Automated $\dot{V}O_{2max}$ calibrator for open-circuit indirect calorimetry systems

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ABSTRACT

GORE, C. J., P. G. CATCHESIDE, S. N. FRENCH, J. M. BENNETT, and J. LAFORGIA. Automated $\dot{V}O_{2max}$ calibrator for open-circuit indirect calorimetry systems. *Med. Sci. Sports Exerc.*, Vol. 29, No. 8, pp. 1095–1103, 1997. The complete calibration of indirect calorimetry systems involves simultaneous checks of gas analyzers, volume device, and software, and this requires a machine that can mimic accurately and precisely the ventilation and expired gases of an athlete. While previous calibrators have been built successfully, none have matched the ventilatory flows produced by athletes during high intensity exercise. A calibrator able to simulate high aerobic power ($\dot{V}O_{2max}$ calibrator) was fabricated and tested against conventional indirect calorimetry systems that use chain-compensated gasometers to measure expired volume ($\dot{V}_E$ systems) and calibrated electronic gas analyzers. The calibrator was also checked against a system that measures inspired volume ($\dot{V}_I$ system) with a turbine ventilometer. The pooled data from both $\dot{V}_E$ and $\dot{V}_I$ systems for predicted $\dot{V}O_2$ ranging from 2.9 to 7.9 L·min^{-1} and ventilation ranging from 89 to 246 L·min^{-1} show that the absolute accuracy (bias) of values measured by conventional indirect calorimetry systems compared with those predicted by the calibrator was excellent. The bias was < 35 mL·min^{-1} for $\dot{V}O_2$ and carbon dioxide production, < 0.50 L·min^{-1} for ventilation ($\dot{V}_{E\ BTPS}$), −0.02% absolute for the percentage of expired O_2, and +0.02% absolute for the percentage of expired CO_2. Overall, the precision of the measured $\dot{V}O_2$, $\dot{V}CO_2$, and $\dot{V}_{E\ BTPS}$ was ~1%. This $\dot{V}O_{2max}$ calibrator is a versatile device that can be used for routine calibration of most indirect calorimetry systems that assess the ventilation and aerobic power of athletes.

GAS EXCHANGE, CALIBRATION, MAXIMUM OXYGEN CONSUMPTION

Integrated calibration of computerized indirect calorimetry systems, which are in widespread use (14,15,20,22,24), is problematic because human subjects are not reliable standards. The traditional approach to system calibration is calibration of individual components. The accuracy and linearity of O_2 and CO_2 gas analyzers are checked with at least three precision gases (9), and the accuracy of the volume device is verified at either constant or pulsatile flows (6) spanning the physiological range up to 220 L·min^{-1} at body temperature and pressure saturated (BTPS).

An integrated approach to system calibration is biological calibration (14). This requires simultaneous (or at least steady-state) measurement of subjects during submaximal exercise against both a computerized system and a "reference" system based on a Tissot tank (27) or Douglas Bag (3). Such tests are time consuming, and it is even more difficult to check the accuracy of a computerized system when subjects are at maximal aerobic power ($\dot{V}O_{2max}$) since the biological variability in $\dot{V}O_{2max}$ is about 5% (16). While submaximal steady-state exercise checks are useful, verification of a computerized system at "maximal" flow rates of as much as 220 L·min^{-1} BTPS are important, since errors in volume measurement are likely to be the greatest source of imprecision in the calculated $\dot{V}O_{2max}$ (9). Therefore, the computerized system should be verified throughout the complete physiological range.

Huszczuk et al. (10) described a gas exchange calibrator with a simulated oxygen consumption ($\dot{V}O_2$) of 0.2–5.0 L·min^{-1} that is based on dilution of a calibration gas (21% CO_2, balance N_2) with room air. However, several features of their calibrator limit its application. First, it can only be used with systems that measure expired ventilation ($\dot{V}_E$), thereby precluding commonly used computerized systems that have a volume device on the inspiratory side of the circuit. Second, data are only provided up to a maximum ventilation of approximately 125 L·min^{-1}, which is much less than the maximum for athletes. Third, the calibrator dead space will alter the composition of expired gas in a "breathing" frequency and tidal volume dependent manner. Despite this potential for dead space to influence precision, it is unclear how Huszczuk et al. (10) dealt with this complex issue.

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The aim of this project was to address these limitations and develop an accurate and precise automated standard for integrated calibration of indirect-calorimetry systems that measures either inspiratory or expiratory volume. In particular, the automated method was designed to simulate exercise ventilation of trained athletes who can attain $\dot{V}_E > 220$ L·min^{-1}$_{(BTPS)}$, as well as $\dot{V}O_2$ and carbon dioxide production ($\dot{V}CO_2$) of > 7.0 L·min^{-1}.

METHODS

General principles. The high capacity calibrator ($\dot{V}O_{2max}$ calibrator) described here is based on the principles of the expiratory calibrator of Huszczuk et al. (10), but adds a "bag-in-a-box" system to maintain ambient barometric pressure ($P_{B\ amb}$) for both inspired and expired volumes. A piston pump is used to deliver a known mass flow of calibration gas (nominally, 21% CO_2 in 79% N_2) into a volume of room air (nominally, 21% O_2 in 79% N_2) to simulate oxygen consumption and carbon dioxide production as a consequence of dilution of room air by calibration gas. The appendix contains details of all components and their calibration.

Prediction equations. Mathematical expressions for the calculation of predicted inspired minute ventilation ($\dot{V}_I$), $\dot{V}_E$, the fractions of expired water vapor (F_EH_2O), oxygen (F_EO_2) and carbon dioxide (F_ECO_2), and subsequently $\dot{V}O_2$ and $\dot{V}CO_2$ were derived from a detailed analysis of the theoretical breath-by-breath flow and consequent volume displacement characteristics of the $\dot{V}O_{2max}$ calibrator, taking dead space into consideration. In broad terms, this was achieved by modeling the flow of gas through the dead space of the manifold (see Fig. 1, item [10]) during a single breath and determining by integration the volume of the gas components from the bag, the piston pump cylinder, and the manifold (see Fig. 1, items [13], [1], and [10], respectively) that contribute to the expirate.

Calibrator versus conventional criterion systems. The validity of the $\dot{V}O_{2max}$ calibrator to predict $\dot{V}O_2$, $\dot{V}CO_2$, $\dot{V}_{E\ BTPS}$, F_EO_2, and F_ECO_2 was tested against two different systems that use chain-compensated gasometers to measure expired volume ($\dot{V}_E$ systems). One $\dot{V}_E$ system was located 130 m above sea level (Laboratory 1) where $P_{B\ amb}$ ~101.3 kPa, while the second was located ~600 m above sea level (Laboratory 2) where $P_{B\ amb}$ ~94.7 kPa. Laboratory 1 has published widely on the accuracy of volume measurement devices (6–8) and, furthermore, continues to validate gas mixtures using Lloyd Haldane techniques (17). This laboratory conducts regular linearity checks of their electronic gas analyzers (Ametek S-3AI O_2, AEI Technologies, Inc., Pittsburgh, PA; Beckman LB-2 CO_2, Beckman Instruments, Fullerton, CA) that are calibrated at two points immediately before and after data collection. Laboratory 2 always uses three gravimetrically prepared gas mixtures (± 0.02% absolute) to check linearity of the electronic analyzers (Ametek S-3AI O_2 and CD-3A CO_2) immediately before and after data collection. Laboratory 2 has two gasometers that both incorporate precision rotary electronic transducers (catalog number 341–597, Radio Spares Components Pty Ltd, Corby, England) to measure volume as a function of linear displacement of the gasometer bell with a resolution of 17 cm^3. The gasometers coupled to customized software allow on-line acquisition of $\dot{V}O_2$ data every 30 s.

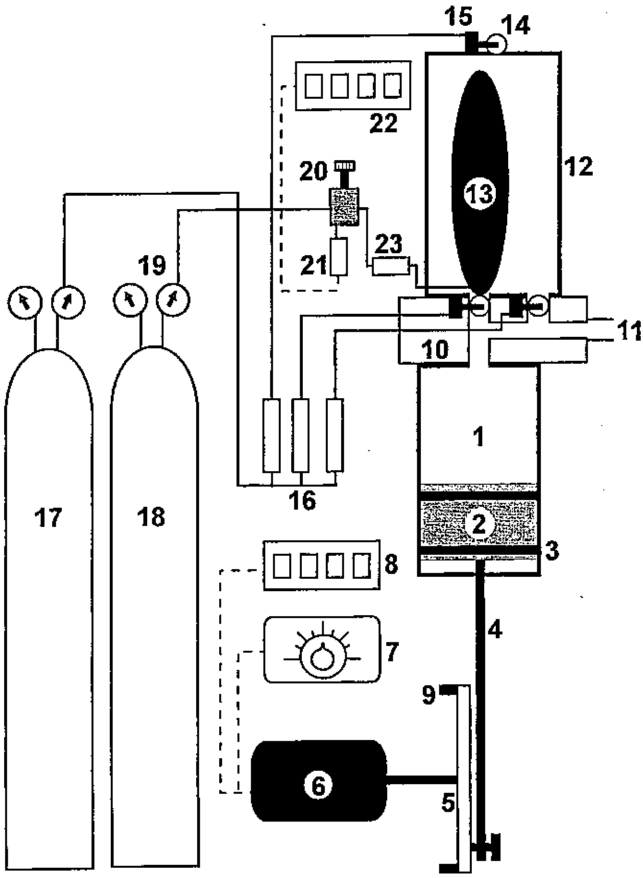

Figure 1—Plan of the key components of the calibrator. Details for each number are contained in the appendix in square parentheses. Note that item [11] is the effective "mouth" of the $\dot{V}O_{2max}$ calibrator and can be used to attach any commercial adult mouthpiece from any indirect calorimetry system.

The integrity of the $\dot{V}O_{2max}$ calibrator to assess $\dot{V}_E$ systems irrespective of ambient pressure was further assessed by running it inside an hypobaric chamber depressurized to 3050 m altitude, where $P_{B\ amb}$ ~ 69.7 kPa. The accuracy and stability of the chamber has been described previously (5). Expired air from the calibrator was collected inside the chamber into evacuated 200-L foil bags (Scholle Industries, Adelaide, Australia), with volumes and gas fractions measured at Laboratory 1. Samples of chamber air were collected into evacuated 4-L foil bags (Scholle Industries) during each run of the calibrator to verify the precise fractions of O_2 and CO_2 in the inspired air that were mixed with the calibration gas.

The validity of the $\dot{V}O_{2max}$ calibrator to assess systems which measure inspiratory volume ($\dot{V}_I$ systems) was also checked at Laboratory 1, using a Morgan Mark II turbine ventilometer (PK Morgan Ltd., Rainham, Kent, UK), which had been shown to have excellent precision and good accuracy (8). A total of 35 tests were conducted on $\dot{V}_E$ systems and 13 tests on $\dot{V}_I$ systems.

Test protocol. A standardized test protocol was used for all calibrator tests. The test system gas analyzers were calibrated and ambient temperature, pressure, and relative humidity in the test laboratory were recorded with identical values entered into the software of the test system and the calibrator. The test system was connected to the $\dot{V}O_{2max}$ calibrator (see Fig. 1, item [11]), and the piston pump was set to the desired tidal volume and frequency. After the flow of calibration gas was initiated with the ambient regulator (see Fig. 1, item [19]), the first 30–60 s of data were ignored in order for the calibrator to achieve a steady state. The next 1–4 min of data for ventilation and expired gas fractions were recorded by the test system, and then the flow of calibration gas was turned off at the regulator. Total time and revolution counts were recorded to calculate precise pumping frequency before turning off the piston pump.

Analysis. The predicted and measured values for $\dot{V}O_2$, $\dot{V}CO_2$, $\dot{V}_{E\ BTPS}$, F_EO_2, and F_ECO_2 from both $\dot{V}_E$ and $\dot{V}_I$ systems were analyzed using Statistica/W (StatSoft, Tulsa, OK). The mean values recorded on each test system during data collection were taken as the "measured values." Accuracy, or bias, of the calibrator was determined for all variables as the mean of the differences between predicted and measured values (1). In addition, 95% confidence intervals ($\pm$ 1.96 SD) of the differences were calculated. The average biases were computed for the pooled data of both $\dot{V}_E$ and $\dot{V}_I$ systems after unpaired Student *t*-tests were conducted to check that there were no significant differences between the measurement systems. Unpaired Student *t*-tests were also used to determine if the average biases were significantly greater than zero.

Pearson's correlation coefficients (r), coefficients of determination (r^2), and SEEs were calculated to determine the degree of association between the values predicted by the $\dot{V}O_{2max}$ calibrator and those measured on well-calibrated conventional indirect calorimetry systems. The slopes and intercepts of the regression lines were also calculated.

The precision of the $\dot{V}O_{2max}$ calibrator for all variables could not be assessed by comparing data from different days because the predicted values change as a function of the ambient barometric pressure, temperature, and relative humidity. Rather, the percent precision was calculated from the measured data collected during one test run with each sapphire orifice (see Fig 1, item [23]) as the (SD/mean) $\times$ 100. This analysis was completed using data from the computerized Tissot $\dot{V}_E$ system at Laboratory 2 and for the turbine $\dot{V}_I$ system at Laboratory 1 which both give values every 30 s (~8 data points per trial). Precision data were also acquired from a breath-by-breath $\dot{V}_E$ system (model CardiO$_2$, Medical Graphics Corporation, St Paul, MN,) which measured 30–55 samples per minute. Precision was calculated for six test runs on each of the three measurement systems for a wide range of simulated $\dot{V}O_2$. Precision results between the three systems were compared with one-way ANOVA and Tukey's *post-hoc* tests to determine significant mean differences. The significance level was set at $P < 0.05$ for all analyses.

RESULTS

Accuracy Overall, there was no significant difference between accuracy of the $\dot{V}O_2$ calibrator on conventional $\dot{V}_E$ or $\dot{V}_I$ systems for any of the measured variables except F_EO_2. When the five data points from the $\dot{V}_E$ tests conducted at 3050 m were excluded from the analyses, there was no difference between the calibrator and conventional indirect calorimetry systems for any of the five variables measured. Data collected at 3050 m could be excluded because the simulator predicted F_EO_2 values as low as 12.61% which is well below the normal physiological limit. Since the calibrator performed with good accuracy within the confines of physiologically normal gas fractions (Fig. 2), there was no justification to suspect that the calibrator lost accuracy, but rather the electronic gas analyzers of the test systems may have incurred a minor loss of linearity. Nevertheless, all data for F_EO_2 were included to calculate average bias because it is a more stringent test of the calibrator if bias is inflated.

Throughout the $\dot{V}O_2$ range of 2.7 to 7.9 L·min^{-1}, the average bias of values measured by conventional indirect calorimetry systems compared with those predicted by the calibrator was 23 mL·min^{-1} for $\dot{V}O_2$, 33 mL·min^{-1} for $\dot{V}CO_2$ (Fig. 3), −0.46 L·min^{-1} for $\dot{V}_{E\ BTPS}$ ranging from 79 to 246 L·min^{-1}, −0.02% (absolute) for F_EO_2 and 0.02% (absolute) for F_ECO_2 (Fig. 2). The average biases were all significantly greater than zero, except for F_ECO_2. The r^2 for predicted versus measured values for $\dot{V}O_2$, $\dot{V}CO_2$, and $\dot{V}_{E\ BTPS}$ were 0.999 ($P < 0.001$), with SEE of 0.063, 0.065, and 1.302 L·min^{-1}, respectively. The r^2 for F_EO_2 and F_ECO_2 were 0.998 and 0.996 ($P < 0.001$), with SEE of 0.05 and 0.07% absolute, respectively. The slope of the regression lines was near 1.00 (range 1.017–0.985) for all variables.

Precision. Precision measures on the breath-by-breath system were significantly higher than the other two systems for $\dot{V}O_2$, F_EO_2, and F_ECO_2, but precision was not generally different between the Tissot $\dot{V}_E$ and turbine $\dot{V}_I$ systems except for F_EO_2 and F_ECO_2. The mean precision for F_EO_2 and F_ECO_2, 0.09% (relative) and <0.37% (relative), respectively, was significantly lower on the turbine $\dot{V}_I$ system than for either of the $\dot{V}_E$

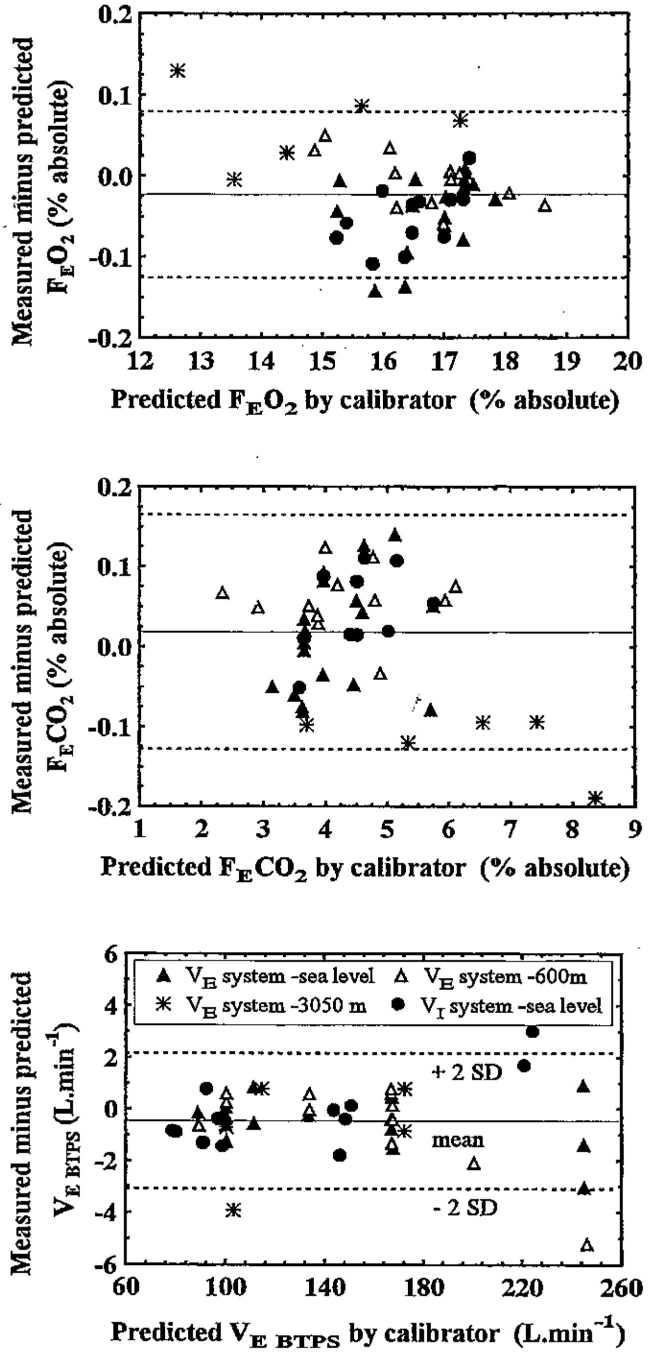

Figure 2—The bias of the calibrator calculated as the difference between values predicted by the calibrator and those measured by "criterion" systems for minute ventilation ($\dot{V}_{E\ BTPS}$), fraction of expired oxygen (F_EO_2), and fraction of expired carbon dioxide (F_ECO_2). Data points are shown for systems that measure expired ventilation ($\dot{V}_E$) and a system that measures inspired ventilation ($\dot{V}_I$) under different conditions. The mean bias and 95% confidence intervals shown in each panel are for the pooled data of both $\dot{V}_E$ and $\dot{V}_I$ systems.

systems. When data from both systems, except the breath-by breath system, were pooled, the mean precision for the measured F_EO_2 and F_ECO_2 was 0.13% and 0.41%, respectively, which equals 0.02% (absolute) at 16.0% F_EO_2 and 0.02% (absolute) at 4% F_ECO_2 (Fig. 4).

The precision for $\dot{V}_{E\ BTPS}$ was 0.7% (Fig. 4) and the corresponding precision for $\dot{V}O_2$ and $\dot{V}CO_2$ was 0.8% for both (Fig. 5). Even when data from the $\dot{V}_E$ breath-by-breath system were included, the mean precision for F_EO_2, F_ECO_2, $\dot{V}_{E\ BTPS}$, $\dot{V}O_2$, and $\dot{V}CO_2$ was 0.17, 0.60, 0.6, 0.9, and 0.9%, respectively.

DISCUSSION

Accuracy and precision. The results demonstrate that throughout and even beyond the physiological exercise range, the $\dot{V}O_{2max}$ calibrator is accurate and precise compared with "criterion" indirect calorimetry systems that measure $\dot{V}_E$ with water-sealed gasometers and F_EO_2/F_ECO_2 with well-calibrated electronic gas analyzers. In general, the accuracy and precision of the calibrator is equally good when tested against a "criterion" system that measured $\dot{V}_I$ with a calibrated turbine volume device and expired gas with electronic analyzers. Even when data were included in the bias and with precision esti-

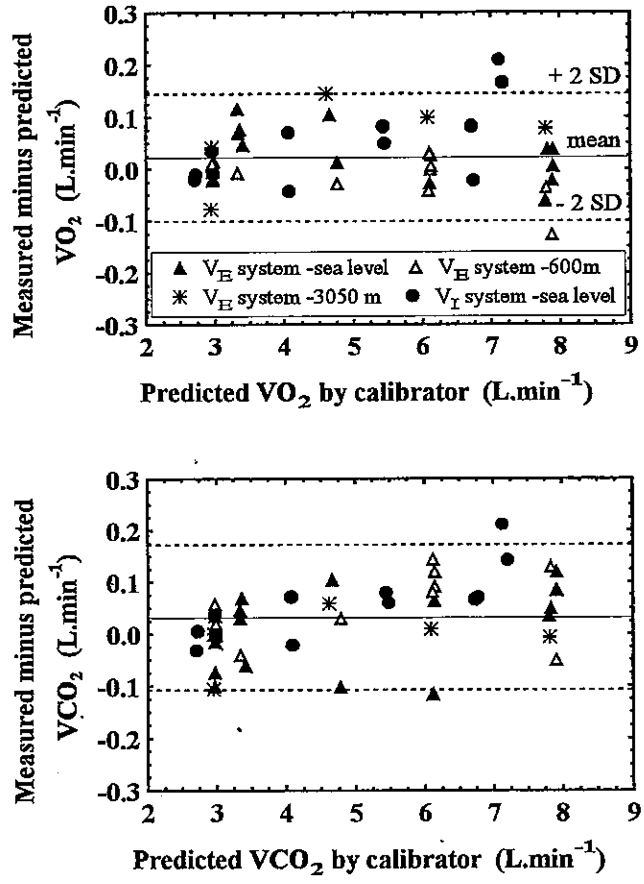

Figure 3—The bias of the calibrator calculated as the difference between values predicted by the calibrator and those measured by "criterion" systems for oxygen consumption ($\dot{V}O_L$) and carbon dioxide production ($\dot{V}CO_L$). Data points are shown for systems that measure expired ventilation ($\dot{V}_E$) and a system that measures inspired ventilation ($\dot{V}_I$) under different conditions. The mean bias and 95% confidence intervals shown in each panel are for the pooled data of both $\dot{V}_E$ and $\dot{V}_I$ systems.

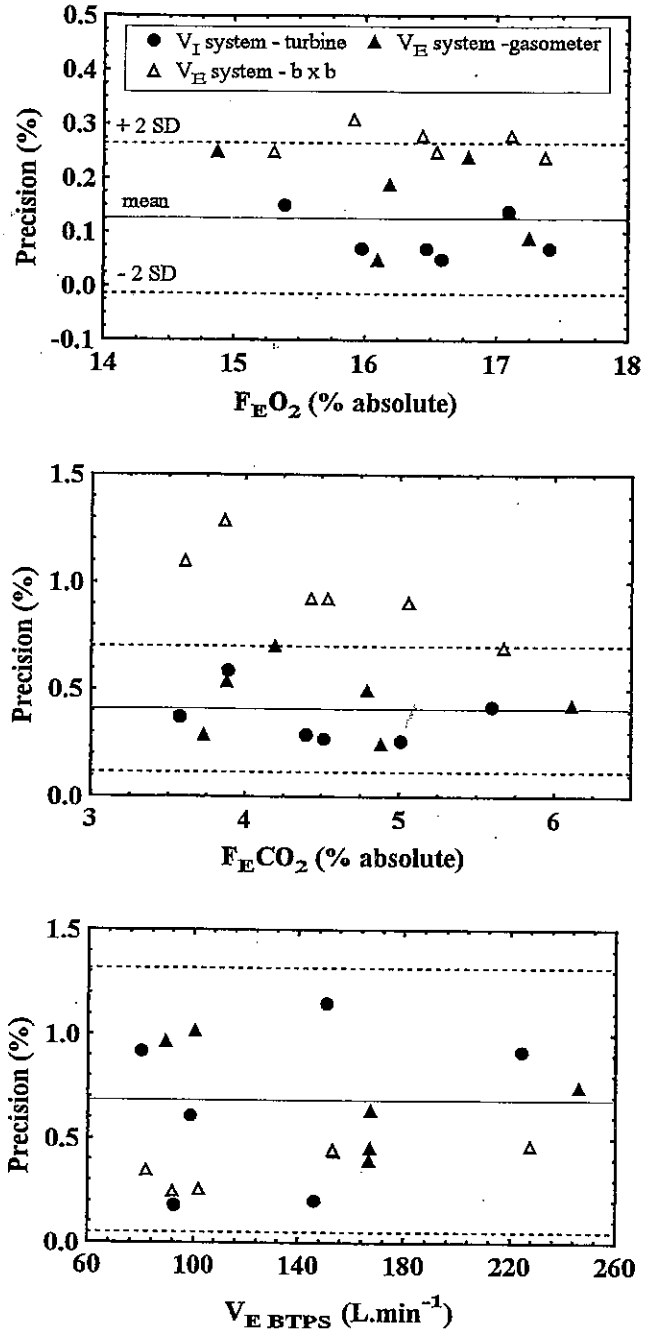

Figure 4—**Precision data for both $\dot{V}_E$ and $\dot{V}_I$ system measurement of minute ventilation ($\dot{V}_{E\ BTPS}$), fraction of expired oxygen (F_EO_2), and fraction of expired carbon dioxide (F_ECO_2) generated by the calibrator. The precision was calculated as the SD as a percentage of the mean, and for each data point, $N \sim 8$ for both the $\dot{V}_I$ system (●) and the $\dot{V}_E$ gasometer system (▲) and ranged from $N = 120$–165 for the $\dot{V}_E$ breath-by-breath system (△). The mean precision and 95% confidence intervals exclude the data for the $\dot{V}_E$ breath-by-breath system which was significantly different from the other two systems for F_EO_2 and F_ECO_2.**

mates that would increase both scores, the bias is < 35 mL·min^{-1} for $\dot{V}O_2$ and $\dot{V}CO_2$, < 0.5 L·min^{-1} for $\dot{V}_E$ and within 0.02% absolute for both F_EO_2 and F_ECO_2. Precision is better than 1% for $\dot{V}O_2$, $\dot{V}CO_2$, and $\dot{V}_E$ and within ~ 0.03% (absolute) for F_EO_2 and F_ECO_2. This calibrator is therefore a versatile device that could be used for routine calibration of most indirect calorimetry systems.

Implications: evaluation of system components. Huszczuk et al. (10) have described the advantages of the principles of operation of their calibrator over those of Boutellier et al. (2) and Foster and Norton (4) because it can provide a fixed level of both $\dot{V}O_2$ and $\dot{V}CO_2$ that is independent of respiration rate. This feature allows their calibrator and the present calibrator to interrogate the accuracy of individual components of the test system as well as the accuracy of the software calculations of $\dot{V}O_2$ and $\dot{V}CO_2$. That is, the linearity of electronic O_2 and CO_2 analyzers, as well as the volume device, can be assessed independently to highlight errors in a single component over a wide range of predicted $\dot{V}O_2$. For example, tests with the $\dot{V}O_{2max}$ calibrator on an "in-house" system revealed that although the O_2 analyzer was accurate and the software was correct, the CO_2 analyzer was progres-

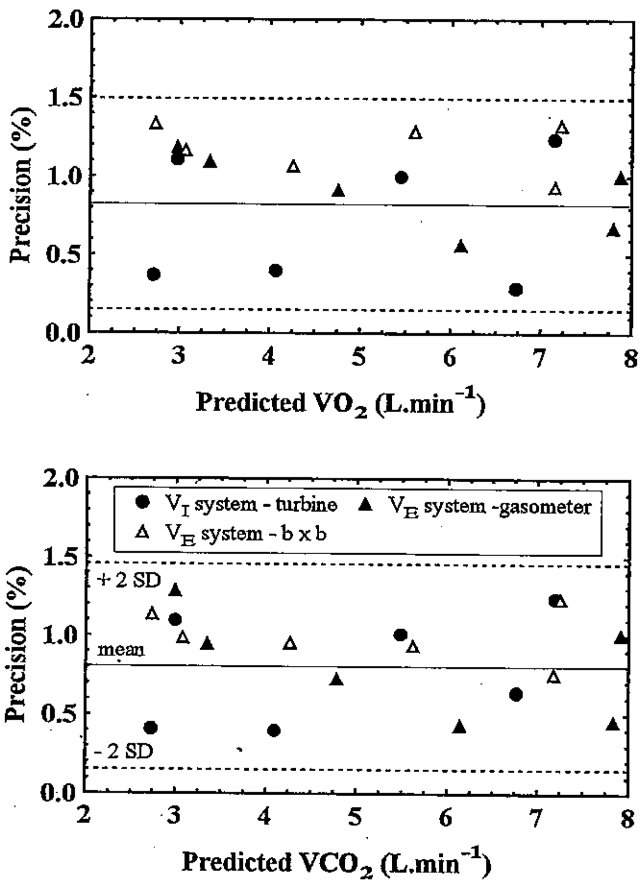

Figure 5—**Precision data for both $\dot{V}_E$ and $\dot{V}_I$ systems measuring oxygen consumption ($\dot{V}O_2$) and carbon dioxide production ($\dot{V}CO_2$) generated by the calibrator. The mean precision and 95% confidence intervals exclude the data for the $\dot{V}_E$ breath-by-breath system which was significantly different from the other two systems for $\dot{V}O_2$. The precision was calculated as the SD as a percentage of the mean, and for each data point, $N \sim 8$ for both the $\dot{V}_I$ system (●) and the $\dot{V}_E$ gasometer system (▲) and ranged from $N = 120$–165 for the $\dot{V}_E$ breath-by-breath system (△).**

sively inaccurate as F_ECO_2 increased from 3 to 6% and the pneumotachograph progressively under-read volumes > 120 L·min^{-1}. The excellent accuracy and precision of the $\dot{V}O_{2max}$ calibrator is critically important when other indirect calorimetry systems are evaluated, and the results demonstrate that such diagnostic capability has been achieved.

Advantages. The $\dot{V}O_{2max}$ calibrator improves on that of Huszczuk et al. (10) in several ways. First, it has the capacity to test indirect calorimetry systems that measure either inspired or expired volume. Second, it uses rapid, pneumatically-actuated valves instead of passive leaflet valves to control accurately the mixture of calibration gas and room air. These valves have response times of < 0.024 s, which is important for consistent performance of the calibrator. Third, the current calibrator can achieve ventilations in excess of 240 L·min^{-1}$_{(BTPS)}$, and correspondingly high $\dot{V}O_2$ and $\dot{V}CO_2$ of > 7.0 L·min^{-1}. While the calibrator of Foster and Norton (4) has pumping specifications that approach 150 L·min^{-1}, none of the previous calibrators (2,4,10) have reported data for respiratory ventilation greater than ~ 130 L·min^{-1}. Large male athletes such as rowers (13) will readily exceed $\dot{V}_{E\ BTPS}$ of 220 L·min^{-1}, and it is therefore relevant to assess the accuracy of an indirect calorimetry system throughout the physiological range if athletes are being assessed routinely. This is important because the accuracy of volume measurement is a key determinant of the calculated $\dot{V}O_2$ (9), with each 1% error in $\dot{V}_E$ resulting in an error in $\dot{V}O_2$ of the same magnitude. This issue is also relevant to some commercial systems such as the Medical Graphics CardiO_2 system, which uses a Pitot tube for volume assessment. While this technology is reproducible and accurate (± 2%) at flows of up to 120 L·min^{-1} (25), its accuracy at twice this level was unsubstantiated until now. The results (Fig. 4, bottom panel) demonstrate that the Pitot tube volume was within 0.5% of the predicted $\dot{V}_{E\ BTPS}$ from 92–228 L·min^{-1}. Fourth, the $\dot{V}O_{2max}$ calibrator uses an absolute pressure regulator (Fig. 1, item [20]) to control mass flow of calibration gas through the sapphire orifices, a cheaper solution than the rotameter approach used by Huszczuk et al. (10). The accuracy of the simulator to predict F_EO_2, F_ECO_2, ventilation, and thus $\dot{V}O_2$ and $\dot{V}CO_2$ is critically dependent on the precise flow of calibration gas through each sapphire orifice. Our results at sea level (Laboratory 1), at ~ 600 m altitude (Laboratory 2) and at simulated 3050 m altitude against $\dot{V}_E$ systems based on water-sealed gasometers demonstrate that the absolute pressure regulator is accurate irrespective of the ambient barometric pressure. The absolute pressure regulator is therefore an effective mechanism to control mass flow of calibration gas.

Limitations. Although the $\dot{V}O_{2max}$ calibrator improves upon the Huszczuk et al. (10) calibrator, it has several limitations. In its current form, the calibrator has been verified only as a steady-state simulator for $\dot{V}O_2$ and $\dot{V}CO_2$. The calibrator can be used to vary the F_EO_2 and F_ECO_2 for a given $\dot{V}O_2$ by changing the piston frequency, and the mathematical model is able to predict the value for the new steady-state F_EO_2 and F_ECO_2 at the new piston frequency. The accuracy of the calibrator to characterize the kinetics of breath-by-breath systems being used to measure rapidly changing $\dot{V}O_2$ has not been verified. However, the dilution calculations model the calibrator response on a breath-by-breath basis taking into consideration the individual dead space components. The demonstrated accuracy of these equations under steady-state conditions suggest that the $\dot{V}O_{2max}$ calibrator may have scope as a calibrator for breath-by-breath indirect calorimetry systems. A second limitation is that the calibrator does not deliver the warm, "fully saturated" expirate of an exercising athlete; rather the calibrator expirate is at room temperature and is a mixture of dry calibration gas and partially saturated ambient air. Therefore, the calibrator does not assess the efficacy of the drying method used by the test system.

An important difference between the calibrator and a human is an inequality between the inspired and expired volumes of the calibrator which can result in the predicted $\dot{V}O_2$ and $\dot{V}CO_2$ being ~20% lower for $\dot{V}_I$ compared with $\dot{V}_E$ systems. Although the difference between inspired and expired volume is taken into account by the prediction algorithms of the calibrator; when testing a $\dot{V}O_2$ system, the operator must know the method of volume measurement and select the relevant set of prediction equations. The algorithms for predicting $\dot{V}_E$ are further complicated because the temperature, pressure, and water vapor of the expired gas that are used by the test system must be known to ensure that both prediction equations and test system equations are using the same correction factors in their respective software. If this information cannot be obtained from the manufacturer and estimates of the appropriate correction factors are made, the accuracy of the predicted $\dot{V}_E$, $\dot{V}O_2$, and $\dot{V}CO_2$ will be reduced.

System calibration by other researchers. The need for a diagnostic calibrator, such as that described here, is apparent when one biological comparison of four metabolic cart systems reported that, while each system was reliable, comparison between systems varied by as much as 22% (21). Pneumotachographs or turbine volume transducers coupled with fast response electronic analyzers and computers have resulted in a number of "metabolic carts" that do not use traditional Douglas bag or gasometers for collection of all the expired gas. Many papers describe calibration of such commercial systems (12,14,15,20,23), and recently a number of reports have examined the accuracy of portable, miniaturized, telemetry indirect calorimetry systems (11,19,22,24,28). However, the calibration of some miniaturized systems has been indirect against metabolic carts (11,24) rather than direct calibrations against first principles devices like gasometers and chemical gas analysis. The $\dot{V}O_{2max}$ calibrator provides a standard that can be used not only to calibrate individual systems but also to compare results between systems. Furthermore, the $\dot{V}O_{2max}$ calibrator has greater precision than biological calibration attempts (16).

CONCLUSION

In summary, the current $\dot{V}O_{2max}$ calibrator has excellent accuracy and precision throughout and beyond the physiological range. The pooled results from both $\dot{V}_E$ and $\dot{V}_I$ systems show that the absolute bias of values measured by conventional indirect calorimetry systems compared with those predicted by the calibrator was < 35 mL·min^{-1} for $\dot{V}O_2$ and $\dot{V}CO_2$, < 0.50 L·min^{-1} for $\dot{V}_{E\ BTPS}$, -0.02% for F_EO_2 and 0.02% for F_ECO_2. Overall, the precision of the measured $\dot{V}O_2$, $\dot{V}CO_2$, and $\dot{V}_{E\ BTPS}$ was ~1%. This calibrator is therefore a versatile device that could be used for routine calibration of most indirect calorimetry system.

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APPENDIX: ($\dot{V}O_{2max}$ CALIBRATOR COMPONENTS AND THEIR CALIBRATION

Key Components

The key components of the $\dot{V}O_{2max}$ calibrator are (a) a pump, (b) a "bag-in-a-box" system, (c) a controlled mass flow of calibration gas and (d) three rotary actuators. Figure 1 is a schematic of the components of the calibrator and the numbers in square parentheses in the following text are included in this figure.

(a) Pump. The pumping system comprises a 160.1-mm internal diameter cylinder [1] (Tufnol Ltd., Birmingham, UK) and a custom-made polyethylene piston [2] (JV Precision Engineering, Canberra, Australia) with dual lip seals [3] (CBC Bearings, Canberra, Australia) at the front and rear of the piston. The piston con rod [4] can be set to different positions on a 23-cm diameter flywheel [5] to alter the piston stroke to deliver "tidal" volumes ranging from 0.5 to 4.0 L by 0.5-L increments. The pump is driven by a 120-W brushless motor [6] (model FBL5120A-30, Oriental Motor Company, Tokyo, Japan) which has variable speed control [7] from 10 to 100 rpm. The pumping frequency of the calibrator is derived from an electronic timing circuit [8] and a simultaneous count of the number of revolutions of the piston is derived from opto-slotted switches [9] (Model H22A1, Quality Technologies Corporation, Groot-Bijgaarden, Belgium) that activate when the piston passes either top or bottom, dead center.

(b) "Bag-in-a-box" system. A PVC plastic manifold [10] with four ports is used to connect the cylinder [1] to a common port [11] and to an acrylic box [12] (JV Precision Engineering, Canberra, Australia) which contains a 3.0 L anesthetic bag [13] (Vacu·Med, Ventura, CA). The common port [11] is the "mouth" of the device and is used as the point to attach a rubber mouthpiece and any indirect calorimetry system that is to be evaluated. The acrylic box is sealed with three custom-made, 22-mm internal aperture, delrin ball-valves [14] (JV Precision Engineering, Canberra, Australia), one of which is located at the top of the acrylic box and the other two are mounted at the base of the box. All three ball valves are driven pneumatically via rotary actuators [15] (model RVS20–90, CKD Corporation, Nagoya, Japan) controlled by solenoid valves [16] (model 4KA210–06, CKD), which are activated by the opto-slotted switches [9]. At the end of "expiration" when the piston passes top-dead center, the bottom two valves open and the top valve closes. This enables calibration gas to be withdrawn from the bag during the subsequent "inspiration." At the end of inspiration, when the piston passes bottom dead center, the bottom two valves close and the top valve opens, allowing calibration gas to enter the bag and an equivalent volume of air to be displaced from the acrylic box during the expiratory cycle. The timing of the opening and closing of the valves allows near ambient barometric pressure to be maintained within the acrylic box throughout inspiration and expiration. Industrial-standard compressed air [17] (BOC Gases, Australia) is used to drive the pneumatics, with all connections between the tank of air and the actuators made with ¼-inch brass, gas-tight fittings (Swagelok Company, Solon, OH) and ¼-inch polypropylene tubing (SMC Corporation, Tokyo, Japan).

(c) Mass flow of calibration gas. A constant mass flow of calibration gas is delivered via precision sapphire orifices to the anesthetic bag from an alpha standard (± 0.02% absolute) cylinder of 21% CO_2 in N_2 [18] (BOC Gases, Sydney, Australia). A dual stage regulator [19] (model 301339T, Veriflo Corporation, Richmond, CA) reduces tank pressure to approximately 720 kPa before the calibration gas reaches an absolute pressure regulator [20] (model 40, Fairchild, Winston-Salem, NC) set to ~410 kPa. The downstream pressure of this regulator is monitored continuously with an absolute pressure transducer [21] (model 891.13.500, 0–6 bar absolute, WIKA Alexander Weigand GmbH & Company, Klingenber, Germany) coupled to a 4½ digit LED display [22] (model 4501, Digital Process Measurement, Randburg, South Africa). The absolute regulator precedes a sapphire orifice [23] (Brockton Jewel Bearing Company, Brockton, MA) and the mass flow of calibration gas is varied by using six different sapphire orifices ranging from 0.36 to 1.04 mm. Connections between the tank of calibration gas and the anesthetic bladder are made with the same gas-tight fittings and tubing as used on the pneumatics lines.

Verifying the Calibrator Components

The performance characteristics of key components of the $\dot{V}O_{2max}$ calibrator were verified against first principles calibration devices where possible before the calibrator was used to check traditional $\dot{V}O_2$ systems. The key components of the $\dot{V}O_{2max}$ calibrator that have greatest effect on the predicted $\dot{V}O_2$ and the predicted fraction of expired oxygen (F_EO_2) and fraction of expired carbon dioxide (F_ECO_2) are the errors of the mass flow of the calibration gas, the concentration of the calibration gas, the volume delivered by the piston pump, and the function of the rotary actuators.

(a) Orifice calibration. Flow was calibrated through the six sapphire orifices by a timed volume measurement conducted against the reference volume standard of a 350-L chain-compensated gasometer (Warren E. Collins Inc., Braintree, MA). All six orifices were calibrated on two separate occasions, 3 months apart, with at least three trials on each occasion, and a minimum gas collection period of 5 min for each trial. The coefficient of variation for the calculated flow rate from multiple trials was less than 0.8% for each sapphire orifice. The difference between the two calibration occasions for a given orifice ranged from −0.7% to +1.6%, and overall averaged 0.7%. The overall uncertainty of orifice flow was thus taken as 1.0%.

(b) Concentration of the calibration gas. Gravimetric techniques (26) are used by BOC Gases (Sydney, Australia) to prepare a mixture of 21% CO_2 in N_2 with an accuracy of CO_2 of ± 0.02% absolute, which is of the same order as can be achieved by a skilled operator of the Lloyd Haldane chemical gas analyzer (18).

(c) Piston calibration. The volumetric accuracy of the calibrator depends on machining of both the piston cylinder and the flywheel for each 0.5-L setting of the stroke volume. The bore of the cylinder and the tidal volume settings each have a machining accuracy of ±0.005 mm.

(d) Rotary actuators. The delay time between top- and bottom-dead center and the response of the rotary actuators was consistently 0.020 and 0.033 s before the valves began to open and close, respectively. The response time of the rotatory actuators was also consistent irrespective of the pumping rate at 15, 30, or 60 rpm. The time to close was 0.018 s and to open was 0.024 s.