Total haemoglobin mass, but not haemoglobin concentration, is associated with preoperative cardiopulmonary exercise testing (CPET) derived oxygen consumption variables

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Abstract (226 words)

Background. Cardiopulmonary exercise testing (CPET) measures peak exertional oxygen consumption ($\dot{V}O_2$ peak) and $\dot{V}O_2$ at anaerobic threshold ($\dot{V}O_2$ at AT- the point at which anaerobic metabolism contributes substantially to overall metabolism). Lower values are associated with excess postoperative morbidity and mortality. A reduced haemoglobin concentration may result from a fall in total haemoglobin mass (tHb-mass), or rise in plasma volume. Thus, tHb-mass may be a more useful measure of oxygen carrying capacity, and may correlate better with CPET-derived fitness measures in preoperative patients, than does circulating haemoglobin concentration ([Hb]). We tested this hypothesis.

Methods. Prior to major elective surgery, CPET was performed, and both tHb-mass (optimised carbon-monoxide rebreathing method, oCOR and circulating [Hb] determined.

Results. In 42 patients (83% male), [Hb] was unrelated to $\dot{V}O_2$ at AT and $\dot{V}O_2$ peak ($r= 0.02$, $p= 0.89$; $r= 0.04$, $p= 0.80$ respectively) and explained none of the variance in either measure. In contrast, tHb-mass was related to both ($r= 0.661$, $p< 0.0001$; $r= 0.483$, $p= 0.001$) for $\dot{V}O_2$ at AT and $\dot{V}O_2$ peak respectively and tHb-mass explained 43.7% of variance in $\dot{V}O_2$ at AT ($p< 0.0001$) and 23.3% in $\dot{V}O_2$ peak ($p= 0.001$).

Conclusions. In contrast to [Hb], tHb-mass is an important determinant of physical fitness prior to major elective surgery. Further studies should determine whether this is reflected in poor outcome, and whether targeted increases in tHb-mass might thus improve outcome.

MESH key words

Physical fitness; Cardiopulmonary Exercise Test; Oxygen Consumption; Anaerobic Threshold; Surgery; Anaemia

Cardiopulmonary exercise testing (CPET) is routinely used to quantify exertional oxygen consumption ($\dot{V}O_2$)- both the peak attained ($\dot{V}O_2$ peak) and that at the anaerobic threshold ($\dot{V}O_2$ at AT: the point during incremental exercise when anaerobic metabolism makes a significant contribution to overall metabolism). Both measures are used as indices of physical fitness prior to major surgery,[1-3] given that reduced values are associated with increased postoperative morbidity and mortality.[4 5]

Haemoglobin (Hb), in circulating red blood cells (RBCs), is the blood's oxygen carrying pigment. Its circulating concentration ([Hb]) reflects both its total circulating mass (tHb-mass) and the volume of plasma in which the RBCs are suspended. Anaemia, defined as [Hb] <130 $g\,l^{-1}$ in men and <120 $g\,l^{-1}$ in women,[6] is associated with decreased aerobic exercise capacity.[7-9] Preoperative anaemia is common (affecting one third of surgical patients) and is associated with an increased risk postoperative morbidity and mortality.[10-11]

Because the proportion of oxygen (O_2) carried in plasma is trivial, the relationship of aerobic fitness with tHb-mass might be stronger than that with [Hb]. Indeed, in *healthy* individuals, [Hb] does not significantly correlate with either $\dot{V}O_2$ peak or $\dot{V}O_{2max}$,[12 13] unlike tHb-mass.[14-15] However, whether this applies to patient populations- and to perioperative patients in particular- is less certain. We thus sought to explore the relationship of [Hb] and tHb-mass with CPET-derived measures of physical fitness in preoperative patients.

Methods

Ethical approval was granted by the London, Camden and Kings Cross Research Ethics Committee (REC reference: 13/LO/1901). We fully adhered to Caldicott guidelines and followed the standards established by the Declaration of Helsinki. All participants provided written informed consent prior to taking part.

Patients

Adult (> 18 years) elective (non-cardiac) surgical patients at University College Hospital (University College London Hospitals NHS Foundation Trust) and Southampton General Hospital (University Hospital Southampton NHS Foundation Trust) were prospectively studied between February and August 2015. Patients were either receiving CPET as part of their routine preoperative assessment, or were (approved) co-recruits to the 'METS' study of the relationship between CPET derived physiological variables and surgical outcome.[16] Age, gender, height, weight, diagnosis, planned surgical procedure, comorbidities (such as diagnosis of diabetes, respiratory or cardiovascular disease) and current medications were documented.

Cardiopulmonary Exercise Testing

CPET was supervised by a clinical exercise physiologist (and clinician where appropriate) and performed in accordance with international guidelines.[17]

Patients cycled on an electromagnetically-braked ergometer (UCLH- Lode BV, Groningen, Netherlands; Southampton- Ergoline 2000, Ergoline GmbH, Bitz, Germany) with respiratory gas analysis made by calibrated metabolic carts [UCLH-Cortex Biophysik, Leipzig, Germany; Southampton- Geratherm Respiratory GmbH (Love Medical Ltd, Manchester, UK)]. Breath-by-breath oxygen uptake ($\dot{V}O_2$) and carbon dioxide (CO_2) output ($\dot{V}CO_2$) were recorded, concurrently with minute ventilation, tidal volume, respiratory rate, and end-tidal gas tensions for O_2 and CO_2.

Patients were connected to appropriate monitoring equipment and rested for an initial 3-minute period, after which 3-minutes of unloaded cycling was completed. Subsequently, patients performed a symptom-limited incremental ramp test set to 10-20 W min^{-1} (based on patient height, weight and age) such as to deliver an intended test duration of 8 to 12 minutes.[18] Test cessation occurred at patient exhaustion, or when cadence fell below 40 rpm for more than 30 seconds in spite of verbal encouragement. CPET was terminated by testing staff on safety grounds if the patient developed a sign or symptom listed in the ATS/ACCP CPET guidelines.[17] After stopping CPET, patients completed a 3-5 minute period of unloaded cycling to 'cool down'.

The anaerobic threshold (ml kg^{-1} min^{-1}) was determined by a clinical exercise physiologist and/or consultant physician, both skilled in CPET analysis, using the modified V-slope method with corroboration by ventilatory equivalents and end-tidal gas tensions for O_2 and CO_2.[19] The highest average $\dot{V}O_2$ over the final 30s of exercise was recorded as the $\dot{V}O_2$ peak (ml kg^{-1} min^{-1}).[20]

Optimised Carbon Monoxide Rebreathing Method (oCOR)

tHb-mass was determined using the optimised carbon monoxide (CO) rebreathing method described in detail by Schmidt and Prommer [21] and was calculated based on the equation in Figure 1. In brief, CO binds avidly to Hb. Carboxyhaemoglobin (COHb) concentration is measured in blood before and after 2 minutes of rebreathing a known CO volume. In this study the CO volume used ranged between 0.5 to 0.9 ml kg^{-1}, depending on gender in the first instance, with fine tuning by [Hb], body mass index and the general condition of the patient. Each participant was seated for 15 minutes to allow stabilisation of plasma volume, after which a mouthpiece containing ~10g 'soda lime' (calcium oxide/sodium hydroxide mixture as a carbon dioxide scrubber) connected them to a spirometer (Spico-CO Respirations-Applikator, Blood Tec, Germany) and a 3 litre anaesthetic bag pre-filled with 100% oxygen. The patient completely exhaled to residual volume and was then instructed to take a deep breath in through the spirometer as the CO dose was administered via a pre-filled 100 ml syringe. To support the diffusion of CO into the blood the patient held their breath for 10 s after the first inspiration, thereafter normal breathing from the spirometer was performed for a further 1 min 50 s. The participant was disconnected after exhaling to residual volume, the exhaled volume being collected and analysed to quantify the CO not absorbed into the bloodstream. Finally, participants fully exhaled to residual volume into a CO gas analyser (Dräger Pac 7000, Drägerwerk AG & Co. KGaA, Germany) before and at minute 4 after CO rebreathing to determine the CO volume exhaled after disconnecting the patient from the spirometer.

Blood sampling

Fingertip capillary samples (200 µl) were collected before and 6- and 8-min after the start of CO rebreathing (Na-heparinized 200 µl RAPIDLyte Multicap Capillary tubes, Siemens Healthcare Diagnostics Inc, Deerfield, USA), with samples analysed within 15 minutes for percent carboxyhaemoglobin (%COHb) using a blood gas analyser (Hemoximeter; Cobas b 221 POC system, Roche Diagnostics Ltd, Switzerland). At Southampton, [Hb] was measured in venous blood. An intravenous cannula was inserted prior to tHb-mass testing at Southampton, allowing venous blood samples (200 µl) to be collected before and at 6- and 8-mins after CO rebreathing via a Na-heparinized blood gas syringe (RAPIDLyte, Siemens Healthcare Diagnostics Inc,

Deerfield, USA). Percent carboxyhaemoglobin was determined at Southampton using the RAPIDPoint 500 Blood Gas System (Siemens Healthcare Diagnostics Inc, Deerfield, USA). Blood sampling from venous, arterial and capillary blood yields an identical Δ%COHb and therefore identical tHb-mass values. [22] All blood samples for the determination of [Hb], Hct and %COHb were collected under the same conditions, from the same site, with patients in a seated position. Blood samples were analysed within 10-15 minutes of one another due to %COHb being analysed after the oCOR test using a blood gas machine (which takes 15 minutes).

Calculations

tHb-mass was calculated using a specifically designed excel spreadsheet (Microsoft Excel 2016 for Apple Macintosh) based on the equation in Figure 1. Also shown in Figure 1 are calculations for blood volume (BV), erythrocyte volume (EV) and plasma volume (PV). When Hct and [Hb] values were acquired from capillary blood at UCLH these values were corrected to venous conditions using the following formulas: [23 24]

$[Hb]\ (g\,dl^{-1}) = [Hbcapillary] \bullet 0.8787 + 1.24$
$Hct\ (\%) = [Hctcapillary] \bullet 0.8425 + 5.23$

Statistical analysis

Statistical analysis was carried out using SPSS Statistics (Version 23.0 for Apple Mackintosh, Chicago, IL). Values are presented as mean (standard deviation, SD), unless otherwise stated. Median and interquartile range (IQR) are reported when variables are not normally distributed and when data transformation (logarithmic, square root or reciprocal) did not result in a normally distributed variable. Categorical variables are presented as frequency (%). Normal (Gaussian) distribution was assessed using a combination of the Kolmogorov-Smirnov test, visual inspection of histogram charts and normal Q-Q plots for each variable. Pearson's correlation coefficient assessed the relationship between [Hb], tHb-mass and exertional $\dot{V}O_2$ allowing adjustment for confounding. Linear regression models assessed the associations between $\dot{V}O_2$ and haematological variables ([Hb] and tHb-mass) and the proportion of variance in $\dot{V}O_2$ explained by [Hb] and tHb-mass, allowing adjustment for confounding. Specifically, age, gender, smoking status, diabetes and the presence of cardiovascular disease (defined if a patient had either ischaemic heart disease, heart failure of previous stroke) were included as confounders in both correlation and regression analyses. [Hb], tHb-mass, exertional $\dot{V}O_2$ at AT and $\dot{V}O_2$ peak were expressed in $g\,l^{-1}$, $g\,kg^{-1}$ and $ml\,kg^{-1}\,min^{-1}$, respectively for both correlation and regression analyses. Elsewhere, $\dot{V}O_2$ is expressed in $ml\,kg^{-1}\,min^{-1}$ and tHb-mass in $g\,kg^{-1}$ unless otherwise stated. All tests were two-sided with statistical significance was accepted as a p-value of < 0.05.

Results

Forty-three patients (24 from University College London Hospital and 19 from Southampton General Hospital) consented to take part in the study. One failed to successfully complete the oCOR protocol and so was excluded. Patient characteristics, including surgical specialty, are shown in Table 1. No major adverse clinical events occurred during CPET. The mean (SD) $\dot{V}O_2$ peak and AT were 15.7 (5.9) and 10.2 (2.3) ml kg^{-1} min^{-1}. AT could not be determined in 3 patients, although their $\dot{V}O_2$ peak data were used in analyses where appropriate. Mean (SD) [Hb] and tHb-mass were 135.6 (15.6) g l^{-1} and 8.2 (1.3) g kg^{-1}. Table 2 shows other haematological variables.

Relationships between haematological variables and oxygen uptake

[Hb] was unrelated to either $\dot{V}O_2$ at AT (r= 0.02, p= 0.89) or $\dot{V}O_2$ peak (r= 0.041, p= 0.796). However, tHb-mass was associated with $\dot{V}O_2$ at AT and $\dot{V}O_2$ peak (r= 0.661 and 0.483, p= 0.001 and < 0.0001 respectively: Figure 2). After adjusting for age, gender, diabetes, smoking status, and the presence of cardiovascular disease, tHb-mass remained correlated with $\dot{V}O_2$ at AT (r= 0.629, p< 0.0001) and $\dot{V}O_2$ peak (r= 0.412, p= 0.011).

Linear Regression Models

[Hb] did not explain any of the variance in $\dot{V}O_2$ at AT (adjusted R^2= -0.027, p= 0.892) or $\dot{V}O_2$ peak (adjusted R^2= -0.023, p= 0.796). In contrast, tHb-mass explained 43.7% of the variance in $\dot{V}O_2$ at AT (p< 0.0001) and 23.3% in $\dot{V}O_2$ peak (p= 0.001). A 1 g kg^{-1} increase in tHb-mass was associated with a 1.0 ml kg^{-1} min^{-1} increase in AT (p< 0.0001), after adjusting for age, gender, smoking status, diabetes, and the presence of cardiovascular disease. Similarly, a 1 g kg^{-1} increase in tHb-mass was associated with a 2.0 ml kg^{-1} min^{-1} increase in $\dot{V}O_2$ peak (p= 0.01) after adjustment for age, sex, smoking status, diabetes, and the presence of cardiovascular disease. A summary of the linear regression analyses can be found in Tables 1 and 2 of the supplementary material.

Discussion

To our knowledge, this is the only study to have explored the relationship between total haemoglobin mass and physical fitness determined by cardiopulmonary exercise testing in patients awaiting major surgery. Whilst [Hb] was unrelated to exertional $\dot{V}O_2$ at either AT or $\dot{V}O_2$ peak (ml kg^{-1} min^{-1}), total haemoglobin mass (g kg^{-1}) was associated with $\dot{V}O_2$ at AT (r= 0.66) and $\dot{V}O_2$ peak (r= 0.48), even after adjusting for measured confounding variables. Some 44% of the variance in $\dot{V}O_2$ at AT was attributable to tHb-mass, which may be of particular importance in the peri-operative setting, given the consistent link between $\dot{V}O_2$ at AT and surgical outcome.

There are few published clinical data with which to compare our results. However, in 12 men with type 1 diabetes (T1DM) and 23 controls, Koponen and colleagues found that [Hb] was not associated with $\dot{V}O_2$ peak, while body mass normalised tHb-mass was strongly related to $\dot{V}O_2$ peak (r= 0.71, p< 0.01, explaining 51% of the variance in $\dot{V}O_2$ peak) in T1DM patients.[25] This study did not, however, report the relationship between tHb-mass and AT. The strength of this correlation between tHb-mass and $\dot{V}O_2$ peak (r= 0.71) was stronger than that which we identified (r= 0.48). This may in part be explained by differences in relatively small sample sizes, differences (albeit small) in measurement error inherent in both oCOR test and CPET measurement, and (perhaps to a greater extent) by $\dot{V}O_2$ peak being dependent upon patient/participant volition and motivation. If this is the case then the relationships between $\dot{V}O_2$ peak and tHb-mass may be underestimated in the current study. In general, the finding that tHb-mass displays a stronger relationship with $\dot{V}O_2$ peak than does [Hb], is consistent with previous reports in healthy volunteers and trained athletes (r= 0.79 in pooled data from 611 subjects;[15] r = 0.48, 0.79 and 0.92 in male runners, and male and female rowers, respectively).[26]

Mean AT measurements of 10 ml kg^{-1} min^{-1} and $\dot{V}O_2$ peak measurements of 16 ml kg^{-1} min^{-1} are in keeping with preoperative data reported by others.[3 27] So, too, is the high prevalence of anaemia (26%).[10 11] Our mean (SD) tHb-mass 677 (146) g, or 8.2 (1.3) g kg^{-1} was lower than that report by Koponen and colleagues [25] for T1DM (722 (121) g and 10.1 (1.5) g kg^{-1}) and healthy controls (898 (96) g and 11.0 (1.1) g kg^{-1} respectively). Furthermore, our values are substantially lower than those reported in endurance athletes, mean (SD) tHb-mass 1285 (123) g and 13.7 (0.5) g kg^{-1} in elite rowers, [28] tHb-mass 958 (123) g in trained cyclists, [24] and in excess of 14.5 g kg^{-1} in elite marathon runners.[12] Such high tHb-mass values only appear to be legally reached by years of intensive training or the influence of strong genetic factors [13] but may also be influenced by elicit use of erythryopoeitin and/or blood transfusion, previously common practice in many endurance

sports. Data from untrained subjects suggest that improvements in $\dot{V}O_2$ peak following only 6 weeks of endurance training are largely due to increases in peak cardiac output and O_2 carrying capacity (quantified by red cell volume and tHb-mass).[29] Given that prolonged endurance training is largely not feasible in patients awaiting major surgery, alternative interventions to increase tHb-mass preoperatively (and thus aerobic fitness and related surgical outcome) are worthy of exploration. In addition, preoperative anaemia is associated with an increased risk of postoperative morbidity and mortality,[10-11] and it would thus perhaps be surprising if a similar relationship was lacking for tHb-mass. Furthermore, as we have shown a close relationship between tHb-mass and preoperative fitness, and given the link between exertional $\dot{V}O_2$ and surgical outcome, it would seem logical to purport that a high tHb-mass may be advantageous to patient outcomes in the perioperative setting, although this remains to be confirmed.

Once tHb-mass has been quantified, other blood volume compartments can be derived providing [Hb] and Hct (%) are measured. Specific formulae shown in Figure 1 allow the calculation of BV, EV and PV. In the context of anaemia, these variables offer additional insight into the haematological and volume status of patients given that the measured [Hb] can be affected by both changes in tHb-mass and PV, independently of one another. As shown in Table 2, tHb-mass (g & g kg^{-1}) and EV (ml & ml kg^{-1}) did not significantly differ between anaemic and non-anaemic patients. However, BV (ml kg^{-1}) and PV (ml & ml kg^{-1}) were expanded in anaemic patients, suggesting to some extent that anaemia in this small sample may in part be related to PV expansion rather than a deficit in oxygen carrying capacity as quantified by tHb-mass. Thus, dilutional anaemia may not have such negative effects on exercise capacity as 'true' anaemia, being characterised by a deficit in tHb-mass. This may to some extent explain the lack of association between [Hb] and exertional $\dot{V}O_2$. In future studies the aetiology of anaemia should therefore also be evaluated. Indeed, in the current study median creatinine concentrations were numerically (but not statistically significantly) higher in anaemic patients, suggesting chronic kidney disease as a potential underlying cause of anaemia. However, it was not the aim of this study to elucidate the underlying aetiology of anaemia, and larger studies (with greater power) would be needed to determine if renal impairment contributed significantly to the prevalence of preoperative anaemia.

Strengths and weaknesses

Strengths of this study include the prospective nature of the use of CPET (considered the gold standard) to determine preoperative exercise capacity, and the use of the oCOR as a reliable and

precise measure of tHb-mass.[30] Indeed, oCOR is a simple, convenient and cheap methodology which doesn't involve the use of radioisotopes (as ^{51}Chromium-labelling of red cells does). This greatly widens its applicability in the clinical setting. Finally, the lack of observed association between [Hb] and exertional $\dot{V}O_2$ is a strength and is in keeping with a number of studies in healthy volunteers and endurance trained athletes.[13 30]

Potential weaknesses of the current study include the relatively small sample size, although this study was adequately powered to explore the relationship between tHb-mass and exertional $\dot{V}O_2$ (achieved power of 0.91, based on sample size of 42, alpha value of 0.05 and correlation of r=0.48 between weight adjusted tHb-mass and AT). In addition, to our knowledge, the current study cohort is the largest in which the relationship between preoperative tHb-mass and CPET-derived physical fitness has been explored and is a larger sample size than the study by Koponen and colleagues.[25] The use of capillary and venous blood across testing sites (UCLH and Southampton) to quantify [Hb] is a weakness, although blood sampling from venous, arterial and capillary blood yields an identical Δ%COHb and therefore identical tHb-mass values. [22] Capillary [Hb] is generally higher than that measured in venous blood.[32-34] Such differences may be related to differences in body posture at the time of sampling, as well as factors affecting fingertip capillary samples such as skin thickness and temperature.[35] In addition, differences in laboratory measures of [Hb] in venous blood may have introduced error. All or some of these potential sources of error may have affected the measured [Hb] value and thus impacted upon the relationship between [Hb] and exertional $\dot{V}O_2$. However, to minimise such confounding factors, [Hb] values obtained from capillary blood were adjusted to venous conditions using established formulas and hospital laboratories subject to rigorous quality control.

In addition, use of different blood gas machines may have introduced error in the measurement of tHb-mass and different metabolic carts across sites may have impacted upon measured $\dot{V}O_2$. However, at UCLH we have previously reported a typical error (TE) of repeat tHb-mass measurements of 1.9 % (95% CI 1.3-3.4%) which is in keeping with the TE commonly reported by other institutions using the oCOR method.[21 37] In addition, both metabolic carts were calibrated prior to every CPET.

Conclusion and further research

In a cohort of patients awaiting major elective surgery, total haemoglobin mass (unlike haemoglobin concentration) was associated with preoperative cardiorespiratory fitness. Future studies may wish to address whether increasing tHb-mass preoperatively leads to improved preoperative cardiorespiratory fitness, and thus outcome.

Details of authors contributions

H.E.M., T.R., M.G., and J.O. conceived the study. J.O., J.O.M.P., D.W., L.L. and E.C., assisted with data collection, J.O., E.C., L.L. and J.O.M.P., collated the data with J.O. performing all statistical analyses and drafting the initial manuscript. H.E.M., T.R., M.G., W.S. and J.O.P.M. helped write the manuscript. All authors read and approved the final version of the manuscript.

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Declaration of interests

MG serves on the Medical Advisory Board of Sphere Medical Ltd. MG has received honoraria for speaking and/or travel expenses from Cortex GmBH (2008 & 2009).

WS is a managing partner of the company "Blood tec GmbH" but he is unaware of any direct or indirect conflict of interest with the contents of this paper.

HEM consults for Google Deepmind on Health technology, and is on the Council of the UK Intensive Care Society but is unaware of any direct or indirect conflict of interest with the contents of this paper or its related fields.

TR is a board member NATA. Network for advancement of Patient Blood Management, Haemostasis and Thrombosis. TR is director of theironclinic.com. UCL and the research program lead by TR has received research funding from a variety of sources including; government, charity and industry sources for research into anaemia, blood transfusion and iron therapy including; NIHR HTA, NHMRC, Health Foundation, Gideon Richter, Vifor Pharma Ltd, Pharmocosmos. TR has also been an invited speaker at conferences and provided consultancy to government and industry on anaemia, blood transfusion and iron therapy in the last 5 years. This does not alter our adherence to the journal policies on sharing data and materials. Please see www.ucl.ac.uk for full list of disclosures.

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References

1. Hennis PJ, Meale PM, Hurst RA, et al. Cardiopulmonary exercise testing predicts postoperative outcome in patients undergoing gastric bypass surgery. *Br J Anaesth* 2012; **109**(4): 566-71
2. Older P, Smith R, Courtney P, et al. Preoperative evaluation of cardiac failure and ischemia in elderly patients by cardiopulmonary exercise testing. *Chest* 1993; **104**(3): 701-4
3. Snowden CP, Prentis JM, Anderson HL, et al. Submaximal cardiopulmonary exercise testing predicts complications and hospital length of stay in patients undergoing major elective surgery. *Ann Surg* 2010; **251**(3): 535-41
4. Levett DZ and Grocott MP. Cardiopulmonary exercise testing for risk prediction in major abdominal surgery. *Anesthesiol Clin* 2015; **33**(1): 1-16
5. Moran J, Wilson F, Guinan E, et al. Role of cardiopulmonary exercise testing as a risk-assessment method in patients undergoing intra-abdominal surgery: a systematic review. *Br J Anaesth* 2016; **116**(2): 177-91
6. World Health Organisation (WHO). Haemoglobin concentration for the diagnosis of anaemia and assessment of severity 2011. Available from http://www.who.int/vmnis/indicators/haemoglobin/en/ (accessed on 20 June 2016)
7. Calbet JA, Lundby C, Koskolou M, et al. Importance of hemoglobin concentration to exercise: acute manipulations. *Respir Physiol Neurobiol* 2006; **151**(2-3): 132-40
8. Otto JM, O'Doherty AF, Hennis PJ, et al. Association between preoperative haemoglobin concentration and cardiopulmonary exercise variables: a multicentre study. *Perioper Med (Lond)* 2013; **2**(1): 18
9. Wright SE, Pearce B, Snowden CP, et al. Cardiopulmonary exercise testing before and after blood transfusion: a prospective clinical study. *Br J Anaesth* 2014; **113**(1): 91-6
10. Baron DM, Hochrieser H, Posch M, et al. Preoperative anaemia is associated with poor clinical outcome in non-cardiac surgery patients. *Br J Anaesth* 2014; **113**(3): 416-23
11. Musallam KM, Tamim HM, Richards T, et al. Preoperative anaemia and postoperative outcomes in non-cardiac surgery: a retrospective cohort study. *Lancet* 2011; **378**(9800): 1396-1407
12. Heinicke K, Wolfarth B, Winchenbach P, et al. Blood volume and hemoglobin mass in elite athletes of different disciplines. *Int J Sports Med* 2001; **22**(7): 504-12
13. Schmidt W and Prommer N. Impact of alterations in total hemoglobin mass on $\dot{V}O_{2max}$. *Exerc Sport Sci Rev* 2010; **38**(2): 68-75
14. Kanstrup IL and Ekblom B. Blood volume and hemoglobin concentration as determinants of maximal aerobic power. *Med Sci Sports Exerc* 1984; **16**(3): 256-62
15. Schmidt W and Prommer N. Effects of various training modalities on blood volume. *Scand J Med Sci Sports* 2008; **18 Suppl**(1): 57-69
16. Wijeysundera DN, Pearse RM, Shulman MA, et al. Measurement of Exercise Tolerance before Surgery (METS) study: a protocol for an international multicentre prospective cohort study of cardiopulmonary exercise testing prior to major non-cardiac surgery. *BMJ Open* 2016; **6**(3): e010359
17. American Thoracic Society and American College of Chest Physicians. ATS/ACCP Statement on cardiopulmonary exercise testing. *Am J Respir Crit Care Med* 2003; **167**(2): 211-77
18. Wasserman K, Hansen JE, Sue DY, Stringer WW, Whipp BJ. Principles of Exercise Testing and Interpretation Including Pathophysiology and Clinical Applications. 4th Edition ed. 2005, Philadelphia: Lippincott Williams & Wilkins.
19. Wasserman K. The anaerobic threshold: definition, physiological significance and identification. *Adv Cardiol* 1986; **35:** 1-23

20. Midgley AW, McNaughton LR, and Carroll S. Effect of the $\dot{V}O_2$ time-averaging interval on the reproducibility of $\dot{V}O_{2max}$ in healthy athletic subjects. *Clin Physiol Funct Imaging* 2007; **27**(2): 122-5
21. Schmidt W and Prommer N. The optimised CO-rebreathing method: a new tool to determine total haemoglobin mass routinely. *Eur J Appl Physiol* 2005; **95**(5-6): 486-95
22. Garvican LA, Burge CM, Cox AJ, et al. Carbon monoxide uptake kinetics of arterial, venous and capillary blood during CO rebreathing. *Exp Physiol* 2010; **95**(12): 1156-66
23. Chaplin H, Jr., Mollison PL, and Vetter H. The body/venous hematocrit ratio: its constancy over a wide hematocrit range. *J Clin Invest* 1953; **32**(12): 1309-16
24. Schumacher YO, Pottgiesser T, Ahlgrim C, et al. Haemoglobin mass in cyclists during stage racing. *Int J Sports Med* 2008; **29**(5): 372-8
25. Koponen AS, Peltonen JE, Paivinen MK, et al. Low total haemoglobin mass, blood volume and aerobic capacity in men with type 1 diabetes. *Eur J Appl Physiol* 2013; **113**(5): 1181-8
26. Gore CJ, Hahn AG, Burge CM, et al. $\dot{V}O_{2max}$ and haemoglobin mass of trained athletes during high intensity training. *Int J Sports Med* 1997; **18**(6): 477-82
27. Wilson RJ, Davies S, Yates D, et al. Impaired functional capacity is associated with all-cause mortality after major elective intra-abdominal surgery. *Br J Anaesth* 2010; **105**(3): 297-303
28. Treff G, Schmidt W, Wachsmuth N, et al. Total haemoglobin mass, maximal and submaximal power in elite rowers. *Int J Sports Med* 2014; **35**(7): 571-4
29. Montero D, Cathomen A, Jacobs RA, et al. Haematological rather than skeletal muscle adaptations contribute to the increase in peak oxygen uptake induced by moderate endurance training. *J Physiol* 2015; **593**(20): 4677-88
30. Gore CJ, Hopkins WG, and Burge CM. Errors of measurement for blood volume parameters: a meta-analysis. *J Appl Physiol (1985)* 2005; **99**(5): 1745-58
31. Schmidt W, Doerfler C, and Wachsmuth N. Influence Of Body Mass, Body Composition, And Performance State On Total Hemoglobin Mass. *Med Sci Sports Exerc* 2009; **41**(5): 541
32. Patel AJ, Wesley R, Leitman SF, et al. Capillary versus venous haemoglobin determination in the assessment of healthy blood donors. *Vox Sang* 2013; **104**(4): 317-23
33. Shahshahani HJ, Meraat N, and Mansouri F. Evaluation of the validity of a rapid method for measuring high and low haemoglobin levels in whole blood donors. *Blood Transfus* 2013; **11**(3): 385-90
34. Ziemann M, Lizardo B, Geusendam G, et al. Reliability of capillary hemoglobin screening under routine conditions. *Transfusion* 2011; **51**(12): 2714-9
35. Baart AM, de Kort WL, van den Hurk K, et al. Hemoglobin assessment: precision and practicability evaluated in the Netherlands-the HAPPEN study. *Transfusion* 2016; **56**(8): 1984-93
36. Schmidt W, Doerfler C, and Wachsmuth N. Influence Of Body Mass, Body Composition, And Performance State On Total Hemoglobin Mass. *Med Sci Sports Exerc* 2009; **41**(5): 541
37. Turner G, Richardson AJ, Maxwell NS, et al. Comparison of total haemoglobin mass measured with the optimized carbon monoxide rebreathing method across different Radiometer ABL-80 and OSM-3 hemoximeters. *Physiol Meas* 2014; **35**(12): N41-9

Tables

Table 1. Patient characteristics. Values are expressed as mean (SD), median (range) or frequency (%).

Variable	**n= 42**
Gender	
Male	35 (83%)
Female	7 (17%)
Age (yr)	66 (24-78)
Height (cm)	172 (9)
Weight (kg)	83 (16)
Surgical specialty	
Urology	19
Hepatology	9
Maxillofacial	2
Upper gastrointestinal	3
Thoracic	4
Vascular	1
Gastrointestinal	4
Morbidities	
Ischaemic heart disease	5 (12%)
Heart failure	1 (2%)
Hypertension	19 (45%)
Stroke	1 (2%)
Diabetes	9 (21%)
COPD	6 (14%)
Smoking history	
Current	6 (14%)
Former	12 (27%)
Never	24 (57%)
Medication	
Beta blocker	6 (14%)
Nitrates	1 (2%)
ACE inhibitor	14 (33%)
Statin	13 (31%)

COPD, chronic obstructive pulmonary disease.

Table 2. Haematological characteristics for all patients and by presence or absence of anaemia. Values are expressed as mean (SD), median (IQR) or frequency (%).

Variable	All patients (n= 42)	Anaemic (n= 11)	Non-anaemic (n= 31)	p-value
[Hb] ($g\,l^{-1}$)	135.6 (15.6)	116.3 (11.7)	142.5 (10.1)	< 0.0001
Hct (%)	41.4 (4.0)	37.4 (4.2)	42.7 (3.0)	< 0.0001
tHb-mass (g)	677 (146)	610 (119)	700 (149)	0.079
tHb-mass ($g\,kg^{-1}$)	8.2 (1.3)	7.8 (1.7)	8.3 (1.2)	0.316
BV (ml)	5495 (1065)	5753 (951)	5404 (1102)	0.356
BV ($ml\,kg^{-1}$)	67.3 (12.2)	74.7 (16.0)	64.6 (9.6)	0.016
PV (ml)	3421 (678)	3775 (560)	3295 (680)	0.043
PV ($ml\,kg^{-1}$)	42.0 (8.2)	49.0 (9.4)	39.5 (6.3)	0.001
PV (%)	62.3 (3.7)	65.9 (3.8)	61.0 (2.7)	< 0.0001
EV (ml)	2074 (462)	1978 (466)	2108 (464)	0.431
EV ($ml\,kg^{-1}$)	25.3 (4.9)	25.6 (7.4)	25.1 (3.9)	0.768
MCV (fL)	90.5 (4.6)	90.6 (4.3)	89.4 (5.0)	0.521
MCH (pg)	30.3 (1.9)	29.7 (1.4)	30.4 (2.1)	0.370
MCHC ($g\,l^{-1}$)	342 (321-349)	320 (314-347)	344 (331-350)	0.096
Creatinine ($\mu mol\,l^{-1}$)	97.5 (50.5)	127.0 (62.7)	88.8 (43.9)	0.055
Albumin ($g\,l^{-1}$)	42 (39-44)	36.0 (7.8)	42.1 (5.0)	0.070

[Hb], Haemoglobin concentration; Hct, haematocrit; tHb-mass, total haemoglobin mass; BV, blood volume; PV, plasma volume; EV, erythrocyte volume; MCV, mean corpuscular volume; MCH, mean corpuscular haemoglobin; MCHC, mean corpuscular haemoglobin concentration. Anaemia defined according to World Health Organisation criteria when ([Hb]) $<130\ g\,l^{-1}$ in men and $<120\ g\,l^{-1}$ in women.

Figures

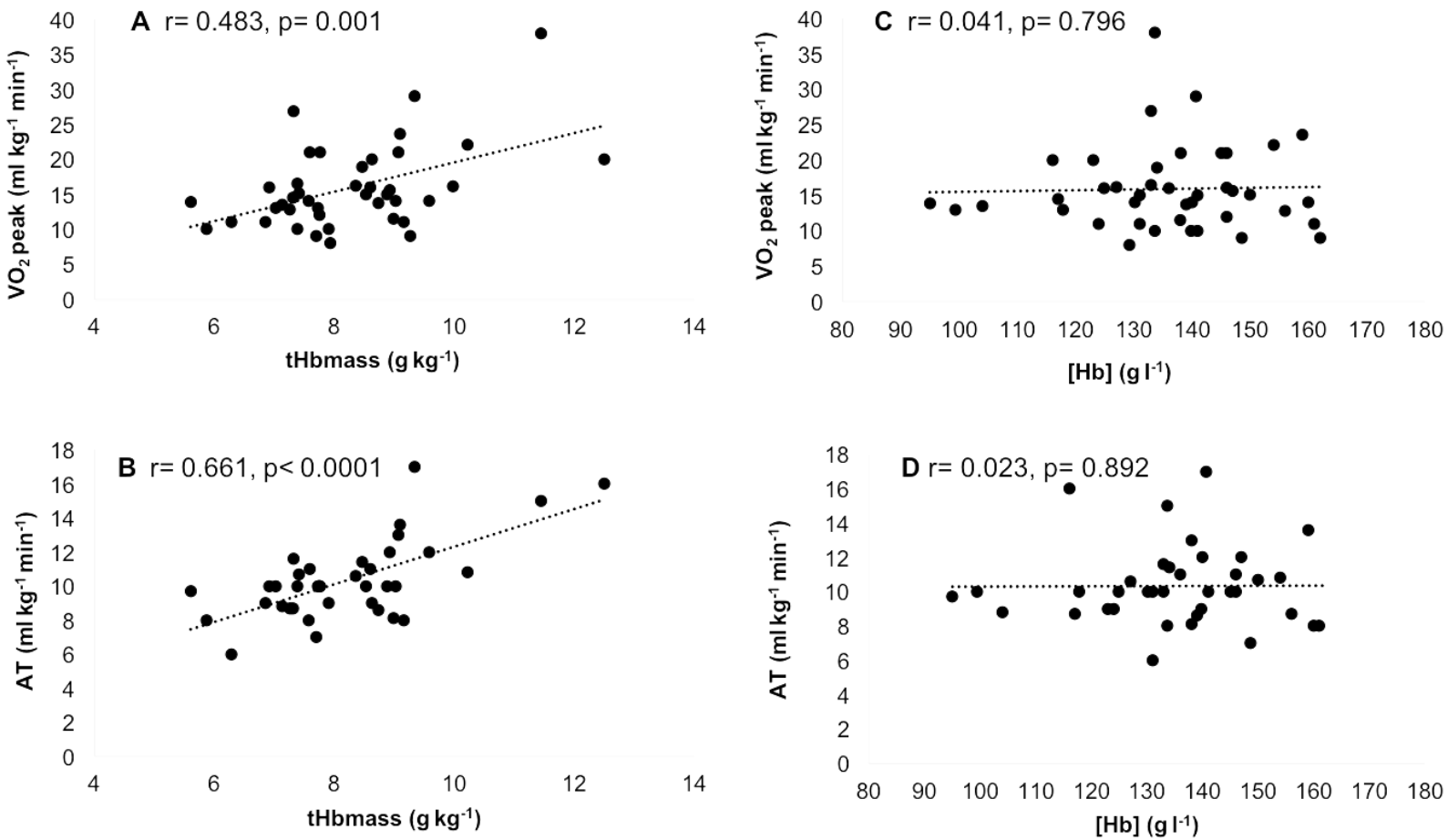
A r= 0.483, p= 0.001
VO2 peak (ml kg-1 min-1)
tHbmass (g kg-1)
B r= 0.661, p< 0.0001
AT (ml kg-1 min-1)
tHbmass (g kg-1)
C r= 0.041, p= 0.796
VO2 peak (ml kg-1 min-1)
[Hb] (g l-1)
D r= 0.023, p= 0.892
AT (ml kg-1 min-1)
[Hb] (g l-1)

Figure 2. Unadjusted relationship between haematological variables and exertional oxygen consumption. Relation of $\dot{V}O_2$ peak (ml kg^{-1} min^{-1}) to tHb-mass (g kg^{-1}) (A) and [Hb] (g l^{-1}) (C) in 42 patients. Relation of AT to tHb-mass (g kg^{-1}) (B) and [Hb] (g l^{-1}) (D) in 39 patients. tHb-mass, total haemoglobin mass; [Hb], haemoglobin concentration; AT, anaerobic threshold; $\dot{V}O_2$ peak, peak oxygen.

$$\text{tHb-mass (g)} = K \times \text{MCO(ml)} \times 100 \times (\Delta\%\text{COHb} \times 1.39)^{-1}$$

K = current barometric pressure x 760^{-1} x [1(0.003661 x current temperature)]
$MCO = CO_{adm} - (CO_{system + lung (after disconnection)} + CO_{exhaled (after disconnection)}$
CO_{adm} = CO volume administered into the system
$CO_{system + lung (after disconnection)}$ = CO concentration in spirometer x (spirometer volume + remaining volume in the lung after rebreathing)
$CO_{exhaled (after disconnection)}$ = end-tidal CO concentration x alveolar ventilation x time
Δ%COHb = difference between baseline %COHb and %COHb post CO administration (average of 6- and 8-min %COHb values)
1.39 = Hüfners number (constant) (ml CO x g Hb^{-1})

$$\text{BV (ml)} = \text{tHb-mass (g)}/[\text{Hb}]\ (\text{g dl}^{-1}) \bullet 100$$

$$\text{EV (ml)} = \text{BV (ml)} \bullet \text{Hct (\%)}$$

$$\text{PV (ml)} = \text{BV} - \text{EV}$$

Figure 1. Equations to calculate total haemoglobin mass, blood, erythrocyte and plasma volumes. CO, carbon monoxide; %COHb, percent carboxyhaemoglobin; [Hb], haemoglobin concentration; Hct, haematocrit; BV, blood volume; EV, erythrocyte volume; PV, plasma volume; lung residual volume, 1500 ml in men, 1200 ml in women; alveolar ventilation, 5000 ml min^{-1}.