

# Validity of a Swimming Snorkel for Metabolic Testing

## Authors

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## Key words

- swimming
- gas analysis
- oxygen uptake
- respiratory valve
- K4 b<sup>2</sup>

## Abstract

Two models of a swimming snorkel connected to a portable metabolic cart (Cosmed K4 b<sup>2</sup>, Rome, Italy) were assessed using a gas exchange simulation system. Four standardized testing protocols were designed to mimic different swimming conditions and were performed similarly in three conditions so that both snorkels could be compared to measured values obtained by connecting the simulator directly with the gas analyzer. Simulated and measured values were highly correlated ( $R^2 = 0.891$  to  $0.998$ ) and in good agreement, with only a small overestimation of expiratory tidal volume (4%,  $p = 0.005$ ), not large enough to

significantly affect the accuracy of ventilation or gas exchange parameters. Values measured using both swimming snorkels also highly correlated with simulated values, particularly for the ventilatory and primary gas exchange variables ( $R^2 = 0.996$  and  $0.998$  in both models for  $\dot{V}O_2$  and  $\dot{V}CO_2$ , respectively). A moderate overestimation of  $F_{E}O_2$  was observed in both models (2.65% and 2.48% relative,  $p = 0.03$ ) and attributed to minimal mixing of inspiratory and expiratory gases, although not affecting  $\dot{V}O_2$  measurements. We conclude that both snorkels are valid devices for measuring pulmonary breath-by-breath gas exchange parameters in connection with the K4 b<sup>2</sup> across a wide physiological range.

## Introduction

Oxygen uptake and related cardiorespiratory variables are important measures of metabolic function during exercise. Moreover, maximal oxygen consumption ( $\dot{V}O_{2max}$ ) has been considered the single best measure of cardiorespiratory capacity and the general indicator of the highest rate at which oxygen can be taken up and utilized by the human body during severe exercise [8]. Open indirect calorimetric methods have been progressively preferred to the classical Douglas bag technique by some investigators for the measurement of expiratory gases to assess oxygen consumption ( $\dot{V}O_2$ ) and energy expenditure (EE) in athletes involved in endurance sports, mostly due to its more advantageous sampling capability and practicality. Requisite machinery to explore human aerobic energetics during field conditions have become available with the improvement of miniaturized metabolic measurement systems. Several studies have shown that these portable systems may serve data within an acceptable level of accuracy [4,5,7,12,13,16,17]. However, in some sport disciplines, particularly

swimming, technical constraints imposed by environmental factors have traditionally hindered the measurement of cardiorespiratory variables within the actual field setting. The Cosmed K4 b<sup>2</sup>® (Cosmed S. r. l., Rome, Italy) is a fully portable gas analysis system that continuously measures expired gases on a breath-by-breath (B × B) basis. It is a lightweight instrument originally designed for the collection and measurement of on-land activities during free movement via a traditional facemask. It can be utilized to assess cardiorespiratory endurance capacity, to predict maximal aerobic power and endurance capacity, and to estimate energy expenditure at rest and during exercise, including different types of sport activities in field conditions [2,3,21]. Previous investigations on the validity and accuracy of the system have been biological calibration studies achieved by comparing the K4 b<sup>2</sup> with various criterion systems such as the Douglas bag method [15] and other laboratory based metabolic carts both with conventional O<sub>2</sub> and CO<sub>2</sub> sensors [4,5,18] and mass spectrometry analysis [16]. They were all performed in human subjects during different types of exercise, while

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none has been carried out using a laboratory calibrator or gas exchange simulation system (GESS) in the laboratory. Moreover, results from these studies show significant discrepancies and current evidence can be considered inconclusive.

The complete calibration and testing of integrated calorimetry systems involves simultaneous checks of gas analyzers, flow and/or volume device, and software [6]. Technical errors combined with biological variability may increase total measurement errors considerably [10,23]. Fortunately, mechanical devices have been developed to calibrate indirect calorimetry systems free of biological variability influences. Huszczuk et al. [9] developed a method for simulating respiratory gas exchange for on-line calibration of metabolic measurement systems. The apparatus can reproduce any range of respiratory and metabolic performance ( $\dot{V}O_2$ : 0.2–3.5 L·min<sup>-1</sup>) accurately (<2% error) in clinical settings. Prieur et al. [19,20] described a gas exchange simulator for quality control of metabolic carts with equal accuracy as compared to Douglas bags. Gore et al. [6] presented an automated  $\dot{V}O_{2\max}$  calibrator with a simulation capacity of  $\dot{V}O_2$  up to 7.9 L·min<sup>-1</sup> and ventilation up to 246 L·min<sup>-1</sup>.

A respiratory snorkel and valve system as described by Toussaint et al. [22] was originally developed to collect respiratory gases in Douglas bags during swimming, although this collection procedure is not easily handled in field testing conditions and requires relatively long steady-state sampling periods (usually more than 30 s) if accuracy has to be guaranteed. Accordingly, this piece of equipment has since then been modified for B × B gas analysis to be used in connection with the K4 b<sup>2</sup> portable metabolic cart in swimming pool conditions, and biologically validated in the laboratory with human subjects [11]. The results showed low to moderate differences between data obtained using the original K4 b<sup>2</sup> facemask and the swimming snorkel, the differences being mainly systematic. The ultimate cause for the discrepancies remained unclear while the investigation could not separate between the actual technical error of measurement and biological variations eventually influencing the results.

The primary aim of this study was to further assess the validity and accuracy of two models of a modified swimming snorkel connected to a portable breath-by-breath metabolic cart using standardized testing protocols generated by a manually operated gas exchange simulation system in the laboratory. The accuracy of the K4 b<sup>2</sup> gas analysis system was also tested using the gas exchange simulator as a criterion method.

## Methods

### Portable metabolic cart (Cosmed K4 b<sup>2</sup>)

The portable metabolic system K4 b<sup>2</sup> (Cosmed, Rome, Italy) was used for all measurements. This miniaturized, open-circuit calorimeter is a lightweight, battery operated, automated system designed for B × B measurements. It integrates a bidirectional flow meter turbine with an opto-electric reader (flow range 0–20 L·s<sup>-1</sup>, linear ventilation range 0–300 L·min<sup>-1</sup> according to the manufacturer), a GFC (gas filter correlation) oxygen sensor (range 7–24% O<sub>2</sub>), and NDIR (non-dispersive infrared) carbon dioxide sensor (range 0–8% CO<sub>2</sub>). Gas samples are obtained from expiratory air straight from the inside of the turbine through a semipermeable Nafion® sampling line (0.75 m in length) and pumped at a predetermined flow rate into the O<sub>2</sub> and CO<sub>2</sub> sensors, which are maintained at constant temperature. Temperature of expired gases is measured via a sensor inside the

turbine unit. Atmospheric barometric pressure (P<sub>b</sub>) is measured by a separate sensor connected to the portable unit of the analyzer. The K4 b<sup>2</sup> proprietary software provides BTPS correction for inspiratory and expiratory parameters, as well as STPD correction for  $\dot{V}O_2$  and  $\dot{V}CO_2$  with conventional equations (for details, see “Technical note: BTPS and STPD corrections for COSMED systems”. Cosmed S.r.l., Italy, 1999). For this experiment, environmental conditions were measured and input into the default correction equations according to the characteristics of the calibration gases used for analysis. The metabolic cart was fully calibrated before every set of measurements at the test laboratory (T<sub>a</sub> = 21.9–22.3 °C, P<sub>b</sub> = 1021–1024 hPa, H<sub>rel</sub> = 42–57%) following the manufacturer’s instructions: 1) turbine calibration with a calibration syringe (V = 3 L); 2) gas sensors calibration with room air (20.93% O<sub>2</sub>, 0.03% CO<sub>2</sub>); 3) gas sensors calibration with reference gas mixture (16.00% O<sub>2</sub>, 4.99% CO<sub>2</sub> in N<sub>2</sub>); and 4) system delay calibration to match the changes in F<sub>E</sub>O<sub>2</sub> and F<sub>E</sub>CO<sub>2</sub>.

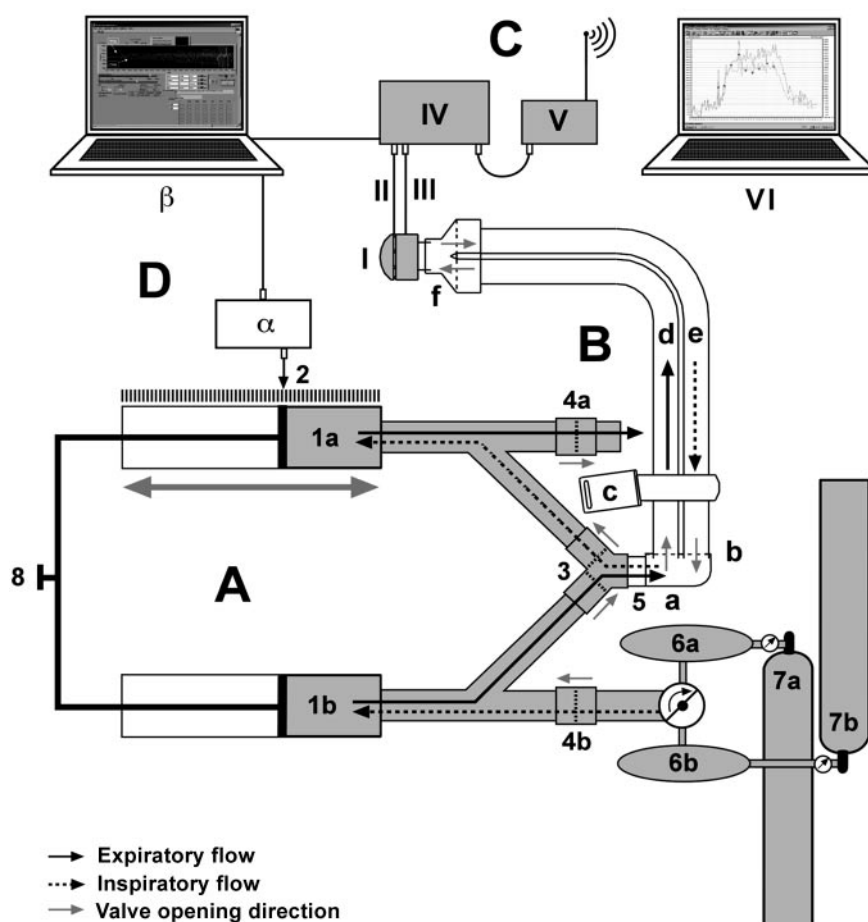
### Swimming snorkels

The low-drag snorkel and valve system originally designed by Toussaint et al. [22] to be used with Douglas bags or metabolic carts with traditional mixing chamber was modified to operate in connection with a B × B gas analysis system by Keskinen et al. [11] and specifically adapted to the K4 b<sup>2</sup>. The original swimming snorkel as developed by Toussaint et al. [22] was mainly used for measurements requiring medium high ventilations leading to  $\dot{V}O_2$  values of approximately 3000 mL·min<sup>-1</sup>; the same laboratory further constructed a system with larger volumes to be used, especially in maximal efforts requiring very high ventilations. In the modified B × B swimming snorkel [11], the connection of the inlet and outlet tubes to the turbine through a connecting unit allows the system to distinguish expiration from inspiration, and tidal volume and respiratory frequency can be defined for B × B analysis. The connection unit permits inspiratory and expiratory gases to mix in small extent in the beginning of both the expiration and inspiration. The temperature of the expiratory gases are measured by a sensor inside the turbine unit and atmospheric pressure is measured by a separate sensor connected to the portable unit of the analyzer. The device consists of a mouthpiece with two non-rebreathing one-way valves, separate tubes for inhalation and exhalation, a head set adjustable to the subject’s head, and a connecting unit to the turbine and the K4 b<sup>2</sup> itself (● Fig. 1). More detailed information about the technical characteristics of this modified respiratory snorkel can be found elsewhere [11].

Following a biological calibration study [11], further modifications were introduced and two models, smaller (SS-S) and larger volume (SS-L) swimming snorkels (SS), were partially redesigned and tested in this study. ● Table 1 shows the volumes of the different compartments of the K4 b<sup>2</sup> directly connected to the gas exchange simulator used to generate the reference gas volumes (REF) according to standardized protocols, as well as connected to both swimming snorkels (SS-S and SS-L).

### Gas exchange simulation system (GESS)

The GESS device used in this investigation consists of two parallel manually operated calibration syringes (V = 3 L) with a computerized position sensor system (sampling frequency = 100 Hz) and apposite software for the monitoring of stroke volumes and waveform patterns, as well as for synchronization with the gas analysis software. Both syringes are operated synchronically



**Fig. 1 A to D** Schematic illustration of the experimental setup: **A** gas exchange simulation system (GESS), **B** respiratory snorkel, **C** K4 b<sup>2</sup> gas analyzer, and **D** flow control and synchronization unit. **A** 1a/1b = expiratory/inspiratory calibration syringes (0–3 L); 2 = computerized position sensor; 3 = connector with two one-way valves; 4a/4b = expiratory/inspiratory one-way valves; 5 = port to gas analyzer (directly, or through swimming snorkel as illustrated); 6a/6b = calibration gas containers (polyethylene bags) with switching valve; 7a/7b = calibration gas cylinders; 8 = pumping handle. **B** a = mouthpiece; b) one-way nonrebreathing valves; c) adjustable head set; d) expiratory tube; e) inspiratory tube; f) connecting unit. **C** I = flow-meter turbine; II = gas sampling line; III = turbine opto-electric reader line; IV = main K4 b<sup>2</sup> unit (gas sensors and flow signal integration); V = battery; VI = computer with K4 b<sup>2</sup> software. **D** α = position sensor transducer; β = computer with on-line flow integration software (synchronizes with K4 b<sup>2</sup> software).

and connected by two separate one-way valves to the gas analysis system through a port. Two different gas containers (polyethylene bags) provide known volumes of calibration gases (nominally 16.00 and 18.05% O<sub>2</sub>, and 3.00 and 4.99% CO<sub>2</sub> ± 2% V<sub>Rel</sub>) continuously obtained from two cylinders. To simulate gas exchange, known volumes of gas are synchronically pumped to a system of tubes and valves that ensured the correct flow direction for inspiratory and expiratory gases. **Fig. 1** shows a schematic plan of the key components and operation principles of the GESS apparatus.

The system allows for calibration and quality control procedures throughout and beyond the physiological range, enabling to vary simulated breathing frequency ( $f_R$ ) up to 100 breaths·min<sup>-1</sup> and tidal volume ( $V_T$ ) up to 3000 mL, which results in minute ventilation ( $\dot{V}_E$ ) values up to 300 L·min<sup>-1</sup>. All measurements were made in the laboratory within controlled environmental conditions. Volumes were corrected to standard BTPS conditions using the same correction equations implemented in the software of the K4 b<sup>2</sup> according to  $P_b$  and  $T$  measured by the system ( $V_t$  for inspiratory volumes, and  $V_t$ ,  $O_{2exp}$  and  $CO_{2exp}$  for expiratory volumes). STPD corrections were also applied to calculated (predicted)  $\dot{V}O_2$  and  $\dot{V}CO_2$ .

### Simulation protocols and data management

Standardized GESS testing protocols were designed to mimic swimming conditions, such as if any given subject would exercise at different gradually increasing and decreasing intensities with  $\dot{V}O_2$  and  $\dot{V}CO_2$  volumes across the physiological range for swimming assessment. Accordingly, the GESS system was used

**Table 1** Volumes of the different compartments of the K4 b<sup>2</sup> gas analyzer directly connected to the gas exchange simulator (REF), and through the smaller (SS-S) and larger (SS-L) swimming snorkels

Volumes (mL)	REF	SS-S	SS-L
GESS dead space	110	83	83
Mouthpiece	0	40	60
Expiration tube	0	480	830
Connecting unit	0	190	110
Total	110	793	1083

GESS = gas exchange simulation system; REF = reference method (GESS directly connected to the K4 b<sup>2</sup>); SS-S = smaller-volume swimming snorkel; SS-L = larger-volume swimming snorkel

to generate various predetermined combinations of ventilatory parameters and expiratory gas fractions grouped in four different standardized protocols (**Table 2**). Each protocol was replicated in the three conditions by connecting the GESS system 1) to the gas analyzer directly through the mouthpiece inlet of the turbine (this was considered as criterion or reference system [REF] for comparisons), and 2) to the gas analyzer through the mouthpiece inlet of each one of the swimming snorkels (SS-S and SS-L). This allowed the assessment of the accuracy of the K4 b<sup>2</sup> metabolic cart against the GESS as a criterion method, as well as the concurrent validity of the metabolic cart by comparing results obtained using each swimming snorkel (SS-S and SS-L) with the reference (REF) method (i.e., the K4 b<sup>2</sup> directly connected to the GESS).

**Table 2** Standardized simulation protocols generated by the gas exchange simulation system (GESS). Each protocol was replicated using the three experimental set-ups

	Strokes (n)	V <sub>t</sub> (L)	f <sub>R</sub> (breaths · min <sup>-1</sup> )	Ṡ <sub>E</sub> (L · min <sup>-1</sup> )	F <sub>E</sub> O <sub>2</sub> (%)	F <sub>E</sub> CO <sub>2</sub> (%)
Protocol #1	10	0.5	10	5	16.00	4.99
	10	0.5	20	10	16.00	4.99
	10	1.0	20	20	16.00	4.99
	10	1.5	20	30	16.00	4.99
	10	1.5	30	45	16.00	4.99
	10	2.0	30	60	16.00	4.99
	10	2.8	30	84	16.00	4.99
	10	2.8	40	112	16.00	4.99
	10	2.8	60	168	16.00	4.99
	10	2.8	20	56	16.00	4.99
Protocol #2	same as for protocol #1				18.05	3.00
Protocol #3	10	1.0	20	20	16.00	4.99
	10	1.0	20	20	18.05	3.00
	10	1.0	20	20	16.00	4.99
	10	2.0	60	120	16.00	4.99
	10	2.0	60	120	18.05	3.00
	10	2.0	60	120	16.00	4.99
Protocol #4	10	1.0	20	20	18.05	4.99
	15	2.0	30	60	16.00	3.00
	20	2.8	40	112	16.00	4.99
	30	2.8	60	168	16.00	4.99
	15	2.0	30	60	18.05	3.00

Strokes = pumping cycles (number); V<sub>t</sub> = tidal volume; f<sub>R</sub> = respiratory frequency; Ṡ<sub>E</sub> = pulmonary minute ventilation; F<sub>E</sub>O<sub>2</sub> = oxygen expiratory fraction; F<sub>E</sub>CO<sub>2</sub> = carbon dioxide expiratory fraction

Simulated (GESS generated) and measured values of each parameter corresponding to seven consecutive pumping strokes for each step of the four simulation protocols (Table 2) were selected by excluding the first two and the last measurements to avoid potentially inaccurate measurements when changing pumping frequency, gas concentration, and/or volume during the manual operation of the GESS, so that each stroke simulated as closely as possible the target values. Output data (i.e., frequencies and volumes actually generated as defined by the position sensor software) were synchronized and compared with metabolic measurements (i.e., K4 b<sup>2</sup> readings) after standard gas corrections according to measured P<sub>b</sub>, T, and H<sub>rel</sub>. All selected values were then pooled for GESS vs. REF comparisons. Averaged values for each step were pooled for comparisons between the three testing conditions (REF, SS-S, SS-L).

### Statistical analysis

First, the accuracy of the K4 b<sup>2</sup> was assessed by comparing values generated by the simulator (GESS) and measured by the portable cart (REF) for the main ventilatory and gas exchange variables: V<sub>t</sub> (L), f<sub>R</sub> (breaths · min<sup>-1</sup>), Ṡ<sub>E</sub> (L · min<sup>-1</sup>), F<sub>E</sub>O<sub>2</sub> (%), F<sub>E</sub>CO<sub>2</sub> (%), ṠO<sub>2</sub> (mL · min<sup>-1</sup>), and ṠCO<sub>2</sub> (mL · min<sup>-1</sup>). Second, the validity of the swimming snorkels was tested by comparing values measured by the K4 b<sup>2</sup> in the three testing set-ups: directly connected to the simulator (REF), and connected through each of the swimming snorkels (SS-S, and SS-L). Agreement between methods was assessed for all parameters by Passing-Bablok regression analysis [17] and Bland-Altman difference plots [1] using ancillary software (Method Validator, ver. 1.19, Metz, France) [14]. Regression parameters (slope and intercept), coefficients of determination (R<sup>2</sup>), and 95% confidence intervals (95% CI) were calculated for the Passing-Bablok regression equations to determine the degree of association between two methods. Accuracy was quantified as the mean of the differences (bias) between two

methods, one of them used as reference or criterion method (GESS or REF), using Bland-Altman plots, which graphically represent the difference scores between pairs of measurements and the mean ± 1.96 standard deviation (SD) of the differences, providing a confidence interval (95% CI) within which 95% of differences between measurements by the two methods are expected to lie [1].

Significant differences between methods of measurement were tested by ANOVA (or Kruskal-Wallis ANOVA on ranks in case of failure of the normality test). Where a significant effect of the setup was obtained, a post hoc pairwise multiple comparison analysis was performed using the Dunn's method to identify differences among methods. Differences between GESS (simulated) and REF (measured) values were tested by paired Student's *t*-test (or Mann-Whitney U-test in non-normal distributions). Precise *p* values are reported and the significance level was set at *p* < 0.05 for all analyses, unless specified data are presented as mean and standard deviation (SD, in parentheses). All tests were performed using SigmaStat® 3.0 (SPSS, Inc., Chicago, IL, USA).

## Results



### Validity of the K4 b<sup>2</sup> portable metabolic cart

Values generated by the simulator (GESS) and measured by the K4 b<sup>2</sup> (REF) for the main gas exchange variables were highly correlated (R<sup>2</sup> = 0.996 to 0.998) and in good agreement (Table 3). No significant bias were found, with the only exception of V<sub>t</sub>, which was slightly overestimated (mean diff. = 0.081 L = 4.0%, *p* = 0.005) (Table 3). Despite minor deviations in Ṡ<sub>E</sub> (mean diff. = 2.9 L · min<sup>-1</sup> = 4.2%, *p* = 0.40), ṠO<sub>2</sub> (mean diff. = 63.9 mL · min<sup>-1</sup> = 3.6%, *p* = 0.61) and ṠCO<sub>2</sub> (mean diff. = -39.5 mL · min<sup>-1</sup> = -2.2%, *p* = 0.75) neither of these differences were significantly different from zero. When looking at the regression and differ-

**Table 3** Agreement values generated by the gas exchange simulator (GESS) and values measured by the K4 b<sup>2</sup> (REF) as assessed by Passing-Bablok regression analysis. The coefficient of determination (R<sup>2</sup>), slope and intercept of the regression equation, as well as the mean difference and p value for these differences (Student's t-test or Mann-Whitney U-test) are shown

GESS vs. REF (N = 166)					
Parameters	R <sup>2</sup>	Slope	Intercept	Mean difference	p
V <sub>t</sub> (L)	0.998	1.053 (1.044 to 1.062)	-0.028 (-0.040 to -0.013)	0.081 (0.069 to 0.093)	0.005
f <sub>R</sub> (breaths·min <sup>-1</sup> )	0.996	1.027 (1.016 to 1.042)	-0.519 (-0.947 to -0.222)	0.193 (0.044 to 0.340)	0.42
Ṡ <sub>E</sub> (L·min <sup>-1</sup> )	0.891	1.045 (1.041 to 1.051)	-0.342 (-0.487 to -0.132)	2.88 (2.41 to 3.35)	0.40
F <sub>E</sub> O <sub>2</sub> (%)	0.988	0.990 (0.972 to 1.007)	0.171 (-0.123 to 0.467)	-0.001 (-0.019 to 0.017)	0.30
F <sub>E</sub> CO <sub>2</sub> (%)	0.986	1.006 (0.986 to 1.026)	-0.030 (-0.103 to 0.043)	-0.005 (-0.024 to 0.013)	0.55
ṠO <sub>2</sub> (mL·min <sup>-1</sup> )	0.998	1.028 (1.018 to 1.036)	18.10 (8.62 to 25.23)	63.9 (47.3 to 80.6)	0.61
ṠCO <sub>2</sub> (mL·min <sup>-1</sup> )	0.998	0.973 (0.965 to 0.982)	0.916 (-13.88 to 8.09)	-39.5 (-51.9 to -27.0)	0.75

V<sub>t</sub> = tidal volume; f<sub>R</sub> = respiratory frequency; Ṡ<sub>E</sub> = pulmonary minute ventilation; F<sub>E</sub>O<sub>2</sub> = oxygen expiratory fraction; F<sub>E</sub>CO<sub>2</sub> = carbon dioxide expiratory fraction; ṠO<sub>2</sub> = oxygen uptake; ṠCO<sub>2</sub> = carbon dioxide production

ence plots in ṠO<sub>2</sub> and ṠCO<sub>2</sub> (● Figs. 2A and B), somewhat larger deviations could be observed in both parameters for values in excess of about 5.5 L·min<sup>-1</sup> with a mean difference of ca. 7% and 3% for ṠO<sub>2</sub> and ṠCO<sub>2</sub>, respectively. These differences were paralleled by a somewhat larger overestimation of Ṡ<sub>t</sub> values at the high range of measurement (ca. > 2.5 L).

### Validity of swimming snorkels

Agreement of gas exchange values generated by the simulator directly connected to the K4 b<sup>2</sup> (REF) and through each of the two swimming snorkels (SS-S and SS-L) is summarized in ● Tables 4 and 5.

### Ventilatory parameters

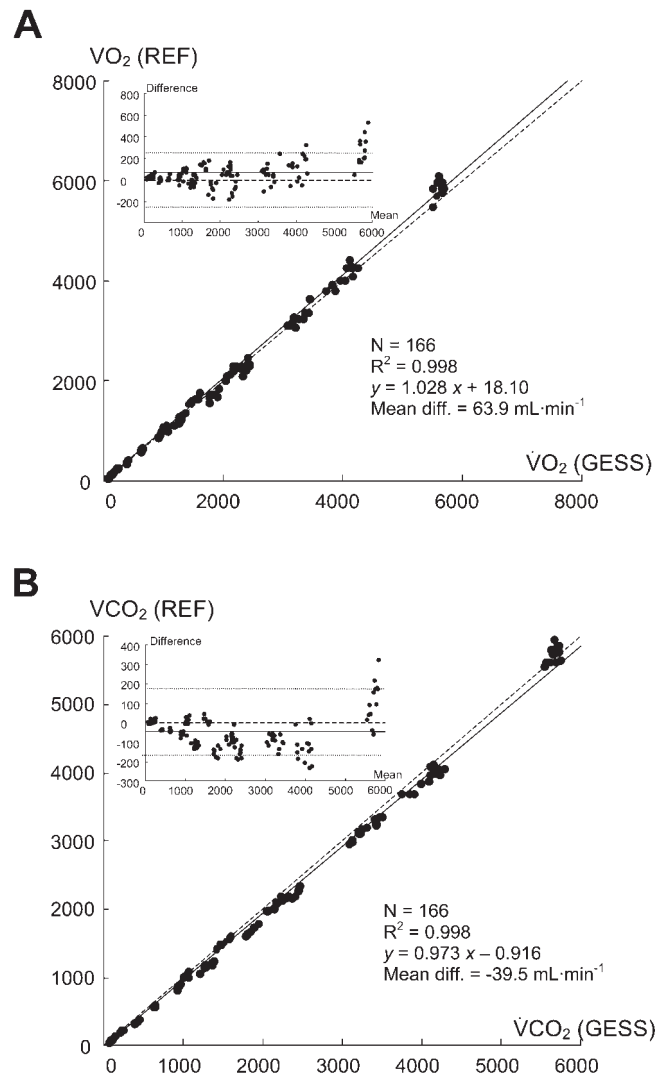
V<sub>t</sub>, f<sub>R</sub>, and Ṡ<sub>E</sub> values measured by the K4 b<sup>2</sup> directly connected to the simulator (REF) and by using both swimming snorkels (SS-S, SS-L) were highly correlated (R<sup>2</sup> = 0.994 to 0.998), the determination coefficients for V<sub>t</sub> and Ṡ<sub>E</sub> being somewhat higher for SS-S (R<sup>2</sup> = 0.996 and 0.998) as compared to SS-L (R<sup>2</sup> = 0.994 and 0.996). Differences between methods were not significantly different from zero (● Tables 4 and 5).

### Expiratory gas fractions

F<sub>E</sub>O<sub>2</sub> and F<sub>E</sub>CO<sub>2</sub> values measured by the three set-ups were somewhat less correlated (R<sup>2</sup> = 0.830 to 0.861) compared to ventilatory parameters. When differences were analyzed (ANOVA), a general significant effect of the set-up was found for F<sub>E</sub>O<sub>2</sub> (p = 0.04) and not for F<sub>E</sub>CO<sub>2</sub> (p = 0.44). When compared pairwise, mean differences between REF and each swimming snorkel for F<sub>E</sub>O<sub>2</sub> significantly differed (0.421 and 0.434% absolute for SS-S and SS-L, respectively, p = 0.03) (● Tables 4 and 5).

### Respiratory gas exchange parameters

ṠO<sub>2</sub> and ṠCO<sub>2</sub> values obtained in the three conditions were highly correlated (R<sup>2</sup> = 0.990 to 0.996), the determination coefficient being somewhat higher for SS-S (R<sup>2</sup> = 0.996 for both parameters) as compared to SS-L (R<sup>2</sup> = 0.988 and 0.990) (● Tables 4 and 5). No significant differences were found between the three set-ups (ANOVA, p = 0.78 and 0.94 for ṠO<sub>2</sub> and ṠCO<sub>2</sub>, respectively). Thus, the overestimation found in the F<sub>E</sub>O<sub>2</sub> values was not large enough to significantly alter the accuracy of ṠO<sub>2</sub> measurements. Despite not being significantly different from zero, mean differences between REF and SS-S (-130 mL·min<sup>-1</sup> and -46 mL·min<sup>-1</sup> for ṠO<sub>2</sub> and ṠCO<sub>2</sub>, respectively) were smaller compared to SS-L (-218 mL·min<sup>-1</sup> and -123 mL·min<sup>-1</sup>). ● Fig. 3



**Fig. 2A and B** Regression and difference plots of oxygen consumption (ṠO<sub>2</sub>, mL·min<sup>-1</sup>) (A) and dioxide production (ṠCO<sub>2</sub>, mL·min<sup>-1</sup>) (B) between values directly measured by the K4 b<sup>2</sup> cart (REF), and generated by the gas exchange simulator (GESS) values. In the Passing-Bablok regression plot, the solid line indicates linear regression (y = ax + b), and the dashed line indicates equality (y = x). In the Bland-Altman plot (inset panel), lines indicate mean difference (solid), equality (dashed), and mean difference ± 1.96 SD (95% CI) intervals (dotted).

**Table 4** Agreement between values measured by the K4 b<sup>2</sup> directly connected to the gas exchange simulator (REF) and using the smaller volume swimming snorkel (SS-S) as assessed by Passing-Bablok regression analysis. The coefficient of determination (R<sup>2</sup>), slope and intercept of the regression equation, as well as the mean difference and p value for these differences (ANOVA, and post hoc test if applicable in parentheses) are shown

REF vs. SS-S (N = 166)					
Parameters	R <sup>2</sup>	Slope	Intercept	Mean difference	p
V <sub>t</sub> (L)	0.996	0.959 (0.941 to 0.993)	0.049 (-0.012 to 0.086)	-0.024 (-0.051 to 0.002)	0.76
f <sub>R</sub> (breaths·min <sup>-1</sup> )	0.998	0.997 (-0.110 to 0.575)	0.219 (-0.132 to 0.493)	0.232 (-0.110 to 0.575)	0.93
Ṡ <sub>E</sub> (L·min <sup>-1</sup> )	0.998	0.980 (0.962 to 1.003)	0.717 (-0.125 to 1.578)	-0.441 (-1.590 to 0.705)	0.25
F <sub>E</sub> O <sub>2</sub> (%)	0.830	1.156 (0.956 to 1.443)	-2.452 (-7.268 to 1.249)	0.421 (0.254 to 0.589)	0.04 (0.03)
F <sub>E</sub> CO <sub>2</sub> (%)	0.850	1.219 (1.011 to 1.714)	-0.878 (-2.853 to -0.246)	-0.307 (-0.468 to -0.146)	0.44
Ṡ <sub>O</sub> <sub>2</sub> (mL·min <sup>-1</sup> )	0.996	0.959 (0.934 to 0.998)	-60.3 (-95.6 to -40.8)	-130 (-180 to -80)	0.78
Ṡ <sub>CO</sub> <sub>2</sub> (mL·min <sup>-1</sup> )	0.996	1.022 (0.987 to 1.049)	-70.1 (-98.2 to -40.6)	-46 (-93.3 to 1.3)	0.94

V<sub>t</sub> = tidal volume; f<sub>R</sub> = respiratory frequency; Ṡ<sub>E</sub> = pulmonary minute ventilation; F<sub>E</sub>O<sub>2</sub> = oxygen expiratory fraction; F<sub>E</sub>CO<sub>2</sub> = carbon dioxide expiratory fraction; Ṡ<sub>O</sub><sub>2</sub> = oxygen uptake; Ṡ<sub>CO</sub><sub>2</sub> = carbon dioxide production

**Table 5** Agreement between values measured by the K4 b<sup>2</sup> directly connected to the gas exchange simulator (REF) and using the larger volume swimming snorkel (SS-L) as assessed by Passing-Bablok regression analysis. The coefficient of determination (R<sup>2</sup>), slope and intercept of the regression equation, as well as the mean difference and p value for these differences (ANOVA, and post hoc test if applicable in parentheses) are shown

REF vs. SS-L (n = 27)					
Parameters	R <sup>2</sup>	Slope	Intercept	Mean difference	p
V <sub>t</sub> (L)	0.994	0.987 (0.970 to 1.010)	-0.003 (-0.035 to 0.031)	-0.038 (-0.067 to -0.008)	0.76
f <sub>R</sub> (breaths·min <sup>-1</sup> )	0.998	1.003 (0.986 to 1.025)	-0.090 (-0.589 to 0.286)	0.249 (-0.121 to 0.620)	0.93
Ṡ <sub>E</sub> (mL·min <sup>-1</sup> )	0.996	0.996 (0.977 to 1.014)	-0.178 (-0.795 to 0.631)	-0.853 (-2.310 to 0.606)	0.25
F <sub>E</sub> O <sub>2</sub> (%)	0.861	1.054 (0.914 to 1.283)	-0.598 (-4.520 to 1.863)	0.434 (0.293 to 0.576)	0.04 (0.03)
F <sub>E</sub> CO <sub>2</sub> (%)	0.856	1.110 (0.962 to 1.382)	-0.542 (-1.494 to -0.172)	-0.284 (-0.428 to -0.140)	0.44
Ṡ <sub>O</sub> <sub>2</sub> (mL·min <sup>-1</sup> )	0.988	0.927 (0.877 to 0.977)	-66.4 (-121.8 to -9.4)	-218 (-305 to -131)	0.78
Ṡ <sub>CO</sub> <sub>2</sub> (mL·min <sup>-1</sup> )	0.990	0.972 (0.912 to 1.025)	-27.8 (-95.2 to 3.1)	-123 (-196 to -50)	0.94

V<sub>t</sub> = tidal volume; f<sub>R</sub> = respiratory frequency; Ṡ<sub>E</sub> = pulmonary minute ventilation; F<sub>E</sub>O<sub>2</sub> = oxygen expiratory fraction; F<sub>E</sub>CO<sub>2</sub> = carbon dioxide expiratory fraction; Ṡ<sub>O</sub><sub>2</sub> = oxygen uptake; Ṡ<sub>CO</sub><sub>2</sub> = carbon dioxide production

illustrates the Passing-Bablok regression and difference plots for Ṡ<sub>O</sub><sub>2</sub> (● 3A) and Ṡ<sub>CO</sub><sub>2</sub> (● 3B).

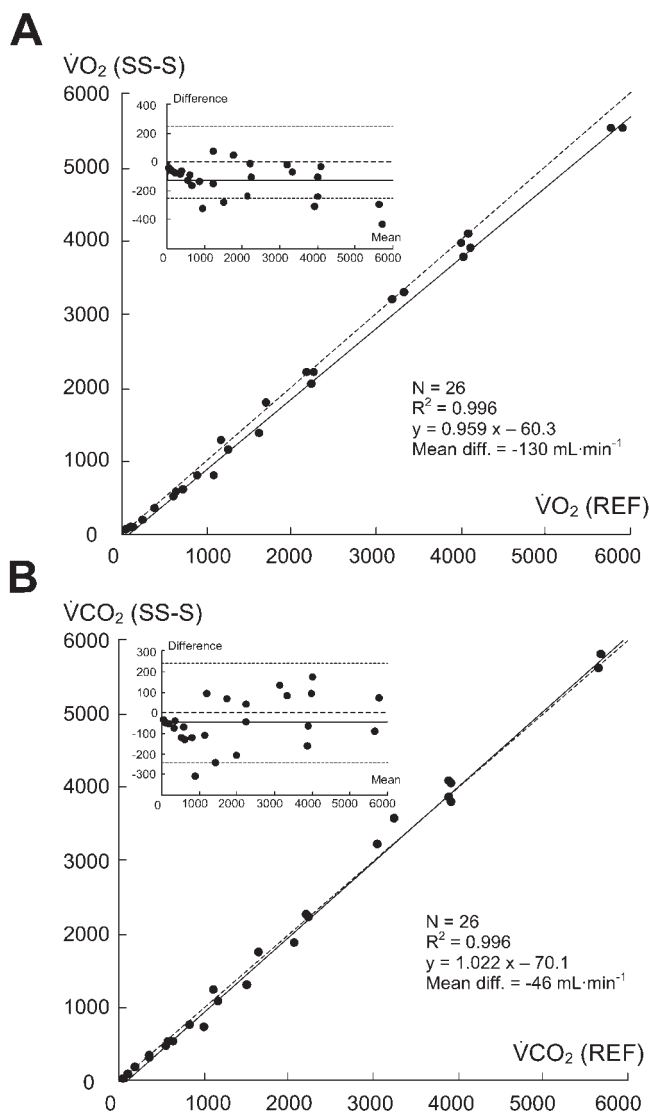
## Discussion

Both models of the swimming snorkel being assessed showed good accuracy in most parameters investigated, and only minor, nonsignificant differences were observed in gas exchange parameters, except a moderate bias in F<sub>E</sub>O<sub>2</sub>, which did not significantly affect the validity of Ṡ<sub>O</sub><sub>2</sub> measurements. SS-S was slightly closer to reference values due to lower dead-spaces and re-breathing volumes. Additionally, present data confirmed the validity of the K4 b<sup>2</sup> portable metabolic cart for B×B gas exchange analysis during exercise throughout a wide physiological range. Finally, the GESS system itself and the validation methodology chosen were proven useful for the purpose in question.

### Utility of the gas exchange simulation system (GESS)

Validation studies with human subjects for the precise assessment of indirect calorimetry systems are problematic mainly because of technical constraints derived from the experimental set-up and biological variability. In fact, although simultaneous data collection would be an ideal experimental design, this is generally not feasible because both systems interfere with each other when operating on a B×B basis. Moreover, human subjects are not considered reliable standards [6]. Integrated simulation devices capable of reproducing both ventilation and gas exchange parameters are a good alternative, and they can be seen as an overall test of hardware, software, and handling of the met-

abolic system being tested. Previous reports have shown the feasibility and utility of different gas exchange simulators for routine calibration and quality control of open-circuit calorimeters [6,9,19,20]. Overall, the GESS used in this investigation proved to be a feasible and useful device for the purpose of generating gas volumes across the range of measurement during swimming. The use of certified calibration gases in the simulation ensured the continuous delivery of expired gas fractions with good reproducibility (average variation coefficients for F<sub>E</sub>O<sub>2</sub> and F<sub>E</sub>CO<sub>2</sub> were 0.46 and 0.87% relative). Nevertheless, the GESS has a number of limitations. First, even if a computerized position sensor and apposite software allowed proper monitoring of the actual "breathing" volume, frequency and waveform, because of manual pumping variability, the system does not allow to check the reliability of ventilatory measurements achieved by the metabolic cart. However, for the purpose of a reliability check for method comparisons, as in this investigation, the quality of the simulation proved to be satisfactory. As an indicator of the repeatability of the ventilatory volumes generated, the variation coefficients for V<sub>t</sub> and Ṡ<sub>E</sub> measurements, which include both manual pumping variability and turbine flow measurement variability, were 2.6% and 3.1%, respectively. A second limitation is that the simulator in its current form does not deliver the warm, vapor saturated expirate of an exercising subject (BTSPS), but rather dry gases (calibration gases come from the cylinder essentially dry but become somewhat humidified by the passage through the Nafion® sampling line) at room temperature. This requires the measurement of temperature and humidity of expired gases, and to ensure that the simulation system and the metabolic cart both use the same algorithms and correction fac-



**Fig. 3A and B** Regression and difference plots of oxygen consumption ( $\dot{V}O_2$ ,  $\text{mL}\cdot\text{min}^{-1}$ ) (A) and dioxide production ( $\dot{V}CO_2$ ,  $\text{mL}\cdot\text{min}^{-1}$ ) (B) as measured by the K4 b<sup>2</sup> cart using the small volume swimming snorkel (SS-S), and directly measured without snorkel (REF). In the Passing-Bablok regression plot, the solid line indicates linear regression ( $y = ax + b$ ), and the dashed line indicates identity ( $y = x$ ). In the Bland-Altman plot (inset panel), lines indicate mean difference (solid), identity (dashed), and mean difference  $\pm 1.96$  SD (95% CI) intervals (dotted).

tors (e.g., to BTPS for inspired and expired volumes, and STPD for  $\dot{V}O_2$  and  $\dot{V}CO_2$ ) in order to make simulated and measured parameters comparable in absolute values. Therefore, further development of the GESS device would ideally include automated (i.e., motor-driven) operation and a system for the measurement and regulation of water vapor content and temperature in expired gases.

#### Accuracy of the K4 b<sup>2</sup> portable metabolic cart

Previous studies have compared the K4 b<sup>2</sup> with laboratory based metabolic carts or Douglas bags in human subjects during exercise only. K4 b<sup>2</sup> measurements have consistently shown to be reliable and highly correlated with those obtained with different metabolic carts [4,5,16,18] and with the Douglas bag method [15]. However, several inaccuracies have been reported in studies in which the system was tested typically during cycling and

treadmill running in human subjects. In summary, the K4 b<sup>2</sup> showed good test-retest reliability [4], but it was found to slightly: a) overestimate [18] or underestimate  $\dot{V}_E$  [15]; b) overestimate  $\dot{V}O_2$  [4,5,15,16]; c) underestimate [15], overestimate [4], or accurately measure [5]  $\dot{V}CO_2$ ; d) underestimate  $F_{E}O_2$  [4,5,18]; and e) underestimate [18] or overestimate [4]  $F_{E}CO_2$ .

For a discussion of the potential errors of an automated gas analysis system, it must be taken into account that any spirometric measurement of this kind relies on appropriate technical design and proper handling of the device. The most important technical characteristics are: 1) the accuracy of the sensors, 2) the dynamics of the gas sensors including its numerical compensations, 3) the synchronization of the signals, and 4) adequate assumptions for expiratory temperature and humidity at the sensors site. This is the first study, to our knowledge, in which this metabolic cart has been tested using a gas exchange simulator. All the above-mentioned technical requisites appear to be adequately fulfilled by the K4 b<sup>2</sup> tested under the carefully controlled conditions of the laboratory. In general, GESS generated (or predicted) and measured values were highly correlated and in good agreement, with only a small but significant overestimation of  $V_t$  (4%), not large enough to significantly influence the accuracy of pulmonary ventilation or gas exchange parameters, although the system showed a nonsignificant trend to overestimate  $\dot{V}_E$  (4.2%) and  $\dot{V}O_2$  (3.6%), and underestimate  $\dot{V}CO_2$  (-2.2%). These results partially agree with those obtained by McLaughlin et al. [15] using the Douglas bag combined with the micro-Scholander technique as a criterion method, who found a slight but significant overestimation of  $\dot{V}O_2$  (ranging from 9.6% at 50 W to 3% at 200 W, with no significant bias at 250 W), and underestimation of  $\dot{V}_E$  (our results showed the opposite trend) and  $\dot{V}CO_2$  at some but not all exercise intensities. However, because of the small magnitude of the discrepancies in the primary variable ( $< 100 \text{ mL}\cdot\text{min}^{-1}$  in  $\dot{V}O_2$  is considered by the authors to be physiologically insignificant for many purposes), they concluded that the K4 b<sup>2</sup> was acceptably accurate for measuring oxygen uptake over a fairly wide range of exercise intensities (up to  $\sim 0$ – $3.5 \text{ L}\cdot\text{min}^{-1}$ ). Our data suggest that one may expect somewhat larger deviations in both parameters at a higher range of measurement (i.e., in excess of about  $5.5 \text{ L}\cdot\text{min}^{-1}$ ). More recently, the K4 b<sup>2</sup> has been compared with a laboratory based mass spectrometer system (Morgan EX670, Morgan Medical Limited, Gillingham, Kent, UK) during cycling at different intensities [16], and found to systematically overestimate both  $\dot{V}O_2$  and  $\dot{V}CO_2$  (3 and 8%, respectively). This bias was close in magnitude (but not in statistical significance) only to that found in the present investigation for  $\dot{V}O_2$  (3.6%,  $p = 0.61$ ), but not for  $\dot{V}CO_2$  (-2.2%,  $p = 0.75$ ). Similarly, present results did not confirm the underestimation of expired gas concentrations ( $F_{E}O_2$  and  $F_{E}CO_2$ ) and  $\dot{V}_E$  overestimation found by Pinnington et al. [18], and neither the overestimation of  $\dot{V}O_2$ ,  $\dot{V}CO_2$ , and  $F_{E}CO_2$  nor underestimation of  $F_{E}O_2$  found by Duffield et al. [4], when comparing serial measurements during exercise in human subjects with laboratory based metabolic carts. Moreover, neither of these could be strictly considered as criterion validation studies, but rather inter-method comparison investigations using biological calibration designs. Overall, the biological variability being excluded in our experimental design, and the values generated by the simulation system being considered as a criterion, present data indicating that the K4 b<sup>2</sup> can be considered as a fairly accurate instrument for measuring  $B \times B$  gas exchange parameters throughout a wide physiological range of measurement during exercise.

### Validity of the swimming snorkels (SS)

The original SS as developed by Toussaint et al. [22] was designed and validated to be used with Douglas bags but not with B × B gas analysis systems. While the original SS-S was mainly used for steady-state measurements at swimming intensities requiring medium high ventilations leading to  $\dot{V}O_2$  values up to approximately 3000 mL·min<sup>-1</sup>, the larger volume device (SS-L) was designed to be used especially in maximal efforts eliciting very high ventilations. In the present study, the highest ventilation attained was 168 L·min<sup>-1</sup>, which was suitable also for the SS-S model. Keskinen et al. [11] further modified the system to make it suitable for B × B measurements and made a biological validation of the system during exercise in laboratory conditions. In the original design of Toussaint [22], the outlet of the expiratory tube was connected to a Douglas bag or to a gas analyzer with a traditional mixing chamber and the inlet of inspired air was separated from the outlet. In the B × B modified snorkel [11], both the outlet and inlet tubes are still separated but they are connected to the turbine of the K4 b<sup>2</sup> via a connecting unit (● Fig. 1), which allows rebreathing to occur through the turbine; with this arrangement expired air can be distinguished from inspired air at the turbine site, and tidal volume and respiratory frequency can be defined for B × B analysis, although allowing inspiratory and expiratory gases to mix to a small extent in the beginning of both the expiration and inspiration. The increase of the air pressure in the turbine marks the start of the expiration and inspiration with a small delay, which had been adjusted by the Cosmed software to exclude the mixed air to be analyzed. Despite these sources of potential technical error, a previous biological validation study [11] showed that values obtained using the new B × B swimming snorkel highly correlated with those obtained using the conventional Hans-Rudolph facemask provided with the K4 b<sup>2</sup> (R<sup>2</sup> values > 0.9). However, differences existed between the two series of measurements so that most ventilatory and gas exchange parameters were lower (3–7%) with the swimming snorkel, the error being mainly systematic along the whole range of measurement. However, the ultimate cause for the discrepancies remained unclear, while the investigation could not differentiate the actual technical error of measurement from the effect of biological variability. Moreover, critical remarks from this previous biological validation study [11] pointed out that a reduction in dead space (inlet and outlet tubes and the connector unit) should be achieved in order to enable more immediate gas sampling and minimize the decrease in expiratory air temperature. Therefore, further modifications were introduced in the design and two models, smaller (SS-S) and larger volume (SS-L) swimming snorkels, were tested in this study.

By using a gas exchange simulator, the present experimental design excluded biological variability and was allowed to focus on the technical error of measurement only. Overall, GESS generated values highly correlated with those obtained using both SS, particularly for the ventilatory parameters ( $V_t$ ,  $f_R$ ,  $\dot{V}_E$ ) and primary gas exchange variables ( $\dot{V}O_2$  and  $\dot{V}CO_2$ ). The only significant discrepancy was a moderate but significant overestimation of  $F_{E}O_2$  in both SS models (2.65% and 2.48% relative in SS-S and SS-L, respectively), which can be attributed to the mixing of inspiratory and expiratory gases in the beginning of both the expiration and inspiration at the connecting unit. Nevertheless, the moderate overestimation of  $F_{E}O_2$  appeared not to be large enough to significantly influence the accuracy of  $\dot{V}O_2$  measurements, which deserves further discussion. Ventilation is com-

puted from the expiratory flow signal only and corrected for temperature and relative humidity at the turbine site. The mouth-to-turbine distance is much longer compared to the conventional facemask setup due to restrictions imposed by the aquatic environment. In field swimming conditions, the K4 b<sup>2</sup> turbine and gas sampling line must be kept well over the surface to avoid the risk of getting water into these key components during the measurements, which would result in serious damage to the system. Further, the length of the out/inlet tubes must be long enough to enable an assistant to follow the swimmer on the pool deck by hanging the K4 b<sup>2</sup> machinery in a basket at the distal end of a hand held lever arm. Despite these technical constraints, ventilatory parameters were accurately measured, and only minor, nonsignificant differences were observed in  $V_t$  and  $\dot{V}_E$  with both SS models compared to REF values. Moreover, the significant overestimation of  $V_t$  found when comparing the GESS values with K4 b<sup>2</sup> (REF) measurements (● Table 3) turned into a minor, nonsignificant underestimation, and the effect on  $\dot{V}_E$  (4.2% bias) became almost negligible when using both snorkels. More complex is the computation of gas exchange parameters ( $\dot{V}O_2$ ,  $\dot{V}CO_2$ ). One may expect that the dead space volumes added to the system at the expiratory pathway by the SS would influence the results in these primary parameters, most obviously because of air rebreathing and mixing. Nevertheless, the excellent correlations and minor, nonsignificant differences found in  $\dot{V}O_2$  values (particularly by SS-S) suggest that the effects of larger dead space volumes actually appeared to compensate for the overestimation of  $F_{E}O_2$ . In fact, compared to REF measurements,  $\dot{V}O_2$  values measured by SS-S, even if not significantly different from values measured by SS-L, showed an even lower bias. In designing the SS-S, a significant shortening of the mouth-to-turbine distance was achieved by reducing the expiration tube length. SS-S has a total dead space volume which is 36% smaller than SS-L (see ● Table 1), and showed 40% lower bias in  $\dot{V}O_2$  values, which confirmed the relevance of this parameter in the accuracy of measurement. Further, it must be reminded that the K4 b<sup>2</sup>, compared to GESS values, showed a small but significant overestimation of  $V_t$  (4%), which resulted in nonsignificant overestimation of  $\dot{V}O_2$  (3.6%). This would further reduce the “true” technical error in  $\dot{V}O_2$  measurements when using the K4 b<sup>2</sup> in connection with both snorkels, which can be estimated to be about 3.1% for SS-S and 7.1% for SS-L. Nevertheless, although certain technical errors of measurement exist, mostly due to moderate air rebreathing and mixing imposed by the technical design for the use of the SS in the water, they actually seem to counterbalance each other. Overall, both SS proved to be valid devices to measure  $\dot{V}O_2$ , although SS-S seemed to produce slightly more accurate measurements.

Despite the clear advantages of using a gas exchange simulator (e.g., excluding biological variability and reliably reproducing gas volumes in a wide range of measurements with different set-ups), a limitation of the present procedure is that, as discussed before, the simulator in its current form does not deliver the warm, saturated expirate of an exercising subject, but rather dry gases at room temperature. Besides the fact that correction factors were applied to ensure the comparability of gas volumes, a previous biological calibration study [11] was indeed performed with human subjects during exercise with comparable results. Moreover, the feasibility and practicality of the system has also been determined in measuring gas exchange parameters and  $\dot{V}O_2$  kinetics during free swimming [21].



In summary, the K4 b<sup>2</sup> portable metabolic cart can be considered as an accurate instrument for measuring B×B gas exchange parameters across a wide physiological range during exercise. When used in connection with two models of a new swimming snorkel, the accuracy of measurement was also good in most parameters, although a moderate bias was observed in the expiratory O<sub>2</sub> fraction, not large enough to affect the accuracy of pulmonary ventilation and oxygen uptake measurements. The smaller snorkel was slightly closer to reference values due to lower dead space and rebreathing volumes. Therefore, we conclude that both respiratory snorkels are valid devices for measuring pulmonary gas exchange parameters during swimming.

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