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ORIGINAL ARTICLE

Cardiac output with modified cardio-impedance against inert gas rebreathing during sub-maximal and maximal cycling exercise in healthy and fit subjects

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Abstract

Purpose We measured cardiac output ($\dot{Q}$) during sub-maximal and supra-maximal exercise with inert gas rebreathing ($\dot{Q}_{IN}$) and modified cardio-impedance ($\dot{Q}_{PF}$) and we evaluated the repeatability of the two methods.

Methods $\dot{V}O_2$ and $\dot{Q}$ were measured twice in parallel with the two methods at sub-maximal (50–250 W) and supra-maximal exercise in 7 young subjects (25 ± 1 years; 74.4 ± 5.2 kg; 1.84 ± 0.07 m).

Results $\dot{Q}_{IN}$ and $\dot{Q}_{PF}$ increased by 3.4 L·min^{-1} and by 5.1 L·min^{-1} per 1 L·min^{-1} of increase in $\dot{V}O_2$, respectively. Mean $\dot{Q}_{PF}$ (23.3 ± 2.5 L·min^{-1}) was 9% lower than $\dot{Q}_{IN}$ (25.8 ± 2.2 L·min^{-1}) during supra-maximal exercise. Bland–Altman analysis showed that: (i) bias ($\dot{Q}_{PF}$–$\dot{Q}_{IN}$) was significantly different from zero (− 0.65 ± 2.61 L·min^{-1}) and; (ii) the ratios $\dot{Q}_{PF} \div \dot{Q}_{IN}$ were linearly related with $\dot{Q}$, indicating that $\dot{Q}_{IN}$ tended to overestimate $\dot{Q}$ in comparison with $\dot{Q}_{PF}$ for values ranging from 10.0 to 15.0 L·min^{-1} and to underestimate it for larger values. The coefficient of variation was similar for sub-maximal values (8.6% vs. 7.7%; 95% CL: ×/÷1.31), but lower for $\dot{Q}_{PF}$ (7.6%; 95% CL: ×/÷ 2.05) than for $\dot{Q}_{IN}$ (27.7%; 95% *CL*: ×/÷2.54) at supra-maximal intensity.

Conclusions $\dot{Q}_{PF}$ seems to represent a valuable alternative to invasive methods for assessing $\dot{Q}$ during sub-maximal exercise. The $\dot{Q}_{PF}$ underestimation with respect to $\dot{Q}_{IN}$ during supra-maximal exercise suggests that $\dot{Q}_{PF}$ might be less optimal for supra-maximal intensities.

Keywords Cycling exercise · Oxygen uptake · Cardiac output · Repeatability · Reliability

Abbreviations

BW	Body weight
C_aO_2	Arterial blood concentration of O_2
(C_aO_2–$C\bar{v}O_2$)	Artero-to-mixed venous blood difference in O_2 concentrations
CV	Coefficient of variation
ΔWL	Net increment of workload during incremental test to exhaustion
ES	Effect size
H	Height
Hb	Haemoglobin
HR	Heart rate
HR_{max}	Maximal heart rate
N_2O	Nitrous oxide
$\dot{Q}$	Cardiac output
$\dot{Q}_{IN}$	Cardiac output assessed by using inert gas rebreathing
$\dot{Q}_{max}$	Maximal cardiac output
$\dot{Q}_{PF}$	Cardiac output assessed using cardio impedance (Physioflow™)

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$\dot{Q}_aO_2$	Systemic oxygen delivery
$\dot{Q}_aO_{2max}$	Maximal systemic oxygen delivery
RC	Repeatability coefficient
SF_6	Sulfur hexafluoride
TE	Typical error
TTE	Time to exhaustion
$\dot{V}CO_2$	Carbon dioxide output
$\dot{V}_E$	Pulmonary ventilation
$\dot{V}O_2$	Oxygen uptake
$\dot{V}O_{2s}$	Oxygen uptake at steady state
$\dot{V}O_{2max}$	Maximal oxygen uptake
$\dot{V}O_{2peak}$	Peak oxygen uptake
WL	Workload
WL_{max}	Maximal workload

Introduction

$\dot{Q}_aO_2$ is the product of $\dot{Q}$ times C_aO_2 and it is mainly set by $\dot{Q}$, as C_aO_2 is usually hardly affected by exercise intensity in normoxia. $\dot{Q}_aO_{2max}$ is the main limiting factor of $\dot{V}O_{2max}$ if one exercises using large muscle mass at sea level (di Prampero and Ferretti 1990; Mortensen et al. 2005). Therefore, the assessment of $\dot{Q}$ is of paramount importance if one wants to quantify the determinants of individual maximal aerobic exercise capacity.

In addition, the evaluation of the $\dot{Q}$ during exercise is essential to quantify the improvements in maximal aerobic power observed after physical training, which usually elicits a remarkable increase of $\dot{Q}_aO_{2max}$. By the same token, the evaluation of $\dot{Q}$ allows us also to gain insightful information on the adaptations brought about by sedentary lifestyle, disuse or aging (Montero et al. 2015; Warburton et al. 1999a).

To assess $\dot{Q}$ in humans, a variety of invasive and non-invasive methods have been proposed and evaluated (Warburton et al. 1999a, b). The formers are usually considered as the most accurate when compared with the latter ones (Warburton et al. 1999a, b).

Despite their high validity and reliability, invasive methods in exercise physiology are not broadly diffused especially because of their practical—technical limitations, costs and the high training level required by the operators (Reuter et al. 2010; Warburton et al. 1999a). These drawbacks have led to the proposal of several non-invasive approaches for assessing $\dot{Q}$ during exercise: the modified cardio-impedance, the inert gas rebreathing methods and the pulse contour analysis, just to cite a few of them (Tam et al. 2004; Warburton et al. 1999a, b).

However, issues concerning their accuracy, reliability and validity have emerged, especially during maximal exercise. Indeed, as shown by Siebenmann et al. (2015), the determination of $\dot{Q}$ during exercise through most popular non-invasive methods (e.g. modified impedance cardiography, inert gas rebreathing and pulse contour analysis) usually generate significantly different values as compared with the ones obtained using the direct Fick's method. Thus, the values of $\dot{Q}$ measured during exercise depend on the applied non-invasive method (Siebenmann et al. 2015). Nonetheless, considering the problems inherent in the invasive determination of $\dot{Q}$ with invasive procedures during exercise, it would be important to evaluate the reliability and repeatability of different non-invasive methods. With regards to this, the determination of $\dot{Q}$ during exercise using inert gas rebreathing with Innocor™ (Innovision, DK) has been compared to the direct Fick method in healthy subjects (Siebenmann et al. 2015). However, repeatability was not investigated and needs further elucidation.

Furthermore, the assessment of $\dot{Q}$ by the modified cardio-impedance has been validated against gold-standard methods in both patients and healthy volunteers during light to severe exercise and at maximal intensity (Charloux et al. 2000; Siebenmann et al. 2015). On the other hand, the determination of $\dot{Q}$ during maximal efforts by using this technology seems to be spoiled by artefacts induced by the excessive movements of the subject (Charloux et al. 2000; Richard et al. 2001).

Hence, we performed the current investigation to compare first inert gas rebreathing by means of Innocor™ (Innovision, DK) and the modified cardio-impedance through Physioflow™ (Manatec Biomed., F) for measuring steady-state $\dot{Q}$ during cycling exercise at sub-maximal intensity in young, fit subjects.

Furthermore, since $\dot{Q}$ could be underestimated and/or overestimated by non-invasive approaches during intense exercise (Siebenmann et al. 2015), we also measured $\dot{Q}$ during cycling exercise of severe and maximal intensity.

Finally, the repeatability of the two methods in the two exercise conditions was evaluated, as "good" repeatability is crucial to evaluate the cardiovascular adaptations induced by interventions and cardiovascular therapies.

Materials and methods

Subjects

Seven young, active and healthy, non-smoker male were investigated after screening for cardiopulmonary diseases, (age: 25 ± 1 years; BW: 74.4 ± 5.2 kg; H: 1.84 ± 0.07 m; $\dot{V}O_{2peak}$: 4643 ± 369 mL·min^{-1}).

The experimental design, methods and procedures of the investigation followed the Declaration of Helsinki and approved by the Ethics Committee of The Norwegian School of Sport Sciences (04-020517) and The Norwegian Centre for Research Data (54117). The subjects gave their written informed consent before participation.

Experimental design

The subjects were investigated in three occasions and they did not perform heavy physical exercise the day before the tests. During the first visit to the laboratory, *BW* (Seca 877, Seca, Hamburg, Germany), *H* (Seca 217, Seca, Hamburg, Germany) and $\dot{V}O_{2peak}$ of the subjects were measured.

On the second day, after a familiarization session with the rebreathing maneuver, $\dot{Q}$ at steady state was measured during four-five submaximal cycling tests between 50 and 250 W (50 W increments). Thereafter, after 5 min of active recovery, and 5 min of rest sitting on the ergometer, $\dot{Q}_{max}$ was measured during supra-maximal exercise. During the third session, performed after a few days, the very same protocol was repeated. All the experiments were performed keeping the environmental conditions strictly controlled (average temperature = 21 °C; average relative humidity = 55–65% and a cooling fan was placed behind the subjects).

Experimental protocol

$\dot{V}O_{2peak}$ determination

In their first visit the subjects performed four sub-maximal exercise tests at the WL of 50, 100, 150 and 200 W pedaling for 6 min at 80–85 revolution-per-minute (rpm) so that the individual linear relationships between WL and: (i) $\dot{V}O_{2s}$; (ii) HR, both averaged in the last 2 min of each step, were obtained.

Then, the linear relationship between WL and HR was extrapolated to the individual HR_{max} (Tanaka et al. 2001) to obtain WL_{max}. Afterwards, we subtracted from WL_{max} the WL of the warm-up preceding the ramp test to obtain ΔWL, which, once divided by 10, yielded the increment in watt per minute (W min^{-1}) of the maximal, ramp test.

The incremental test started with 3 min of warm up at 100 W followed by a stepwise increase in WL every minute until exhaustion and it was terminated when the subject was not able to keep the selected pedaling rate of 80 rpm. The rates of increase of the WL was able to induce exhaustion in all the subjects in 10–12 min. HR, $\dot{V}O_2$, $\dot{V}CO_2$ and $\dot{V}_E$ were measured continuously and averaged on a 30-s base. Peak oxygen uptake ($\dot{V}O_{2peak}$) was defined as the highest $\dot{V}O_2$ measured in a 30-s epoch.

Cardiac output during sub-maximal exercise

The subject started pedaling at 50 W maintaining a fixed pedaling frequency of 80–85 rpm. The sub-maximal steps were carried out at the workloads indicated above and, if feasible, also at 250 W. After 5 min of exercise at each step, $\dot{Q}$ at steady state was measured using inert gas rebreathing ($\dot{Q}_{IN}$). Afterwards, the WL was increased and the procedure repeated.

$\dot{Q}$ and HR with Physioflow™ were recorded throughout experiment; $\dot{V}O_2$, $\dot{V}CO_2$ and $\dot{V}_E$, were assessed from the third to the fifth minute at each WL until the beginning of the rebreathing maneuver. Once the subject had completed the last sub-maximal step, he cooled down pedaling for 5 min at 50 W and then remained seated for 5 min on the saddle.

Cardiac output during supra-maximal exercise

Knowing the relationship between WL and $\dot{V}O_{2s}$ and the $\dot{V}O_{2peak}$, the WL corresponding to 105% of individual maximal aerobic mechanical power was calculated for the supra-maximal test. The subject started to pedal for 3 min at 50 W, thereafter the WL was quickly increased to the calculated supra-maximal WL and the subject kept pedaling until exhaustion.

Also during this test, $\dot{V}O_2$, $\dot{V}CO_2$ and $\dot{V}_E$ were measured. Approximately one minute before exhaustion, the mouthpiece of the metabolic cart was removed and $\dot{Q}_{IN}$ was assessed during the last 30 s of exercise before exhaustion. As during sub-maximal exercise, $\dot{Q}_{PF}$ with Physioflow™ was continuously recorded.

Methods

$\dot{V}O_2$, $\dot{V}CO_2$ and $\dot{V}_E$ were measured every 30 s using mixing chamber, open-circuit indirect calorimetry (Oxycon Pro; Jaeger Instrument, Hoechberg, Germany) during all the experiments. Before each test, the gas analyzers and ventilatory flow transducer were calibrated according to the instruction manual. $\dot{Q}_{IN}$ at steady state during sub-maximal and at maximal exercise was assessed via the inert gases rebreathing using Innocor™ (Innovision, DK) (Clemensen et al. 1994), which was calibrated according to the instruction manual. During the rebreathing (5–6 breaths, 10 to 15 s), the subject rebreathes a gas mixture from a rubber bag filled with 5.0% of N_2O, blood soluble gas, 1% SF_6, blood non-soluble gas used to calculate the variations of the volume of the closed system, 94% of O_2 and ambient air so that at the beginning of the rebreathing an O_2 enriched mixture containing abut 0.5% N_2O and 0.1% sulfur hexafluoride SF_6 in the filled bag exists. The volume of the ambient air diluting the concentrated mixture, and hence the volume of the bag, are calculated based on tidal volume and $\dot{V}O_2$ preceding rebreathing. Pulmonary N_2O transfer is estimated from the rate of disappearance of N_2O over three expirations after a stable SF_6 concentration is attained. The rate of disappearance of

N_2O gas from alveoli is proportional to the blood flow of the ventilated parts of the lungs.

Beat-by-beat $\dot{Q}_{PF}$ and HR were measured using a modified cardio-impedance method using Physioflow™ (Manatec Biomed., F) (Charloux et al. 2000). Electrode placement and skin preparation were carried out according to the instructions of the operating manual. Moreover, the subjects wore a tight mesh t-shirt to avoid the displacement of the electrodes. After instrumentation, the subject sat quietly on the bike and after 3–5 min blood pressure was measured three times (Spot Vital Signs® LXi, Welch Allyn, USA). The mean values of systolic and diastolic pressures were fed to the software to calibrate the Physioflow™.

To reposition the electrodes in the very same positions in the following experimental session, skin landmarks were traced on a transparent plastic sheet.

Statistics and data analysis

Data are reported as mean ± standard deviation. The averages of the beat-by-beat $\dot{Q}_{PF}$ measured during the fifth and sixth minutes of sub-maximal exercise were computed and compared with the ones assessed in parallel with rebreathing. During supra-maximal exercise, $\dot{Q}_{PF}$ recorded during the rebreathing was averaged. (C_aO_2 – $C\bar{v}O_2$) was calculated as the ratio between $\dot{V}O_2$ and $\dot{Q}$; values of (C_aO_2–$C\bar{v}O_2$) larger than 200 $mL \cdot L^{-1}$ were considered not plausible and this criterion was utilized to identify impossible $\dot{Q}$ values (Siebenmann et al. 2015).

Linear regressions were computed by means of least–square methods and the significance between of the differences between slopes and intercepts were evaluated (Zar 1999). Paired data were analyzed using a Student's *t* test; effect size (ES) for paired data was also calculated (Cohen 1988).

The agreement between the two sets of $\dot{Q}$ data (cardio-impedance and inert gas rebreathing) was evaluated by using Bland–Altman analysis (Bland and Altman 2003) plotting the differences ($\dot{Q}_{PF}$–$\dot{Q}_{IN}$) and the ratios ($\dot{Q}_{PF} \div \dot{Q}_{IN}$) against their corresponding averages.

The repeatability was evaluated by computing the typical error (TE) and the coefficient of variation (CV) as measures of the absolute and relative error, respectively (Hopkins 2000). TE and CV can be considered as the variation one can expect from one trial to another if subjects perform multiple trials.

Repeatability coefficient (RC), namely the difference that will be exceeded by only 5% of pairs of measurements performed on the same subject, was also calculated (Bland and Altman 2003).

Correlation analyses were conducted with Pearson's product-moment correlation and correlation coefficients (r) were classified as small ($0.1 \le r < 0.3$), moderate ($0.3 \le r < 0.5$), high ($0.5 \le r < 0.7$), very high ($0.7 \le r < 0.9$), and almost perfect ($r \ge 0.9$) (Hopkins et al. 2009).

Data were analyzed by using MedCalc Ver 17.6 (MedCalc Software bvba, Ostend, Belgium) and a Microsoft Office Excel spreadsheet prepared for the purpose (MO, Microsoft, Seattle, USA).

Results

One subject was not able to perform the supra-maximal workload in one of the two experimental sessions. In another subject, two replicated measurements of $\dot{Q}$ during the supra-maximal protocol using inert gas rebreathing were lost due to technical errors.

$\dot{V}O_2$ during sub-maximal exercise increased linearly as a function of WL from 1.21 $L \cdot min^{-1}$ ± 0.04 at 50 W, 1.78 $L \cdot min^{-1}$ ± 0.05 at 100 W, 2.39 $L \cdot min^{-1}$ ± 0.05 at 150 W, 3.05 $L \cdot min^{-1}$ ± 0.08 at 200 W and 3.62 $L \cdot min^{-1}$ ± 0.06 at 250 W.

Likewise, $\dot{Q}$ increased linearly as a function of exercise intensity: $\dot{Q}_{PF}$ from 10.4 ± 1.7 $L \cdot min^{-1}$ at 50 W to 22.9 ± 2.5 $L \cdot min^{-1}$ at 250 W and $\dot{Q}_{IN}$ from 12.6 ± 1.0 $L \cdot min^{-1}$ at 50 W to 20.8 ± 1.4 $L \cdot min^{-1}$ at 250 W.

The individual linear regressions between sub-maximal, steady–state $\dot{Q}$ and $\dot{V}O_2$ are reported for all the subjects in Fig. 1. Both $\dot{Q}_{PF}$ and $\dot{Q}_{IN}$ increased linearly as a function of $\dot{V}O_2$. However, the average slopes of the regressions obtained using the two methods were significantly different. In the first experimental run, $\dot{Q}_{PF}$ increased, on the average, by 4.7 ± 0.7 $L \cdot min^{-1}$ per $L \cdot min^{-1}$ of increase in $\dot{V}O_2$, whereas $\dot{Q}_{IN}$ increased by 3.3 ± 0.6 $L \cdot min^{-1}$ per $L \cdot min^{-1}$ ($n = 7$; $P < 0.01$; large ES). In the second run, the slope for $\dot{Q}_{PF}$ turned out to be 5.7 ± 1.17 $L \cdot min^{-1}$ per $L \cdot min^{-1}$ of increase in $\dot{V}O_2$, whereas it was 3.4 ± 0.6 $L \cdot min^{-1}$ per $L \cdot min^{-1}$ for $\dot{Q}_{IN}$ ($n = 7$; $P < 0.01$; large *ES*). The grand averages were 5.1 ± 1.0 $L \cdot min^{-1}$ and 3.4 ± 0.6 $L \cdot min^{-1}$ per $L \cdot min^{-1}$ for $\dot{Q}_{PF}$ and $\dot{Q}_{IN}$, respectively ($n = 14$; $P < 0.001$; ES = 1.33).

Average WL during the supra-maximal tests was 343 ± 34 W, which corresponded to about ~ 106% of mean maximal aerobic mechanical power (324 ± 32 W). The mean time to exhaustion (TTE) was 220 ± 41 s for the supra-maximal test. $\dot{V}O_2$ attained the value of 4.32 ± 0.30 $L \cdot min^{-1}$ during supra-maximal exercise. These $\dot{V}O_2$ values were about 7% smaller ($P = 0.013$, large *ES*) than the $\dot{V}O_{2peak}$ assessed during the preliminary maximal test (4.64 ± 0.37 $L \cdot min^{-1}$), due to the switch of mouthpieces ~ 1 min before exhaustion (from indirect calorimetry to inert-gas rebreathing).

During supra-maximal test, inert gas rebreathing generated only one implausible value of $\dot{Q}_{IN}$; modified cardio impedance generated three implausible values of $\dot{Q}_{PF}$. When only the plausible values were considered, $\dot{Q}_{PF}$ during

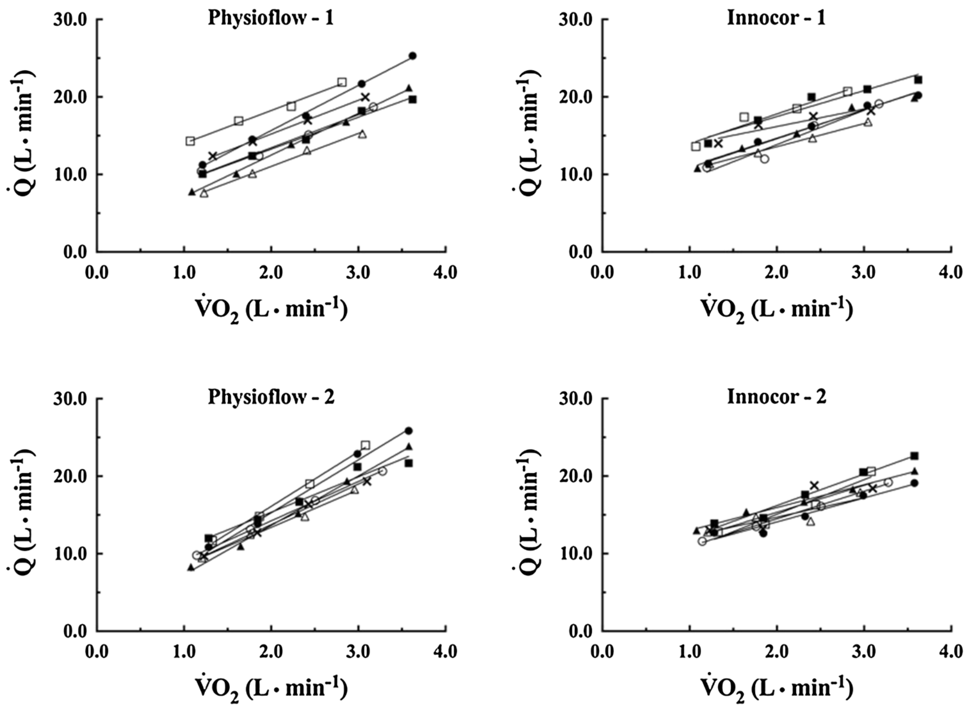

Fig. 1 The individual steady-state $\dot{Q}$ values of the seven subjects measured during sub-maximal cycling exercise with modified cardio impedance (left column) and inert gas rebreathing (right column) in the first (top diagrams) and in the second (bottom diagrams) experimental trial are represented as a function of the corresponding $\dot{V}O_{2s}$. The graphs also report the corresponding individual regression lines. Please see text for further details

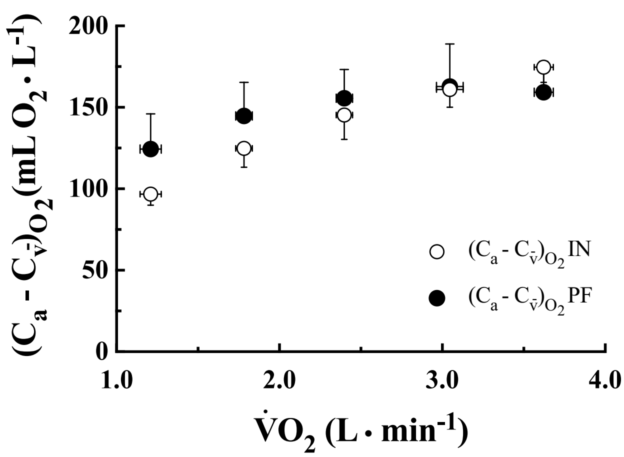

Fig. 2 Steady-state values of the artero-mixed venous O_2 difference calculated form the corresponding values of $\dot{Q}$ and $\dot{V}O_{2s}$. White dots refer $\dot{Q}_{IN}$, and black dots refer to $\dot{Q}_{PF}$

supra-maximal test was equal to 23.3 ± 2.5 L·min^{-1} and $\dot{Q}_{IN}$ to 25.8 ± 2.2 L·min^{-1}, i.e. about 9% larger, on the average, than the one assessed with cardio impedance.

During sub-maximal exercise, (C_aO_2–$C\bar{v}O_2$) calculated as the ratio between $\dot{V}O_2$ and $\dot{Q}_{PF}$ increased on the average from 124 ± 22 mL·L^{-1} at 50 W to 159 ± 19 mL·L^{-1} at 250 W; the one obtained from $\dot{Q}_{IN}$ increased from 96.8 ± 6.8 mL·L^{-1} to 174.7 ± 9.3 mL·L^{-}1 within the same WL range (Fig. 2). During supra-maximal test, when only the plausible values of $\dot{Q}$ were considered, (C_aO_2–$C\bar{v}O_2$) calculated from $\dot{Q}_{PF}$ was 182.3 ± 10.3 mL·L^{-1} and the one corresponding to $\dot{Q}_{IN}$ was 169.3 ± 17.3 mL·L^{-1}.

The results of the Bland–Altman plot are shown in Fig. 3 where the average of the differences ($\dot{Q}_{PF}$–$\dot{Q}_{IN}$) obtained at all the exercise intensities are plotted against their corresponding means (top diagram). Average $\dot{Q}_{PF}$–$\dot{Q}_{IN}$ amounted to − 0.65 L·min^{-1} (bias) and was significantly different from zero ($P = 0.044$,); SD (precision) was 2.61 L·min^{-1} and the 95% limits of agreement ranged from − 5.8 to 4.5 L·min^{-1}. Since the distribution of the difference was markedly heteroscedastic—the absolute values of $\dot{Q}_{PF}$–$\dot{Q}_{IN}$ increased with the average of the two measurements—we also plotted the ratios ($\dot{Q}_{PF} \div \dot{Q}_{IN}$) vs. the corresponding means. Mean $\dot{Q}_{PF} \div \dot{Q}_{IN}$ amounted to 0.95 and it was significantly different from 1 ($P = 0.0157$). SD was 0.16 and the 95% limits of agreement of the discrepancy of the ratio form 1 were 0.68/1.36).

($\dot{Q}_{PF}$–$\dot{Q}_{IN}$) were moderately and linearly related with their corresponding means ($n = 68$; $y = -3.95 + 0.19 \cdot \times$; $P = 0.057$; $r^2 = 0.11$, $F = 8.16$). Similarly, also ($\dot{Q}_{PF} \div \dot{Q}_{IN}$) were moderately and linearly related to the means ($n = 68$; $y = 0.68 + 0.017 \cdot \times$; $P < 0.0001$; $r^2 = 0.21$, $F = 20.06$). This indicates a linear relationship between the amplitude of the error and the absolute values of the measurements so that $\dot{Q}_{IN}$ tended to overestimate $\dot{Q}$ in comparison with $\dot{Q}_{PF}$ for values of $\dot{Q}$ ranging between 10.0 and 15.0 L·min^{-1} and to underestimate it for larger values corresponding o higher sub-maximal exercise intensities.

For $\dot{Q}_{PF}$, *TE* was 1.17 L·min^{-1} (95% confidence limits, CL: ×/÷ 1.29; $n = 31$) and 1.64 L·min^{-1} (95% CL: ×/÷1.98;

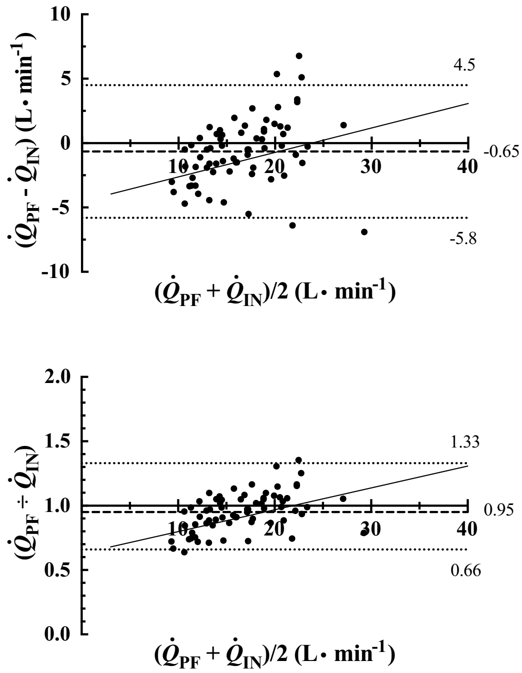

Fig. 3 Top: Bland–Altman diagram of the differences $\dot{Q}_{PF}$–$\dot{Q}_{INt}$ plotted as a function of their averages. Bottom: Bland–Altman diagram of the ratios $\dot{Q}_{PF} \div \dot{Q}_{INt}$ plotted as a function of their averages. Dotted lines in the two diagrams refer to biases and to errors, i.e. 95% CI of the bias. Continuous thin lines describe the linear relationships between the variables at stake. Please see text for more details

$n=6$) at sub-maximal and supra-maximal workloads, respectively. This corresponded to CVs of 8.6% (95% CL: ×/÷1.31) and 7.6% (95% CL: ×/÷2.05), respectively. For $\dot{Q}_{IN}$, *TE* was 1.10 L·min^{-1} (95% CL: ×/÷1.29; $n=31$) and 5.90 L·min^{-1} (95% CL: ×/÷2.19; $n=5$) at sub-maximal and supra-maximal workloads, respectively. This corresponded to *CV*s of 7.7% (95% CL: ×/÷ 1.31) and 27.7% (95% CL: ×/÷2.54), respectively. Finally, RC amounted to 3.79 L·min^{-1} and to 4.81 L·min^{-1} for $\dot{Q}_{PF}$ and $\dot{Q}_{IN}$, respectively.

Discussion

$\dot{Q}$ measured with cardio impedance and inert gas rebreathing increased during sub-maximal exercise by 5.1 L·min^{-1} and 3.4 L·min^{-1} per L·min^{-1} of increase in $\dot{V}O_2$, respectively.

Second, Bland–Altman analysis showed that: (i) bias between $\dot{Q}_{PF}$ and $\dot{Q}_{IN}$ amounted to − 0.65 L·min^{-1} and was significantly different from zero; (ii) ($\dot{Q}_{PF}$–$\dot{Q}_{IN}$) and ($\dot{Q}_{PF} \div \dot{Q}_{IN}$) were correlated with the absolute values of $\dot{Q}$, implying that $\dot{Q}_{IN}$ overestimated $\dot{Q}$ in comparison with $\dot{Q}_{PF}$ when $\dot{Q}$ was lower than about 15.0 L·min^{-1}, whereas it underestimated $\dot{Q}$ for values above 15.0 L·min^{-1}.

Finally, $\dot{Q}_{PF}$ was characterized by a good repeatability in comparison with $\dot{Q}_{IN}$, since it showed a coefficient of variation close to 8% regardless the intensity of the exercise.

The $\dot{Q}$ determination by the Physioflow™ has been validated against the Fick method during exercise in patients and in healthy subjects (Charloux et al. 2000; Richard et al. 2001). $\dot{Q}$ is expected to increase by ~ 5–6 L·min^{-1} per L·min^{-1} increase in $\dot{V}O_2$ when it is measured with invasive, gold-standard methods. Therefore, the average slope of the linear $\dot{Q}_{PF}$ vs. $\dot{V}O_2$ relationship (5.1 ± 1.0 L·min^{-1}) is within the range of values mentioned above and it is also practically identical to the one measured with direct Fick method during sub-maximal cycling (4.9 ± 0.3 L·min^{-1}) (Siebenmann et al. 2015). However, the slope of the $\dot{Q}_{PF}$ vs. $\dot{V}O_2$ regression line appeared to be lower when compared to other studies: Siebenmann et al. (2015) have reported an increase of $\dot{Q}_{PF}$, determined by Physioflow of 6.0 ± 0.4 L·min^{-1} per L·min^{-1}; $\dot{Q}_{PF}$ increased by 7.2 L·min^{-1} per L·min^{-1} increase in $\dot{V}O_2$ in patients with chronic obstructive pulmonary disease and sleep apnea syndrome likely because of the lower (C_aO_2–$C\bar{v}O_2$) prevailing in these patients (Charloux et al. 2000).

$\dot{Q}$ has been simultaneously assessed with Innocor™ and the direct Fick method in healthy subjects during sub-maximal and maximal cycling exercise (Siebenmann et al. 2015). In that occasion, the average increase of $\dot{Q}$ per L·min^{-1} increase in $\dot{V}O_2$ turned out to be 3.9 ± 0.2 L·min^{-1}, lower than expected and in agreement with the findings of the present investigation. Moreover, also in studies where Innocor™ was used in patients (Agostoni et al. 2005), this method underestimated $\dot{Q}$ when compared to invasive method, as $\dot{Q}$ increased by 3.5 L·min^{-1} per L·min^{-1} of increase in $\dot{V}O_2$. In conclusion, a bulk of evidence seems to suggest that Innocor™ may underestimate $\dot{Q}$ during cycling exercise in healthy, young subjects by about 20%.

Bland–Altman plots confirmed the poor level of agreement between $\dot{Q}_{PF}$ and $\dot{Q}_{IN}$ during sub-maximal exercise. First, bias was significantly different from 0; second, $\dot{Q}_{PF} \div \dot{Q}_{IN}$ were moderately and linearly related with the absolute values of $\dot{Q}$ indicating that $\dot{Q}_{IN}$ in respect to $\dot{Q}_{PF}$ overestimated $\dot{Q}$ below values of approximately 15 L·min^{-1}, corresponding to a $\dot{V}O_2$ of 2.4–2.5 L·min^{-1} (about 55% of $\dot{V}O_{2max}$ in our subjects), and underestimated $\dot{Q}$ at higher exercise intensities.

During supra-maximal exercise, $(C_aO_2$–$C\bar{v}O_2)_{PF}$ turned out to be, on the average, remarkably high: 182.3 ± 10.8 mL·L^{-1}. In addition, three $(C_aO_2$–$C\bar{v}O_2)_{PF}$ values out of seven indicated not plausible $\dot{Q}$. These findings suggest that modified cardio impedance may underestimate $\dot{Q}$ in this condition. Indeed, strenuous cycling exercise against a constant supra-maximal WL (~ 106% of maximal aerobic mechanical power) may have negatively affected cardio impedance recoding because of excessive movements, respiratory artefacts due the broad excursion of the thoracic cage due to hyperventilation and to accumulation of fluid in the lungs, thereby causing the underestimation of $\dot{Q}_{PF}$ at maximal exercise (Charloux et al. 2000; Kemps et al. 2008; Warburton et al. 1999b).

Recent data (Siebenmann et al. 2015) tended to show that also inert gas rebreathing underestimated $\dot{Q}$ during maximal cycling exercise in young, active men: Innocor™ generated a higher number of not plausible $\dot{Q}$ values than Physioflow™ and maximal $(C_aO_2–C\bar{v}O_2)_{IN}$ was about 180 mL·L^{-1} (Fig. 1a in Siebenmann et al. 2015). The results reported in the present investigation partially agree with the ones summarised above: maximal $(C_aO_2–C\bar{v}O_2)_{IN}$ calculated using $\dot{V}O_{2peak}$ was indeed about 4% larger than the value measured using direct Fick method (170 ± 20 mL·L^{-1}) at the end of maximal cycling exercise in active, young men (Siebenmann et al. 2015), but only one $\dot{Q}$ during supra-maximal exercise turned out to be frankly unrealistic.

As reported by Jarvis et al. (2007), Innocor™ may underestimate $\dot{Q}$ because of N_2O recirculation that occurs already after 8.5 s at a $\dot{V}O_2$ of 2.5 L·min^{-1} and in less than 8 s at a $\dot{V}O_2$ of 3 L·min^{-1} (Rigatto et al. 1968). Therefore, recirculation of N_2O may prevent further uptake of the soluble gas, contributing to the observed underestimation of $\dot{Q}$. Moreover, as N_2O only dissolves into the liquid phase of the whole blood, the progressive hemoconcentration occurring from rest to maximal exercise and resulting in a 5–10% increase in Hb concentration and in a reduction of plasma volume, may limit the uptake of N_2O.

So, it seems that during the supra-maximal tests both the two non-invasive techniques may underestimate $\dot{Q}_{max}$ and that $\dot{Q}_{PF}$ may be characterized by a larger underestimation of $\dot{Q}$, whereby few implausible values of $(C_aO_2–C\bar{v}O_2)_{PF}$ were observed at supra-maximal intensity. It is worth noting that, by performing ramp exercise to exhaustion, and not constant work rate of supra-maximal intensity, some of the causes of artifacts that undermine the measurements obtained with $\dot{Q}_{PF}$ may be avoided, especially if the subjects keep their thorax as still as possible approaching exhaustion. This is also partially confirmed by the findings of other investigators (Siebenmann et al. 2015) who found higher values of $\dot{Q}_{max}$ with Physioflow™ by using an incremental test to exhaustion.

The modified cardio-impedance benefits from "good" repeatability. Therefore, when multiple $\dot{Q}$ assessments are required on the same subject, $\dot{Q}_{PF}$ would vary within an "acceptable" range due to random errors. Regarding Innocor™, its *TE, CVs* and *RC* suggest that this method may be not suitable when $\dot{Q}$ has to be measured repeatedly during maximal exercise. However, these results should be interpreted with a pinch of salt because of the limited number of subjects evaluated during supra-maximal exercise with inert gas rebreathing, and of the short duration of the exercise bout. Indeed, it has been shown that Innocor™ was characterized by a CV of 7.0%, 95% CL: 5.5 to 9.5 (Fontana et al. 2009) during repetitions of maximal exercise in a group of 30 young men and women.

A clear limitation of the study was the low number of tested subjects (7 healthy males) and the absence of a gold-standard method as a reliable comparison. Furthermore, during the supra-maximal test the participants might have interrupted the exercise before attaining maximal $\dot{Q}$. Furthermore, as already outlined, the change of mouthpiece necessary for carrying out the measurement of $\dot{Q}$ by Innocor™ prevented us to measure $\dot{V}O_2$ using mixing chamber during the last minute of the supra-maximal effort.

Conclusions

The modified cardio-impedance might be a non-invasive alternative to the invasive approaches for the determination of $\dot{Q}$ in healthy population, at least during exercise at sub-maximal intensity. In addition, it is attractive because its implementation does not need trained personnel. However, the application of Physioflow™ during supra-maximal effort seems to be less indicated.

Moreover, the "good" repeatability showed by the Physioflow™ makes this technique suitable when the changes brought about by interventions such as training/de-training and the efficacy of specific therapies on the cardiovascular responses have to be evaluated and quantified.

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Compliance with ethical standards

Conflict of interest The authors declare no conflicts of interest.

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