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RESEARCH ARTICLE

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Determinants and reference values for blood volume and total hemoglobin mass in women and men

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Abstract

Blood volume (BV) is an important clinical parameter and is usually reported per kg of body mass (BM). When fat mass is elevated, this underestimates BV/BM. One aim was to study if differences in BV/BM related to sex, age, and fitness would decrease if normalized to lean body mass (LBM). The analysis included 263 women and 319 men (age: 10–93 years, body mass index: 14–41 kg/m^2) and 107 athletes who underwent assessment of BV and hemoglobin mass (Hb_{mass}), body composition, and cardiorespiratory fitness. BV/BM was 25% lower (70.3 ± 11.3 and 80.3 ± 10.8 mL/kg^{BM}) in women than men, respectively, whereas BV/LBM was 6% higher in women

(110.9 ± 12.5 and 105.3 ± 11.2 mL/kg^{LBM}). Hb_{mass}/BM was 34% lower (8.9 ± 1.4 and 11.5 ± 11.2 g/kg^{BM}) in women than in men, respectively, but only 6% lower (14.0 ± 1.5 and 14.9 ± 1.5 g/kg^{LBM})/LBM. Age did not affect BV. Athlete's BV/BM was 17.2% higher than non-athletes, but decreased to only 2.5% when normalized to LBM. Of the variables analyzed, LBM was the strongest predictor for BV ($R^2 = .72$, $p < .001$) and Hb_{mass} ($R^2 = .81$, $p < .001$). These data may only be valid for BV/Hb_{mass} when assessed by CO re-breathing. Hb_{mass}/LBM could be considered a valuable clinical matrix in medical care aiming to normalize blood homeostasis.

1 | INTRODUCTION

The assessment of blood volume (BV) is a critical factor in the understanding of the pathophysiology of cardiovascular and renal disease.[1,2] In chronic renal disease,[3] including dialysis patients,[4] a decreased hemoglobin concentration [Hb] (anemia) can be the result of a decreased red blood cell volume (RBCV; true anemia) or an increased plasma volume (PV; dilutional anemia), or a combination of the two. Moreover, erythrocytosis in polycythemia vera can be masked by an elevated PV and anemia by a decreased PV. Hence, implementing the measurement of total BV, PV, and RBCV apart from exclusively assessing [Hb], is vital for the diagnosis and therapy of numerous patient populations.

BV was early related to size across sex and race,[5,6] and in 1962, Nadler et al. established the first equation to predict BV based on body mass (BM) and height, by means of infusion of I^{131}-human serum albumin[7] and until today, BV and intravascular volumes are commonly reported in mL per kg of BM. However, this method of expressing BV results in low values for obese individuals, presumably because adipose tissue is hypovascular.[8,9] This confounding influence of body fat may be avoided by normalizing BV to lean body mass (LBM), as suggested by the International Council for Standardization in Hematology,[10] and also highlighted previously.[11] Accordingly, our first aim was to test the hypothesis that known differences in BV related to sex, age, and aerobic fitness decrease if BV is normalized to LBM, rather than BM.

The complexity of assessing BV using radioactive tracers has limited their application in the clinical routine.[1] An alternative approach for determining BV is carbon monoxide (CO) rebreathing,[12] which is valid and relatively simple and implementation in the clinic is strongly advocated for.[13–15] Although the approach has been used extensively by physiologists for over 100 years,[8] only recently have clinical studies emerged that are based on this method and a medical device (MDR/EU certified) has also been commercialized. Furthermore, with the CO rebreathing method, the primary variable determined is Hb_{mass}, from which BV, RBCV, and PV are derived from the simultaneous assessment of [Hb] and hematocrit. In contrast to the clinically obtained [Hb], the clear advantage of determining Hb_{mass} is that its quantification is independent of PV status, and hence invaluable when trying to distinguish true anemia from dilutional anemia.[3,4] For this to materialize, however, reference values for Hb_{mass} need to be established. Accordingly, our second aim was to establish reference values for absolute and relative Hb_{mass} in healthy women and men across a wide age range to facilitate its clinical use.

2 | METHODS

This is a retrospective, observational study for which we used data from earlier published ($n = 15$) and unpublished ($n = 6$) studies conducted by CL and collaborating groups (see Table 1). Investigations that reported both body composition and Hb_{mass} by CO rebreathing were analyzed to establish reference values for Hb_{mass} and BV over a wide age range. Data previously published were amassed and unpublished data were also included (Table S3). The studies that provided unpublished data were approved by the local ethics committee in Copenhagen, Denmark (nos. H-18013069, H-17029966, H-17036662, and H-18016341), by the local ethics committee at the Lillehammer University College, Norway (MRU04062017), the local ethics committee at Inland University, Norway (20/03749), Conjoint Health Research Ethics Board (REB18-1654) of the University of Calgary and the Regional Committee for Medical and Health Research Ethics, Norway (255 224 and 2018/739). Seven elite athletes who are routinely measured by CL gave their informed consent for their data to be included in the analysis.

2.1 | Data source

A total of 689 individuals (41.2% women) were included in the analysis. The study population comprises a heterogeneous group of sedentary, recreationally active, and athletic participants. The participants were non-smoking and healthy, except for 43 obese (but otherwise healthy) individuals (body mass index [BMI] >30 kg·m^{-2}).[9] The anthropometric characteristics of the 582 non-athletic individuals included (689 individuals—107 athletes, see below) are displayed in Table 2. The majority of the individuals were adults, with 26 girls and 30 boys also included (between 9.5 and 17.9 years old). Body fat and LBM were examined by dual-energy x-ray absorptiometry (DXA), bioelectrical impedance analysis (BIA), or skinfold measurements. To compute body surface area (BSA), the Dubois and Dubois formula was applied.[16] Aerobic fitness assessed as maximal oxygen uptake (VO_{2max}) was included in the data analyses if available. Table 1 provides information on which method was used in which studies.

TABLE 1 Hematological reference values for adult, non-athletic women, and men.

	Women	*N*	Men	*N*	*p* Value
BV (mL)	4682 ± 738	235	6279 ± 829	288	<.001
BV (mL/kg body mass)	70.3 ± 11.3	233	80.3 ± 10.8	288	<.001
BV (mL/kg^{LBM})	110.9 ± 12.5	196	105.3 ± 11.2	215	<.001
BV (mL/m^2 BSA)	2482 ± 361	233	2949 ± 386	288	<.001
RBCV (mL)	1823 ± 299	235	2708 ± 411	288	<.001
RBCV (mL/kg^{BM})	27.4 ± 4.8	233	34.6 ± 5.0	288	<.001
RBCV (mL/kg^{LBM})	43.2 ± 5.3	196	45.1 ± 5.5	215	<.001
RBCV (mL/m^2 BSA)	967 ± 159	233	1273 ± 196	288	<.001
PV (mL)	2861 ± 480	235	3570 ± 510	288	<.001
PV (mL/kg^{BM})	43.0 ± 7.2	233	45.7 ± 6.9	288	<.001
PV (mL/kg^{LBM})	67.7 ± 8.6	196	60.2 ± 7.7	215	<.001
PV (mL/m^2 BSA)	1516 ± 226	233	1677 ± 233	288	<.001
Hb_{mass} (g)	595 ± 91	235	901 ± 123	288	<.001
Hb_{mass} (g/kg^{BM})	8.9 ± 1.4	233	11.5 ± 1.5	288	<.001
Hb_{mass} (g/kg^{LBM})	14.0 ± 1.5	196	14.9 ± 1.6	215	<.001
Hb_{mass} (g/m^2 BSA)	315 ± 42	233	423 ± 53	288	<.001
Hb (g/dL)	13.4 ± 0.9	235	14.7 ± 1.1	288	<.001
Hematocrit (%)	40.3 ± 2.7	235	43.7 ± 2.9	288	<.001

Note: Mean ± SD. Shaded area in Table indicates the higest value between Women and Men.
Abbreviations: BSA, body surface area; BV, blood volume; Hb, hemoglobin concentration; Hb_{mass}, hemoglobin mass; LBM, lean body mass; PV, plasma volume; RBCV, red blood cell volume.

TABLE 2 Multivariable linear regression analysis with the predictor variable and sex as independent variables and blood volume and hemoglobin mass as dependent variables.

		Predictor		Sex		Overall model	
	Intercept	$\beta_{predictor}$	*p* Value	β_{Women}	*p* Value	Adjusted R^2	*p* Value
Blood volume							
Lean mass (kg)	1401	82	<.001	−172	.106	.72	<.001
Body mass (kg)	3670	33	<.001	−1228	<.001	.61	<.001
Height (m)	−2834	5074	<.001	−952	<.001	.60	<.001
Body surface area (m^2)	2490	1776	<.001	−1169	<.001	.58	<.001
BMI (kg/m^2)	4830	59	<.001	−1587	<.001	.54	<.001
VO_{2max} (mL/min)	5676	0.18	<.001	−1440	<.001	.51	<.001
Hb (g/dL)	7674	−95	.005	−1724	<.001	.51	<.001
Hemoglobin mass							
Lean mass (kg)	188	12	<.001	−94	<.001	.81	<.001
Body mass (kg)	535	5	<.001	−254	<.001	.74	<.001
Height (m)	−453	754	<.001	−210	<.001	.73	<.001
Body surface area (m^2)	281	291	<.001	−236	<.001	.73	<.001
Hb (g/dL)	343	38	<.001	−255	<.001	.70	<.001
VO_{2max} (mL/min)	691	0.06	<.001	−239	<.001	.70	<.001
Age (years)	919	−0.42	.084	−307	<.001	.66	<.001

Note: Data originate from non-athletic adults. The predictors were ordered according to the strength of the association (Adjusted R^2). Only modelsthat were significant were included in the table. Women served as reference.
Abbreviations: BMI, body mass index; Hb, hemoglobin concentration; VO_{2max}, maximal oxygen uptake.

Participants were defined as athletes if they had either participated in the Olympics or world championships in an endurance event and/or had a VO_{2max} of ≥62 mL/min/kg for women and ≥70 mL/min/kg for men. Athletes ($n = 107$; 21 women and 86 men) were included to investigate whether there were differences in intravascular volumes between the athletic and non-athletic populations. Athletes included cyclists, rowers, triathletes, and cross-country skiers.

2.2 | CO rebreathing

Hb_{mass} was determined using the CO rebreathing technique where BV, RBCV, and PV were derived by integration of [Hb] and non-F-cell corrected hematocrit.[12] The included participants were either assessed with the manually operated CO rebreathing method[17] (10 min manual), with an automated device version thereof (10 min Detalo) (Detalo Performance, Detalo Health, Denmark), or with a 2-min manually operated variation[18] (Table 1). Although the measurement results between these approaches vary,[19,20] this variation is largely considered non-clinical relevant.[12] Analyzing the data included in this investigation revealed that Hb_{mass}/LBM differed between the three CO rebreathing methods (Figure S1), the values being 14.09, 14.46, and 15.05 $g \cdot kg^{-1}$ for the 2 min, 10 min manual, and 10 min Detalo, respectively, and which is also be related to differences in methodology and included subject population.

2.3 | Body composition

Body fat and LBM mass were assessed by DEXA in data from 16 out of 21 studies (Table S3). One study used skin fold thickness to estimate LBM which was measured at four representative body sites (biceps brachialis, triceps brachialis, subscapular, and suprailical) using a precision caliper.[21] In another study, BIA was applied to assess fat mass.[22] To calculate LBM for these individuals, bone mineral content was averaged over all adult individuals who were assessed by DEXA, and the average (2.8 kg) was used to calculate the missing LBM (fat-free mass minus bone mineral content).

2.4 | Maximal oxygen uptake

VO_{2max} was assessed on treadmills or bike-ergometers with an incremental test to exhaustion using open-circuit indirect calorimetry.

2.5 | Data analyses and statistics

Statistical analysis was performed, and figures were made using R version 4.2.1 and R studio version 2022.02.0 (packages[23–33]). Data were evaluated for normality and equal variance by graphical inspection of normal Q-Q and residual plots. Results are reported in mean ± SD unless otherwise indicated. Hematological differences between women and men or between the athletic and non-athletic populations were evaluated by independent t tests. Multivariable linear regression analyses assessed the relationship between absolute BV and Hb_{mass} with body composition (BM, LBM, and fat mass), height, sex, age, and VO_{2max}. When indicated that an analysis was adjusted for a specific factor, an independent variable, for example, sex or LBM, was added to the regression analysis. Best subset multiple regression analysis was performed to find the best combination of predictors for BV and Hb_{mass}. For this purpose, the combination of predictors with a high adjusted R^2 and low Akaike Information Criteria (AIC) and Mallow's Cp were chosen to create a formula from (i) a clinical set of predictors (sex, age, weight, height, LBM, VO_{2max}) and ii) simple predictors (sex, age, weight, height) for BV and Hb_{mass}. A 10-fold cross-validation was used to internally validate the models that best predicted BV and Hb_{mass}, and the root mean square error, the mean absolute error, and the mean absolute percentage error were computed to derive the accuracy of the models. The accuracy in the suggested formulas and the widely used formula by Nadler et al.[7] was visualized by plotting the measured BV and the predicted BV in a Bland–Altman plot.[34] Statistical significance was set to a 2-tailed p value <.05.

3 | RESULTS

3.1 | Participants general characteristics

The anthropometric characteristics of the 582 non-athletic adults and children are displayed in Table S4. BM, height, BSA, body fat (%), LBM, and VO_{2max} were significantly different between women and men ($p < .001$). The anthropometrics of the 107 athletes was as follows: 21 women, 86 men, age: 23.0 ± 3.8 years, body fat: 13.4 ± 4.0%, VO_{2max}: 74.7 ± 7.5 mL/min/kg^{BM}) (see Table S1).

3.2 | Hematological characteristics

3.2.1 | Reference values

Absolute and normalized reference values for BV, RBCV, PV, and Hb_{mass} in non-athletic adult individuals are depicted in Table 1. Men had significantly higher values than women for both absolute and normalized values (kg and BSA) for BV, RBVC, PV, and Hb_{mass}. Absolute values for BV, RBCV, PV, and Hb_{mass} were 25%, 33%, 20%, and 34% higher in men than in women (all $p < .001$). These differences persisted after normalization to BM (13%, 21%, 6%, and 23%, respectively, all $p < .001$) (Figure 1) or BSA (m^2) (16%, 24%, 10%, and 26%, respectively, all $p < .001$). BV, RBVC, PV, and Hb_{mass} per BMI are shown in Figure S6.

3.2.2 | Sex differences and LBM

After normalization to LBM, BV, and PV were 6% and 11% higher in women than in men (all $p < .001$) (Figure 2), whereas RBCV and Hb_{mass} were 4% and 6% higher in men than in women (all $p < .001$), respectively.

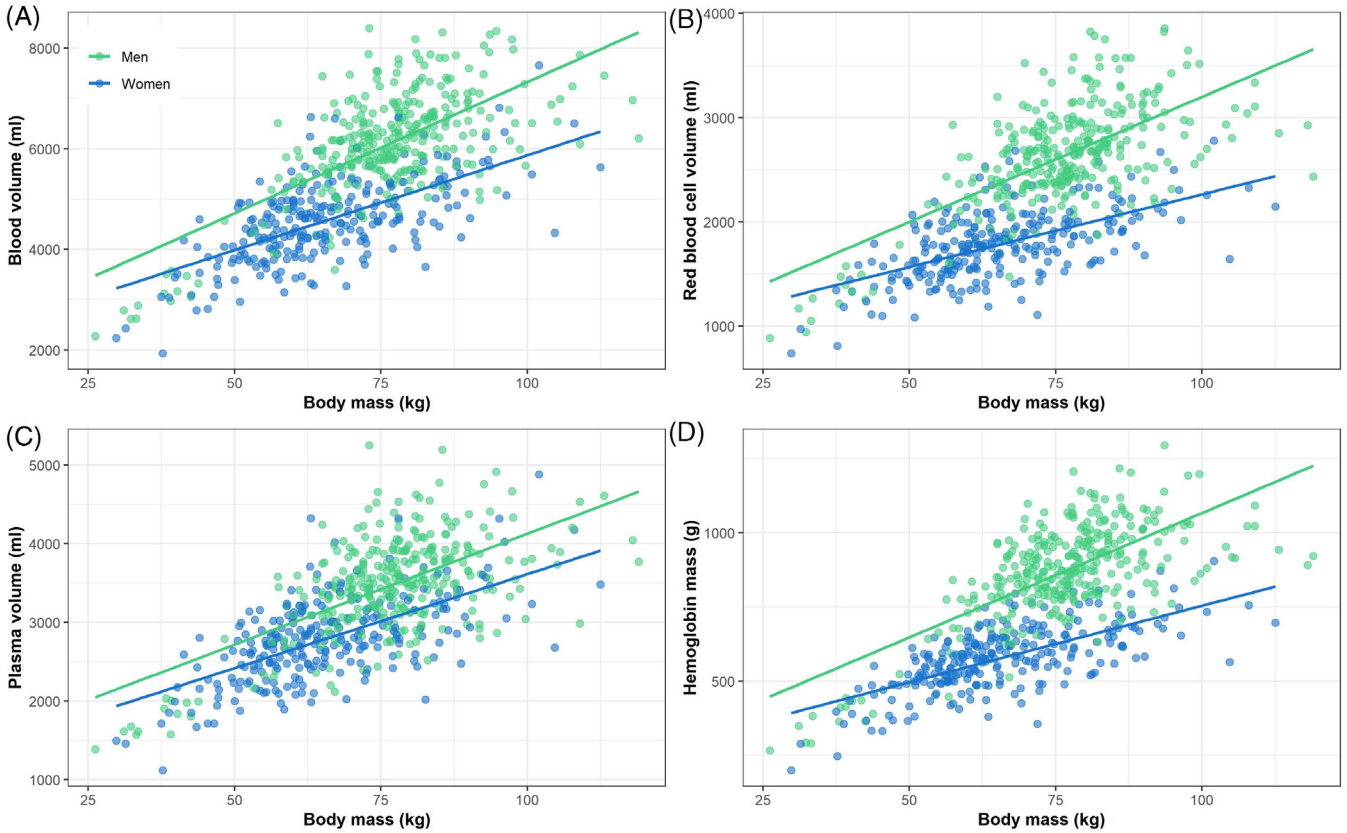

FIGURE 1 Linear relationship between body mass and intravascular volumes/hemoglobin mass in all non-athletic individuals across ages (children and adults). (A) Blood volume, $R^2_{Women} = .40$, $R^2_{Men} = .44$. (B) Red blood cell volume, $R^2_{Women} = .35$, $R^2_{Men} = .42$. (C) Plasma volume, $R^2_{Women} = .38$, $R^2_{Men} = .36$. (D) Hemoglobin mass, $R^2_{Women} = .45$, $R^2_{Men} = .47$. All $p < .001$. Each dot represents one individual. [Color figure can be viewed at wileyonlinelibrary.com]

3.2.3 | Age

Absolute BV was positively related to age in children and adolescents between 9 and 18 years (girls: +207 mL/year, $R^2 = .39$, $p < .001$; boys: +385 mL/year, $R^2 = .45$, $p < .001$), but did not change with age in adult women (+0.6 mL·year^{-1}, $R^2 = .00$, $p = .780$) or men (+3.7 mL·year^{-1}, $R^2 = .00$, $p = .14$) (Figure 3 and Figure S2). Conversely, BV normalized to LBM increases with age across the entire age range (Figure 3), and also in adult individuals only (women: +0.11 mL/kg^{LBM}/year, $R^2 = .03$, $p = .012$, men: +0.12 mL/kg^{LBM}/year, $R^2 = .05$, $p < .001$), due to a decrease in LBM (women: −0.04 kg^{LBM}/year, $R^2 = .03$, $p = .015$; men: −0.05 kg^{LBM}/year, $R^2 = .02$, $p = .021$). Total RBCV remained similar with age in adult individuals, while when normalized to LBM, it increased in women (+0.07 mL/kg^{LBM}/year, $R^2 = .06$, $p < 0.001$) and men (+0.04 mL/kg^{LBM}/year, $R^2 = .02$, $p = .024$). There was a small statistical tendency for a positive correlation between PV and age in adult men (+3.00 mL/year, $R^2 = .01$, $p = .053$), but not in women. Also, when normalized to LBM, PV increases with age in men (+0.08 mL/kg/year, $R^2 = .05$, $p = .001$), but not in women. Finally, absolute Hb_{mass} was not correlated with age in women (−0.04 g/year, $R^2 = .00$, $p = .903$), whereas it decreased weakly with age in men (−0.74 g/year, $R^2 = .01$, $p = .044$). Conversely, Hb_{mass} normalized to LBM increased with age in women (+0.01 g/kg^{LBM}/year, $R^2 = .03$, $p = .006$), but not in men. Sex did not interact with alterations in intravascular volumes and Hb_{mass} with age, neither when normalized to kg of LBM, except for Hb_{mass} normalized to LBM, for which being female tends to lead towards a greater increase with age than being male (+0.01 g/kg^{LBM}/year, $p = .095$). In adult men, [Hb] and hematocrit were stable throughout the age range, while in adult women, [Hb] tended to increase slightly with age (+0.006 g/dL/year, $R^2 = .01$, $p = .068$), and hematocrit remained unaltered (Figure S3). Sex did not interact with alterations in intravascular volumes and Hb_{mass} with age, neither when normalized to kg of LBM, except for Hb_{mass} normalized to LBM, for which being female tends to lead toward a greater increase with age than being male (+0.01 g/kg^{LBM}/year, $p = .095$).

3.2.4 | Aerobic fitness

BV and Hb_{mass} per kg of BM were higher in athletes (Δ +16.3 mL/kg^{BM} [14.3–18.4], $p < .001$; Δ +3.0 g/kg^{BM} [2.7–3.3], $p < .001$) than in non-athletes (Figure 4, Figures S4 and S5, and Table S1). The higher intravascular volumes and Hb_{mass} in the athletes were largely paralleled by a higher LBM, but also remained elevated when normalized to LBM (BV: Δ + 2.9 mL/kg^{LBM} [0.8–5.0], $p = .007$; RBCV: Δ +3.6 mL/kg^{LBM} [2.5–4.6],

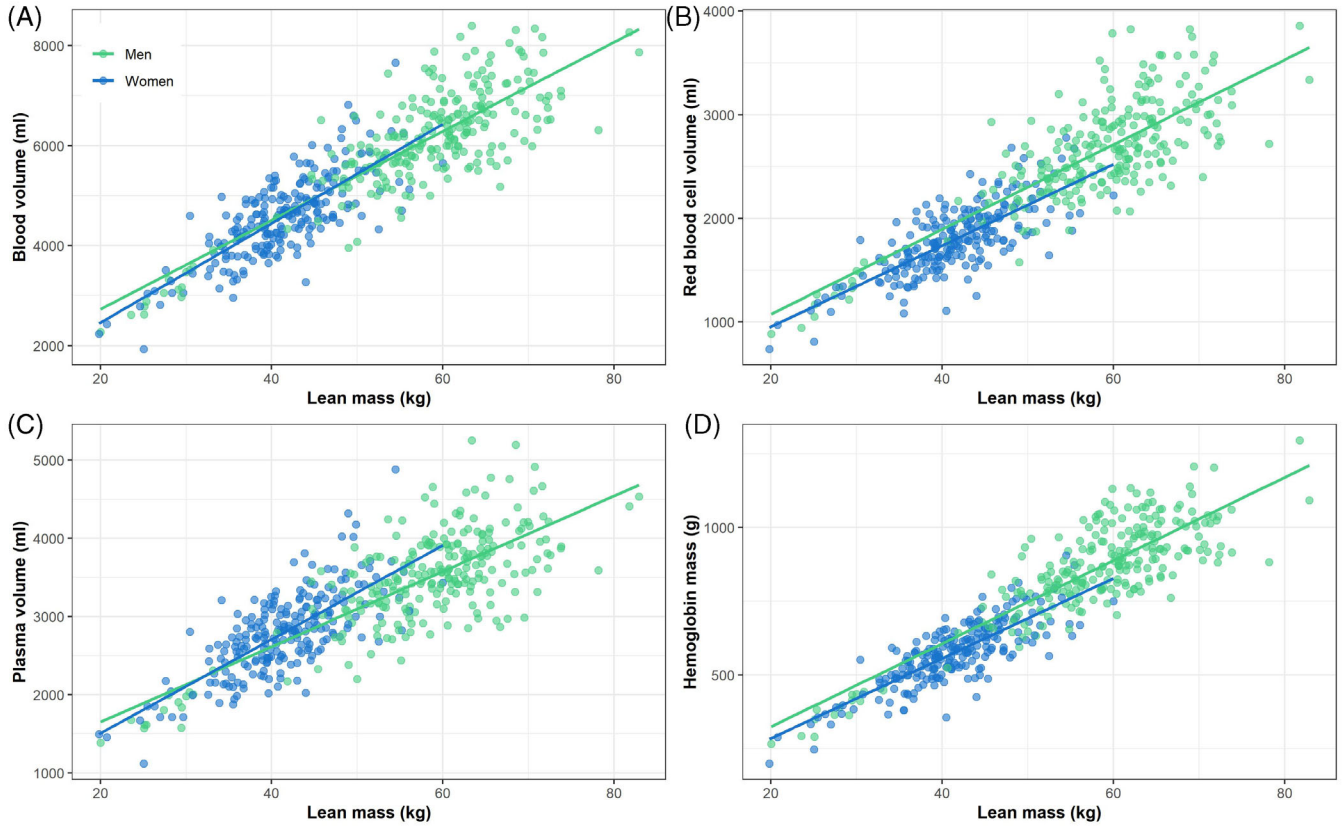

FIGURE 2 Linear relationship between lean body mass and intravascular volumes/hemoglobin mass in all non-athletic individuals across ages (children and adults). (A) Blood volume, $R^2_{Women} = .59$, $R^2_{Men} = .67$. (B) Red blood cell volume, $R^2_{Women} = .56$, $R^2_{Men} = .62$. (C) Plasma volume, $R^2_{Women} = .53$, $R^2_{Men} = .56$. (D) Hemoglobin mass, $R^2_{Women} = .65$, $R^2_{Men} = .71$. All $p < .001$. Each dot represents one individual. [Color figure can be viewed at wileyonlinelibrary.com]

$p < .001$; Hb_{mass}: Δ +1.5 g/kg^{LBM} [1.2–1.8], $p < .001$), except for PV per LBM (Δ −0.7 mL/kg^{LBM} [−2.3 to 0.9], $p = .412$).

3.3 | Multivariable linear regression analyses

In non-athletic adults, there was a strong positive correlation between LBM and BV ($R^2 = .71$, $p < .001$) and LBM and Hb_{mass} ($R^2 = .79$, $p < .001$). The association between LBM and BV was little affected by adjustment for sex (Tables 2 and S2). Not shown in Table 2, LBM was also associated with BV independently of BM ($\beta_{LBM} = 77$, $p_{LBM} < .001$) or fat mass ($\beta_{LBM} = 82$, $p_{LBM} < .001$). The positive association between LBM and Hb_{mass} improved further when adjusted for sex (to $R^2 = .81$), and LBM is also associated with Hb_{mass} independently of BM ($\beta_{LBM} = 11$, $p_{LBM} < .001$) or fat mass ($\beta_{LBM} = 12$, $p_{LBM} < .001$). Of note, when adjusted for sex and LBM, the positive association between BV and absolute VO_{2max} disappears ($\beta_{VO2max} = -0.04$, $p_{vo2max} = .51$), whereas Hb_{mass} is positively associated with absolute VO_{2max} independently of LBM ($\beta_{VO2max} = 0.04$, $p_{vo2max} < .001$). Best subset multiple regression analyses revealed that age, height and LBM was the best combination of the available variables (here termed "clinical" and used in formulas 1, 4, and 5 below) to predict BV (adjusted $R^2 = .73$, $p < .001$, $n = 411$, for AIC and Mallow's Cp, see Table 2A–D), whereas sex, height and LBM was the best combination to predict Hb_{mass} (adjusted $R^2 = .81$, $p < .001$, $n = 411$). For the "simple" set of variables (variables assumed accessible to all, and used in formulas 2, 3, 6, and 7 below), sex, height, weight, and age are the best combination of variables to predict BV (Adjusted $R^2 = .67$, $p < .001$, $n = 523$), whereas it is sex, weight, and height for Hb_{mass} (adjusted $R^2 = .77$, $p < .001$, $n = 523$). From this combination of predictors, formulas 1–7 were established to predict BV and Hb_{mass} (for BV calculations, see Supporting Information: Spreadsheet). Measures of internal validation of these models are presented in Table S2.

1. All, clinical: BV (mL) = 6.4*age (years) + 1639*height (m) + 77.4*LBM (kg) – 1565
2. Women, simple: BV (mL) = 7.7*age (years) + 4390*height (m) + 23.8*weight (kg) – 4555
3. Men, simple: BV (mL) = 7.7*age (years) + 4390*height (m) + 23.8*weight (kg) – 3799
4. Women, clinical: Hb_{mass} (g) = 166.8*height (m) + 10.8*LBM (kg) – 139.9
5. Men, clinical: Hb_{mass} (g) = 166.8*height (m) + 10.8*LBM (kg) – 50.3

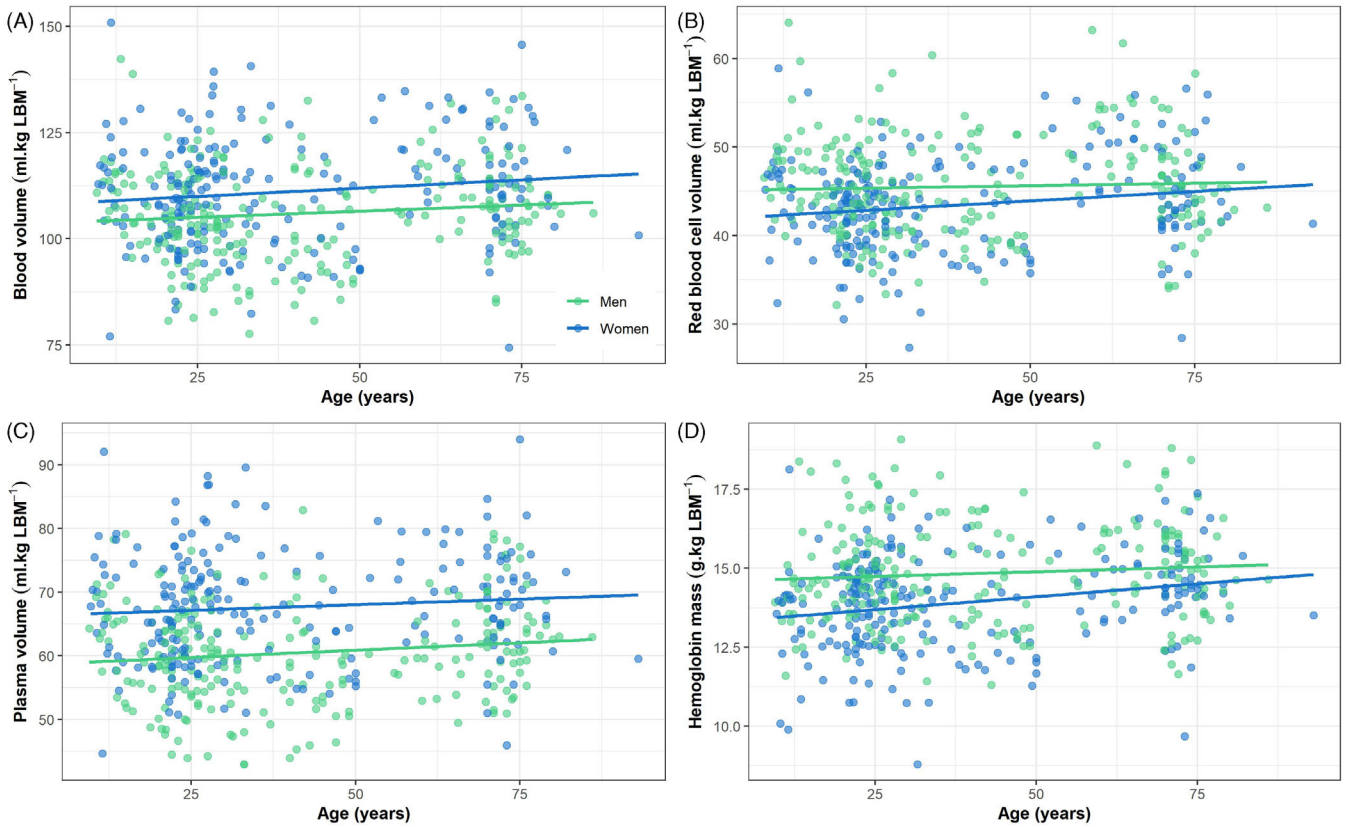

FIGURE 3 Linear relationship between age and intravascular volumes/hemoglobin mass per kg of lean mass in all non-athletic individuals across ages (children and adults). (A) Blood volume per lean mass, $R^2_{Women} = .01$ ($p = .05$), $R^2_{Men} = .01$ ($p = .08$). (B) Red blood cell volume per lean mass, $R^2_{Women} = .03$ ($p = .01$), $R^2_{Men} = .00$ ($p = .49$). (C) Plasma volume per lean mass, $R^2_{Women} = .00$ ($p = .20$), $R^2_{Men} = .01$ ($p = .04$). (D) Hemoglobin mass per lean mass $R^2_{Women} = .05$ ($p < .001$), $R^2_{Men} = .00$ ($p = .20$). Each dot represents one individual. LBM, lean body mass. [Color figure can be viewed at wileyonlinelibrary.com]

6. Women, simple: Hb_{mass} (g) = 543.7*height (m) + 3.5*weight (kg) – 547.5
7. Men, simple: Hb_{mass} (g) = 543.7*height (m) + 3.5*weight (kg) – 349.0

3.4 | Prediction of BV

When the measured BV from the present data set was plotted against the predicted BV by Nadler, the mean difference corresponded to 819 mL with 95% limits of agreement being −536 and 2174 mL (Figure S7). For the present, simple formula (formulas 2 and 3) for BV, the mean difference corresponded to −3.6 mL with 95% limits of agreement being −1264 and 1256 mL, whereas for the clinical formula (formula 1), the mean difference corresponded to −1.9 mL with 95% limits of agreement being −1156 and 1152 mL.

4 | DISCUSSION

The present study provides reference values for BV, intravascular volumes and Hb_{mass} in healthy individuals between 9 and 93 years of age. We demonstrate that intravascular volumes and Hb_{mass} are strongly correlated to LBM, and that sex differences for these variables are greatly diminished when data were normalized to LBM rather than to BM. As normalization to LBM explained 71% and 79% of the variability in BV and Hb_{mass}, LBM thus seems critical for the evaluation of BV and Hb_{mass} in clinical settings. The data presented here may only be considered valid for comparison to assessments conducted with similar methodology.

4.1 | Sex differences in intravascular volumes and hemoglobin mass

Our data illustrate that women have ~70 mL BV/kg^{BM} and that the equivalent value is ~80 mL/kg^{BM} for men (Table 1). However, when normalizing BV to LBM, the difference is in female favor (~111 and 105 mL/kg^{LBM}, respectively), but the difference in BV between sex was nonetheless much reduced. The greater female BV/LBM is driven by a greater PV/LBM in females compared to males. In terms of Hb_{mass}, females have ~9 g/kg^{BM}, whereas males have 11.5 g/kg^{BM}. When normalized to LBM, the sex difference was diminished to 14 g/kg^{LBM} for females and ~15 g/kg^{LBM} for males. Our study confirms that women have lower BV per BM and BSA than men[11,35] but also reveals a strong association between LBM with BV and Hb_{mass} that was independent of sex. Indeed, the current data set reveals that LBM explains 71% of the variability in BV and that adding sex to

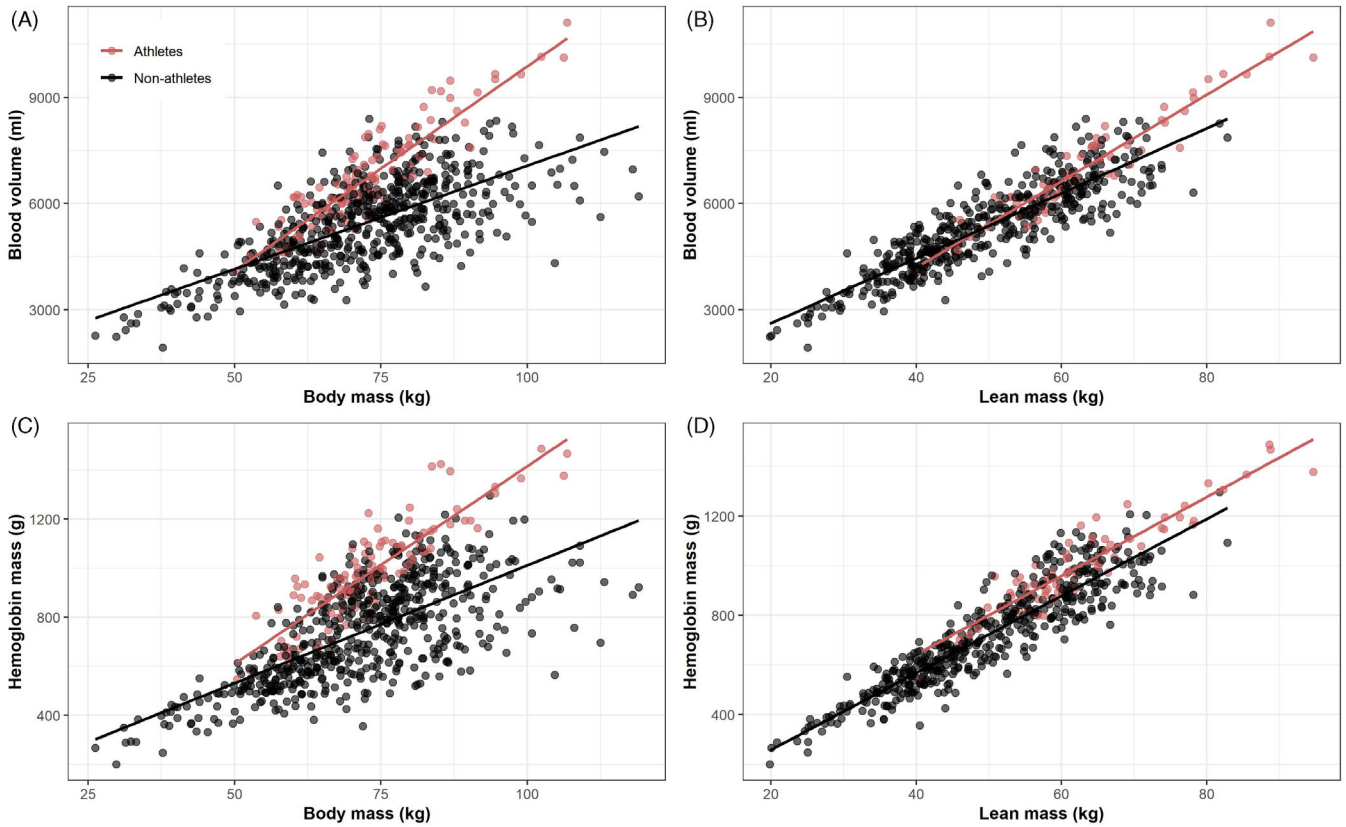

FIGURE 4 Linear relationship between body mass or lean mass with blood volume and hemoglobin mass in adult athletes and non-athletes. (A) Blood volume and body mass, $R^2_{\text{Athletes}} = .81$, $R^2_{\text{Non-athletes}} = .37$. (B) Blood volume and lean mass, $R^2_{\text{Athletes}} = .91$, $R^2_{\text{Non-athletes}} = .71$. (C) Hemoglobin mass and body mass, $R^2_{\text{Athletes}} = .76$, $R^2_{\text{Non-athletes}} = .37$. (D) Hemoglobin mass and lean mass, $R^2_{\text{Athletes}} = .87$, $R^2_{\text{Non-athletes}} = .79$. All $p < .001$. Each dot represents one individual. [Color figure can be viewed at wileyonlinelibrary.com]

the regression analysis only adds 1% explanatory power to the prediction (increasing it to 72%). In a similar manner, LBM explains 79% of the variability in Hb_{mass} and that adding sex to the regression analysis only increases this by 2% to 81%. Accordingly, the lower BV per kg BM in women mainly originates from the lower LBM to total BM ratio. Of note, while there are still statistical differences in BV, RBCV, PV, and Hb_{mass} per LBM between women and men, these are of a relatively small magnitude and their clinical importance is likely to be considered negligible. Our results are in line with an earlier study, where BV/BM differed between young, active women and men (80 vs. 94 mL/kg^{BM}), but where the sex differences were diminished after normalization to LBM (105 vs. 109 mL/kg^{LBM}).[11] The larger Hb_{mass} in men has previously been attributed to higher circulating testosterone[36,37] and indeed testosterone treatment accelerates erythropoiesis and increases [Hb] and hematocrit.[38] The higher testosterone could directly increase Hb_{mass} by stimulating erythropoiesis and/or indirectly by increasing LBM.[39]

4.2 | BV across age span

Our data indicates that absolute BV is stable across a wide age span, whereas BV/kg^{LBM} in marginally elevated with age in both sexes (women: 0.11 mL/kg^{LBM}/year and men: 0.12 mL/kg^{LBM}/year, respectively), mainly as a result of a progressive decrease in LBM. Although we acknowledge the statistically significant increase over time, the clinical relevance hereof is unlikely to have meaningful functional outcomes.

4.3 | BV, intravascular volumes, Hb_{mass}, and aerobic fitness

Endurance exercise performance is strongly related to BV, RBCV, and Hb_{mass}.[40–43] We[40,44] and others[45,46] have previously reported on intravascular volumes and Hb_{mass} from national team athletes and attributed that their higher BV, which may exceed 10 L or 110 mL/kg^{BM}, may be training-induced, genetically determined, or a combination of both.[47] The present analysis, however, suggests that one main driver for the elevated intravascular volumes or Hb_{mass}/kg^{BM} in elite athletes relates to a higher LBM and lower body fat mass in athletes than in non-athletes. Our results are in line with previous work based on fewer participants.[48] When normalized to LBM, the athletes included in our study cohort only have ~57 g more ($p < .001$) Hb_{mass} than healthy individuals. Intriguingly, endurance training may increase Hb_{mass} of untrained individuals by ~60 g

within 8 weeks without concomitantly changing LBM,[47] suggesting that the higher Hb_{mass} per LBM observed in athletes may not solely be related to genetic endowment and natural selection to sports.

4.4 | The use of formulas to predict BV and the data generated here in contrast to previous data

Although we observed a strong association between body composition and BV and developed novel algorithms to accurately predict the BV based on CO in a population of a given body composition, it should also be emphasized that individual values vary considerably around the predicted values. Generally, in individuals with high body fat, or a higher-than-normal contribution of LBM to BM, it is more accurate to use a formula based on LBM to estimate BV. Today, LBM may be assessed easily and at low cost by impedance technology, which most clinical facilities with a nephrology department can access. Although the comparison between BV values predicted by the widely used Nadler formula[7] and the measured BV from the current study generates a similarly large 95% confidence interval as the comparison with our novel formulas, the Nadler formula underestimates BV by 15.6% in the current study (Figure S7). Where this discrepancy stems from is uncertain but could be related to the difference in used methodology (CO rebreathing vs. dual-isotope infusion)[49] and as highlighted below the estimation formulas may only be valid for CO rebreathing determined BV.

4.5 | Limitations

The main limitation of the present study may be that the collected data and the derived estimation formulas for BV and Hb_{mass} in humans may only be valid if compared to similar methodology, for example, if BV is assessed by CO rebreathing, as the determined parameters may vary according to methods used. The difference between CO rebreathing and dual isotope methodology is that volumes (but not Hb_{mass}) with CO rebreathing are based on integration with hematocrit and which hence requires valid assessment hereof. Since a peripheral determined hematocrit typically varies from whole-body hematocrit, F-cell correction may be applied,[50] but this was not performed in the present study which may emphasize that the present dataset is valid for CO rebreathing only. This being said, excellent correlations have been reported without the application of an F-cell correction for both manually[51] and medical device[49] CO determined BVs and dual isotope methodology, although in absolute terms, these may vary somewhat. This limitation aside, the values presented here are the first large-scale data using CO methodology. BV also provides Hb_{mass} and could be a promising clinical candidate. The CO rebreathing-based data may also be considered relevant when seen in light of continuing problems associated with acquiring relevant isotopes and the lack of medical devices for its use in parts of the world. In terms of other limitations, it should also be noted that 9% of body compositions were not determined by DXA but by either impendence or skin caliper. Another important limitation is that the data are derived from an all Caucasian study population and future studies on other population groups are thus warranted.

4.6 | Conclusions

In summary, BV and Hb_{mass} are strongly associated with LBM and when LBM is taken into account, most previously observed differences in BV/BM between sex, aerobic fitness, and aging are to a large extent is minimized. We propose BV/kg^{LBM} and Hb_{mass}/kg^{LBM} as strong reference points in clinical treatment situations aiming to normalize BV status.

AUTHOR CONTRIBUTIONS

Laura Oberholzer: Data collection, analysis of data, figure preparation, and revising and approving the manuscript. David Montero, Paul Robach, Christoph Siebenmann, Camilla Koch Ryrsøe, Thomas C. Bonne, Andreas Breenfeldt Andersen, Jacob Bejder, Trine Karlsen, Elisabeth Edvardsen, Bent R. Rønnestad, Håvard Hamarsland, Ana C. Cepeda-Lopez, Jörn Rittweger, Gunnar Treff, Christoph Ahlgrim, Nicki Winfield Almquist, Jostein Hallén: Data collection and revising and approving the manuscript. Carsten Lundby: Study conception, data collection, and drafting of the manuscript.

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CONFLICT OF INTEREST STATEMENT

None of the authors have any conflict of interest to declare.

DATA AVAILABILITY STATEMENT

Data will be made available upon reasonable request to CL.

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REFERENCES

1. Thijssen S, Kappel F, Kotanko P. Absolute blood volume in hemodialysis patients: why is it relevant, and how to measure it? *Blood Purif*. 2013;35(1–3):63-71.
2. Miller WL. Fluid volume overload and congestion in heart failure: time to reconsider pathophysiology and how volume is assessed. *Circ Heart Fail*. 2016;9(8):e002922.

3. Lundby C, Ponte B, Lundby AK, Robach P, de Seigneux S. Red blood cell volume is not decreased in ESA-naive anemic chronic kidney disease patients. *Physiol Rep*. 2018;6(21):e13900.
4. Bomholt T, Larsson S, Rix M, et al. Intravascular volumes evaluated by a carbon monoxide rebreathing method in patients undergoing chronic hemodialysis. *Hemodial Int*. 2020;24(2):252-260.
5. Brown E, Hopper J Jr, Hodges JL Jr, Bradley B, Wennesland R, Yamauchi H. Red cell, plasma, and blood volume in the healthy women measured by radiochromium cell-labeling and hematocrit. *J Clin Invest*. 1962;41(12):2182-2190.
6. Wennesland R, Brown E, Hopper J Jr, et al. Red cell, plasma and blood volume in healthy men measured by radiochromium (Cr51) cell tagging and hematocrit: influence of age, somatotype and habits of physical activity on the variance after regression of volumes to height and weight combined. *J Clin Invest*. 1959;38(7):1065-1077.
7. Nadler SB, Hidalgo JU, Bloch T. Prediction of blood volume in normal human adults. *Surgery*. 1962;51(2):224-232.
8. Haldane J, Smith JL. The mass and oxygen capacity of the blood in man. *J Physiol*. 1900;25(5):331-343.
9. Cepeda-Lopez AC, Zimmermann MB, Wussler S, et al. Greater blood volume and Hb mass in obese women quantified by the carbon monoxide-rebreathing method affects interpretation of iron biomarkers and iron requirements. *Int J Obes (Lond)*. 2019;43(5):999-1008.
10. Pearson TC, Guthrie DL, Simpson J, et al. Interpretation of measured red cell mass and plasma volume in adults: expert panel on radionuclides of the International Council for Standardization in Haematology. *Br J Haematol*. 1995;89(4):748-756.
11. Falz R, Fikenzer S, Hoppe S, Busse M. Normal values of hemoglobin mass and blood volume in young, active women and men. *Int J Sports Med*. 2019;40(4):236-244.
12. Siebenmann C, Keiser S, Robach P, Lundby C. CORP: the assessment of total hemoglobin mass by carbon monoxide rebreathing. *J Appl Physiol*. 2017;123(3):645-654.
13. Ahlgrim C, Birkner P, Seiler F, et al. Applying the optimized CO rebreathing method for measuring blood volumes and hemoglobin mass in heart failure patients. *Front Physiol*. 2018;9:1603.
14. Strobeck JE, Feldschuh J, Miller WL. Heart failure outcomes with volume-guided management. *JACC Heart Fail*. 2018;6(11):940-948.
15. Otto JM, Plumb JO, Clissold E, et al. Hemoglobin concentration, total hemoglobin mass and plasma volume in patients: implications for anemia. *Haematologica*. 2017;102(9):1477-1485.
16. Dubois D, Dubois E. A formula to estimate the approximate surface area if height and weight be known. *Arch Intern Med*. 1916;17:863-871.
17. Burge CM, Skinner SL. Determination of hemoglobin mass and blood volume with CO: evaluation and application of a method. *J Appl Physiol*. 1995;79(2):623-631.
18. Schmidt W, Prommer N. The optimised CO-rebreathing method: a new tool to determine total haemoglobin mass routinely. *Eur J Appl Physiol*. 2005;95(5):486-495.
19. Keiser S, Meinild-Lundby AK, Steiner T, et al. Detection of blood volumes and haemoglobin mass by means of CO re-breathing and indocyanine green and sodium fluorescein injections. *Scand J Clin Lab Invest*. 2017;77(3):164-174.
20. Kellenberger K, Steiner T, Wehrlin JP. Comparison of the automatised and the optimised carbon monoxide rebreathing methods. *Scand J Clin Lab Invest*. 2022;82:474-480.
21. Treff G, Schmidt W, Wachsmuth N, Völzke C, Steinacker JM. Total haemoglobin mass, maximal and submaximal power in elite rowers. *Int J Sports Med*. 2014;35(7):571-574.
22. Lundgren KM, Aspvik NP, Langlo KAR, et al. Blood volume, hemoglobin mass, and peak oxygen uptake in older adults: the generation 100 study. *Front Sports Act Living*. 2021;3:638139.
23. Gerds T, Ozenne B. Publish: Format Output of Various Routines in a Suitable Way for Reports and Publication. R package version 2020.12.23 ed; 2021.
24. Dowle M, Srinivasa A. data.table: Extension of 'data.frame'. R package version 1.14.4 ed; 2022.
25. Wickham H, Hester J, Bryan J. readr: Read Rectangular Text Data. R package version 2.1.3 ed; 2022.
26. Fox J, Weisberg S. *An {R} Companion to Applied Regression*. 3rd ed. Sage; 2019.
27. Wickham H. *ggplot2: Elegant Graphics for Data Analysis*. Springer-Verlag; 2016.
28. Rudis B. hrbrthemes: Additional Themes, Theme Components and Utilities for 'ggplot2'. R package version 0.8.0 ed; 2020.
29. Hebbali A. olsrr: Tools for Building OLS Regression Models. R package version 0.5.3 ed; 2020.
30. Wickham H, Francois R, Henry L, Müller K. dplyr: A Grammar of Data Manipulation. R package version 1.0.10 ed; 2022.
31. Wilke C. cowplot: Streamlined Plot Theme and Plot Annotations for 'ggplot2'. R package version 1.1.1 ed; 2020.
32. Yee TW. VGAM: Vector Generalized Linear and Additive Models. R package version 1.1-7 ed; 2022.
33. Kuhn M. caret: Classification and Regression Training. R package version 6.0-93 ed; 2022.
34. Bland JM, Altman D. Statistical methods for assessing agreement between two methods of clinical measurement. *Lancet*. 1986;327(8476):307-310.
35. Feldschuh J, Enson Y. Prediction of the normal blood volume. Relation of blood volume to body habitus. *Circulation*. 1977;56(4 Pt 1):605-612.
36. Handelsman DJ, Hirschberg AL, Bermon S. Circulating testosterone as the hormonal basis of sex differences in athletic performance. *Endocr Rev*. 2018;39(5):803-829.
37. Mancera-Soto E, Ramos-Caballero DM, Magalhaes J, Chaves Gomez S, Schmidt WFJ, Cristancho-Mejía E. Quantification of testosterone-dependent erythropoiesis during male puberty. *Exp Physiol*. 2021;106(7):1470-1481.
38. Calof OM, Singh AB, Lee ML, et al. Adverse events associated with testosterone replacement in middle-aged and older men: a meta-analysis of randomized, placebo-controlled trials. *J Gerontol A Biol Sci Med Sci*. 2005;60(11):1451-1457.
39. Landgraff HW, Hallén J. Longitudinal training-related hematological changes in boys and girls from ages 12 to 15 yr. *Med Sci Sports Exerc*. 2020;52(9):1940-1947.
40. Lundby C, Robach P. Performance enhancement: what are the physiological limits? *Phys Ther*. 2015;30(4):282-292.
41. Lundby C, Montero D, Joyner M. Biology of VO2max: looking under the physiology lamp. *Acta Physiol*. 2017;220(2):218-228.
42. Martino M, Gledhill N, Jamnik V. High VO2max with no history of training is primarily due to high blood volume. *Med Sci Sports Excerc*. 2002;34(6):966-971.
43. Zelenkova IE, Zotkin SV, Korneev PV, Koprov SV, Grushin AA. Relationship between total hemoglobin mass and competitive performance in endurance athletes. *J Sports Med Phys Fitness*. 2019;59(3):352-356.
44. Jelkmann W, Lundby C. Blood doping and its detection. *Blood*. 2011;118(9):2395-2404.
45. 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):504-512.
46. Zelenkova I, Zotkin S, Korneev P, Koprov S, Grushin A. Comprehensive overview of hemoglobin mass and blood volume in elite athletes across a wide range of different sporting disciplines. *J Sports Med Phys Fitness*. 2019;59(2):179-186.
47. Montero D, Breenfeldt-Andersen A, Oberholzer L, et al. Erythropoiesis with endurance training: dynamics and mechanisms. *Am J Physiol Regul Integr Comp Physiol*. 2017;312:R894-R902.

48. Schumacher YO, Ahlgrim C, Pottgiesser T. Evaluation of anthropometrical reference parameters for hemoglobin mass in endurance athletes. *J Sports Med Phys Fitness*. 2008;48(4):509-514.
49. Breenfeldt Andersen A, Bonne TC, Hansen J, Oturai P, Lundby C. Validation of a clinically applicable device for fast and accurate quantification of blood volume. *J Clin Lab Anal*. 2023;37:e24928.
50. Orth VH, Rehm M, Haller M, Thiel M, Finsterer U. Die Messung des Blutvolumens—aktueller Stand. *Anaesthesist*. 2001;50(8):562-568.
51. Thomsen JK, Fogh-Andersen N, Bülow K, Devantier A. Blood and plasma volumes determined by carbon monoxide gas, 99mTc-labelled erythrocytes, 125I-albumin and the T 1824 technique. *Scand J Clin Lab Invest*. 1991;51:185-190.

SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

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