumotachometer and turbine flowmeter, compares well with both the results of the criterion method and previous investigations. Based on these findings, we conclude that the reliability of the Moxus metabolic system is acceptable and applicable for metabolic measurements at most common conditions in a wide range.

Validity

The present results show that the Moxus metabolic system, with both devices to determine ventilation, compares well overall with the DBM. Significant differences were detected, however, related primarily to the determination of ventilation. The results obtained for the metabolic variables and V_E are discussed below in relation to the two different devices for measuring ventilation, the criterion DBM and other published results.

The present differences between the DBM and the Moxus metabolic system with the pneumotachometer were small but were statistically significant at the two lowest and the highest submaximal powers and at VO_{2max}. Noticeably, the relative differences were almost identical for VO_2 and VCO_2 and followed closely the relative differences in V_E. This strongly indicates that an inaccuracy in the determination of V_E caused the differences in VO_2 and VCO_2. The differences noted in the determination of V_E with the Moxus pneumotachometer in relation to the DBM indicate that this variable was not determined entirely accurately throughout the measuring range with the pneumotachometer in the present human study. According to the manufacturer's general documentation for the pneumotachometer used in the Moxus system (Hans Rudolph 4813), it is designed for athlete spirometry applications and has a flow range of 0–800 L min^{-1}. However, after the data collection, we received the results from a delivery check of the individual unit and found that these did not include any measurements below 80 L min^{-1}. Since this is the same range as that of our submaximal powers (24–76 L min^{-1}), it is likely that a lack of linearization explains the imprecise values noted at some of these work rates. Interestingly, in line with this finding, Bassett et al. (2001) pointed out that the Hans Rudolph 3813 pneumotachometer is nonlinear in the lower flow range (0–80 L min^{-1}) and needs correction with the "Yeh algorithm" (Yeh et al. 1982, 1987).

The differences in metabolic variables between the Moxus turbine flowmeter and the DBM were small but statistically significant at all powers except VO_{2max}. The average relative differences at submaximal powers did not, like the results from the pneumotachometer, vary across the measurement range with a positive difference at the lower work rates that successively changed to a negative difference at the higher submaximal powers and at VO_{2max}. Like the data from pneumotachometer, the relative differences were almost identical for VO_2 and VCO_2 narrowly following the relative differences in V_E, indicating that inaccuracy in the determination of V_E caused the differences in VO_2 and VCO_2. We could not obtain any technical documentation on the individual turbine flowmeter (VMM-400), but according to the manufacturer's general information, it has a linear range of 6–720 L min^{-1} with a relative accuracy ±1.5 %. Thus, the present results show that when the individually used VMM-400 flowmeter is compared to the DBM, it is within the accuracy limits at two of the present exercise levels and slightly outside at the other four. Concerning previous validity evaluations of the Moxus Modular metabolic system with a turbine flowmeter, Medbø et al. (2012) recently reported that VO_2 was on average 3 % higher than that obtained by the DBM in the range of 1.2–3.75 L min^{-1}. The bias was caused partly by the ventilation being 1.4 % too high and partly by the O_2 extraction being 2 % too high (Medbø et al. 2012).

In a thorough investigation by Foss and Hallén (2005) another model of turbine flowmeter (the Triple V flow turbine, Carefusion GmbH, Hoechberg, Germany) was included, while the Oxycon Pro metabolic system with mixing chamber was being evaluated. In that study, the measured ventilation was 1.8 % lower with Oxycon Pro than with the DBM while on average VO_2 was 0.8 % low

because of concomitant differences in the expired gas fractions (see below). In addition, the same type of turbine in a study of the second generation of the Oxycon Mobile breath-by-breath metabolic system compared overall well with the DBM at submaximal powers, while slightly lower values were found for VO_{2max} without a significant different VO_{2max} (Rosdahl et al. 2010).

Concerning the accuracy of determining the expired fractions of O_2 and CO_2, we obtained small but significant differences at submaximal powers with both sensors as compared to the DBM. The basic algorithms for VO_2 and VCO_2 calculations show that these differences affect VO_2 and VCO_2 and concur with the direction of the differences seen versus the DBM. Since F_EO_2 was higher and F_ECO_2 was lower with the DBM than with Moxus, it is appealing to speculate these differences are caused by a dilution of the expired air with room air either from the unavoidable small dead space located in the three-way valve used for switching between the bags and timing the collections, or from some room air being trapped within the Douglas bags at small places. Concerning the latter factor, we minimized the influence by flushing the Douglas bags with expired air before they were used for the first time each day. Furthermore, since it would require a very large volume of room air to explain the difference (of about 0.1 % for FEO_2) seen in the present study, other factors must be involved as well. As an example, 1 L of room air would be required to dilute FEO_2 from 15.50 to 15.57 % if 75 L of expired air is collected in a Douglas bag.

Since the Moxus gas analyzers also were used for the Douglas bag analysis, it can be excluded that the differences in the gas fractions were related to differences in the gas analysis equipment. Interestingly, the dilution phenomenon was not detectable at VO_{2max} with considerably higher ventilation and subsequent changes in the expired fractional concentrations of O_2 and CO_2. A few recent studies report the expired fractional concentrations of O_2 and CO_2 (Crouter et al. 2006; Bassett et al. 2001; Foss and Hallén 2005), while Bassett et al. (2001) reported no significant difference. Interestingly, the data by Crouter et al. (2006) and Foss and Hallén (2005) show the same results as in the present study, i.e., a higher F_EO_2 and lower F_ECO_2 in the Douglas bags than in the automated metabolic system with a mixing chamber. Nevertheless, causes of the fractional differences were not discussed by these authors and since separate gas analyzers were used in their experimental setup it cannot be established whether the discrepancy was caused by room air dilution in the Douglas bags or because the analyzers measured the gas fractions slightly different. Similarly, although the data were not given as the expired fractional concentrations, Medbø et al. (2012) reported the O_2 and CO_2 extraction per volume of air breathed being 0.08 and 0.14 % points higher than those of the DBM, respectively. These authors briefly discussed some possible causes to the discrepancy with no final conclusion concerning the cause of the observed bias. Although the DBM has been in routine use for many years and the diffusion of gas through the wall of the bag was early critically examined (Shephard 1955), the influence of room air dilution in the bags has, to the best of our knowledge, not been scrutinized in previous studies.

Limitations and strengths of the present study

The present study was designed to obtain the most favorable conditions for comparing the automated system against the DBM with respect to reliability and to validity. To optimize the evaluation of reliability, we included well-trained participants only, as this is known to minimize biological variation. Additionally, to minimize the influence of variation in mechanical work we used a well controlled cycle ergometer and carefully checked the cadence and brake force during the test. Nevertheless, if we had used an electromagnetically braked and cadence independent cycle ergometer instead, the variation attributable to fluctuations in workload may have been further reduced. A further refinement may also be achieved by excluding the influence of biological variation, either by using a metabolic calibrator (Huszczuk et al. 1990) or by evaluating the variation against a criterion method used simultaneously in a serial connection as in Foss and Hallén (2005). However, since the expired gas from a metabolic simulator is not humidified to physiological conditions and the ventilation pattern is not identical to physiological breathing, this approach could neither be considered comprehensive. For validity evaluation, the ideal would be to analyze precisely the same breaths with both methods, collected with both methods simultaneously through a serial connection in each test, this as an alternative to separate tests on different days. In the present study, however, expired air was sampled over 30–240 s and averaged, thus a lag from the mixing chamber to the DB is negligible and no systematic differences introduced.

Future perspectives

Although the present study altogether shows adequate validity and reliability for common applications, it seems that the overall performance of the Moxus system can be further improved by fine-tuning the measurement of ventilation. During the writing of this paper, we have been informed by the manufacturer of a recent upgrade offering a larger range of linearization of the pneumotachometer, a new interface with improved accuracy and resolution and the A/D conversion increased from 12 to 16 bits, with more accurate electronics, and also various software improvements. Future

studies should, therefore, be performed to ascertain whether the newer version of the Moxus system or other brands of automated system will reach the same reliability and validity as the criterion DBM.

Although the DBM is generally accepted as the criterion method, we suggest that future studies may also critically examine this method and specific laboratory setups from various aspects of importance. Hence, still warranted are studies similar to Shephard's (1955) study "A critical examination of the Douglas bag technique" and the recent study by Hopker et al. (2012) in which potential errors in the DBM were examined. For example, the collection of expired air in our and in other laboratories can possibly be further refined by lowering the small volume of room air trapped in the three-way valve and the Douglas bags. Additionally, the match of start and stop of collecting expired air during inspiration would be improved with computerized detection of the breathing cycle and a loop that triggers an automatic switch in the three-way valve. We believe that the DBM is still needed as the most suitable reference method for metabolic measurements as it allows all incorporated variables to be traced and controlled at the detailed level necessary with a gold standard.

Conclusions

The Moxus metabolic system shows high and adequate reliability and reasonable validity over a wide measurement range when compared to the criterion DBM. Validity can be further improved by refinements in the measurement of ventilation, as both the pneumotachometer and turbine flowmeter differ slightly compared to the criterion method at few powers, and this results in concomitant changes in $V\mathrm{O}_2$ and $V\mathrm{CO}_2$. With further refinements of the experimental setup for validation, and with further refinements of the Moxus system or other automated metabolic systems, future studies may show complete agreement between the criterion DBM and an automated metabolic system with a mixing chamber.

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Conflict of interest The authors declare no conflicts of interest.

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