Replicate Measurements of Haemoglobin Mass during a Single Day are Feasible and Precise

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Replicate Measurements of Haemoglobin Mass during a Single Day are Feasible and Precise

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Key word
- CO-rebreathing method
- altitude training
- athletes
- Hb_{mass}
- reproducibility
- technical error

Abstract

Duplicate haemoglobin mass (Hb_{mass}) measurements are recommended before and after altitude training sojourns to identify individual adaptations in athletes with a high level of certainty. Duplicate measurements reduce typical error (TE) and disclose measurement outliers, but are usually made on separate days, which is not a practical protocol for routine services in elite sport settings. The aim of this study was therefore to investigate whether it is safe (carboxyhaemoglobin < 10%) to measure Hb_{mass} twice on the same day and to compare TE with measurements made on separate days. 18 healthy men completed 3 different procedures to measure Hb_{mass} twice a day with the carbon monoxide rebreathing method: A (Hb_{mass} measured twice within 6 h), B (dito A, combined with 1 h of hyperoxic training between the tests), C (dito B, within 2 h). First Hb_{mass} measurements of the 3 test days served as procedure D. Carboxyhaemoglobin did not exceed 10% in any procedure. TE and confidence limits for procedures A, B, C and D were 1.4% (1.0–2.1%), 1.1% (0.8–1.7%), 1.3% (1.0–2.0%) and 1.5% (1.2–2.1%), respectively. Duplicate measurements of Hb_{mass} on the same day are feasible and show TE similar to triplicate measurements on separate days.

Introduction

Total haemoglobin mass (Hb_{mass}) is a key factor related to maximal oxygen uptake ($\dot{V}O_2$max) and is strongly related to endurance performance in healthy subjects [6,12,18]. Accordingly, elite endurance athletes have an increased blood volume and an increased Hb_{mass} by up to 50% compared to sedentary people [18]. Hb_{mass} is reported to be very stable in elite athletes over time [2,6,15,17,18], but even small changes of Hb_{mass} can affect $\dot{V}O_2$max in athletes. It is estimated that a change in Hb_{mass} of 1 g is associated with a $\dot{V}O_2$max increase of about 4 mL/min [18]. As Hb_{mass} can be increased with altitude training [17], altitude sojourns are often used as a legal performance enhancement method by endurance athletes. The challenge is that changes in Hb_{mass} are relatively small (~1.1%/100 h of exposure to adequate altitude [8]) and are often not much larger than the usual typical error (TE) [11] of the carbon monoxide (CO)-rebreathing method, which has been reported to be between 1.4–2.2% [3,7,8,15]. Double measurements can improve the analytical precision, as they reduce TE by almost 30% [11], and hence enhance the potential to identify a change in Hb_{mass}. Therefore, double or even triplicate measurements are recommended [5] to identify small physiological effects of e.g. altitude training. As it takes more than 6 h to exhale administered CO completely [16,21], it has been recommended to perform repeated measurements at intervals of at least 12 h [16]. In practice, repeated measurements of Hb_{mass} are, therefore, usually done on separate days. However, double or triplicate measurements on separate days are not always feasible in elite endurance athletes' daily routines. Therefore, we tried to find a procedure allowing the measurement of Hb_{mass} twice on the same day. A critical issue is the sufficient reduction of carboxyhaemoglobin (%HbCO) to allow safe (%HbCO should not exceed 10%; [20]) and valid replication of the CO-rebreathing method. It has been shown that increased ventilation with O_2-enriched gas or hyperbaric O_2 can increase the clearance of CO from the blood [4,21]. In line with these results, Schmidt and Prommer (2005) [16] showed a reduction of the %HbCO half-time after the CO-rebreathing method when executing a bout of heavy exercise in comparison to rest.

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We hypothesized that moderate exercise in combination with breathing hyperoxic air should speed up CO clearance and allow a double measurement of Hb_{mass} on the same day. The aim of this study was first to investigate whether Hb_{mass} can be measured twice a day combined with or without one hour of hyperoxic training resulting in a %HbCO below 10%, and second to compare the reproducibility of these twice a day procedures with triplicate measurements on separate days.

Material and Methods

Subjects

18 healthy, recreationally active men (characterised at the first test day as follows (mean ± SD): age 34 ± 10 years, height 179 ± 6 cm, weight 76.7 ± 8.3 kg, Hb_{mass} 910 ± 101 g, Hb 14.1 ± 0.7 $g \cdot dL^{-1}$, Htc 42.0 ± 1.7%) volunteered and gave written, informed consent to participate in this study. The study was approved by the Institutional Review Board of the Swiss Federal Institute of Sports Magglingen and was performed in accordance with the ethical standard of the International Journal of Sports Medicine [9]. All subjects were non-smokers, did not donate blood prior to and during the study, had no injuries or severe illness, and did not stay at an altitude higher than 1 500 m for more than 2 consecutive days during the study period.

Study design

Fig. 1 gives an overview over the study's design. Each subject completed 3 different procedures (A, B and C) to measure Hb_{mass} with the CO-rebreathing method [16] twice a day. All procedures were separated by at least 48 h and were performed in a randomised order within 15 days. For randomisation every possible sequence of the 3 different procedures (in total 6 possible sequences) was randomised to 3 subjects. Procedure A: The first and second measurements of Hb_{mass} were separated by 6 h of normal daily activity (no exercise). Procedure B: In addition to procedure A, subjects trained for 60 min by breathing hyperoxic air. Procedure C: Same as procedure B, but the second measurement of Hb_{mass} started 2 h after the CO administration of the first measurement of Hb_{mass}. The first Hb_{mass} measurements of each of the 3 test days represent procedure D (triplicate measurements on separate days).

CO-rebreathing

Hb_{mass} was measured with the CO-rebreathing method, as described by Schmidt and Prommer [14, 16]. Briefly (for a more detailed description see Steiner and Wehrlin [19]) subjects rested for 5 min in a sitting position before 3 capillary blood samples (35 µL) from the right earlobe were collected and analysed immediately for the baseline %HbCO values (ABL 800flex; Radiometer A/S, Copenhagen, Denmark). Subjects then rebreathed a mixture of 3.5 L O_2 and a body weight-related dose of CO (1.2 mL/kg of body mass) through a glass spirometer (BloodTec, Bayreuth, Germany) for 2 min whilst wearing a nose clip. A portable CO gas detector (CO Single Gas Detector; BW Technologies, Calgary, Canada) was used during the rebreathing period to check for possible CO leakage at the nose, mouthpiece, and spirometer. Post %HbCO in capillary blood was measured 6 and 8 min after commencing the CO rebreathing (and averaged as a 7-min value). A portable CO analyser (Dräger PAC 7000, Dräger Safety, Lübeck, Germany) was used to detect parts per million CO in the expired air before and 2 min after the rebreathing period. Hb_{mass} was calculated from the mean change in %HbCO before and after CO rebreathing (Δ%HbCO), as described previously by Steiner and Wehrlin [19]. All measurements of Hb_{mass} were performed by the same investigator.

Hyperoxic training

The 1-h hyperoxic training (2 × 30 min with a 5-min break in between) was performed at a low exercise intensity (Borg scale [1] values 12 to 13) on either an electronically braked cycle ergometer (Ergometrics 800, Ergoline, Bitz, Germany) or on a treadmill (HP Cosmos Venus, H/P/Cosmos Sports & Medical GmbH, Nussdorf-Traunstein, Germany) 30 min after the CO administration of the first Hb_{mass} measurement. The hyperoxic condition (FiO_2 = 0.46) was simulated by a modified AltiTrainer200® (SMTEC, Nyon, Switzerland). The device enables a constant desired FiO_2 by mixing 100% medical grade oxygen with ambient air at a constant ratio. Subjects inhaled hyperoxic air through a face mask, which was connected by a plastic hose to the mixing chamber of the AltiTrainer. The AltiTrainer was calibrated before each training session according to the current barometric pressure.

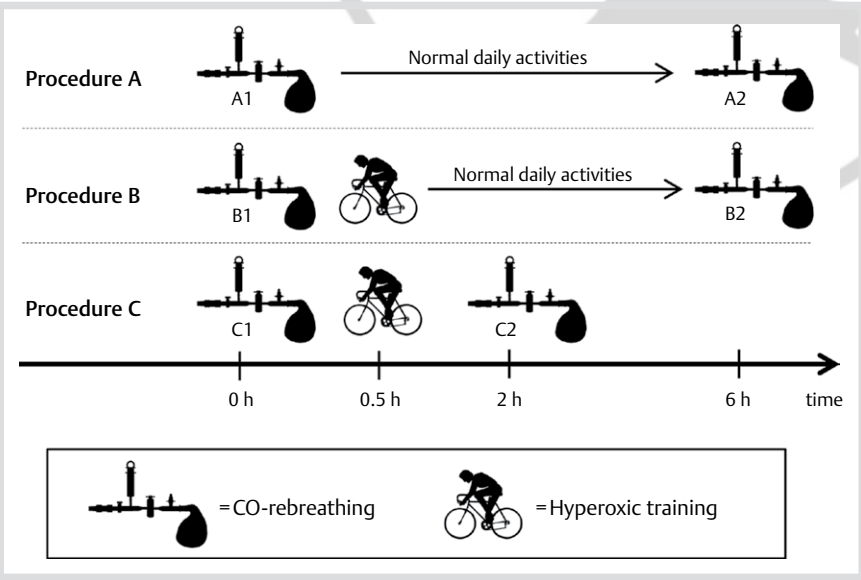

Fig. 1 Schematic illustration of the study's design.

Statistics

Descriptive statistical analyses were performed using Microsoft Office Excel 2007 (Microsoft Corporation, Washington, USA). Values are expressed as mean ± standard deviation unless otherwise indicated. Differences in Hb_{mass} and %HbCO were calculated with a one-way repeated measures analysis of variance (ANOVA) using Systat (Systat Software, Inc., San Jose, USA, Version 10.2). Bonferroni adjustment was used for multiple comparisons. The reliability of the Hb_{mass} measured with the different procedures was evaluated according to Hopkins [11]. Briefly, the TE for Hb_{mass} was calculated as the standard deviation of the log-transformed difference scores divided by √2 and expressed as back-transformed percentage TE. TE is expressed with the associated 95% confidence limits (CL). Significance was set as $p < 0.05$.

Results

Effect of different procedures on %HbCO values

The baseline %HbCO values of the second measurements on a test day were higher compared to the first measurement for all 3 procedures ($p < 0.001$) and differed between procedures ($p < 0.001$). Procedure B showed the lowest %HbCO baseline levels of the second measurement. %HbCO measurements after 7 min also differed between the first and second measurements ($p < 0.001$) as well as between the procedures for the second measurement ($p < 0.001$). At minute 7, %HbCO of all 3 procedures stayed below 10% and ranged from 5.3–9.3% for procedure A, 5.5–7.6% for procedure B and 5.5–8.3% for procedure C. The Δ%HbCO values neither differed between procedures nor between the first and second measurements. Baseline %HbCO values did not differ for the first measurement on a test day (**Table 1**).

Reproducibility of the different procedures

Neither the procedure nor the baseline %HbCO had an effect on the calculated Hb_{mass} (**Table 2**). The TE of procedures A, B and C amounted to 1.4, 1.1 and 1.3%, respectively, while the TE of procedure D was 1.5% (**Table 2**).

Discussion

This study investigated 2 main issues: first, whether it is safe to measure Hb_{mass} twice a day combined with or without 1 h of hyperoxic training; and second, to compare the reproducibility of these twice a day procedures to triplicate measurements on separate days.

The main finding of the present study is that Hb_{mass} can be measured twice a day in a safe manner in healthy subjects, when a break of 6 h is provided between measurements. This interval can be further minimised when hyperoxic exercise is used between the tests. With the selected procedures, the removal of the CO residues from the first CO-rebreathing measurement was sufficient to avoid excessive high %HbCO values during the second measurement. Although CO removal was incomplete and corresponding baseline %HbCO values were higher for the second measurements for all 3 procedures, the values were low enough to not exceed the recommended %HbCO peak value of about 10% during a CO-rebreathing measurement [20]. This criterion was already fulfilled when the second Hb_{mass} measurement was performed 6 h after the CO administration of the first measurement with just normal daily activity in between the 2 measurements (procedure A). However, to shorten the time between the 2 measurements in order to make the entire procedure more athlete-friendly, we assumed that with exercise [10, 13, 16] as well as breathing hyperoxic air [21], %HbCO degradation happens faster. This was confirmed when comparing the baseline %HbCO levels of the second measurements between the procedures including exercise and breathing hyperoxic air (procedures B and C) with procedure A including just daily physical activity and breathing environmental air. This is in line with the results of Zavorsky et al. [21], who showed that CO removal is significantly sped up with even light physical activity of 45 W compared to rest. Hyperoxic conditions (100% O_2) sped up CO removal even more and were assessed as more relevant for CO clearance than exercise intensity [21], as CO removal (half-life time) was not significantly different between exercise intensities from 45 to 100 W. This indicates that precise control of exercise intensity is not necessary and that increased ventilation with even low exercise intensity combined with hyperoxic breathing is sufficient to significantly speed up CO removal.

The assumption that the procedure with the longest time frame between the 2 measurements combined with breathing hyperoxic air (procedure B) is the most effective in CO removal was confirmed. However, procedure C shows that even a 2 h interval between 2 repetitive tests allows sufficient CO removal for the second CO-rebreathing measurement, provided that hyperoxic training is included in the "rest" period.

The slightly increased baseline %HbCO values due to the proceeding CO-rebreathing measurement neither deteriorated the TE of the testing procedure nor influenced the measured Hb_{mass}. The TE for all within-one-day procedures (A, B and C) as well as for procedure D was low compared to those reported in the literature [3, 7, 8, 15]. It can be assumed that the TE of the 3 within-one-day procedures approximates the true analytical error of the CO-rebreathing method since most biological variation

Table 1 Carboxyhaemoglobin values (%HbCO) at baseline and minute 7 of the different procedures.

	First measurement			Second measurement		
Procedure	HbCO_baseline (%)	HbCO_7min (%)	ΔHbCO (%)	HbCO_baseline (%)	HbCO_7min (%)	ΔHbCO (%)
A	0.5 ± 0.1	6.2 ± 0.4	5.7 ± 0.4	2.5 ± 0.3 *†	8.1 ± 0.6 *†	5.6 ± 0.4
B	0.6 ± 0.1	6.3 ± 0.4	5.7 ± 0.4	1.1 ± 0.2 *#	6.7 ± 0.4 *#	5.7 ± 0.4
C	0.5 ± 0.2	6.2 ± 0.4	5.7 ± 0.4	1.5 ± 0.2 *	7.2 ± 0.5 *	5.7 ± 0.4

HbCO_baseline = %HbCO levels before CO-rebreathing; HbCO_7min: averaged %HbCO levels 6 and 8 min after starting the CO-rebreathing; ΔHbCO = difference between HbCO_baseline and HbCO_7min

* Significantly different than values of the first measurement at the same time point (at baseline or at minute 7; $p < 0.001$). † Significant difference compared to %HbCO levels in procedures B and C ($p < 0.001$). # Significantly different than procedure C ($p < 0.001$). Values are means ± SD

Table 2 Individual total haemoglobin mass values measured at different time points and with different procedures including the typical error (in %) of all procedures.

	Procedure A		Procedure B		Procedure C	
Subject	Hb_{mass}_A1 (g)	Hb_{mass}_A2 (g)	Hb_{mass}_B1 (g)	Hb_{mass}_B2 (g)	Hb_{mass}_C1 (g)	Hb_{mass}_C2 (g)
1	973	961	978	965	939	923
2	713	748	747	721	725	722
3	950	978	953	957	985	961
4	965	963	973	968	965	957
5	888	901	889	894	905	890
6	753	758	739	736	752	769
7	996	986	986	968	994	981
8	840	863	857	852	834	827
9	816	791	790	802	795	814
10	943	928	949	950	968	932
11	1070	1087	1083	1085	1075	1078
12	997	997	1018	989	1018	980
13	873	881	873	882	875	882
14	1044	1045	1030	1040	1015	1027
15	811	816	794	808	827	821
16	902	901	902	901	902	901
17	854	840	794	811	830	822
18	1002	1024	1001	1020	973	993
Mean	911	915	909	908	910	905
SD	100	99	104	103	99	95
Change in mean (g, 95% CL)	4.3 (−3.8–12.5)		−0.4 (−7.3–6.5)		−5.4 (−13.9–3.1)	
TE (%, 95% CL)	1.4 (1.0–2.1)		1.1 (0.8–1.7)		1.3 (1.0–2.0)	
TE of procedure D * (%, 95% CL)	1.5 (1.2–2.1)					

Hb_{mass} = total haemoglobin mass; 1 = first measurement; 2 = second measurement; SD = standard deviation; TE = typical percentage error; CL = confidence limits; * The TE of procedure D was calculated with the first measurements of procedures A, B and C (A1, B1, and C1) using the randomised order of procedures for every athlete

might be excluded. As soon as the 2 measurements are conducted more than a few days apart, an additional biological variation contributes to the TE [3,8]. The biological variation increases progressively the longer apart the 2 measurements are done, and the analytical and biological components seem to be independent and additive [8]. This could explain why our TE for the within-one-day procedures was slightly lower compared to other studies and compared to procedure D. In both cases an additional biological variation comes into play since measurements usually are made 1 to several days apart. The fact that double measurements within one day are feasible with a very high level of accuracy signifies a noticeable improvement for elite athletes. Athletes typically have to come to the laboratory on 2 separate days to have a double measurement and achieve the same level of measurement precision. Due to the focus on elite sport services, we did not test a protocol with hyperoxic application without exercise, what probably would be a favourable protocol in patient settings. Further studies might address this issue, as well as fine tune the test interval lengths or the effect of different FiO_2 on CO removal and the related effect on the reproducibility of repetitive measurements on the same day.

Conclusion

It is possible to measure Hb_{mass} twice in the same day with carboxyhaemoglobin not reaching levels above 10% with the second measurement. When combined with hyperoxic training lasting about 1 h, the time between the 2 measurements can markedly be reduced to 2 h without impairing the accuracy of the CO-rebreathing method. Therefore, if it is important to have a high level of certainty about an athlete's true Hb_{mass} level, we recommend measuring Hb_{mass} twice a day within 2–6 h, including 1 h of hyperoxic training.

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Conflict of interest: The authors have no conflict of interest to declare.

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