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The simultaneous comparison of acetylene or carbon dioxide flux as a measure of effective pulmonary blood flow in children

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The simultaneous comparison of acetylene or carbon dioxide flux as a measure of effective pulmonary blood flow in children. M. Rosenthal, A. Bush. ©ERS Journals Ltd 1997.
ABSTRACT: Both acetylene (Ac) and carbon dioxide can be used to measure effective pulmonary blood flow (Q'eff) noninvasively. They are safe and reasonably accurate in adults during rest and exercise, but there have been no simultaneous comparisons in children.

One hundred and six healthy children (55 males and 51 females, aged 8–17 yrs) were studied using an Innovision quadrupole mass spectrometer. They all underwent five rebreathing manoeuvres at rest, and then single measurements were again taken after 9 min of bicycle exercise. Mixed venous CO_2 levels were calculated either by a linear (L) or curvilinear (C) extrapolation method.

At rest, the coefficients of variation for Q'eff were Ac 8%, L 20%, and C 16% (p<0.001). The median resting values were: Ac 3.2 (95% confidence interval (95% CI) 3.1–3.4) L 5.1 (95% CI 4.6–5.4) and C 4.7 (95% CI 4.3–5.1) $L \cdot min^{-1} \cdot m^{-2}$, (p<0.001). Compared to Ac, only 14 and 17% of L and C values, respectively, were ±0.5 $L \cdot min^{-1} \cdot m^{-2}$, whilst 41 and 29%, respectively were more than ±2 $L \cdot min^{-1} \cdot m^{-2}$.

During exercise, median values were: Ac 6.7 (95% CI 6.3–7.0); L 8.0 (95% CI 7.3–8.4); and C 7.2 (95% CI 6.5–7.9) $L \cdot min^{-1} \cdot m^{-2}$. L was significantly greater than C (p<0.001), but C was similar to Ac (p=0.06). More than 50% of L and C values could not be calculated for various reasons, whereas all 106 Ac values could be calculated.

Neither carbon dioxide method is sufficiently reliable to be used in children in a clinical setting. Acetylene was safe, reliable, accurate and preferred.
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The noninvasive measurement of effective pulmonary blood flow (Q'eff) in humans using gas exchange and the Fick principle has been available since 1912 [1]. The Fick principle states that Q'eff can be calculated from the ratio of soluble gas consumption to its arteriovenous content difference, assuming that the end-capillary arterial tension equals that in the alveolus (after BORNSTEIN [2]). If all measurements are gathered noninvasively, this is termed an indirect Fick method.

Several candidate gases have been used since nitrous oxide in the original study [1]. Acetylene (Ac) is used [3–8] because it is 50% more water soluble than nitrous oxide, reducing measurement error [9], and less lipophilic, so that results are less affected by dissolution in the erythrocyte membrane. Similarly, freon has also been used but is now less popular, because, as a chlorofluorocarbon, it is potentially damaging to the environment and also its high molecular weight changes its behaviour compared to Ac.

Carbon dioxide is used as an alternative to nonnative gases by applying the Fick principle in reverse, namely the ratio of CO_2 production to its venoarterial content difference [10, 11]. CO_2 is claimed to be a superior marker to acetylene in obstructive lung disease, as the delayed disappearance of Ac into poorly-ventilated regions of the lung would lead to a spuriously raised result for pulmonary blood flow [12]. However, both a theoretical model [13] and *in vivo* [14] evidence have disproved this. Nevertheless, CO_2 was chosen when investigating pulmonary blood flow in adults with cystic fibrosis [15]. Simultaneous, invasive direct Fick measurements and noninvasive indirect Fick CO_2 rebreathing studies on 16 adult subjects [16] found that CO_2 values overestimated the direct Fick result by 0.5 (range +2.5 to -1.5) $L \cdot min^{-1}$ at rest, but underestimated by only 0.16 $L \cdot min^{-1}$ during exercise. Repeatability testing in one subject [17] showed a coefficient of variation (CV) of 24% at rest and 8% during exercise for CO_2 rebreathing methods.

As invasive methods of gaining routine measurements in children are unacceptable, the best indirect method needs to be determined. We have used respiratory mass spectrometry to compare the noninvasive, simultaneous assessment of effective pulmonary blood flow using both Ac and CO_2 during rest and exercise in 106 normal children.

Methods

The study received approval from the Royal Brompton Hospital Ethics Committee and informed, written consent was obtained from all parents and children. The methods and study population have been described in detail previously [18].

The study population

One hundred and six healthy children (55 males and 51 females) were recruited from the three London state schools closest to the study venue to maximize recruitment. Of the parents invited, 239 out of 312 gave consent and 106 of the 239 were chosen, at random, to ensure even representation across the age range for each sex, once entry criteria were satisfied. The entry criteria were: 1) aged more than 7.5 yrs, the minimum age which, based on a pilot study, ensured co-operation; b) more than 125 cm tall, the minimum height for using the exercise bicycle; and 3) no history of recent (3 weeks) acute or significant chronic respiratory or other conditions, and not receiving medications.

Twenty three of the children were aged 8.0–10.5 yrs, 24 were aged >10.5–12.5 yrs, 37 were aged >12.5–14.5 yrs, and 22 were aged >14.5–16.9 yrs. Thirty of the children were prepubertal [19], 36 in early and 38 in late puberty, and two boys refused to be examined.

Measurements

Children were studied in pairs and fasted for at least 1 h prior to study. On arrival, their date of birth, race, height (Harpenden height stadiometer; Holtain Ltd, Crymmych, UK), weight (SECA, Birmingham, UK), and two-site skinfold measurements (triceps and subscapular; Holtain Ltd, Crymmych, UK) to estimate fat mass [20] were recorded. They then underwent a physical examination to exclude unexpected disease. Vitallography was used to exclude unexpected airways obstruction, but no child failed this screening procedure.

The physiological measurements have been described previously [3–11, 21–23]. Rebreathing from a bag containing nonnative gases allows equilibrium with the resident lung gas. From this closed system, using the Fick principle, the rate of disappearance of the soluble gas, Ac, into the pulmonary bloodstream is used to determine Ac uptake. The arteriovenous content difference is the arterial Ac content which is calculated from its known plasma solubility and the end-tidal alveolar concentrations. The venous content is assumed to be zero. The ratio is the Q'eff, namely that part of the right ventricular output in contact with ventilated alveoli. The formula is:

$$Q'\text{eff} = -\beta_{Ac} \cdot \frac{V\text{RB} \cdot \dfrac{F_i^0}{F_i^{eq}} \cdot \dfrac{1}{\text{int}_{Ac}} \cdot \dfrac{760}{P\text{B} - 47}}{\alpha_b}$$

where: β_{Ac} is the slope of the natural logarithm of the disappearance of Ac with time, corrected for changes in the total volume of gas within the lung/rebreathing bag system calculated from changes in the fractional concentration of a nonsoluble inert gas, sulphur hexafluoride (F_i) at the start (0) of an experiment and at equilibrium (eq) of the test gas mixture with the child's lung gas; VRB is the total volume of the test gas mixture in the rebreathing bag at the start of the experiment; int_{Ac} is the intercept of the disappearance curve of Ac with time, extrapolated back to time zero once mixing of the test and native gases has occurred; PB is ambient atmospheric pressure in mmHg; and α_b is the plasma solubility constant for acetylene; *i.e.* 0.740 mL (standard temperature and pressure dry (STPD))·mL blood^{-1}·atmosphere^{-1}.

CO_2 efflux into the same closed system that allows solution of Ac is also measured during the manoeuvre. The arterial content is assumed to be proportional to the end-tidal CO_2 level and calculated knowing the CO_2 dissociation characteristics of normal blood. The venous CO_2 content is calculated by two methods, that of FARHI *et al.* [21] and DA SILVA's iterative modification [22] of the method of DEFARES [23]. The Fick equation is again applied to determine pulmonary blood flow.

To allow simultaneous Q'eff calculation in children by the three methods (one Ac and two CO_2), a methodological compromise was necessary, namely that there was no additional CO_2 (usually 4%) in the test gas. This is normally added in the method of DA SILVA *et al.* [22], to hasten alveolar CO_2 equilibrium with mixed venous blood. The reasons were: firstly, that a high CO_2 level at the end of a rebreathing manoeuvre during exercise was subjectively so unpleasant to the children during a pilot study that the main study would have been impractical [24], and, secondly that the added CO_2 prevented calculation of mixed venous CO_2 content by the method of FARHI *et al.* [21] by shortening the pre- and intratest time interval between the points of equal CO_2 tensions. A consequence of this compromise was that the time (20 s), to which extrapolation of end-tidal CO_2 levels occurs to determine mixed venous content in the method of DA SILVA *et al.* [22], then proved to be too short. After the pilot study, 30 s was found to be more appropriate.

Mass spectrometry measures the fractional concentrations of the components of a gaseous mixture on the basis of their mass:charge ratio alone [25]. An Innovision 2000 quadrupole mass spectrometer (Innovision, Odense, Denmark) continuously sampled the subject's ventilated gas at a rate of 5 mL·min^{-1} through 0.2 mm internal diameter Teflon tubing. The rebreathing test gas comprises 35% oxygen, 5% sulphur hexaflouride, 0.3% Ac, 0.3% carbon-18 monoxide (^{18}CO), and the balance nitrogen (BOC, London, UK). The mass spectrometer measured the above gas concentrations, as well as the argon, helium and CO_2 levels present in lung gas; eight gases in all. The individual components of the mixture were analysed semicontinuously, with each component gas allocated ≥10 ms for analysis; the first 2 ms being discarded to eliminate errors due to machine equilibration. Ac was accorded 15 ms to improve accuracy due to its low concentration of, at most, 0.3% compared to end-tidal CO_2 levels of >3%. At 0.3% concentration, acetylene is odourless, nonexplosive and gives repeatable results [26]. The stable isotope ^{18}CO was present in the rebreathing gas to measure transfer factor, but the data do not form part of the present study.

All gases in this study were, therefore, measured 9.7 times·s^{-1}. The delay from sampling to measurement was 450 ms. During each study, the spectrometer underwent a two-point calibration three times by exposure both to a calibration gas and zero gas (vacuum), and a one-point calibration at least eight times. All errors due to changes in gas viscosity, non-measurement of rare gases (e.*g.* xenon), the addition of exhaled water vapour or electrical drift, were avoided by ensuring that the total electrical signal represented 100% of the gas (known and unknown), with the known components correctly apportioned: automatic total pressure correction. The ideal rebreathing bag gas volume was found, from a pilot study, to be 40% of the subject's predicted forced vital capacity (FVC). This allowed complete emptying with minimum effort at rest, but was sufficiently large for all stages of exercise.

A rebreathing manoeuvre requires the accurate detection of the change from expiration to inspiration at functional residual capacity (FRC), so that the rebreathing bag can be activated to allow the subject to subsequently breathe from a closed system. A breath-activated pneumatic valve was employed, with a calibrated pneumotachograph providing the activation signal. The signal delay was 75 ms, the dead space of the valve apparatus was 106 mL, and the pneumotachograph zero flow "offset" voltage was calibrated more than three times per study.

Measurement protocol

A standard protocol was used for all children. They arrived at the laboratory having fasted for 1 h. Following anthropometric and flow-volume loop measurements, the children practised with the equipment. Once they were confident with the technique, they rested for 10 min and then, from FRC performed, five 20 s rebreathing manoeuvres every 3 min for 15 min. A metronome was used to control the respiratory rate to 40 breaths·min^{-1}.

During the manoeuvres, the subject had continuous pulse rate and arterial oxygen saturation measured using a surface oximeter (Nellcor, Hayward, CA, USA) placed over the right supraorbital artery. When the resting measurements had been recorded, the subject exercised using an electromagnetic bicycle (Seca 100, Birmingham, UK), which produces a constant workload independent of pedal speeds over the range 6–150 revolutions per minute (rpm). The bicycle was electrically calibrated before every study. After a 3 min rest, the subject performed a 12 s rebreathing manoeuvre (the shortened time ensured that CO_2 did not build up sufficiently during rebreathing to cause the subject distress during exercise), and then began cycling, initially backwards at zero load to loosen up, and then forwards, at 25 W·m^{-2}, increasing in 15 W·m^{-2} increments every 3 min until exhaustion. During the last 20 s of each 3 min stage, the children performed a 12 s rebreathing manoeuvre whilst continuing to pedal. For consistency, the children were encouraged to pedal at 50–70 rpm during the entire duration of exercise.

Data analysis

The mass spectrometer software uses algorithms from the above methods to calculate the data. However, to minimize error, all traces were scrutinized for the following confounding factors: 1) was the point of "complete" gaseous mixing between the test gas and resident lung air correctly identified? 2) was there evidence of pulmonary blood recirculation of acetylene? 3) was there evidence of any leak, *i.e.* had the subject inhaled room air during rebreathing? and 4) were there other artefacts (*e.g.* coughing) distorting the validity of the result? All such problems were easily identifiable and corrected. Less than 1% of traces were unusable because of uncorrectable artefact.

The data were analysed using Statistical Package for the Social Sciences (SPSS) for windows V6.0 (SPSS, Chicago, USA). Resting values were determined from the average of the last three out of five rebreathing measurements at rest, the first two being discarded [4]. BLAND and ALTMAN [27] statistics were used for comparison of methods.

Results

Rest

The results are summarized in table 1. The CVs for the three methods (one Ac and two CO_2) at rest were median 8% for Ac, 20% for the CO_2 linear (L) method of FARHI *et al.* [21] and 16% for the CO_2 curvilinear (C) method of DA SILVA *et al.* [22]. The latter two were both significantly greater than Ac ($p<0.001$), and L was also greater than C ($p<0.001$). There was no significant age effect (Kruskal-Wallis test: all p-values >0.3) for any of the three methods, although the CV for the youngest age group was always slightly higher (median for Ac 9%, L 25% and C 20%). Using BLAND and ALTMAN [27] methodology, both CO_2 methods showed a consistent positive bias compared to Ac at rest (median excess; Farhi

Table 1. – Comparison of acetylene and both carbon dioxide methods in the measurement of effective pulmonary blood flow at rest and after 9 min increasing exercise, having completed 3 min of exercise at 40 W·m^{-2}

	Acetylene (n=106)	Carbon dioxide (Farhi) (n=106)	Carbon dioxide (Da Silva) (n=106)
Rest			
Evaluable results	106	97	98
Median (95% CI) L·min^{-1}·m^{-2}	3.2 (3.1–3.4)	5.1 (4.6–5.4)***	4.7 (4.3–5.1)***+++
Median CV (95% CI) %	8 (6–9)	20 (16–28)***	16 (13–22)***+++
(range) %	(0.3–23)	(0.5–157)	(0.5–167)
Median difference (95% CI)		1.58 (1.2–2.4)***	1.16 (0.8–1.7)***+++
(95% limits) L·min^{-1}·m^{-2}		(0.24–21)	(0.03–8.1)
Percentage ±0.5, or >±2 L·min^{-1}·m^{-2}		13, 41	17, 29
Exercise			
Evaluable results	106	52	50
Median (95% CI) L·min^{-1}·m^{-2}	6.7 (6.3–7.0)	7.98 (7.3–8.4)***	7.24 (6.5–7.9)+++
Median difference (95% CI) to		1.05 (0.6–1.5)***	0.28 (-0.1–0.7)+++
acetylene (95% limits) L·min^{-1}·m^{-2}		(-3.3–12.0)	(-2.8–9.0)
Percentage ±0.5 or >±2 L·min^{-1}m^{-2}		11, 33	34, 32

CV: coefficient of variation; 95% CI: 95% confidence interval. 95% limits: 95% of results lie between quoted values. ***: $p<0.001$, compared to acetylene; +++: $p<0.001$, compared to CO_2 (Farhi).

(L) 1.58 $L \cdot min^{-1} \cdot m^{-2}$ Da Silva; and (C) 1.17 $L \cdot min^{-1} \cdot m^{-2}$). The limits of agreement for CO_2 results against a given Ac result were very wide (Farhi (L) 95% CI 0.24–21.0 $L \cdot min^{-1} \cdot m^{-2}$; Da Silva (C) 95% CI 0.03–8.1 $L \cdot min^{-1} \cdot m^{-2}$). Only 13% of Farhi (L) results and 17% of Da Silva (C) results were within ±0.5 $L \cdot min^{-1} \cdot m^{-2}$ of their respective Ac result.

Exercise

In table 1, the results following 9 min of increasing exercise and having completed 3 min of exercise at 40 $W \cdot m^{-2}$ are summarized. This time was chosen, as every child achieved this exercise level. It was not possible to calculate a CV, as only one value was obtained for each child. The bias for both CO_2 methods improved compared to Ac (median excess: Farhi (L) 1.05 $L \cdot min^{-1} \cdot m^{-2}$; and Da Silva (C) 0.28 $L \cdot min^{-1} \cdot m^{-2}$), and the scatter of CO_2 results *versus* Ac values was reduced compared with rest. Thirty four per cent of Da Silva (C) results were now within ±0.5 $L \cdot min^{-1} \cdot m^{-2}$, as opposed to only 11% of the Farhi (L) results. It was not possible to calculate CO_2 results during exercise for >50% of the children by either CO_2 method, although Ac values were calculable for all children (see Discussion).

Discussion

Any method of physiological measurement should be simple, accurate, repeatable, robust to confounding errors, and relevant to the true variable. Invasive measurements of cardiac output are indeed accurate and robust to errors. Nevertheless, the result in a catheterized child lying still - and probably terrified - will never be a measure of a true resting measurement, as studies have demonstrated [28–30]. Noninvasive methods certainly have the advantage of being less frightening to a child, but must also conform to the other criteria. All indirect methods assume that the lung behaves as a single alveolus perfused by a single blood vessel of constant flow. Ac has the advantage that mixed venous levels can indeed be assumed to be zero, and is robust to departures from these ideal assumptions [13, 14]. No gaseous method will be immune to errors due to severe obstructive lung disease hampering mixing, but with moderate obstructive lung disease, the Ac method's error in Q'_{eff} at rest was only 6% (0.18 $L \cdot min^{-1} \cdot m^{-2}$) [14]. The method is also robust, at least in dogs, to rapid changes in body temperature, haematocrit and an unrestrained breathing pattern [31], the last being particularly relevant for younger children.

In contrast, the mathematical complexity involved in estimating mixed venous CO_2 levels will always lead to error. Attention has also been paid to refining the calculation of the arterial CO_2 content [11], the reason being that it is calculated from the end-tidal CO_2 levels prior to the rebreathing test. Non-steady state conditions leads to the situation where end-tidal values may not, therefore, reflect alveolar values.

A further practical problem was the inability to calculate CO_2 derived Q'_{eff} during exercise in half of the children. By far the most common reason for this was that the CO_2 concentration during rebreathing reached the end-tidal CO_2 concentration of the breath prior to the rebreathing manoeuvre within the first rebreathing breath. This rendered calculation of CO_2 production at equal tissue concentrations too inaccurate during this very short period (<3 s). More rarely, there were often less than four breaths in a 12 s rebreathing test, and thus only three points to either L or C extrapolate to determine mixed venous levels, again rendering calculation inaccurate. The natural log-transformed Ac slope is linear, not extrapolated for slope calculations, and is measured 10 times per second. As a result, even though only the middle portion of each expiration is examined, reflecting the best approximation to average alveolar values, the resulting trace has at least 12 points, greatly increasing the robustness of the slope measurement. Critics of the Ac method may argue that the calculation of Ac's solution in lung tissue (lung tissue volume), which is part of the Q'_{eff} formula, requires extrapolation and is, thus, prone to error. This is indeed the case, nevertheless its effect on the calculation of Q'_{eff} is small. A critique of all sources of measurement error in the Q'_{eff} formula with acetylene demonstrates a typical error of only 2% [32].

Clearly, the ideal study ought to have involved simultaneous direct Fick measurements of cardiac output, together with the indirect measures, but this would be ethically impossible. Both Ac and CO_2 in adults under ideal conditions give similar results to the direct Fick. This study, therefore, begs the question, which method is the most accurate? Table 2 summarizes some of the studies that have determined normal values, and exemplifies the difficulties of determining a true comparison

Table 2. – Examples of studies assessing normal resting pulmonary blood flow/cardiac output in children and adults

First author	[Ref.]	Age group	n	Method(s)	Q'_{eff} (rest) $L \cdot min^{-1} \cdot m^{-2}$
Current study		8–17 yrs	106	Ac/CO_2 (sitting)	3.2 (Ac), 5.1 (CO_2), 4.8 (CO_2)
Bowyer	[3]	5–14 yrs	98	Freon (sitting)	2.7
Sackner	[6]	24–60 yrs	11	Ac (sitting)	3.1
Esperson	[33]	Young adults	11	CO_2/Fi/ThD/DOP (sitting)	4.8 (CO_2), 4.8 (Fi), 6.7 (ThD), 5.1 (DOP)
Cander	[5]	29–36 yrs	4	Ac/N_2O/ether (sitting)	3.3 (Ac), 3.3 (N_2O), 2.3 (ether)
Reybrouck	[16]	Mean 29 yrs	6	CO_2	Mean difference between duplicates 24%
Klausen	[17]	21 yrs	1	Ac/CO_2	3.3 (Ac), 3.4 (CO_2)
Lock	[29]	5–16 yrs	10	Fi (supine)	4.5
Cumming	[28]	Mean 12 yrs	60	Fi (supine)	4.0
Thadani	[34]	32–46 yrs	10	Fi (sitting and supine)	2.8 (sitting), 3.5 (supine)
Ericksson	[30]	13–14 yrs	8	Dye (sitting)	3.4

Q'_{eff}: effective pulmonary blood flow; Ac: acetylene; CO_2: carbon dioxide; N_2O: nitrous oxide; Fi: invasive direct Fick; ThD: thermodilution; DOP: Doppler; Dye: indicator dye dilution and the small number of subjects available for comparison. For example, the study by KLAUSEN [17] is often quoted as that which compares Ac to CO_2 methods. However, the results are obtained in only a single subject and five of the 17 rest results were discarded for technical reasons. In general, supine measurements are agreed to be 5–20% greater [3] than sitting measurements, and in children all direct Fick measurements are supine. Secondly, the stress of a direct Fick measurement may alter resting values, and, thirdly, adult measurements cannot be extrapolated to children.

The purpose of the present study was to determine the most suitable method for routine use in children, who are rarely constrained by ideal conditions! At a concentration of 0.3%, acetylene is odourless and nonexplosive, and gives accurate, relevant and, at rest, repeatable results. The carbon dioxide methods were more variable at rest, and did not allow a result to be calculated reliably during exercise. We therefore recommend acetylene as the preferred gas for noninvasive pulmonary blood flow measurements in children.

References

1. Krogh A, Lindhard J. Measurements of the blood flow through the lungs in man. *Scand J Physiol* 1912; 27: 100–125.
2. Bornstein A. Eine Methode zur vergieichenden Messung des Herzschlagvolumens beim Menschen. *Pflügers Arch* 1910; 132: 307–318.
3. Bowyer JJ, Warner JO, Denison DM. Effective pulmonary blood flow in normal children at rest. *Thorax* 1988; 43: 972–977.
4. Bush A, Busst C, Johnson S, Denison DM. Rebreathing method for the simultaneous measurement of oxygen consumption and effective pulmonary blood flow during exercise. *Thorax* 1988; 43: 268–275.
5. Cander L, Forster RE. Determination of pulmonary parenchymal tissue volume and pulmonary capillary flow in man. *J Appl Physiol* 1959; 14: 541–551.
6. Sackner MA, Greeneltch D, Heiman MS, Epstein S, Atkins N. Diffusing capacity, membrane diffusing capacity, capillary blood volume, pulmonary tissue volume, and cardiac output measured by a rebreathing technique. *Am Rev Respir Dis* 1975; 111: 157–165.
7. Chapman CB, Taylor HL, Borden C, Ebert RV, Keys A. Simultaneous determinations of the resting arteriovenous oxygen difference by the acetylene and direct Fick methods. *J Clin Invest* 1950; 29: 651–659.
8. Teichman J, Adaro F, Veicsteinas A, Cerretelli P, Piiper J. Determination of pulmonary blood flow by rebreathing of soluble inert gases. *Respiration* 1974; 31: 296–309.
9. Grollman A. The determination of the cardiac output of man by the use of acetylene. *Am J Physiol* 1929; 88: 432–445.
10. Driscoll DJ, Staats BA, Beck KC. Measurement of cardiac output in children during exercise: A review. *Pediatr Exer Sci* 1989; 1: 102–115.
11. Heigenhauser GJF, Jones NL. Measurement of cardiac output by carbon dioxide rebreathing methods. *Clin Chest Med* 1989; 10: 255–264.
12. Coates AL. Measurement of cardiac output during exercise. *Chest* 1992; 102: 985–986.
13. Petrini MF, Peterson BT, Hyde RW. Lung tissue volume and blood flow by rebreathing: theory. *J Appl Physiol: Respirat Environ Exercise Physiol* 1978; 44: 795–802.
14. Kalley MC, Hyde RW, Smith RJ, Rothbard RL, Schreiner BF. Cardiac output by rebreathing in patients with cardiopulmonary diseases. *J Appl Physiol* 1987; 63: 201–210.
15. Lands LC, Heigenhauser GJF, Jones NL. Cardiac output determination during progressive exercise in cystic fibrosis. *Chest* 1992; 102: 1119–1123.
16. Reybrouck T, Fagard R. Assessment of cardiac output at rest and during exercise by a carbon dioxide rebreathing method. *Eur Heart J* 1990; 11 (Suppl. I): 21–25.
17. Klausen K. Comparison of carbon dioxide rebreathing and acetylene methods for cardiac output. *J Appl Physiol* 1965; 20: 763–766.
18. Rosenthal M, Bush A, Deanfield JE, Redington A. A comparison of cardiopulmonary function in children undergoing the atriopulmonary or total cavopulmonary connection Fontan procedure. *Circulation* 1995; 91: 372–378.
19. Tanner JM. *In*: Growth at Adolescence. 2nd Edn Oxford, Blackwell Scientific Publications, 1962.
20. Tanner JM, Whitehouse RH. Revised standards for triceps and subscapular skinfolds in British children. *Arch Dis Child* 1975; 50: 142–145.
21. Farhi LE, Nesarajah MS, Olszowka AJ, Metildi LA, Ellis AK. Cardiac output determination by a simple onestep rebreathing technique. *Respir Physiol* 1976; 28: 141–159.
22. Da Silva GA, El-Manshawi A, Heigenhauser GJF, Jones NL. Measurement of mixed venous carbon dioxide pressure by rebreathing during exercise. *Respir Physiol* 1985; 59: 379–392.
23. DeFares JG. Determination of the mixed venous carbon dioxide tension from the exponential carbon dioxide rise during rebreathing. *J Appl Physiol* 1958; 13: 159–164.
24. Washington RL. Measurement of cardiac output. *In*: Rowland TW, ed. Paediatric Laboratory Exercise Testing: Clinical Guidelines. Champaign, Illinois, USA. Human Kinetics. 1993; pp. 131–140.
25. Schied P. Respiratory mass spectroscopy. *In*: Laszlo G, Sudlow MF, eds. Measurement in Clinical Respiratory Physiology. London, Academic Press, 1983; pp. 163–190.
26. Nielson OW, Hanson S, Christensen P, Gronlund J. Repeatability of the acetylene rebreathing method in measuring cardiac output: influence of acetylene concentration. *Acta Anaesthesiol Scand* 1990; 34: 354–357.
27. Bland JM, Altman DG. Statistical methods for assessing agreement between two methods of clinical assessment. *Lancet* 1986; i: 307–310.
28. Cumming GR. Haemodynamics of supine bicycle exercise in 'normal' children. *Am Heart J* 1977; 93: 617–622.
29. Lock JE, Einzig S, Moller JH. Haemodynamic responses to exercise in normal children. *Am J Cardiol* 1978; 41: 1278–1284.
30. Eriksson BO, Grimby G, Saltin B. Cardiac output and arterial blood gases during exercise in pubertal boys. *J Appl Physiol* 1970; 31: 348–352.
31. Hsia CCW, Herazo LF, Ramanathan M, Johnson RL. Cardiac output during exercise measured by acetylene rebreathing, thermodilution, and Fick techniques. *J Appl Physiol* 1995; 78: 1612–1616.
32. Rosenthal M. The assessment of cardiorespiratory function by respiratory mass spectrometry in children. MD Thesis, Manchester, UK, 1994.
33. Esperson K, Jenson W, Thmsen JK, Eliasen K, Olsen NV, Kanstrup IL. Comparison of cardiac output measurement techniques: thermodilution, Doppler, CO_2-rebreathing and the direct Fick method. *Acta Anaesthesiol Scand* 1995; 39: 245–254.
34. Thadani U, Parker JO. Haemodynamics at rest and during supine and sitting bicycle exercise in normal subjects. *Am J Cardiol* 1978; 41: 52–59.