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Determination of blood volume by Pulse CO-Oximetry

S Lalande, JW Kelsey, MJ Joyner, and BD Johnson

Division of Cardiovascular Diseases, Department of Medicine, Mayo Clinic, Rochester, MN, 55905, USA

Abstract

Aim—The objective of this study was to determine whether changes in carboxyhaemoglobin (COHb) saturation following carbon monoxide (CO) rebreathing can be accurately detected by Pulse CO-Oximetry in order to determine blood volume.

Methods—Noninvasive measurements of carboxyhaemoglobin saturation (SpCO) were continuously monitored by Pulse CO-Oximetry before, during and following 2 minutes of CO rebreathing. Reproducibility and accuracy of noninvasive blood volume measurements were determined in 16 healthy non-smoking individuals (15 males, age: 28 ± 2 years, body mass index: 25.4 ± 0.6 kg/m²) through comparison with blood volume measurements calculated from invasive measurements of COHb saturation.

Results—The coefficient of variation for noninvasive blood volume measurements performed on separate days was 15.1% which decreases to 9.1% when measurements were performed on the same day. Changes in COHb saturation and SpCO following CO rebreathing were strongly correlated ($r=0.90$, $p<0.01$), resulting in a significant correlation between invasive and noninvasive blood volume measurements ($r=0.83$, $p=0.02$).

Conclusion—Changes in SpCO following CO rebreathing can be accurately detected by Pulse CO-Oximetry, which could potentially provide a simplified, convenient and reproducible method to rapidly determine blood volume in healthy individuals.

Keywords

Carbon monoxide; carboxyhaemoglobin saturation; rebreathing

Introduction

A convenient determination of blood volume represents a valuable tool in clinical and exercise physiology. Commonly used techniques for determination of blood volume are radioactive indicators, dye-dilution methods or carbon monoxide labelling of haemoglobin (Hb) following carbon monoxide rebreathing (Ertl et al. 2007). Carbon monoxide rebreathing has been shown to have similar accuracy, sensitivity and variability as the gold

Corresponding author: Sophie Lalande, PhD, Human Integrative and Environmental Physiology Laboratory, Division of Cardiovascular Diseases, Joseph 4-221, 200 First Street SW, Mayo Clinic, Rochester, Minnesota, 55905, USA, Phone: (507) 255-8791, Fax: (507) 266-7929, lalande.sophie@mayo.edu.

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standard radioactive methods (Burge and Skinner 1995; Ohki et al. 2000). The accuracy of the carbon monoxide rebreathing method stands on the reliability and precision in measuring changes in carboxyhaemoglobin (COHb) saturation (Gore et al. 2005). Multiple blood draws and expensive medical equipment required to measure changes in COHb saturation make this method somewhat impractical for clinical and research purposes.

Recent advances in Pulse CO-Oximetry allow the noninvasive measurements of COHb saturation, a measurement referred to as SpCO. This new technology uses more than seven wavelengths of light to measure several species of human haemoglobin including COHb. The ability of the Rad-57 (Masimo Corp., Irvine, CA), the first commercially available Pulse CO-Oximeter measuring SpCO, to detect changes in COHb saturation levels was examined by having individuals breathe small doses of carbon monoxide (Barker et al. 2006). When compared to COHb saturation values obtained through blood samples from a radial artery cannula, the Pulse CO-Oximeter estimated absolute SpCO with an uncertainty of $\pm 2.0\%$ (one standard deviation) within the range of 0 to 15 % (Barker et al. 2006). While the variability in the absolute measurement of SpCO has been investigated, the ability of Pulse CO-Oximetry to detect relative changes in SpCO in a given individual on a given session remains to be determined. The importance of determining blood volume has been demonstrated in various fields of research such as aging, blood doping or adaptation to altitude (Davy and Seals 1994; Mancini et al. 1997; Parisotto et al. 2000). Determination of blood volume is also of great importance when studying different disease status as it allows the investigators to account for left ventricular loading conditions (e.g. heart failure). Therefore, the objective of the present study was to determine whether a relative change in SpCO following brief carbon monoxide rebreathing can be detected reproducibly and accurately by Pulse CO-Oximetry in order to develop a simple and rapid, noninvasive method for the determination of blood volume.

Methods

A total of 16 healthy and physically active individuals were recruited to participate in the study (15 males, age: 28 ± 2 years, height: 180 ± 1 cm, weight: 82.0 ± 2.1 kg, body mass index: 25.4 ± 0.6 kg/m²). Participants were excluded from the study if they had a known heart condition or pulmonary disease or if they were smokers. The study was approved by the Mayo Clinic Institutional Review Board and all participants provided written informed consent.

Study protocol

The carbon monoxide rebreathing protocol used in this study was based on the optimized carbon monoxide rebreathing method developed by Schmidt and Prommer (2005), which showed similar validity and reliability to the longer carbon monoxide rebreathing method performed by Burge and Skinner (1995). Briefly, the optimized carbon monoxide rebreathing method consists in inhaling a bolus of carbon monoxide added to a low volume closed-circuit filled with pure oxygen and to rebreathe for a period of 2 minutes (Schmidt and Prommer 2005). Changes in COHb saturation are determined 5 minutes following the start of the carbon monoxide rebreathing (Schmidt and Prommer 2005). The carbon

monoxide rebreathing method causes a short-term increase in COHb saturation by approximately 6.5 % (Burge and Skinner 1995) and COHb saturation can be raised to 15 % without any perceived effects in healthy individuals (Stewart 1975).

Participants were supine for at least 20 minutes before the procedure to allow the movement of fluid from interstitial to intravascular spaces to stabilize (Jacob et al. 2005). The closed-circuit rebreathing system consisted of a 2-liter anesthesia bag connected to a pneumatic valve, and subsequently connected to a mouthpiece. Participants were switched from room air to the rebreathing system at end-exhalation. A bolus of pure carbon monoxide was immediately added to the rebreathing system which was previously filled with room air. The volume of carbon monoxide added to the rebreathing system was calculated for each individual from their body surface area and haemoglobin levels and ranged from 75 to 90 ml in our participants (Burge and Skinner 1995). Due to the short rebreathing time of 2 minutes, soda lime was not used to absorb carbon dioxide, and we observed no significant decrease in arterial oxygen saturation even though there was no oxygen supply to the rebreathing system. Participants were instructed to breathe normally during the 2 minutes of carbon monoxide rebreathing and for an additional 8 minutes following the rebreathing. Two finger pricks were performed before the rebreathing to determine haematocrit and haemoglobin levels (Autoread QBC Diagnostics, Port Matilda, PA) and an average value was used in the calculation of blood volume. The accuracy of this method of haemoglobin determination is reported to be within 0.5 g/dL of the cyanmethaemoglobin method, which is considered the gold standard method (Gehring et al. 2002).

SpCO was continuously monitored by Pulse CO-Oximetry (Radical-7, SET software version 7601, Masimo Corp., Irvine, CA, accuracy of $\pm 3\%$ within the range of 1-40%) using adult ReSposable sensors (rev E) placed on the middle finger of the right hand. SpCO was recorded at a sampling frequency of 1 Hz (Automated Data Collection, Masimo Corp., Irvine, CA) for a period of 4 minutes preceding the start of the rebreathing and for 10 minutes following the start of the rebreathing. In order to obtain a precise measurement of blood volume, it is necessary to correct for the amount of carbon monoxide left in the rebreathing system as it is not diffused into the circulation and could result in an overestimation of blood volume of up to 200 ml. We used labelled carbon monoxide ($C^{18}O$) to monitor the amount of carbon monoxide in the system by mass spectrometry (Perkin Elmer MGA 1100, St Louis, MO) throughout the rebreathing (Figure 1). The concentration of carbon monoxide remaining in the system measured in 3 individuals after 2 minutes of rebreathing ranged from 1.9 to 2.8 % with an average value of 2.1 %. We consequently used a correction factor of 2.2 % for all individuals, as reported by Thomsen *et al.* (1991) and used by others (Burge and Skinner 1995; Gore et al. 2006), for the amount of carbon monoxide remaining in the rebreathing system. The following equations were used to calculate blood volume (Burge and Skinner 1995):

$$nCO = 1000 \times \left[\frac{\frac{\text{barometric pressure}}{760} \times \text{volume of CO added to the rebreathing system}}{0.08206 \times (273 + \text{temperature})} \right]$$

$$nHb = \frac{nCO \times 25}{\text{changes in SpCO}}$$

$$VRBC = \left[\frac{644 \times Hct}{Hb} \right] \times nHb$$

$$\text{Blood volume} = \frac{VRBC \times 100}{Hct \times F_{\text{cell ratio}}}$$

where blood volume was calculated from red blood cell volume (VRBC) and haematocrit (Hct) corrected for the whole body phenomenon (Fcell ratio, or venous-to-body Hct correction factor, of 0.91). nHb is haemoglobin mass, Hb is haemoglobin and nCO is the molar amount of carbon monoxide added to the rebreathing system. The value 25 is to account for changes in SpCO being reported as a percentage and to convert millimoles of monomeric haemoglobin to tetrameric haemoglobin while the value 644 is to correct for the haematocrit being reported as a percentage, to express red blood cell volume in milliliters, and to convert haemoglobin from grams per liter to millimoles per liter (Burge and Skinner 1995). As mentioned above, nCO was corrected by 2.2% to account for carbon monoxide remaining in the rebreathing apparatus. When obtained by finger prick, haematocrit values were corrected by a factor of 0.96 to account for trapped plasma. Plasma volume was calculated as the difference between blood volume and VRBC.

To quantify the reproducibility of the SpCO measurements obtained by Pulse CO-Oximetry, blood volume was determined on 7 individuals on 3 separate occasions. Consecutive visits were separated by an average time of 4 days, ranging from 24 hours to 2 weeks. Participants were instructed to maintain their habitual physical activities and hydration levels throughout the duration of the study. Reproducibility was also determined on 8 individuals during 2 consecutive measurements performed during the same hour. A fixed dose of 70 ml of carbon monoxide was used during the consecutive measurements. The accuracy of blood volume calculated from changes in SpCO was determined in 7 individuals through comparison with blood volume measurements calculated from changes in COHb saturation obtained by analysis of antecubital venous blood samples with laboratory CO-oximetry (ABL 720, Radiometer, Denmark). Blood samples were collected 2 minutes before and 10 minutes after the start of rebreathing and were analyzed for COHb saturation, haematocrit and total haemoglobin.

Data and statistical analysis

Changes in SpCO were calculated as the difference between a pre-rebreathing average value determined from minute -2 to minute 0 and the following values: peak value observed near minute 3, an average value from minute 4 to 6, an average value from minute 7 to 10 and the absolute value obtained at minute 10. These changes in SpCO were compared to the changes in COHb saturation determined invasively by venous blood samples. Coefficients of

variation were calculated to report the reproducibility of blood volume measurements. Pearson's correlation coefficient was used for the analysis of associations between variables. Results are presented in mean $\pm$ standard error of the mean.

Results

Figure 2 shows the typical response to carbon monoxide rebreathing as measured by Pulse CO-Oximetry. There was a rapid increase in SpCO in the first minutes of rebreathing which reached a peak value at minute 3 before decreasing and reaching a plateau. Of all the possibilities in calculating the change in SpCO following carbon monoxide rebreathing (peak value at minute 3, average value from minute 4 to 6 (Schmidt and Prommer 2005), average value from minute 7 to 10 (Gore et al. 2005) and the absolute value obtained at minute 10 (Burge and Skinner 1995)), the best correlation between changes in SpCO and changes in COHb saturation was obtained when using the peak value at minute 3 (peak value minute 3: $r = 0.90$, $p < 0.01$, average minute 4 to 6: $r = 0.61$, $p = 0.15$, average minute 7 to 10: $r = 0.53$, $p = 0.22$ and absolute minute 10: $r = 0.56$, $p = 0.20$), therefore all changes in SpCO were calculated using the peak SpCO at minute 3. There was a significant correlation between blood volume measurements derived from invasive COHb saturation and SpCO ($r = 0.83$, $p = 0.02$). Direct comparison between absolute values of invasive COHb saturation and SpCO obtained at minute -2 and minute 10 had a mean error and standard deviation of 0.4 ± 2.1 %. Figure 3 shows a Bland Altman plot of the changes in COHb saturation and the difference between changes in SpCO and changes in COHb saturation in response to carbon monoxide rebreathing. Participants' characteristics, haemoglobin mass, red blood cell volume, plasma volume, blood volume and blood volume scaled to body weight are presented in Table 1 for day-to-day measurements and same day measurements (Reproducibility of Pulse CO-Oximetry) as well as the comparison between SpCO and invasive measurements of COHb saturation (Accuracy of Pulse CO-Oximetry). The coefficient of variation for blood volume measurements determined from changes in SpCO performed on different days was 15.1 % while the coefficient of variation of the consecutive blood volume measurements was 9.1 %.

Dicussion

SpCO measurements from Pulse CO-Oximetry provided noninvasive, reliable measurements of changes in COHb saturation. In our study, SpCO by Pulse CO-Oximetry had the same degree of uncertainty as previously reported by Barker *et al* (2006) (2.1 vs. 2.0%). However, the accuracy of the blood volume measurements with the carbon monoxide rebreathing method stands on the reliability and precision in measuring changes in COHb saturation (Gore et al. 2005), and changes in SpCO were strongly correlated to changes in COHb saturation obtained invasively ($r = 0.90$, $p < 0.01$). Although our blood volume values were high, they are similar to the blood volumes reported in healthy physically active men with similar height and body mass (Gore et al. 2006).

In general, determination of blood volume using carbon monoxide rebreathing has a low error ($\sim 2.2\%$), which is comparable to the error observed for the radioactive labeled ^{51}Cr technique ($\sim 2.8\%$) and lower than the error reported when using the Evans blue dye-dilution

method (~6.7%) (Gore et al. 2005). We observed coefficients of variation of 15% and 9% when performing day-to-day measurements and same day measurements, respectively. Day-to-day variability is introduced by physiological variation such as hydration status, activity levels, haematocrit, and haemoglobin levels as well as the analytic variation of haemoglobin and haematocrit measurements (Fraser et al. 1989; Thirup 2003). Therefore, the variability of the technique was reduced when performing blood volume measurements on the same day. The variation reported for determination of blood volume by Pulse CO-Oximetry showed similar variation to the 7.9% day-to-day variation obtained by Christensen *et al* (1993) who used a smaller bolus of carbon monoxide and venous blood sampling during a rebreathing period lasting 10 to 15 minutes. Therefore, our noninvasive determination of blood volume has similar variability as other carbon monoxide rebreathing techniques as well as the Evans blue dye-dilution method (Christensen et al. 1993; Gore et al. 2005).

Following carbon monoxide inhalation, COHb saturation measured by venous blood samples progressively increases until reaching a plateau approximately 8 minutes after the start of the rebreathing (Burge and Skinner 1995; Schmidt and Prommer 2005). Time for circulatory mixing has previously been estimated to take 7.5 minutes and 8.9 minutes (Gibson and Evans 1937; Noble et al. 1946). Therefore, venous blood samples used to calculate the changes in COHb saturation are usually collected 10 minutes into the rebreathing to ensure complete mixing of carbon monoxide into the circulation. In contrast, COHb saturation measured in arterialised blood increases rapidly in the first minute of rebreathing before reaching a peak value and decreasing back to a plateau approximately 4 minutes following the start of the rebreathing (Schmidt and Prommer 2005). Haemoglobin mass calculated from an average of the arterialized samples obtained at minute 4 and 6 was identical to the haemoglobin mass derived from venous blood samples obtained at minute 9.5 (Schmidt and Prommer 2005). However, it was suggested that sampling capillary blood 5 minutes after the start of rebreathing may be too premature and that capillary blood should instead be sampled at minutes 8 and 10 following the start of the rebreathing to allow for complete circulatory mixing of carbon monoxide (Gore et al. 2006). On the other hand, it has also been reported that the administration of a bolus leads to a faster uptake and mixing within the cardiovascular system, with complete mixing observed after 80 to 150 seconds (Bruce and Bruce 2003; Kisch et al. 1995). Our SpCO measurements are the first representations of continuous monitoring of the arterial COHb saturation response to carbon monoxide rebreathing. Similar to Schmidt and Prommer (2005), we observed a peak at minute 3 following the start of the rebreathing before values decreased to a plateau. Schmidt and Prommer (2005) determined COHb saturation as an average of values from arterialized blood samples obtained at minute 4 and 6 following the start of rebreathing; Burge and Skinner (1995) used the COHb saturation from venous blood samples obtained at minute 10 following the start of rebreathing, while Gore *et al* (2006) suggested that capillary blood should be sampled at minute 8 and 10 following the start of the rebreathing. Therefore, multiple data analyses were performed to determine the optimal analysis of SpCO in relation to invasive values. Of all the possibilities in calculating the change in SpCO, the best correlation between changes in SpCO and changes in COHb saturation was obtained when using the peak value at minute 3. Therefore, as supported by the findings of Kisch *et al*

(1995), we used the peak value in SpCO as representing complete mixing of carbon monoxide in arterial blood in order to calculate blood volume.

Conclusions

In conclusion, determination of blood volume by Pulse CO-Oximetry is relatively simple, reproducible, safe, convenient to the individual, and does not require cumbersome and expensive blood gas analyzers. Further technical advances in Pulse CO-Oximetry could potentially reduce the variability in SpCO measurements resulting in the increased accuracy of noninvasive blood volume estimations. Currently, the accuracy of blood volume estimations may be improved by performing two consecutive measurements of blood volume and reporting the average of both values for each individual.

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Figure 1. Breath-by-breath labelled carbon monoxide ($C_{18}O$) response to 2 minutes of rebreathing as assessed by mass spectrometry in one individual

Figure 2.
Timeline of the SpCO response to carbon monoxide rebreathing in one individual.
Rebreathing starts at minute 0

Figure 3. Bland Altman plot of the changes in COHb saturation and the difference between changes in SpCO and changes in COHb saturation in response to carbon monoxide rebreathing

Table 1

Participants' characteristics and vascular volumes

	Age (years)	Height (cm)	Weight (kg)	BMI (kg/m ²)	Hbmass (mmol)	BV (ml)	Vrbc (ml)	PV (ml)	BV scaled (ml/kg)
<i>Reproducibility of Pulse CO-Oximetry</i>									
Day-to-day group (n=7)	22 ± 1	180 ± 2	79.7 ± 1.3	24.6 ± 0.7	16.60 ± 0.87	7302 ± 393	3042 ± 158	4260 ± 240	91.9 ± 4.9
Same day group (n=8)	31 ± 3	180 ± 2	82.8 ± 3.8	25.5 ± 0.7	14.36 ± 0.27	6653 ± 128	2664 ± 49	3989 ± 80	81.0 ± 1.9
<i>Accuracy of Pulse CO-Oximetry</i>									
Blood gases (n=7)	28 ± 1	179 ± 1	82.4 ± 2.0	25.8 ± 0.9	15.08 ± 0.93	7065 ± 348	2703 ± 158	4362 ± 205	85.3 ± 3.6
Pulse CO-Oximetry (n=7)	28 ± 1	179 ± 1	82.4 ± 2.0	25.8 ± 0.9	15.15 ± 2.19	7053 ± 954	2709 ± 380	4344 ± 578	84.5 ± 10.4

BMI: body mass index, Hbmass: hemoglobin mass, BV: blood volume, VRBC: red blood cell volume, PV: plasma volume.