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Published in: Proceedings of the Institution of Mechanical Engineers, Part P: Journal of Sports Engineering and Technology

DOI: 10.1177/1754337120919609

Publication date: 2020

Document version: Submitted manuscript

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Citation for pulished version (APA): Ainegren, M., Jensen, K., & Rosdáhl, H. (2020). Breathing resistance in metabolic systems: Its effects on pulmonary ventilation and oxygen uptake in elite athletes with high aerobic power. Proceedings of the Institution of Mechanical Engineers, Part P: Journal of Sports Engineering and Technology, 234(3), 217-226. https://doi.org/10.1177/1754337120919609

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Breathing resistance in metabolic systems: its effects on pulmonary ventilation and oxygen uptake in elite athletes with high aerobic power

Journal:	Part P: Journal of Sports Engineering and Technology
Manuscript ID	JSET-19-0096.R2
Manuscript Type:	Original Article
Date Submitted by the Author:	19-Mar-2020
Complete List of Authors:	Ainegren, Mats; Mid Sweden University, Department of Quality Technology and Mechanical Engineering Jensen, Kurt; The University of Southern Denmark, Department of Sport Science and Clinical Biomechanics, Muscle Physiology and Biomechanics Rosdahl, Hans; The Swedish School of Sport and Health Sciences, Sweden, Sport and Health Sciences
Keywords:	Automated metabolic systems, breathing resistance, Douglas Bag system, oxygen uptake, ventilation, pulmonary gas fractions
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Title

Breathing resistance in metabolic systems: its effects on pulmonary ventilation and oxygen uptake in elite athletes with high aerobic power

Preferred running head/ short title

Physiological effects of breathing resistance

Authors

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Abstract

The aim of the present study was to investigate the effects on pulmonary ventilation and oxygen uptake ($\dot{V}O_2$) in athletes with a very high maximal oxygen uptake ($\dot{V}O_2$ max) and corresponding high ventilation capacity when using a modern metabolic system with relatively high resistance to breathing (HIGH_{RES}), compared to a traditional system with low resistance to breathing (LOW_{RES}). Four rowers and three cross-country skiers (without asthma), competing at a high international level, performed in experimental conditions with LOW_{RES} and HIGH_{RES} using a rowing ergometer and roller skis on a treadmill. The results showed that $\dot{V}O_2$, blood lactate, heart rate and respiratory exchange ratio were not different between the LOW_{RES} and HIGH_{RES} test conditions during both submaximal and maximal

exercise. Also, the athlete's time to exhaustion (treadmill) and mean power (rowing ergometer) from maximal tests were no different between the two conditions. However, ventilation and expiratory O_2 and CO_2 concentrations were different for both submaximal and maximal exercise.

Thus, the authors have concluded that the differences in resistance to breathing of metabolic systems influence elite endurance athletes \dot{V}_E at low to very high workloads, thus affecting the expired gas fractions, but not the submaximal $\dot{V}O_2$, $\dot{V}O_2$ max and performance in a laboratory setting at sea level.

Keywords

Automated metabolic systems, breathing resistance, Douglas Bag system, oxygen saturation, oxygen uptake, ventilation

Introduction

Indirect calorimetry is a method that determines whole body metabolic rate via the measurement of pulmonary gas exchange ¹. The golden standard for indirect calorimetry is still considered to be the Douglas Bag method, which involves collecting exhaled air in sealed bags, followed by an analysis of the content in terms of volume and gas fractions ^{2, 3}. Since the 1960s, automated metabolic systems that aim to facilitate practical measurements and the presentation of data in real time have been introduced to the commercial market. These systems are either based on mixing chamber, breath-by-breath or hybrid methodology (through micro-sampling into a miniature mixing chamber) and are available both as stationary systems for the laboratory and portable systems for measurements in the field ^{1, 4-7}. Custom-designed portable Douglas bag systems have also been built for measurements in the field ⁸⁻¹⁰.

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Automated metabolic systems are commonly used to investigate athletes' maximal oxygen uptake ($\dot{V}O_2 max$) during sport-specific performance in various exercise activities. However, only a few studies have validated these systems using highly skilled endurance athletes with pulmonary ventilation corresponding to nearly 200 L/min during maximal exercise ^{5, 6, 11}. Furthermore, because highly trained athletes may ventilate more than 200 L/min during maximal exercise ¹², the resulting increased resistance may further limit the accuracy of some systems ¹¹. The capacity for this kind of extreme breathing is likely a challenge for many breath-by-breath systems and even for systems with mixing bag technology. In these cases with very high ventilation, another factor that should be considered is the resistance to breathing (RES) that is attributable to the metabolic system's hardware. Effects on pulmonary ventilation would be expected due to increased airway resistance caused by the dimensions of the hoses, breathing valves, flowmeters, and mixing chambers¹³.

To minimize RES when using a Douglas Bag system, it has been recommended that the hose should be 30 mm or greater in internal diameter (ID), but without stating the hose's maximum length ¹⁴. Saltin and Åstrand ¹⁵ noted that in a Douglas Bag system with a hose ID of 35 mm and length of 0.5 m, the pressure difference (Δp) measured between the ambient air and inside of the system hardware was 1, 3, 6 and 10 cmH₂O (98, 294, 588 and 981 Pa) at air flow rates of 100, 200, 300 and 400 L/min, respectively. Gore et al. ¹⁶ recommended that the Δp should be less than 6 cmH₂O at flows up to 300 L/min and hoses should be greater than 30 mm in ID and no longer than 1.5 m on either the inspiratory or expiratory side. However, the standard lengths of hoses delivered with common metabolic systems (Jaeger Oxycon Pro, Carefusion Germany 234 GmbH, Hoechberg, Germany; Moxus Modular Metabolic System, AEI Technologies Inc., Pittsburg, USA; and AMIS 2001, Innovision A/S, Odense, Denmark) are often 1.7 to 2.7 m (Hans Rudolph Inc., Shawne, USA and Flexible ducting U62, Senior Aerospace BWT, Adlington, UK).

A recent study ¹³ investigated the RES in three commonly used automated metabolic systems and the results showed that the RES was much higher than in previous recommendations. Significant differences were found between the systems and their individual breathing valves, hoses, flowmeters and mixing chambers that were included as parts. Interestingly, the lowest resistance was found with a custom made Douglas Bag system, which had about half the resistance of the automatic metabolic systems. Another interesting observation is that Δp for two of the tested breathing valves was found to be higher than in the information on the manufacturer's website (Hans Rudolph Inc., Shawne, USA).

However, a difference in Δp can be expected, depending on the circumstances in which it is measured. For instance, manufacturers usually measure the Δp in their two-way nonrebreathing valves during a static (constant) one-direction flow, while human pulmonary ventilation entails a dynamic intermittent flow. In the study by Ainegren¹³, the Δp was measured in the breathing valve mouthpiece adapter and the inspiratory and expiratory flow were obtained by a metabolic simulator. The metabolic simulator provides a dynamic intermittent flow, similar to human breathing, allowing for more relevant conditions when measuring Δp compared to a constant flow condition. The alternating flow between the breathing valve's inspiratory and expiratory ports must pass both in and out through the mouthpiece and adapter; in practice this is a prolongation of the upper airways and, together with the valve volume, there is an increase in anatomical dead space, which accelerates the flow back and forth through the mouthpiece and valve. This intermittent flow produces twice the mean flow rate (during the flow) and a much higher peak flow than in a constant flow. The accelerations and higher flow rates lead to more turbulence and a higher overall mean Δp than for a constant flow at the same ventilation: both manufacturers and researchers present an exponential increase in Δp for a linear increase in flow rate. Further, the opening-closing procedure of the breathing valve's inspiratory and expiratory ports may also result in different

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 Δp compared to when they are continuously open to a flow. The air density also varies, therefore Δp may not be normalized against a standard pressure. However, the extent of a likely difference in Δp due to different measurement circumstances is so far unknown.

The oxygen cost of the respiratory muscles' work of breathing and the effects of different RES have been studied by using a proportional assist ventilator and installing obstacles that increase RES in the hardware that distributes the air flow ¹⁷⁻²². The results show that the oxygen cost for the work of breathing constitutes a significant part of measured whole body \dot{V} O_2 (10-15%) and the large differences in RES have a great influence on physiological and performance measures: e.g. submaximal exercise $\dot{V}O_2$ follows a change in RES, while ventilation changes in the opposite direction. During maximal exercise, ventilation is still changed, as is the subject's performance level, while any effect on $\dot{V}O_2 max$ seems more unclear.

The significance of RES for various types of face masks has also been studied by manipulating the inspiratory and/or expiratory resistance in the parts used for distribution of the air flow ²³⁻²⁷. The results showed both unchanged and increased $\dot{V}O_2$ and heart rate (HR) and unchanged or decreased ventilation on submaximal exercise due to increased RES. For maximal exercise tests, decreased $\dot{V}O_2$ max and performance level were found in those studies where this was measured, while ventilation and HR were either decreased or unchanged due to increased RES. However, these studies had other purposes than to study the effects of the RES of commercial metabolic systems and the subjects were mostly untrained or poorly endurance trained with a low $\dot{V}O_2$ max.

In a recently published paper ²⁸, the physiological effects of different RES in two types of two-way non-rebreathing valves were studied using endurance-trained and recreationally active subjects of both genders. The Δp of the complete metabolic systems hardware was unfortunately unknown, while the difference in pressure between the two tested valves was

 known by the information given by the manufacturers. This study found significantly higher submaximal $\dot{V}O_2$ and energy expenditure for all tested groups with the use of the valve with higher RES, while HR and rated perceived exertion (RPE) remained unchanged. Also, submaximal ventilation was higher for the endurance-trained groups, but not for the recreational groups. Further, on an incremental maximal test, peak oxygen uptake, ventilation, HR and RPE were similar, while time to exhaustion (TTE) was significantly shorter with the use of the valve with higher RES. The reason for the higher submaximal $\dot{V}O_2$ and ventilation and shorter TTE was ascribed to an increase in breathing work and reduced blood flow to the locomotor muscles. However, the subjects RER only reached ~1.00 on the maximal test, a level that is far below the adopted end criterion for a maximal aerobic performance, which means that the validity of the measuring equipment and experiments can be questioned.

To gain knowledge of the energy cost needed for breathing through the hardware of metabolic systems, estimations can be done in the same way as for a fan or a pump that drives a fluid through a pipe system, using Eq. (1):

$$P_{REO} = \Delta p \dot{V} / \eta \tag{1}$$

where P_{REQ} is the required power (watts), Δp is the pressure (Pa) difference measured between the ambient air and the inside of the system hardware, \dot{V} is the volumetric air flow rate (m³/s) and η is the mechanical efficiency, which in this case is the ratio of the obtained power to the athlete's metabolic rate (a unit analysis for the P_{REQ} equation gives: $\frac{N}{m^2} \cdot \frac{m^3}{s} = \frac{Nm}{s}$ $= \frac{J}{s} = watt$). The metabolic rate can be calculated from the caloric equivalent (CE), which is based on the respiratory exchange ratio (RER) and is well known for a given $\dot{V}O_2$ (CE: 4.686 - 5.047 kCal per L of $\dot{V}O_2$ at RER 0.707 - 1.00), and converted to power (e.g. 1 watt = 0.01433 kCal/min). Applying the P_{REQ} equation in the study by Kim ²⁸, using the difference in Δp between the two valves (1.8 cmH₂O, 176.5 Pa), a mean of the inspiratory and expiratory volumetric flow in the valve of 0.0067 m³/s (ventilation 200 L/min), a η of 20% and a CE of 5

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kCal per L of $\dot{V}O_2$, gives a P_{REQ} of 5.9 watts, 0.08 kCal/min and a $\dot{V}O_2$ of 0.02 L/min. Further, calculating the P_{REQ} from the study on RES in hardware for automated metabolic systems ¹³, using the same mean flow in the breathing valve as above and a mean Δp of 856.5 Pa (inspiratory and expiratory Δp of 913 and 800 Pa), which was measured for a complete system hardware (Moxus Modular Metabolic System, AEI Technologies Inc., Pittsburg, USA) supplied with the same type of two-way non-rebreathing valve (2700, Hans Rudolph Inc., Shawne, USA) as one of the valves used in Kim et al. ²⁸, gives a P_{REQ} of 28.6 watts, 0.4 kCal/min and a $\dot{V}O_2$ of 0.08 L/min.

Thus, even if the RES in the hardware of modern automated metabolic systems has been found to be higher than earlier systems and recommendations, it seems unlikely that there are any significant effects on oxygen cost and energy expenditure due to RES in the hardware of present day automatic metabolic systems. However, with the increased use of automated metabolic systems, instead of Douglas Bag systems with much lower RES ¹³, and longer hoses for the distribution of inhaled and exhaled air to the measurement system's sensors, experiments investigating the influence of RES on elite athletes' ventilation and aerobic energy expenditure have an increasingly greater relevance.

Against this background, the aim of the present study was to investigate the effects of a relatively higher RES, typical of some modern metabolic systems on pulmonary ventilation and oxygen uptake in athletes with a very high $\dot{V}O_2$ max and correspondingly high ventilation capacity. Based on the present calculations of energy cost needed for breathing through the hardware, the present hypothesis is that the measurement of oxygen uptake is not affected by the RES previously found in modern stationary metabolic systems.

Methods

Measurements of breathing resistance

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The resistance to breathing was calculated as the ratio of delta (driving) pressure to the rate of air flow as shown in Eq. (2):

$$RES = \Delta p / \dot{V}$$
(2)

where RES (Pa/L/s) is the resistance to breathing, Δp (Pa) is the pressure difference measured between the ambient air and the inside of the metabolic systems hardware and \dot{V} (L/s) is the air flow rate.

The Δp was measured (-2500 to 2500 Pa, GMSD25 MR, Swedish Thermo Instrument AB, Täby) at a rate of 100 Hz and filtered at 8 Hz using a Butterworth-filter in Microsoft Excel. For both inspiratory (\dot{V}_I) and expiratory (\dot{V}_E) flows, the Δp in the systems' hardware should be greatest near the subject's mouth. Thus, the Δp was measured in the adapter between the mouthpiece and the breathing valve by replacing the regular adapter with a custom-made adapter manufactured from ABS plastic, using additive manufacturing (Mid Sweden University). The custom-made adapter's geometry was equivalent to the manufacturer's original adapter, but supplemented with connections for measuring negative and positive Δp during \dot{V}_I and \dot{V}_E , respectively. Since the measured Δp is negative compared to the ambient air during inspiration and positive during expiration, a negative sign is reported before the values for the inspiratory RES (RES_I). Further, the RES_I and expiratory RES (RES_E) were calculated from flows in at ambient (ATPS) and body (BTPS) temperature, pressure and water vapor saturation conditions, respectively.

Setup for metabolic measurements

Two different hardware setups were used to achieve experimental conditions with low RES (LOW_{RES}) and high RES (HIGH_{RES}). For the LOW_{RES}, a Douglas Bag system described in an earlier paper ¹³ was used. This system was found to have approximately half the RES of three

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investigated automated metabolic systems and only one-third of the RES of the automated system with the highest RES. This LOW_{RES} hardware setup included the same type of breathing valve and hose as an automated metabolic system (AMIS 2001, Innovision A/S, Odense, Denmark) and a custom-built three-way valve (Håkan Eriksson, Karolinska University Hospital, Stockholm, Sweden) to distribute the expired air, either to the ambient surroundings or for collection to bags (130 L, PU coated fabric, C. Fritze Consulting, Svedala, Sweden). The HIGH_{RES} experiments were carried out using components (and resistance) added to the Douglas Bag system described above. The standard setup was supplemented with the same type of hose on the inspiratory side and a valve mounted on the outer end of the hose. This extra valve was a two-way T-shape breathing valve (2700, Hans Rudolph Inc., Shawne, USA) with the opening plugged where the mouth piece is mounted in normal use, which made it a one-way valve with the air flow able to pass straight through the inlet and outlet of the valve. The same type of modified breathing valve was also mounted on the expiratory side between the hose and the inlet to the three-way valve.

In order to acquire knowledge of the resistance in the LOW_{RES} and HIGH_{RES} setups prior to experiments with athletes, a study was carried out using a metabolic simulator (Metabolic Simulator No 17056, Vacumed, Ventura, CA, USA) with the ability to select standardized \dot{V} by mimicking different tidal volumes (V_T) and breathing frequencies (f_B) ¹³. A V_T of 3 L and f_B of 15, 30, 45, 60 and 75 V_T/min to give the mean \dot{V} of 45-225 L/min, with the corresponding mean \dot{V}_I and \dot{V}_E (during flow) of 1.5, 3.0, 4.5, 6.0, and 7.5 L/s, was created using the simulator. Five adjacent curves of Δp and \dot{V} formed the results, which showed that within the range of tested air flows, LOW_{RES} was -46.1 ± 7.3 and 53.2 ± 12.9 Pa/L/s, while HIGH_{RES} was -155.6 ± 13.4 and 154.7 ± 15.7 Pa/L/s, for the inspiratory and expiratory sides, respectively. Hence, this result shows a large difference in resistance between LOW_{RES} and HIGH_{RES} experimental conditions, with the HIGH_{RES} similar to that reported for automated

metabolic systems ¹³. Consequently, it was stated that this experimental setup should provide a valid and representative range of RES to study the influence of resistance to breathing.

The validity of the mechanical lung simulator was also checked using both LOW_{RES} and HIGH_{RES} setups and the same \dot{V} as mentioned above. After sampling a number of 43 V_T (129 L) into a 130 L Douglas Bag, it was emptied in a water-sealed spirometer (custom made and enlarged copies of a Collins-Tissot). This showed that the volume from the mechanical lung simulator was like the volume determined in the spirometer: 128.1 ± 1.5 L (P > 0.05), coefficient of variation = 1.14%. The laboratory air pressure (*p*), temperature (T), relative humidity (RH) and density (*p*) were 983 hPa, 22.5° C, 38% and 1.16 kg/m³ during the measurements with the mechanical lung simulator.

The Douglas Bag's content of expired gas fractions was measured using O₂ and CO₂ gas analyzers (Skiers: AEI Technologies Inc., Pittsburg, USA; Rowers: Vacumed Gold Edition, Ventura, USA and AMIS sport, Innovision A/S, Odense, Denmark), while the bag's gas volume (BTPS) was measured in the spirometer, which was equipped with a temperature sensor for calculations of STPD gas volume. The gas analyzers were calibrated with the following O₂ and CO₂ concentrations: AEI Techn., $16\% \pm 0.02$ rel.% and $4\% \pm 1\%$; Vacumed, $15\% \pm 0.04\%$ and $6\% \pm 0.1\%$; AMIS, $15\% \pm 0.02$ rel.% and $5\% \pm 0.1$ rel.%. *Subjects and exercise protocol*

A total of seven endurance trained athletes, including four rowers and three cross-country skiers (age, 26.3 ± 6.3 yr; body height, 1.91 ± 0.04 m; body weight, 91.6 ± 9.7 kg), who compete at a high international level volunteered to take part in the study. The study was approved by the Regional Ethical Review Board, reg no. 2016-418-32M. Before the experiments started, the subjects signed their voluntary approval to participate in the study. The study. The study.

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All subjects participated in both experimental conditions, where three of the subjects started with LOW_{RES} and four started with the $HIGH_{RES}$ setup. A rowing ergometer (Concept 2 Inc, Morrisville, USA) was used for the rowers' experiments while the skiers roller skied on a treadmill (Rodby Innovation AB, Vänge, Sweden) using the classical style diagonal stride technique. The time between the two experimental conditions was 4.5 h for the rowers and one to two days for the skiers.

Before the data collection began, there was a 10-minute warm-up period on the initial submaximal workload. The exercise protocol included three submaximal workloads (Sub 1, Sub 2 and Sub 3, corresponding to 57 ± 6 , 66 ± 4 and 75 ± 3 % of \dot{VO}_2 max) for four minutes each, with a one-minute break between them, followed by a 10-minute break before a maximal test (Max) was performed. The rowing ergometer power (P) of the three submaximal workloads was predetermined at 230, 260 and 290 W, while the maximal test was a 6-minute all-out test, where the mean P was registered. The skiers performed the three submaximal workloads on treadmill inclinations and speeds of 4° at 2.5 m/s, 5° at 2.64 m/s and 6° at 2.78 m/s. Max was a ramp test with constant speed (3.06 m/s) and an increase in treadmill inclination by 1° every minute, starting at 3°, until voluntary exhaustion. If the skier performed more than 8 minutes (completed 10° inclination), the speed was increased by 0.14 m/s every minute until exhaustion. The skier's time to exhaustion (TTE) was also registered from the maximal tests. The roller skis' (Swix Classic Roadline C2, Lillehammer, Norway) rolling resistance coefficient (μ_R) was carefully investigated in another study, conducted during the same period, using special equipment for this purpose ²⁹. This study showed that μ_R did not change during the period the experiments were carried out ($\mu_R = 0.025$).

Data collection and analysis

The data collection took place at low altitude (< 300 m above sea level) at three different laboratories. The skiers performed tests at Mid Sweden University, Östersund, Sweden ³⁰ and the rowers performed tests at the laboratory at Swedish Sports Confederation, Bosön, Sweden or the Department of Sports Science and Clinical Biomechanics, University of Southern Denmark, Odense, Denmark. The laboratories' p, T, RH and ρ were 990 ± 26 hPa, 18.8 ± 1.0° C, 45 ± 12% and 1.18 ± 0.03 kg/m³ during the experiments with the athletes.

Heart rate (HR) (Polar Electro OY, Esbo, Finland) and Δp were recorded during the last minute of each submaximal workload, while a Douglas Bag was filled with expired air. During the maximal test, HR, Δp and Douglas Bags were sampled throughout the test (filling time per bag 30-40 s). Directly after each submaximal workload and the maximal test, the subjects rated their perceived exertion in breathing (RPE_B 6-20) and a capillary blood sample was taken from a fingertip to analyse blood lactate (La) (Biosen S-line Lab+, EKF-Diagnostic, Cardiff, UK).

The results of HR, \dot{V}_E , f_B , $\dot{V}O_2$, ventilatory equivalent ($\dot{V}_E/\dot{V}O_2$), RER, RES_I and RES_E were calculated as mean and standard deviation (SD) from the last minute for each submaximal workload. From the maximal test, the results were calculated from 30 s of sampling and from the same time during the test as when the highest $\dot{V}O_2$ ($\dot{V}O_2$ max) was obtained. The subjects' f_B was determined by counting the number of Δp curves and the V_T from the ratio of \dot{V}_E/f_B . The \dot{V}_I and \dot{V}_E were calculated at current ATPS and BTPS conditions, respectively, while the $\dot{V}O_2$ and $\dot{V}O_2$ max were calculated according to STPD conditions.

Due to the volume of CO₂ produced not being the same as the O₂ consumed, except when RER is 1.00, \dot{V}_I and \dot{V}_E are not exactly the same ³¹. Since the Douglas Bag method measures the athlete's \dot{V}_E only, to calculate RES_I, \dot{V}_I was calculated using Eq. (3), which is known as the Haldane transformation:

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$$\dot{V}_I = \dot{V}_E (F_E N_2 / F_I N_2)$$
(3)

where F_1N_2 is the fraction (%) of nitrogen in inspired air and F_EN_2 is the fraction of nitrogen in expired air computed from the measured gas fractions of expired oxygen and carbon dioxide as shown in Eq. (4):

$$F_E N_2 = 1 - (F_E O_2 + F_E C O_2)$$
(4)

Furthermore, the \dot{V}_I and \dot{V}_E flow rates (L/s) are also dependent on the relative time (%) between inspiration and expiration, which was calculated for each flow direction (t_I and t_E) by the number of samples for each flow (S_I and S_E , respectively) divided by the total sum of samples as shown in Eq. (5):

$$t_{I} = S_{I} / (S_{I} + S_{E}); t_{E} = S_{E} / (S_{I} + S_{E})$$

(5)

Statistics

The statistical analyses were performed in SPSS for Windows statistical software release 24.0 (SPSS Inc., Chicago, Illinois, USA). Statistical differences (P < 0.05) between LOW_{RES} and HIGH_{RES} test conditions were evaluated for HR, La, RER, RPE_B, \dot{V}_I (L/s), \dot{V}_E (L/min and L/s), $\dot{V}_E/\dot{V}O_2$, V_T , f_B , $\dot{V}O_2$, $\dot{V}O_2$ max, RES_I, RES_E, TTE and P, using the paired t-test. On Sub 1 for LOW_{RES}, the Δp curve for one of the subjects was unfortunately not satisfactorily registered. Thus, the results for RES_I and RES_E on Sub 1 are reported for six subjects only.

Results

No significant (P > 0.05) difference was found in \dot{VO}_2 between the LOW_{RES} and HIGH_{RES} experimental conditions either at Sub 1, Sub 2, Sub 3 or Max (see Fig. 1). On the contrary, \dot{V}_E (L/min) was significantly lower (< 0.05) for the HIGH_{RES} test conditions at all workloads (see Fig. 2). The lower \dot{V}_E for HIGH_{RES} was due to a lower f_B , while V_T was unchanged for all workloads, except the lowest (see Table 1). Also, due to the lower pulmonary ventilation per minute, the \dot{V}_E/\dot{VO}_2 , \dot{V}_I and \dot{V}_E (L/s) were significantly lower using HIGH_{RES}. Further, the F_EO_2 and F_ECO_2 were significantly different for all workloads, with lower F_EO_2 and higher F_ECO_2 for the HIGH_{RES} experiments. Significant differences were also found for both RES₁ and RES_E between the LOW_{RES} and HIGH_{RES} experiments for all workloads (see Table 1). However, no differences were found between conditions with regard to La, HR, RER, t_I, t_E, TTE, P and RPE_B, except for RPE_B on Sub1 (see Table 1).

Figure 1 near here please

Figure 2 near here please

Table 1 near here please

Discussion

To the best of the authors' knowledge, this is the first study to evaluate the influence of different RES in metabolic systems on pulmonary ventilation and oxygen uptake in elite athletes with very high aerobic power.

The authors hypothesized that in athletes with a very high aerobic power and ventilation capacity, the high resistance against breathing, which has previously been estimated in modern stationary metabolic systems, would not affect the measurement of $\dot{V}O_2$ when compared to the minor breathing resistance that exists in the traditional Douglas Bag method. Results showed that the measured variables $\dot{V}O_2$, La, HR and RER were unchanged between

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the LOW_{RES} and HIGH_{RES} experimental conditions during both submaximal and maximal
exercise. Also, there were no differences in TTE and P between the two test conditions.
However, breathing frequency and pulmonary ventilation were affected during both
submaximal and maximal exercise.
Calculations of the P_{REQ} and oxygen cost, based on the measured Δ*p* and volumetric flow

of the seven subjects for the LOW_{RES} and HIGH_{RES} testing conditions, are presented in Table 2. Mechanical efficiency ($\eta = 17.9 \pm 0.7\%$) was established from the three submaximal workloads using the ratio of the power of the rowing machine and the rower's metabolic rate calculated from their RER, CE and $\dot{V}O_2$. The calculated result assumes that the gas flow is not compressed: the relatively small Δp between the inside of the hardware and the ambient air (0.2 - 2%) shows that the air is likely to be minimally compressed. Further, mechanical efficiency can vary by a few percent between individuals, different types of sports and activity levels and may also be different for the respiratory muscles in comparison to the muscles used for locomotion. However, these possible causes of error are not likely to dramatically change the calculated results of P_{REO} and oxygen cost.

Table 2 near here please

As can be seen in Table 2, the P_{REQ} and oxygen cost is very small. Even at maximal exercise at the HIGH_{RES} test condition, the calculated cost for breathing through the hardware is less than 1.5% of the measured energy expenditure and oxygen uptake. This should be less than any day-to-day variation measured with metabolic systems, including the Douglas Bag method. Even though the difference in hardware resistance between LOW_{RES} and HIGH_{RES} is both large, valid and representative for today's metabolic systems, the calculated energy cost appears to be too small, and therefore would not be expected to have a significant influence

on the results of $\dot{V}O_2$. Thus, it is not surprising that the actual measurements of the athletes' \dot{V} O_2 were not significantly different between the LOW_{RES} and HIGH_{RES} conditions. As a comparison, Wetter et al. ²⁰ calculated the oxygen cost for the respiratory muscles' work of breathing using an equation by Aaron et al. ¹⁷ at 0.18, 0.30 and 0.61 L/min for exercises of 50%, 75% and $\dot{V}O_2$ max, and the oxygen cost of a large difference in RES (<10 cmH₂O/L/s (981 Pa/L/s)) to 0.11, 0.20 and 0.54 L/min, which was verified in their experiments on 50 and 75% of $\dot{V}O_2$ max. However, this study used a proportional-assist ventilator to reduce work on the inspiratory muscles during unloading and added mesh screens during loading with many times larger RES than in the present study.

Nevertheless, the difference in RES between the two experimental conditions clearly affected the athletes' pulmonary ventilation, even at relatively low flow rates. Increasing the RES from low to a high RES similar to that of hardware used by manufacturers of automated metabolic systems significantly reduced the \dot{V}_E by 5.9 ± 1.1% (see Fig. 2). The reduction was due to decreased f_B , while the V_T was unchanged, although there was a trend to a larger V_T for the HIGH_{RES} test condition. Thus, it might be that a possible increase in submaximal oxygen and energy consumption and a decrease in TTE and P, due to increased RES, was cancelled out due to the decreased f_B and \dot{V}_E , resulting in similar work of breathing and $\dot{V}O_2$. However, the difference in RES and \dot{V}_E was not perceived by the athletes, which is shown by similar ratings of perceived exertion for breathing between the two test conditions.

Despite the very high $\dot{V}O_2 max$ (5.8 – 6.8 L/min) for the athletes taking part in this study, the ventilation at $\dot{V}O_2 max$ (< 206 L/min) was not as high as that reported in some other studies ¹². The tests were carried out on low land (less than 300 m above sea level), even though the air density was lower than at standard sea level conditions. With an even lower air density, the ventilation and $\dot{V}_E/\dot{V}O_2$ can be expected to be higher than in this study. Even though the viscosity and friction against the hardware inner surfaces will be a little lower with

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decreased density, resistance to breathing in the hardware of a metabolic system being used at high altitude should be addressed.

There were also differences in the measured F_EO_2 and F_ECO_2 between the LOW_{RES} and $HIGH_{RES}$ test conditions, where the former decreased and the latter increased in the $HIGH_{RES}$ test condition. This is well in line with the decrease in breathing frequency and pulmonary ventilation found for $HIGH_{RES}$ since When the breathing frequency changes, the duration for each breath in the lungs also changes. was Thus, with an increased duration, and thereby the time for the diffusion of gases to proceed between the pulmonary capillaries and alveoli is extended. This which should be the reason why a greater difference occurred for $HIGH_{RES}$ between the inspiratory and expiratory gas fractions for O_2 and CO_2 . A changed V_T also entails changed expired gas fractions, since the gas volumes in the lower respiratory tract are changed proportionally to a changed V_T (while anatomical dead space remains relatively constant). However, the athlete's V_T was not different between the two experimental conditions.

One important concern of this finding is that researchers and manufacturers should be aware that breathing resistance differences between an automated metabolic system and the reference method being used to validate the system can imply changes in \dot{V}_E that are somehow compensated for by changes in F_EO₂ and F_ECO₂, resulting in the same $\dot{V}O_2$, as shown by Jensen ⁷. In addition, during progressive submaximal work, $\dot{V}_E/\dot{V}O_2$ and $\dot{V}_E/\dot{V}CO_2$ are often used to determine the so-called ventilatory thresholds. These provisions could thus depend on whether a system with high or low RES is used. Prior to the study's implementation, the option of using a representative automated metabolic system for the HIGH_{RES} setup was discussed, but this option was rejected due to possible validity problems with the comparative flow measurements. If an automated system had been used for the HIGH_{RES}, speculation would have arisen at this stage about whether the established difference

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in ventilation was due to a validity problem with the automated system flow sensor or the difference in breathing resistance.

The $\dot{V}_E/\dot{V}O_2$ was lower for HIGH_{RES} due to the lower pulmonary ventilation and similar oxygen uptake compared to LOW_{RES}. The $\dot{V}_E/\dot{V}O_2$ increased from Sub 1 to Max from 21.0 to 30.7 and 19.7 to 28.8 for LOW_{RES} and HIGH_{RES}, respectively. Increasing the $\dot{V}_E/\dot{V}O_2$ and thereby the oxygen cost of breathing when hyperventilating is significant ¹⁷. Saltin ³² and Dempsey ^{33, 34} discuss how a reduction in the oxygen saturation of the arterial blood (SaO₂) that occurs in highly trained athletes might be a cost benefit between lowering the energy demands needed for the increased hyperventilation at the expense of a small reduction in SaO₂. In the present study, the $\dot{V}_E/\dot{V}O_2$ for the submaximal workloads was within the lower range of the critical level (20 to 25) indicated by Saltin and above this level for the maximal tests, but lower than that seen in some other studies for maximal exercise (30 to 40) ^{35, 36}. Another aspect contributing to lowering $\dot{V}_E/\dot{V}O_2$ would be that \dot{V}_E increases less proportional $(M^{0.55})$ with body weight (M) than $\dot{V}O_2$ max ($M^{0.73}$), which would limit maximal ventilation more in relatively heavy athletes (as in this case)¹². However, the $\dot{V}_E/\dot{V}O_2$ and oxygen partial pressure gradient between the alveoli and pulmonary capillaries were all apparently still sufficient to cause an effective gas exchange and similar oxygen carrying capacity in the blood, which is indicated by the changes in F_EO₂ and F_ECO₂ that are discussed above and similar metabolic and performance measures for the test with high breathing resistance in comparison to the test with low resistance.

The results of \dot{V}_E , $\dot{V}O_2$, $\dot{V}O_2max$ and TTE in this study exhibit both similarities and deviations from the results of other studies investigating the influence of breathing resistance (see Introduction). The reason for this may consist of several factors, such as the participant's different ventilation, aerobic and performance capacity, protocols, equipment and of course the differences in RES of the hardware being studied. In many of the other studies, the RES

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was much higher than in this study and large differences in inspiratory vs. expiratory RES were also common.

The results of this study should be most useful for researchers, clinicians and test leaders who work with studies of healthy endurance trained athletes who are active at a very high performance level. Also, both users and manufacturers should consider resistance differences between systems in the validation processes, which may result in differences in \dot{V}_E , but not necessarily in $\dot{V}O_2$. Even though the athletes' performances and oxygen uptake were unaffected by the difference in RES in the hardware being used, the authors recommend being vigilant with metabolic systems with a high RES. A conscious choice of hardware and its components can make a great difference to RES. Also, if extremely long hoses are necessary, e.g. for tests on a very large treadmill, there should be consideration about using a Douglas Bag system with very low RES instead of an automated system with higher RES due to the system sensors and mixing chambers.

Conclusion

The results of this study show that the differences in RES of metabolic systems influence elite endurance athletes' \dot{V}_E at low to very high workloads and this affects the expired gas fractions, but not the submaximal $\dot{V}O_2$, $\dot{V}O_2$ max and performance in a laboratory setting at sea level.

Acknowledgements

Many thanks go to Glenn Björklund, Tobias Elgh and Thomas Lindberg (the Swedish Sports Confederation, Bosön, Sweden) for great assistance with the physiological measurements carried out on the rowers.

Declaration of conflicting interests

The author(s) declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article

Funding

The author(s) received no financial support for the research, authorship, and/or publication of

this article.

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Table legends

Table 1. Compilation of measured data from three submaximal workloads and a maximal test for test conditions with low (LOW_{RES}) and high (HIGH_{RES}) breathing resistance.

Table 2. Calculated required power (P_{REQ}) and oxygen cost ($\dot{V}O_2$) for breathing through the two types of hardware with low (LOW_{RES}) and high (HIGH_{RES}) breathing resistance.

Figure legends

Figure 1. Oxygen uptake with low (LOW_{RES}) and high (HIGH_{RES}) breathing resistance at submaximal and maximal exercise.

Figure 2. Ventilation with low (LOW_{RES}) and high (HIGH_{RES}) breathing resistance at submaximal and maximal exercise. *Significant (P < 0.05) difference from LOW_{RES}.

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44 45 46 **Table 1**. Compilation of measured data from three sub maximal workloads and a maximal test for test conditions with low (LOW_{RES}) and high (HIGH_{RES}) breathing resistance.

		St	ub 1	S	ub 2	S	ub 3	Ν	/lax
		LOW _{RES}	HIGH _{RES}	LOW _{RES}	HIGH _{RES}	LOW _{RES}	HIGH _{RES}	LOW _{RES}	HIGH _{RES}
RESI	Pa/L/s	-40.7 ± 6.7	$-164.1 \pm 21.2*$	-41.1 ± 7.6	$-158.3 \pm 25.9*$	-42.1 ± 6.3	$-151.5 \pm 24.5*$	-52.3 ± 5.5	$-163.4 \pm 16.2*$
RESE	Pa/L/s	$53.9.0\pm7.9$	$161.7 \pm 7.1*$	55.6 ± 9.7	$157.4 \pm 9.6*$	57.4 ± 7.5	$156.5 \pm 14.9*$	71.0 ± 4.9	$168.3 \pm 5.4*$
₿,	L/s	2.54 ± 0.46	2.34 ± 0.73	2.93 ± 0.43	$2.77\pm0.46*$	3.40 ± 0.60	$3.25 \pm 0.51*$	6.08 ± 0.52	$5.66 \pm 0.38*$
₿ _E	L/s	2.47 ± 0.53	$2.27\pm0.40\texttt{*}$	2.99 ± 0.52	$2.78\pm0.46*$	3.47 ± 0.55	3.31 ± 0.41	6.48 ± 0.63	$6.12\pm0.60*$
t _I	%	0.50 ± 0.02	0.49 ± 0.02	0.51 ± 0.03	0.50 ± 0.03	0.51 ± 0.02	0.51 ± 0.02	0.51 ± 0.02	0.52 ± 0.01
t _E	%	0.50 ± 0.02	0.51 ± 0.02	0.49 ± 0.03	0.50 ± 0.03	0.49 ± 0.02	0.49 ± 0.02	0.49 ± 0.02	0.48 ± 0.01
VT	L	2.26 ± 0.21	2.68 ± 0.59	2.50 ± 0.38	2.62 ± 0.32	2.63 ± 0.37	2.77 ± 0.37	3.18 ± 0.37	3.21 ± 0.46
\mathbf{f}_{B}	V_T / min	33.6 ± 8.4	26.9 ± 8.9	36.8 ± 9.2	$32.3 \pm 8.1*$	40.1 ± 9.9	$36.4 \pm 8.9*$	59.9 ± 8.1	$55.8\pm7.6*$
F_EO_2	%	15.79 ± 0.50	$15.48\pm0.45*$	15.93 ± 0.61	$15.61\pm0.65*$	16.07 ± 0.69	$15.81\pm0.63*$	17.19 ± 0.38	$16.95\pm0.38*$
F_ECO_2	%	4.50 ± 0.32	$4.85\pm0.28*$	4.48 ± 0.39	$4.79\pm0.40*$	4.45 ± 0.46	$4.73\pm0.46*$	4.07 ± 0.36	$4.36\pm0.46*$
V _E /VO	2	21.0 ± 2.1	$19.7 \pm 1.6 *$	21.7 ± 2.4	$20.4\pm2.5*$	22.5 ± 2.9	$21.3 \pm 2.5*$	30.7 ± 3.1	$28.8\pm2.2*$
HR	b/min	130.3 ± 13.9	130.6 ± 12.0	144.9 ± 9.4	144.0 ± 9.1	158.0 ± 5.5	157.0 ± 6.1	182.7 ± 7.3	182.1 ± 8.1
La	mMol/L	0.98 ± 0.17	1.27 ± 0.80	1.09 ± 0.34	1.09 ± 0.28	1.60 ± 0.51	1.57 ± 0.47	12.0 ± 2.4	12.6 ± 2.6
RER	VCO_2/VO_2	0.84 ± 0.04	0.86 ± 0.04	0.87 ± 0.05	0.87 ± 0.05	0.89 ± 0.05	0.90 ± 0.04	1.11 ± 0.05	1.11 ± 0.06
RPE _B	6-20	9.1 ± 1.6	9.4 ± 1.7	11.0 ± 2.0	$11.7 \pm 1.7*$	13.1 ± 1.1	13.7 ± 1.5	19.1 ± 1.2	19.2 ± 1.2
Р	watts							481.0 ± 28.3	480.0 ± 30.9
TTE	min.sec							8.23 ± 0.41	8.16 ± 0.35

* Significant (P<0.05) difference from LOW_{RES}. RES_I and RES_E (inspiratory and expiratory resistance), \dot{V}_I and \dot{V}_E (inspiratory and expiratory flow rates), t_I and t_E (inspiratory and expiratory relative time), V_T (tidal volume), f_B (breathing frequency), F_EO_2 and F_ECO_2 (expiratory gas fractions), V_E/VO_2 (ventilatory equivalent), HR (heart rate), La (blood lactate), RER (respiratory exchange ratio), RPE_B (ratings of perceived exertion in breathing), P (mean power from 6-min all-out test on rowing ergometer), TTE (time to exhaustion from the treadmill ramp maximal roller skiing test).

Table 2. Calculated required power (P_{REC}) and oxygen cost (\dot{VO}_2) for breathing through the two types of hardware with low (LOW_{RES}) and high (HIGH_{RES}) breathing resistance.

Condition	Workload	I Watts	Watts P_{REQ} kCal·min ⁻¹	
LOW _{RES}	Sub 1	1.67	0.024	0.005
	Sub 2	2.42	0.035	0.007
	Sub 3	3.31	0.047	0.010
	Max	13.60	0.195	0.039
HIGH _{RES}	Sub 1	4.85	0.069	0.014
	Sub 2	6.79	0.097	0.020
	Sub 3	9.25	0.133	0.027
	Max	32.00	0.459	0.091

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