Non-invasive prospective targeting of arterial P_{CO_2} in subjects at rest

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Accurate measurements of arterial P_{CO_2} (P_{a,CO_2}) currently require blood sampling because the end-tidal P_{CO_2} (P_{ET,CO_2}) of the expired gas often does not accurately reflect the mean alveolar P_{CO_2} and P_{a,CO_2}. Differences between P_{ET,CO_2} and P_{a,CO_2} result from regional inhomogeneities in perfusion and gas exchange. We hypothesized that breathing via a sequential gas delivery circuit would reduce these inhomogeneities sufficiently to allow accurate prediction of P_{a,CO_2} from P_{ET,CO_2}. We tested this hypothesis in five healthy middle-aged men by comparing their P_{ET,CO_2} values with P_{a,CO_2} values at various combinations of P_{ET,CO_2} (between 35 and 50 mmHg), P_{O_2} (between 70 and 300 mmHg), and breathing frequencies (f; between 6 and 24 breaths min^{-1}). Once each individual was in a steady state, P_{a,CO_2} was collected in duplicate by consecutive blood samples to assess its repeatability. The difference between P_{ET,CO_2} and average P_{a,CO_2} was 0.5 ± 1.7 mmHg ($P = 0.53$; 95% CI −2.8, 3.8 mmHg) whereas the mean difference between the two measurements of P_{a,CO_2} was −0.1 ± 1.6 mmHg (95% CI −3.7, 2.6 mmHg). Repeated measures ANOVAs revealed no significant differences between P_{ET,CO_2} and P_{a,CO_2} over the ranges of P_{O_2}, f and target P_{ET,CO_2}. We conclude that when breathing via a sequential gas delivery circuit, P_{ET,CO_2} provides as accurate a measurement of P_{a,CO_2} as the actual analysis of arterial blood.

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Accurate measurement of arterial P_{CO_2} (P_{a,CO_2}) is important for the clinical assessment of patients and, in physiological studies, for the assessment of control of breathing and cerebral blood flow. Currently, the reference standard for measuring P_{a,CO_2} is analysis of arterial blood via direct arterial puncture. This invasive approach has a number of disadvantages for both the subject (discomfort and potential arterial wall damage) and investigator (restricted mobility of the catheter insertion site, cost, time delay for blood analysis, and limited temporal resolution of changes in P_{a,CO_2}). As a result, investigators have long sought a suitable non-invasive method to measure P_{a,CO_2}.

Non-invasive methods of predicting P_{a,CO_2} from alveolar P_{CO_2} (P_{A,CO_2}) consider the lung to be a tonometer in which CO_2 equilibrates between alveolar gas and capillary blood. In reality, however, the lung is not a single homogeneous time-invariant gas exchange compartment. Rather, P_{CO_2} varies in different regions of the lung as a result of differences in ventilation-to-perfusion matching ($\dot{V}_A/\dot{Q}$) throughout the lung and, in each lung region, throughout the respiratory cycle (Dubois *et al.* 1952; Lenfant, 1967). The contribution to the P_{a,CO_2} of blood passing each alveolus reflects the average P_{CO_2} in that alveolus during the respiratory cycle (Jones *et al.* 1979; Robbins *et al.* 1990). P_{a,CO_2}, then, reflects the time- and flow-weighted averages of all alveolar ventilatory fluctuations in all $\dot{V}_A/\dot{Q}$ regions throughout the lung, i.e. the mean P_{A,CO_2} (Lenfant, 1967). As a result, the relation between the P_{CO_2} in the exhaled gas and the P_{a,CO_2} is so obscured that one cannot calculate the P_{a,CO_2} from the P_{A,CO_2}.

We reasoned that if the regional variations of P_{CO_2} in the lung could be reduced, then (a) the end-tidal P_{CO_2} (P_{ET,CO_2}) would accurately reflect the mean P_{A,CO_2} and (b) the P_{a,CO_2} would not be affected by the distribution of pulmonary blood flow. In other words, P_{ET,CO_2} should equal mean P_{A,CO_2}, and, as mean P_{A,CO_2} is equal to P_{a,CO_2}

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Table 1. Subject anthropomorphic and pulmonary function data

Subject	Age (years)	Weight (kg)	Height (cm)	VC (l,% predicted)	FEV1/V_C (% predicted)	FRC (l,% predicted)	DL_{CO} (% predicted)
1	45	77	176	5.1 (113)	77 (104)	2.9 (78)	30 (93)
2*	33	81	177	4.4 (90)	73 (95)	3.1 (81)	29 (91)
3§	49	89	174	5.0 (116)	66 (90)	3.5 (98)	33 (103)
4	59	81	177	6.0 (144)	65 (92)	4.6 (122)	32 (90)
5	53	76	175	4.7 (113)	77 (107)	3.2 (88)	26.5 (88)

*History of mild asthma, occasional use of inhaled beta agonists, occasional smoker of cigarettes.
§History of smoking of about one package of cigarettes per week for 20 years.

(Jones *et al.* 1979; Robbins *et al.* 1990), P_{ET,CO_2} should equal P_{a,CO_2}.

In our laboratory, we have experimented with a method of controlling P_{ET,CO_2} by providing specific flows and concentrations of CO_2 to a sequential gas delivery circuit (Slessarev *et al.* 2007). To the extent that minute ventilation ($\dot{V}_E$) exceeds such gas flow, previously expired gas, stored in an expiratory gas reservoir, enters the lung (Somogyi *et al.* 2005). This gas, we hypothesized, reduces both the regional variations and respiratory fluctuations of P_{A,CO_2} towards a mean P_{A,CO_2} (see Fig. 2 in Prisman *et al.* 2007). We tested this hypothesis using the method of Slessarev *et al.* (2007) to prospectively target a series of P_{ET,CO_2} values and measuring mean P_{A,CO_2} by analysing contemporaneously drawn arterial blood samples for P_{CO_2}. To test the robustness of the relation between P_{ET,CO_2} and P_{a,CO_2}, we also varied the end-tidal P_{O_2} values (P_{ET,O_2}) and breathing frequencies (f) at each target P_{ET,CO_2}.

Methods

Participants

The study was approved by the University Health Network Research Ethics Board. Informed written consent was obtained from five middle-aged male subjects. Their anthropomorphic and pulmonary function test data performed at the clinical pulmonary function laboratory at the Toronto General Hospital are presented in Table 1. Subjects were healthy except as noted in the caption to Table 1.

Setting P_{ET,CO_2} and P_{ET,O_2}

Measurements of O_2 consumption ($\dot{V}_{O_2}$) and CO_2 production ($\dot{V}_{CO_2}$) were used to target P_{ET,CO_2} and P_{ET,O_2}. Subjects were seated comfortably at room temperature breathing via a sequential gas delivery circuit and mask (Fig. 1 in Slessarev *et al.* 2007). Adhesive tape (Tegaderm, 3M Health Care, St Paul, MN, USA) was applied as necessary to prevent leaks between the face and the mask. Gas was sampled continuously from inside the mask. $\dot{V}_{CO_2}$ and $\dot{V}_{O_2}$ were determined for each subject as follows:

$$\dot{V}_{CO_2} = \dot{G}_1 \times F_{E,CO_2}$$
$$\dot{V}_{O_2} = \dot{G}_1 \times (0.21 - F_{E,O_2})$$

where F_{E,CO_2} and F_{E,O_2} are the fractional concentrations of CO_2 and O_2 of mixed expired gas (sampled from the expiratory reservoir) and $\dot{G}_1$ in this instance is the flow of air. $\dot{V}_{CO_2}$ and $\dot{V}_{O_2}$ were measured every 45 s until three consecutive values differed by less than 10%. The last such readings were taken as the $\dot{V}_{CO_2}$ and $\dot{V}_{O_2}$ for calculating the target end-tidal values. $\dot{V}_A$, $\dot{V}_{CO_2}$ and $\dot{V}_{O_2}$ were then used to calculate the required gas flow to the circuit and its inspired fractional concentrations of CO_2 and O_2 to obtain the targeted P_{ET,CO_2} and P_{ET,O_2} (see Slessarev *et al.* 2007 for details).

We used a custom made gas blender fitted with P_{CO_2} and P_{O_2} sensors to blend gases in response to computer instructions (Respiract^{TM}, Thornhill Research Inc., Toronto, Canada). The rapid response CO_2 sensor (Ir3107, Servomex Group Ltd, Sugar Land, TX, USA) is accurate within $\pm$0.1% CO_2 in the range of 0–10% CO_2 and the O_2 sensor (UFO 130, Teledyne Analytical Instruments, City of Industry, CA, USA) to within 1% with a resolution of 0.1%. Both sensors underwent a two-point calibration before every experiment and were configured to report P_{CO_2} and P_{O_2} at BTPS. Expiratory flows were monitored continuously via a turbine (Universal Ventilation Meter, Vacu-Med, Ventura, CA, USA) placed in the expiratory limb of the circuit, and analysed for tidal volume (V_T) and $\dot{V}_E$. All analog data were digitized, analysed and recorded on a computer after analog-to-digital conversion with a customized commercial data acquisition program (LabVIEW, National Instruments, Austin, TX, USA).

A 22-gauge catheter was inserted aseptically under local anaesthesia into a radial artery and maintained with a continuous slow flush of 0.9% saline solution. During the last minute of each 3 min target condition, two consecutive arterial blood samples (~2 ml) were drawn over approximately 20 s into heparinized syringes. Blood was analysed within 20 min via a clinically maintained

point-of-care blood gas analyser (RapidPoint® 405: Bayer HealthCare Diagnostics, Medfield, MA, USA). Analyser results on test samples are analysed daily and certified to be within 4% of reading for P_{CO_2} values less than 55 mmHg and within 6% of reading for P_{O_2} values less than 150 mmHg and 10% for P_{O_2} values greater than 150 mmHg. Quality control included automatic one-point calibrations every 30 min and two-point calibrations every fourth calibration. All blood gas values were reported at 37 °C.

Experimental protocol

To evaluate the relations between P_{ET,CO_2} and P_{a,CO_2}, we targeted and measured P_{ET,CO_2} during two experimental phases: (1) P_{O_2} was held constant; and (2) P_{O_2} was varied. In Phase I (Fig. 1), f was held constant at 6, 12, 18, or 24 breaths min^{-1} while P_{ET,CO_2} was targeted to three of four partial pressures (35, 40, 45, 50 mmHg) for 3 min each, at each f, and P_{ET,O_2} was kept constant at 100 mmHg.

Phase II was similar to Phase I except that P_{ET,CO_2} was held constant at 40 mmHg while P_{ET,O_2} was targeted to three different partial pressures (70, 150, 300 mmHg) at each of the four f. Breathing frequency was held constant and the target P_{ET,CO_2} cycled (*versus* vice versa) to minimize the duration of hypercapnia, which some subjects found uncomfortable. The different values of f were applied in a randomized order in each subject and maintained by instructing the subject to breathe in time with a metronome. These tests resulted in comparisons of measured P_{ET,CO_2} averaged over the last 60 s of each breathing period with the average P_{a,CO_2} from both arterial blood samples, for six different combinations of targeted P_{CO_2} and P_{O_2}, at each of the four f, for a total of 24 comparisons per subject.

Statistics

All data are expressed as the mean ± S.D. unless otherwise noted. To assess the variability of P_{a,CO_2} between the two blood samples for each subject during each experimental condition, we calculated an intra-class correlation coefficient (ICC); values approaching 1.0 indicate a strong degree of agreement between the two assessments, while values near 0 indicate little or no relation between the measured pairs. To assess the differences between targeted P_{ET,CO_2} values and measured P_{ET,CO_2} values and P_{a,CO_2} values, a series of repeated measures ANOVAs was performed to determine whether these differences were significant, and whether experimental phase or f significantly affected the magnitudes of the differences. Subject identifiers were included as a random effect in these models to account for the relatedness of observations from the same subject. The analyses were performed using the SAS System v.9.1 software package. Statistical significance was set at the $P = 0.05$ level. Bland–Altman analysis (Bland & Altman, 1986) was used to calculate the limits of agreement between P_{ET,CO_2} and average P_{a,CO_2}. We also calculated the repeatability coefficient (as defined by the British Standards Institution), referred to by Bland & Altman (1986), which refers to the 95% confidence intervals of differences between two repeated measures of the same quantity by the same method.

Results

All subjects completed the protocol without difficulty. Figure 2 shows the results obtained during a typical experiment. With subjects synchronizing f to a

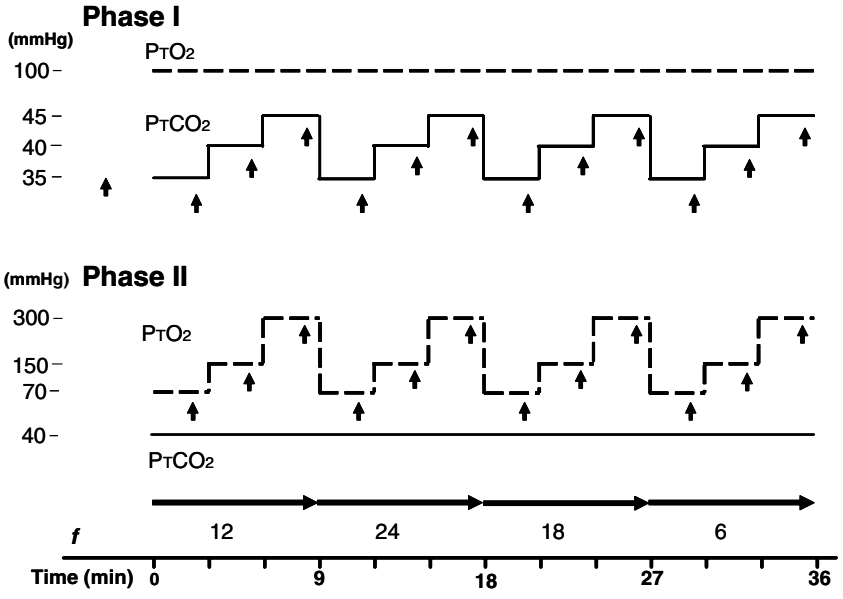

Figure 1. Experimental protocol
Time course showing target end-tidal P_{O_2} (P_{T,O_2}) and target end-tidal P_{CO_2} (P_{T,CO_2}). In Phase I, P_{T,O_2} was kept constant and P_{T,CO_2} was set to three different levels for each of four different frequencies (f). In Phase II, P_{T,CO_2} was kept constant and P_{T,O_2} was set to three different levels for each of four different f. Upward pointing arrowheads indicate time for duplicate arterial blood sampling. Horizontal arrows indicate period of constant f.

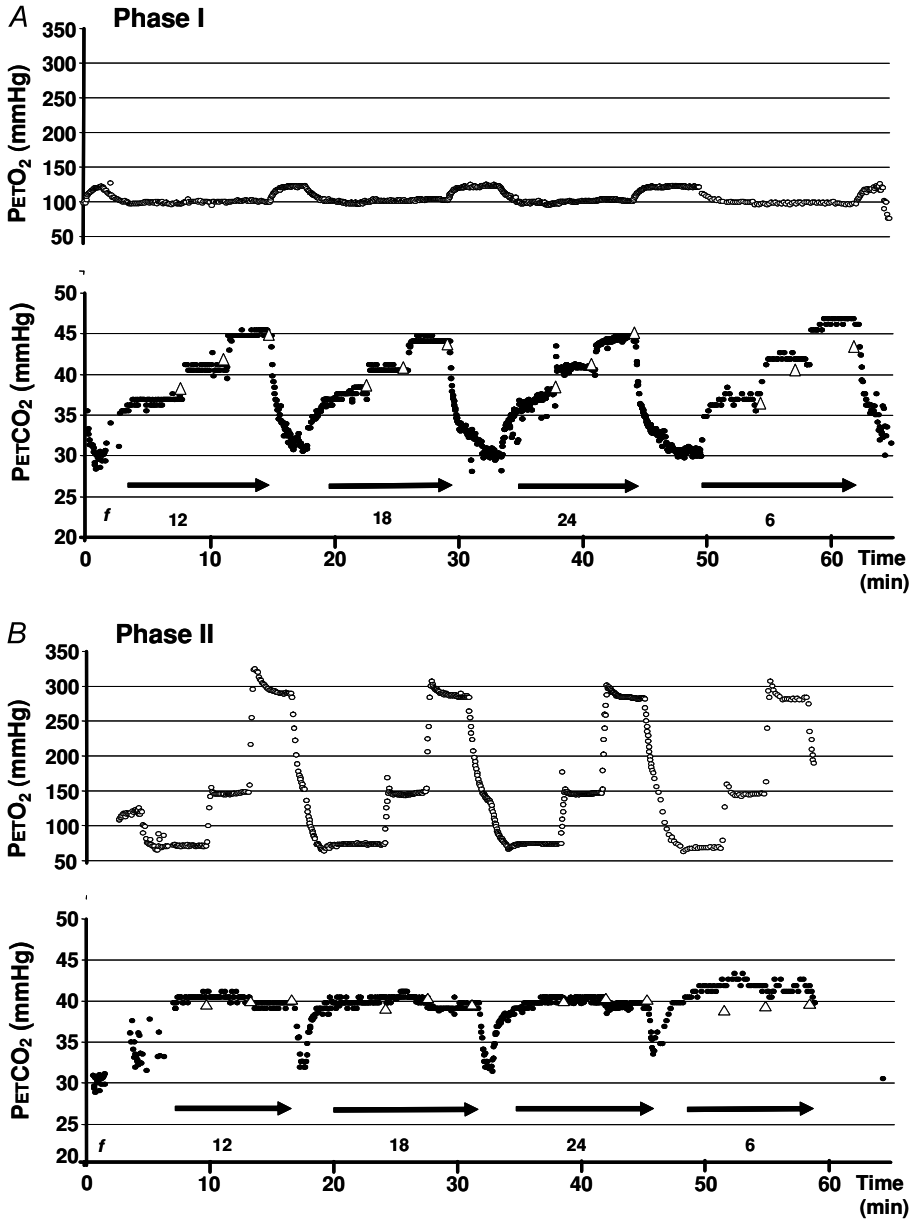

Figure 2. Example data

A, P_{ET,O_2} and P_{ET,CO_2} from one subject during Phase I of the protocol. Each end-tidal value is represented by a filled circle. Averages of two P_{a,CO_2} values at end of each 3 min test interval are represented by open triangles. Horizontal arrows designate the time during which *f* was maintained by breathing in synchrony with a metronome. Subjects were administered room air in the intervals between fixed *f*. Reductions in P_{CO_2} between tests can be partly attributed to a rebound hyperventilation after hypercarbia, as was previously reported by Wise *et al.* (2007) and partly to artifact as the face mask was flooded with air at high flows between tests. In Phase I, before each period of breathing at controlled *f*, subjects were encouraged to hyperventilate for about 1–2 min to below a P_{ET,CO_2} of 35 mmHg so they could undergo a sharp step up to the new target P_{CO_2}. This was done to minimize the time to attain target end-tidal values and thereby shorten the duration of the protocol. P_{ET,CO_2} measurements and arterial blood sampling were performed only after a steady state was achieved. The increases in P_{ET,O_2} between periods of fixed *f* were the result of hyperventilation on room air. *B*, P_{ET,O_2} and P_{ET,CO_2} from one subject during Phase II of the protocol. Between periods of fixed *f*, subjects breathed room air without restriction.

Table 2. Summary of differences between target end-tidal (P_{T,CO_2}), end-tidal (P_{ET,CO_2}) and arterial (P_{a,CO_2}) P_{CO_2}, reported as mean ± S.D. (95% CI) and categorized by breathing frequency and experimental phase

Breaths min^{-1}	6	12	18	24	(12, 18, 24)
Phase I: P_{T,O_2} constant, P_{T,CO_2} varied					
$P_{ET,CO_2} - P_{T,CO_2}$	2.38 ± 0.99*	0.98 ± 1.43	1.20 ± 1.55	0.62 ± 1.67	0.93 ± 1.53
	(1.83, 2.93)	(0.19, 1.77)	(0.34, 2.06)	(−0.30, 1.54)	(0.47, 1.39)
$P_{a,CO_2} - P_{T,CO_2}$	1.86 ± 2.52	1.61 ± 2.19	2.10 ± 2.10	1.44 ± 2.33	1.72 ± 2.17
	(0.46, 3.25)	(0.40, 2.82)	(0.94, 3.26)	(0.15, 2.73)	(1.06, 2.37)
Phase II: P_{T,O_2} varied, P_{T,CO_2} constant					
$P_{ET,CO_2} - P_{T,CO_2}$	2.11 ± 1.93*	0.94 ± 1.71	0.75 ± 1.64	0.83 ± 1.75	0.84 ± 1.66
	(1.04, 3.18)	(−0.01, 1.89)	(−0.16, 1.66)	(−0.14, 1.79)	(0.34, 1.34)
$P_{a,CO_2} - P_{T,CO_2}$	1.24 ± 2.49	1.48 ± 2.04	1.14 ± 2.41	1.27 ± 2.64	1.30 ± 2.33
	(−0.14, 2.61)	(0.35, 2.61)	(−0.19, 2.48)	(−0.20, 2.73)	(0.60, 2.00)
Combined Phases					
$P_{ET,CO_2} - P_{T,CO_2}$	2.24 ± 1.51*	0.96 ± 1.55	0.97 ± 1.59	0.72 ± 1.68	0.89 ± 1.59
	(1.68, 2.81)	(0.39, 1.54)	(0.38, 1.57)	(0.10, 1.35)	(0.55, 1.22)
$P_{a,CO_2} - P_{T,CO_2}$	1.55 ± 2.48	1.55 ± 2.08	1.62 ± 2.27	1.36 ± 2.45	1.51 ± 2.25
	(0.62, 2.47)	(0.77, 2.32)	(0.77, 2.47)	(0.44, 2.27)	(1.04, 2.00)

Data show P_{CO_2} differences in mmHg. *$P < 0.02$.

metronome (at constant $\dot{G}_1$), V_T was inversely related to f (1.96 ± 0.36, 1.19 ± 0.28, 0.98 ± 0.2 and 0.85 ± 0.19 l at $f = 6, 12, 18$ and 24 breaths min^{-1}, respectively; $P < 0.01$).

For all f and P_{ET,O_2} values, the difference between P_{ET,CO_2} and average P_{a,CO_2} was 0.5 ± 1.7 mmHg ($P = 0.53$; 95% CI −2.8, 3.8 mmHg) (Fig. 3*A*). There were no significant differences between P_{ET,CO_2} and average P_{a,CO_2} regardless of P_{O_2}, f, or target P_{ET,CO_2} (repeated measures ANOVAs). Differences between target P_{ET,CO_2} and actual P_{ET,CO_2} and between target P_{ET,CO_2} and P_{a,CO_2} are presented in Table 2. The only significant difference between target P_{ET,CO_2} and P_{ET,CO_2} occurred at $f = 6$ breaths min^{-1}.

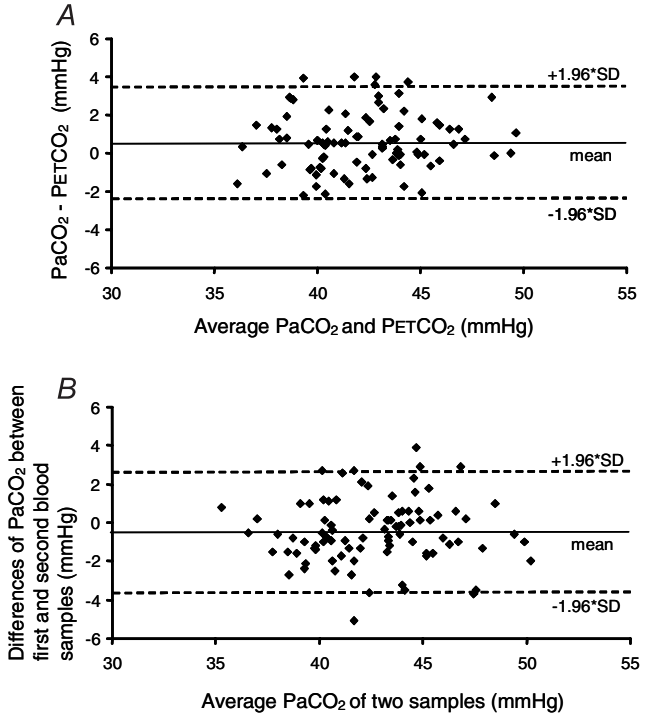

Figure 3. Bland–Altman plots
The limits of agreement between P_{ET,CO_2} and P_{a,CO_2} (*A*) and the repeatability coefficient for repeat P_{a,CO_2} measurements (*B*).

A duplicate analysis of P_{a,CO_2} was performed to establish the repeatability coefficient of the reference standard measure as the benchmark for declaring P_{ET,CO_2} and P_{a,CO_2} interchangeable. The ICC between blood measurements was 0.94 (95% CI: 0.91, 0.96, $P < 0.01$) and the mean difference between two consecutive P_{a,CO_2} measurements was −0.1 ± 1.6 mmHg (mean absolute difference 1.4 ± 1.1 mmHg).

The repeatability coefficient for P_{a,CO_2} was −3.7, 2.5 ($P = 0.47$) (Fig. 3*B*), practically identical to that between P_{ET,CO_2} and average P_{a,CO_2} (S.D. 1.7 *versus* 1.6 mmHg, respectively). This was true even when the data were analysed separately for f of 6 (0.70 ± 1.94, 95% CI: −0.03, 1.42; $P = 0.15$) and for f of 12–24 breaths min^{-1} (−0.62 ± 1.49, 95% CI: −0.93, −0.31; $P = 0.76$). In other words, measurement of P_{ET,CO_2} was interchangeable with measurement of P_{a,CO_2}.

Discussion

This is the first study to show consistent and close agreement between P_{ET,CO_2} and P_{a,CO_2} over a wide range of P_{ET,CO_2}, P_{ET,O_2} and f. The designation of agreement is taken from the approach advocated by Bland & Altman (1986) for assessing the interchangeability of measures of a physiological quantity – in this case, P_{a,CO_2}. The accepted reference standard is analysis of invasively

acquired arterial blood. Therefore, as part of this study, we tested the repeatability of P_{a,CO_2} of duplicate consecutively drawn blood samples during steady state conditions of P_{ET,CO_2}. The mean difference between blood samples was −0.1 mmHg, indicating no systematic bias. The repeatability coefficient reflects the range of differences between readings for 95% of duplicate readings, and this variability reflects the sum of machine (±1.6 mmHg), handling and actual blood P_{CO_2} variability (Lenfant, 1967). Because the limits of agreement between P_{ET,CO_2} and P_{a,CO_2} were the same as between any two readings of the reference standard, we can designate them as being interchangeable under these experimental conditions. The methodology we used therefore extends the ability to perform physiological research when the P_{a,CO_2}, as the independent variable, must be both controlled and measured accurately.

The need for a new method of using P_{ET,CO_2} to assess P_{a,CO_2} may be questioned since P_{ET,CO_2} has been deemed to be an acceptable estimate of P_{a,CO_2} at rest (Jones *et al.* 1979; Robbins *et al.* 1990). However, closer scrutiny identifies multiple exceptions and uncertainties in this relation. In subjects breathing room air, P_{ET,CO_2} consistently underestimates P_{a,CO_2} at rest (Robbins *et al.* 1990) and overestimates it during exercise (Matell, 1963; Jones *et al.* 1979; Williams & Babb, 1997; Benallal *et al.* 2002). Various factors affect the difference between P_{ET,CO_2} and P_{a,CO_2}; these include V_T (Jones *et al.* 1979), age (Miller & Tenney, 1956; Holland *et al.* 1968), the presence of obstructive lung disease (Liu *et al.* 1995; Prause *et al.* 1997), and gravity (Barr, 1963). Most of these factors are not taken into account in the calculation of P_{a,CO_2} with the above-mentioned methods, resulting in uncertainty. We therefore investigated if the sequential gas delivery method of attaining a target P_{ET,CO_2} (which also does not take any of these conditions into account) establishes conditions in the lung that ensure that P_{ET,CO_2} equals mean P_{A,CO_2} (as measured by P_{a,CO_2}).

A previously reported method, dynamic end-tidal forcing (DEF), targets end-tidal values using an integral-proportional feedback loop to make breath-by-breath corrections to inspired gases, but it does not actually target P_{a,CO_2}. In studies of the control of breathing, Robbins *et al.* (1990) allowed subjects to breathe freely in response to targeted changes in P_{ET,CO_2} over the range of 40–50 mmHg using DEF and compared the P_{CO_2} in exhaled gas to measured P_{a,CO_2}. At rest, P_{ET,CO_2} significantly underestimated P_{a,CO_2} with a large S.D. (mean ± S.D., −1.35 ± 2.64 mmHg). St Croix *et al.* (1995) used DEF in older subjects with a protocol (but not a method of targeting P_{ET,CO_2}) similar to ours; they found that the difference between P_{ET,CO_2} and P_{a,CO_2} varied with the fractional concentration of inspired CO_2. Despite studying subjects with greater alveolar deadspace than younger subjects (Robbins *et al.* 1990), they also found that P_{ET,CO_2} significantly overestimated P_{a,CO_2} (+2.9 ± 1.7 mmHg) and explained this finding by noting that DEF targets P_{ET,CO_2} regardless of its effect on mixed venous P_{CO_2} and mean P_{A,CO_2}. They concluded that caution must be exercised when inferring the degree of stimulation (i.e. the P_{a,CO_2}) at the chemoreceptors based on measurements of P_{ET,CO_2}. This is particularly true under hypercapnic conditions because P_{ET,CO_2}-to-P_{a,CO_2} differences '... were often very large, indicating that end-tidal forcing is not useful for deriving individual values' of P_{a,CO_2}. In contrast, we observed that targeted P_{CO_2}, P_{ET,CO_2} and P_{a,CO_2} were independent of both the inspired fractional concentration of CO_2 and V_T (repeated measures ANOVAs), except at $f = 6$ breaths min^{-1}, a finding discussed in greater detail below.

A characteristic of the sequential gas delivery circuit is that the difference between $\dot{V}_E$ and $\dot{G}_1$ is made up with rebreathed gas from the expiratory reservoir. The higher P_{CO_2} in the previously exhaled gas entering high $\dot{V}_A/\dot{Q}$ lung regions raises their P_{CO_2} values towards those of better perfused regions (Swenson *et al.* 1994; Brogan *et al.* 2004). This raises the P_{CO_2} in alveoli with high $\dot{V}_A/\dot{Q}$ towards the P_{CO_2} of those with better matched $\dot{V}_A/\dot{Q}$, resulting in a more homogeneous P_{A,CO_2}. In addition, larger V_T and previously exhaled gas also reduces the intrabreath P_{CO_2} fluctuations in a given alveolus without changing the net equilibrium P_{CO_2} (see Somogyi *et al.* 2005). As a result, the distribution of P_{CO_2} throughout the lung and over the course of a breath cycle approaches the mean P_{A,CO_2}. An important consequence of having very similar P_{CO_2} values in all alveoli is that the P_{a,CO_2} is no longer dependent on the regional distribution of blood flow. We propose this as the most likely explanation for our observation that for f between 12 and 24 breaths min^{-1}, the mean P_{A,CO_2} – i.e. P_{a,CO_2} – and targeted P_{ET,CO_2} are identical.

The same proposed mechanism accounts for the discrepancy between P_{ET,CO_2} and P_{a,CO_2} at $f = 6$ breaths min^{-1}. Consider that the amplitude of fluctuations of P_{CO_2} varies inversely with inspiratory duration and f and directly with V_T; the effect of the latter is the most influential (Jones *et al.* 1979). At $f = 6$ breaths min^{-1}, V_T values were maximal, resulting in larger fluctuations of P_{CO_2} in the alveoli. The end-inspiratory P_{CO_2} falls because the lower f allows a greater accumulation of 'fresh' gas in the inspiratory reservoir during prolonged exhalation. In addition, prolonged dwell time in the alveoli allows the exhaled P_{CO_2} to equilibrate more completely with the P_{CO_2} in mixed venous blood (Dubois *et al.* 1952; Matell, 1963). Nevertheless, as already noted, the mean P_{A,CO_2} is maintained equal to the targeted P_{ET,CO_2}.

We included changes in P_{O_2} as part of the protocol to examine the effect of P_{O_2} on the gradient between P_{ET,CO_2} and P_{a,CO_2}. In our study, P_{ET,O_2} had no effect on P_{ET,CO_2} or the difference between P_{ET,CO_2} and P_{a,CO_2}.

Larson & Severinghaus (1962) reported that changing from air to O_2 breathing increased the mean end-tidal to arterial P_{CO_2} gradient by 1.5 mmHg. They suggested that O_2 breathing may divert much of the pulmonary flow from non-dependent relatively poorly perfused parts of the lung to dependent areas of the lung, thereby increasing the alveolar dead space. If this effect was present in our study, it was too small to be observed, despite our many tests. It is also possible that the rebreathing of exhaled gases, hypercapnia, or both, may have masked this effect of increased alveolar dead space.

Limitations

Our subject sample was limited to five middle-aged male subjects at rest and therefore cannot be used to predict the response of a population. Our aim was limited to testing whether respiratory rate, changes in P_{ET,CO_2} and P_{ET,O_2} affects the prediction of P_{a,CO_2} from P_{ET,CO_2} when breathing with a sequential gas delivery system. Like St Croix *et al.* (1995) who carried out a very similar protocol using DEF, we assumed that pooling multiple data from each subject does not preclude the independence of our observations and approximates a larger sample size. All of our subjects were male; this is also consistent with other reported studies. Our subjects had a wide age range, and two had mild obstructive lung disease (Table 1), but there were no intersubject differences detected in the results. While we did not test elderly subjects, the rationale of our method with respect to distribution of previously exhaled gas to areas of alveolar deadspace predicts that the gradient between P_{ET,CO_2} and P_{a,CO_2} will be less in subjects with somewhat larger anatomic and physiological deadspace but near-normal, age-adjusted ventilatory capacities. Although there is no theoretical reason that precludes using the method in subjects exercising moderately in a steady state, we have not yet attempted this.

Safety

In addition to standard physiological monitors such as end-tidal gas monitoring, electrocardiography and pulse oximetry, our main safety feature is that all source gases contain at least 10% O_2 so that it is not possible to provide a hypoxic mixture if one or more of the gas sources suddenly fail.

Other potential applications

This method of targeting end-tidal gases with a sequential gas delivery circuit (Slessarev *et al.* 2007) is suitable for studies of the control of breathing because P_{ET,CO_2} and P_{ET,O_2} can be controlled independently, and independent of $\dot{V}_E$. Such precise control of P_{a,CO_2} can be also used to provide continuous assessment of the stimuli to CO_2- and O_2-responsive vascular beds in the brain (Vesely *et al.* 2001; Mikulis *et al.* 2005; Prisman *et al.* 2008) and eye (Gilmore *et al.* 2004; Venkataraman *et al.* 2005; Gilmore *et al.* 2007). While the method of Slessarev *et al.* (2007) has not yet been used to study coronary, renal and other vascular beds, it should be possible with the same sequential gas delivery system.

Conclusion

It is now possible to target P_{a,CO_2}, in addition to P_{ET,CO_2}, using a sequential gas delivery system. For respiratory rates greater than 12 breaths min^{-1}, the resulting P_{ET,CO_2} will provide as accurate a measurement of P_{a,CO_2} as the analysis of an arterial blood sample.

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Conflict of interest

J.D., L.F., S.I. and J.F. have made contributions to the intellectual property related to the development of the automated gas blender and the methodology of targeting end-tidal gases. Patent applications have been filed according the IP policies of the University Health Network (UHN) and the University of Toronto. All rights to the patent have been assigned to TSI, a company formed under the auspices of the Business Development Office of the UHN. J.D., L.F., and J.F. are minor share holders in TSI.