International Journal of General Medicine

Dovepress
Taylor & Francis Group

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ORIGINAL RESEARCH

Hemoglobin Mass and Blood Volume at Moderate Altitude: Establishing Lean-Body-Mass-Adjusted Reference Values for Clinical Use

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Background: Residing at high altitude is associated with increased red blood cell volume (RBCV) and reduced plasma volume (PV), changes that complicate interpretation of hemoglobin (Hb) and hematocrit (Hct). Here, we assess blood volume (BV), RBCV, PV, and hemoglobin mass (Hbmass) in individuals living at moderate altitude with the aim of deriving reference range for use in clinical practice.

Methods: One hundred and fifty-eight moderate-altitude residents (ALT; 51 women) from Abha, Saudi Arabia (2250 m above sea level) were recruited and compared to 40 control subjects (CON; 0 women). Blood volumes and Hbmass were determined by carbon monoxide rebreathing. Lean body mass (LBM) was quantified by dual-energy X-ray absorptiometry and adjusted for.

Results: When normalized to LBM, ALT men displayed an 8% increase in BV (109 vs 101 mL, $p = 0.003$), 14.8% increase in RBCV (54 vs 47 mL, $p < 0.001$), and 16.2% increase in Hbmass (18 vs 15 g, $p < 0.001$) compared to CON. ALT women exhibited lower BV (3928 vs 5476 mL), RBCV (1684 vs 2690 mL), PV (2276 vs 2767 mL), and Hbmass (558 vs 886 g), than ALT men, ($p < 0.001$ for all). When expressed per body weight, ALT men have higher RBCV (33 vs 30 g), lower PV (34 vs 40 mL) and no different BV (68 vs 69 mL, $p = 0.443$). When expressed per LBM, gender differences in RBCV and Hbmass disappeared (RBCV: 54 vs 51 mL/kg^{LBM}, $p = 0.164$; Hbmass: 18 vs 17 g/kg^{LBM}, $p = 0.201$). Women have significantly higher BV (120 vs 109 mL/kg^{LBM}) owing to higher PV (72 vs 55 mL/kg^{LBM}).

Conclusion: Moderate altitude is associated with a higher BV owing to a higher RBCV, which also explains the elevated Hb. A reference range based on the 5% and 97.5% percentiles of blood volumes and Hbmass adjusted for LBM is suggested for use in clinical practice.

Keywords: hemoglobin, high altitude, red blood cell volume, carbon monoxide rebreathing, lean body mass, altitude

Introduction

The blood volume (BV) is mainly comprised of two compartments, plasma volume (PV) and red blood cell volume (RBCV), and constitutes a controlled milieu that can be altered by both physiological and pathological processes. In clinical practice, Hemoglobin and hematocrit are easy-to-obtain indirect measurements of red cell volume; however, both have many shortfalls and need to be interpreted carefully.[1] Since these measures are concentration-based, a patient with acute bleeding can have a Hb value that is minimally changed despite significant blood loss. Meanwhile, reduced plasma volume, like in a patient using diuretics, could lead to an erroneous polycythemia diagnosis, ie, spurious polycythemia. Conversely, expansion of plasma volume as occurs in cases of heart failure and chronic kidney disease leads primarily to dilutional anemia, with actual anemia occurring in lesser degree.[2–4]

Received: 14 September 2025
Accepted: 26 November 2025
Published: 2 December 2025

The effect of plasma expansion on Hb and Hct is also noticed in cases of polycythemia vera, where expanded plasma can mask polycythemia, which subsequently might escape an early diagnosis.[5] In the 2016 revision of polycythemia vera diagnosis criteria, Hb and Hct thresholds were lowered with the aim of capturing more cases that would perhaps otherwise be missed under the former cutoffs. However, these thresholds now overlap with reference range values, resulting in over-workup of otherwise normal individuals.[6,7] This overlap is exaggerated at high altitudes, where physiological adaptations can complicate interpretation of Hb. When sea level residents' sojourn to altitude, plasma volume decreases within 24 hours, and RBCV increases within weeks.[8,9] Combined, these changes consistently elevate Hb, but in a manner that may not be uniform, as the contribution of RBCV change is initially absent, then increases over time. In the case of a concomitant pathological process, it cannot be determined which process is responsible for altering Hb. Furthermore, while greater hemoglobin mass is associated with greater Hb concentration, these parameters do not have a linear relationship; two individuals with equal Hb values might differ in hemoglobin mass and blood volume.[10]

At present, anemia is diagnosed as Hb below 120 g/L for females and 130 g/L for males based on reference values at sea level. However, since Hb and Hct are altered by altitude exposure, both anemia and polycythemia diagnosis criteria should be reevaluated for high-altitude contexts.[11] How permanent residence at moderate altitude affects RBCV and PV remains poorly understood, as do the respective contributions of these parameters to altitude-induced increase in Hb.

The measurement of red cell blood volume and Hbmass has long been a key diagnostic tool, especially in patients with suspected polycythemia vera. Historical radioisotope-derived methods of measurement, such as with ^{51}Cr and ^{125}I, show equivalency and acceptable precision,[12] but are cumbersome, costly, and suboptimal in diagnostic accuracy, and have lost favor in clinical practice.[13] Many hematologists have shifted towards using genetic tests, bone marrow histology, and serum erythropoietin level.[13] In addition, newer methods of assessing Hbmass and blood volumes in clinical practice, like the CO rebreathing method, are now available and have become widely utilized in a variety of patient populations.[14–17] The CO rebreathing method especially has been demonstrated effective in various settings with a very low margin of error,[18] and could be used to confirm true increases in Hbmass, thereby minimizing the number of individuals subjected to unnecessary evaluation for polycythemia.

We previously reported on blood volume and Hbmass measurements at moderate altitude, showing a possible increase of Hbmass in long-term dwellers when compared to the historical sea-level cohort.[19] That study was limited by the absence of local controls and by not all subjects having available lean body mass assessment. Since a reliable reference range is a prerequisite for proper utility in clinical practice. The present study aimed to establish reference values for hemoglobin mass and blood volumes in a moderate-altitude population. These reference ranges are essential for future studies evaluating anemia, polycythemia, and other conditions affecting blood volume—such as cardiac, renal, and hepatic diseases—as well as pharmacological effects, including those of SGLT inhibitors.

Methods

The study protocol was approved by the Institutional Review Board at King Khalid University (ECM#2023-3219) and was conducted according to the principles outlined in the Declaration of Helsinki. Participants were recruited through directed advertisements targeting university students and clinic visitors. A total of 220 were enrolled (170 altitude residents and 46 international controls). Ultimately, 158 residents and 40 international controls were included in the final analysis. Sea-level controls were limited to men due to operational and safety constraints related to CO-rebreathing and DXA imaging; consequently, cross-altitude comparisons were prespecified for men only.

Inclusion and Exclusion Criteria

Healthy participants 18 years or older with hemoglobin ≥120 g/L for females and ≥130 g/L for males who are long-term dwellers at altitude were considered eligible for this study. All participants were provided with an informational video about the procedure and signed informed consent forms. Brief interviews were conducted to obtain information about any medical conditions, drug use, or smoking. A control cohort was recruited from healthy international university students originally from sea-level East African countries. The control cohort was recruited before traveling back to their countries of origin, where they stayed for a minimum of two months, after which measurements were collected within 48 hours of their return to Saudi Arabia.

Data Collection and Blood Volume Measurements

Lean body mass was assessed using dual-energy X-ray absorptiometry (Lunar iDXA, GE healthcare). Blood volumes were measured according to a published and validated CO rebreathing protocol[20] also validated for clinical use.[21] Briefly, the participant was seated in the recumbent position and an IV line secured in the dorsum of one hand for collection of venous blood. Hemoglobin (g/L), hematocrit (%), and carboxyhemoglobin (%) were determined by blood gas analyzer (Siemens Rapidpoint 500e analyzer or Radiometer ABL90). A CO rebreather device (Detalo Performance, Detalo Health, Denmark) was used to administer CO gas (99.9% pure, 1 mL/kg) according to the four-minute protocol. Venous samples were collected before CO administration and at minute six post-administration. CO-Hb% was determined immediately and hemoglobin mass (Hbmass) was calculated from the change in CO-Hb%. BV, RBCV, and PV were derived from Hb and HCT.

All CO-rebreathing assessments were performed using the Detalo Health device (Detalo, Denmark) with automated calibration and dose algorithms; primary outputs (HbCO kinetics, Hbmass, PV, RV) were device-calculated and exported directly, minimizing operator influence. Phlebotomy and body-composition technicians were not informed of participants' control vs altitude status, and data cleaning and RI construction were conducted with group labels masked until the analysis plan was frozen. Order of testing was randomized within sessions, and device calibration logs were archived. While complete blinding is not feasible in physiological testing, these steps limit measurement and analytical bias.

Statistical Analysis

Participant characteristics were summarized as median (IQR). Hemoglobin mass (Hbmass) and intravascular volumes—red blood cell volume (RBCV), plasma volume (PV), and total blood volume (BV)—were analyzed as both absolute values and normalized per kilogram of body weight (BW) or lean body mass (LBM).

RI derivation followed CLSI EP28-A3c with ≥120 per partition whenever feasible. Our altitude cohort comprised 158 adults (107 men, 51 women), enabling nonparametric percentile RIs with bootstrap CIs. For male altitude vs sea-level comparisons (n = 107 vs n = 40), observed standardized effects for Hbmass (d = 0.77) and RBC volume (d = 0.68), and for LBM-normalized metrics (Hbmass/LBM d = 1.12; RBC-V/LBM d = 1.02) yielded >95% post-hoc power at $\alpha = 0.05$; parameters with small effects appropriately showed low power.

Reference intervals (RIs) were derived from the altitude residents using nonparametric quantiles (2.5th–97.5th percentiles) with 90% confidence intervals based on 20,000 bias-corrected and accelerated (BCa) bootstrap replications. The Clinical & Laboratory Standards Institute (CLSI) EP28-A3c guideline was followed,[22] and robustness was evaluated using 5th–95th and 5th–97.5th intervals. No winsorization was applied.

Sex partitioning was assessed using the CLSI "gate" procedure, which evaluates whether either sex shows disproportionate tail exceedance when using combined-sex RIs. One-sided exact binomial tests were used by design, since only upward deviation from the nominal 2.5% tail probability is relevant.

Altitude effect analysis was restricted to men and analyzed using three linear regression models with HC3 robust standard errors: (i) absolute outcomes adjusted for age and LBM, (ii) absolute outcomes adjusted for age and BW, and (iii) normalized outcomes adjusted for age. Results are reported as adjusted mean differences (AMEs) with 95% confidence intervals. Quantile regression at $\tau = 0.25$, 0.50, and 0.75 was used to assess distributional shifts, with bootstrap-based variance estimation.

Sensitivity analyses included exclusion of high-residual leverage points, alternate RI tail definitions, and normalization contrasts between BW and LBM. Additionally, generalized linear models with Gamma distribution and log link were used to account for potential mean–variance coupling.

All tests were two-sided except for the CLSI gate procedure, which used one-sided testing as specified. Complete-case analysis was used throughout, with no imputation. Statistical analyses were performed in StataNow 19.5 (StataCorp, College Station, TX), with a fixed seed (12345) to enhance reproducibility.

Results

After initial inclusion, 12 altitude participants were excluded from analysis: 9 due to hemoglobin levels outside the reference range and 3 due to errors in measurements. In total, 158 high-altitude residents were included in the final analysis, with 51 (32%) being females. Among 46 initially recruited international students, 6 were excluded due to errors in measurements, leaving 40 to be included in the final analysis. LBM measurements were available for 60 altitude men, 30 altitude women and 31 control men. Most high-altitude participants (n = 151) were healthy college students aged 18–24 years; the other 7 were healthy men recruited from outpatients' visitors. The median age was 21 years for altitude men, 22 years for altitude women, and 26 years for control men. The median Hb and Hct were 165 g/L (49.1%) in altitude men, 141 g/L (42.8%) and in altitude women, and 152 g/L (46.5%) in control men (Table 1).

Blood Volumes Measurement

Altitude men had higher absolute values for BV, RBCV, and PV compared to women (Table 1). The median BV was 5476 mL in men compared to 3928 mL in women ($p < 0.001$), PV: 2758 vs 2276 mL, and RBCV: 2677 vs 1684 mL, ($P < 0.001$ for all). Altitude men had higher RBCV per body weight (33.2 vs 29.9 mL/kg, $p < 0.001$) and lower PV (34 vs 39.7 mL/kg, $p < 0.001$) whereas BV was not statistically different between men and women when expressed per body weight (67.8 vs 69 mL/kg, $p = 0.443$). When expressed per LBM, BV was higher in females (120 vs 109 mL/kg^{LBM}, $p < 0.001$) due to higher plasma volume (71.5 vs 55.2 mL/kg^{LBM}, $p < 0.001$). The gender difference in RBCV vanished when expressed per LBM (54 vs 51 mL/kg^{LBM}, $p = 0.206$).

Table 1 Participant Characteristics

Variable	Altitude Residents			Sea Level Cohort	
	Men (n = 107)	Women (n = 51)	P-value*	Men (n = 40)	P-value†
Demographics					
Age	21 (19–23)	22 (20–22)	0.386	26 (23–29)	<0.001
Height (cm)	169.5 (166–175)	158 (153–162)	<0.001	169.8 (166–174)	0.794
Body Composition					
Weight (kg)	82 (70–95)	56.9 (50–68)	<0.001	69.7 (57–78)	<0.001
Lean Body Mass (kg)	47 (37–53)	31.3 (29–36)	<0.001	51.6 (44–54)	0.045
Body Mass Index (kg/m^2)	28.4 (24–32)	22.9 (21–27)	<0.001	23.2 (20–27)	<0.001
Hematological Parameters					
Hemoglobin (g/L)	165 (157–171)	141 (134–149)	<0.001	152 (146–163)	<0.001
Hematocrit (%)	49.1 (47–51)	42.8 (40–45)	<0.001	46.5 (45–50)	<0.001
Absolute Volumes					
Hemoglobin Mass (g)	886 (774–1014)	558 (498–647)	<0.001	777.5 (636–856)	<0.001
Blood Volume (mL)	5476 (4684–6193)	3928 (3534–4659)	<0.001	4994 (4332–5714)	0.009
Plasma Volume (mL)	2767 (2377–3199)	2276 (1996–2832)	<0.001	2543 (2303–3037)	0.172
RBC Volume (mL)	2690 (2342–3100)	1684 (1497–1989)	<0.001	2387 (1952–2585)	<0.001
Normalized Volumes					
Body Weight Normalized					
Hemoglobin Mass per Body Weight (g/kg)	11.2 (9.8–12.8)	9.9 (8.2–11.4)	<0.001	11.4 (10.1–12.7)	0.572
Blood Volume per Body Weight (mL/kg)	67.8 (60.2–77.9)	69 (61–80.3)	0.443	77.8 (65.7–81.8)	0.004
Plasma Volume per Body Weight (mL/kg)	34 (30.1–38.8)	39.7 (35.1–46)	<0.001	40.6 (34–45)	<0.001
Red Blood Cell Volume per Body Weight (mL/kg)	33.2 (29.5–38.9)	29.9 (24.2–33.8)	<0.001	35.2 (31–38)	0.261
Lean Body Mass Normalized					
Hemoglobin Mass per Lean Body Mass (g/kg)	17.9 (16.4–19.6)	16.9 (14.8–19.7)	0.201	15.3 (14.1–16.4)	<0.001
Blood Volume per Lean Body Mass (mL/kg)	109.2 (101.3–115.8)	119.9 (113.9–138.1)	<0.001	101 (93.3–107.6)	0.003
Plasma Volume per Lean Body Mass (mL/kg)	55.3 (48.8–60.3)	71.6 (64.6–79.9)	<0.001	53.9 (48.5–58.3)	0.364
Red Blood Cell Volume per Lean Body Mass (mL/kg)	54 (49.1–59.5)	51.1 (45.2–58.4)	0.164	47.1 (43.5–50.2)	<0.001

Notes: Data presented as median (IQR). *Comparisons: Altitude Men vs Altitude Women (Mann–Whitney *U*-test). †Comparisons: altitude Men vs sea level control Men (Mann–Whitney *U*-test).

Hemoglobin Mass

Hbmass was higher in altitude men compared to women both in absolute terms (885 vs 558 g, $p < 0.001$) and per total body weight (11.2 vs 9.8 g/kg, $p < 0.001$). However, normalization of Hbmass to lean body mass abrogated the difference between males and females (17.9 vs 16.9 g/kg^{LBM}, $p = 0.201$).

Effect of Altitude on Hbmass and Blood Volumes

To explore the moderate altitude effect on blood volumes and Hbmass, altitude cohort were compared with recently relocating sea level cohort. The control group had a mean BMI of 23.7 ± 4.3 and LBM of 49.6 ± 7.6 kg, while altitude men had a mean BMI of 28.4 ± 5.7 kg and LBM of 38.9 ± 21.1 kg. Controls exhibited a lower mean Hb and Hct compared to altitude men (Hb 152.4 ± 12.1 g/L, Hct 46.6 ± 3.7% vs Hb 163.7 ± 11 g/L, Hct 49.1 ± 3.5%, $p < 0.001$ for both). Altitude men had significantly higher BV (5476 vs 4994 mL, $p = 0.009$) and when expressed per LBM (109 vs 101 mL/kg^{LBM}, $p = 0.003$) whereas BV was higher in control group when reported per body weight (77.8 vs 67.8 mL/kg, $p = 0.004$). The RBCV was also higher in altitude men when expressed as total (2690 vs 2387 mL, $p < 0.001$) or per LBM (54 mL vs 47.1 mL/kg^{LBM}, $p < 0.001$), but not when expressed per body weight (33.2 vs 35.2 mL/kg, $p = 0.261$). PV was not different between the two cohorts when expressed as total (2767 mL vs 2543, $p = 0.172$) or per LBM (55.3 mL vs 53.09 mL/kg^{LBM}, $p = 0.364$) but was significantly higher in the control cohort when expressed per body weight (34 vs 40.6 mL/kg^{LBM}, $p < 0.001$).

Hbmass was significantly higher in altitude men both as an absolute value (886 vs 777.5 g, $p < 0.001$) and when expressed per LBM (17.9 vs 15.3 g/kg^{LBM}, $p < 0.001$) but not when expressed per body weight (11.2 g vs 11.4 g/kg, $p = 0.625$). The LBM assessment proves important for comparison due to the discrepancy between lean mass and body weight in the two groups, with the altitude cohort showing less lean body mass and more fat compared to the controls, underscoring the importance of LBM assessment when comparing two cohorts with different body composition (Table 1).

The net effect of altitude on blood volumes and Hbmass was presented in (Table 2) In men, the adjusted effect of moderate altitude on blood volume parameters varied according to the normalization framework. Under the LBM-adjusted framework, altitude was associated with significantly higher values for blood volume (601 mL, 95% CI: 1009–192 mL; $p = 0.004$), hemoglobin mass (145 g, 95% CI: 221–69 g; $p < 0.001$), and red blood cell volume (393 mL, 95% CI: 624–162; $p = 0.001$). Plasma volume also tended to be lower (208 mL, 95% CI: 428–12 mL; $p = 0.064$), though this did not reach statistical significance.

By contrast, in the BW-adjusted framework, altitude effects were attenuated, with only hemoglobin mass showing a significant expansion (59 g, 95% CI: 115 to 2; $p = 0.041$). Other parameters, including blood volume, plasma volume, and red blood cell volume, were not significantly different. Overall, the LBM-based adjustment demonstrated greater sensitivity, detecting altitude-related changes in 3 of 4 parameters, compared with only 1 of 4 under the BW framework.

Table 2 Adjusted Effect of Moderate Altitude on Blood Volume Parameters in Men

Parameter	Framework	Effect (95% CI)*	P-value
Blood Volume (mL)	BW-adjusted	95.9 (416.6 —224.8)	0.555
	LBM-adjusted	600.95 (1009.5 — 192.4)	0.004
Hemoglobin Mass (g)	BW-adjusted	58.7 (115 — 2.4)	0.041
	LBM-adjusted	145.2 (221.1 — 69.3)	<0.001
Plasma Volume (mL)	BW-adjusted	−35.4 (151.98 — -222.9)	0.709
	LBM-adjusted	207.9 (428.1 — 12.3)	0.064
Red Blood Cell Volume (mL)	BW-adjusted	131.3 (-302.3 — 39.7)	0.131
	LBM-adjusted	392.95 (624.2 — 161.8)	0.001

Notes: Sample: Men only (n = 96; altitude n = 60, sea level n = 36) from both altitude groups. * Effect = Adjusted mean differences (altitude minus Sea level men) with 95% CI. Models: LBM framework adjusted for age+lean body mass; BW framework adjusted for age+body weight. Framework sensitivity: LBM detected altitude effects in 3/4 vs BW in 1/4 parameters.
Abbreviations: LBM, lean body mass; BW, body weight; CI, confidence interval.

Table 3 Comparison of LBM vs BW Normalization Approaches for Reference Intervals (5–97.5 Percentiles)

Parameter	n	Δ Median (LBM–BW, 95% CI)	Δ Lower Limit (95% CI)	Δ Upper Limit (95% CI)
Hemoglobin Mass (g/kg)				
Combined	96	7.5 (7—7.98)	6.6 (5.9—7.8)	8.99 (6.7—11.7)
Men	60	6.97 (5.9—7.7)	5.99 (5.1—7.1)	8.2 (6.4—9.1)
Women	36	7.3 (6.5—8.6)	6.5 (4.4—7.7)	13.2 (7.6—13.2)
RBC Volume (mL/kg)				
Combined	96	21.8 (20—23)	19.8 (17.7—24)	28.1 (22.2—36.2)
Men	60	21.7 (19.3—23.9)	17.8 (14.7—21.2)	25.3 (18.2—27.9)
Women	36	21.5 (19.1—24.6)	19.2 (12.9—22.2)	38.8 (23.1—38.8)
Plasma Volume (mL/kg)				
Combined	96	23.4 (20.4—26)	20.7 (18—22)	46.9 (42.3—51.5)
Men	60	22.6 (20.3—24.7)	19.6 (17.4—21.6)	25.8 (19.8—30.9)
Women	36	31.9 (26.3—34.4)	25.9 (23.4—29.5)	44.8 (29.4—48.4)
Blood Volume (mL/kg)				
Combined	96	46.2 (42.1—49)	40.2 (36.5—43.1)	70.6 (52.10—89.1)
Men	60	42.7 (38.9—46.8)	39.5 (35.5—42.8)	49.1 (35.2—57.9)
Women	36	52.3 (48—61.3)	43.9 (34.5—51.4)	87.1 (71.7—90.3)

Notes: Reference intervals calculated using robust nonparametric methods. 90% CI = 90% confidence interval for reference interval limits. Full numeric RIs appear in Supplementary Tables S1–S2; CLSI gate decisions (LBM-normalized endpoints) are shown in Supplementary Table S3.
Abbreviations: LBM, lean body mass; BW, body weight; RBC, red blood cell.

Suggested Reference Ranges for Blood Volumes and Hbmass

The complete altitude-specific reference intervals are presented in Supplementary Table S1, which provides the primary nonparametric 2.5th–97.5th percentiles with 90% bootstrap confidence intervals. Because the study inclusion criteria—based on sea-level hemoglobin and hematocrit cutoffs—could admit participants with borderline anemia at altitude, Supplementary Table S2 also reports the 5th–95th percentiles as a robustness check. Table 3 summarizes the comparison between the LBM- and BW-normalized frameworks rather than the raw intervals. The LBM-based reference range is preferred, particularly when comparing groups with differing body composition, as it minimizes the influence of adiposity on blood-volume estimates. For interpretive purposes, the 5th percentile was adopted as the lower bound because it provides greater clinical specificity in this population. Alternative cutoffs (2.5% and 95%) representing varying sensitivity thresholds are detailed in the Supplementary material (supplement Tables S1-S6). Graphical summaries of the reference intervals, gate-analysis results, and robustness checks are presented in Supplementary Figures S1–S5.

Discussion

In this work, we determined intravascular blood volumes and Hbmass in healthy residents residing at moderate altitude in order to derive an altitude specific reference range to be used in future clinical practice. We employed a CO rebreathing technique that is safe and easy to use both in clinic and in other contexts. Our results demonstrate that in this population of healthy moderate-altitude residents, the blood volume, red cell volume, and Hbmass are increased compared to sea level residents. Moreover, our data also corroborate that normalization to lean body mass rather than total body weight is of greater value when interpreting blood volumes and Hbmass.[23] The absence of female sea-level controls limits cross-altitude inference for women; we therefore report female RIs at altitude without cross-altitude contrasts and plan a dedicated female sea-level validation.

As expected, we observed that BV, RBCV, and Hbmass are higher in men compared to women both in absolute terms and when normalized to total body weight. However, consistent with a previous report,[23] these sex-related differences not only diminished when normalizing to LBM but women in fact displayed a higher total BV per lean body mass, which was explained by higher PV. Hence, consideration of LBM is important for obtaining reliable and useful blood volume measurements. Indeed, given that fat tissue is hypovascular, such normalization should provide more clinically valid

measurements, especially in patients with obesity. It is accordingly recommended that BV be reported as LBM-normalized values, particularly in the setting of obesity.[24–26]

To explore the effect of high altitude on Hbmass and intravascular volumes, we compared altitude residents with a newly arrived international sea level cohort. Lean body mass assessment proved valuable here, since the two groups differed in body composition: the controls had higher lean body mass and lower body fat compared to the altitude participants. The altitude cohort exhibited higher absolute baseline hemoglobin and hematocrit and significantly higher total Hbmass and which persisted when adjusted per lean mass compared to the controls (17.9 vs 15.3 g/kg^{LBM}) Blood volume was increased in the altitude cohort on account of an increase in red blood cell volume, whereas the plasma volume was not different between groups. Since plasma volume contracts 24 h upon arrival to high altitude,[27] we may have missed a true difference in PV between altitude and control subjects as the latter were measured only 24–48 h upon arrival to altitude, and which may accordingly lead to erroneous conclusions regarding the effects of RBCV and PV contribution to the altitude induced increase in Hb. Overall, the findings of this work indicate that moderate altitude residents have increased blood volume, red blood volume, and Hbmass, which poorly correlated with hemoglobin and hematocrit values. Therefore, clinical decisions for patients residing at altitude should be based on direct measurement of blood volume and Hbmass rather than relying on Hb or Hct alone.

The occurrence of altitude-associated increase in red blood cell volume complicates the diagnosis of pathological polycythemia at altitude. Furthermore, changes in both plasma volume and red blood cell volume are established to occur alongside polycythemia vera, leading to so-called masked polycythemia in which the patient presents with normal Hb and Hct.[28] To account for such situations, the criteria for considering polycythemia have been lowered to Hb and Hct of 16 g/dL and 48% for females and 16.5 g/dL and 49% for males,[29] values well into the normal ranges of these parameters at altitude. Hence, more specific screening methods are needed when selecting individuals for polycythemia evaluation at high altitudes. One diagnostic criterion for polycythemia vera is Hbmass >25% over predicted value, but applying this criterion depends on having a validated reference range. For our population, we suggest using a reference range based on the 5% and 97.5% percentiles (Table 3). The lean-body-mass-adjusted reference range is best as this adjustment also abolishes sex and limits obesity effects on RBCV and Hbmass. Furthermore, previous studies have shown that age is not an important factor when blood volumes and Hbmass are adjusted to lean mass, implying that the aging-associated decreases in blood volume and Hbmass are related to decreased muscle mass.[23] Thus, our reference range can be used in an adult population of any age. Accordingly, regardless of Hb and HCT values, an adult high-altitude resident with Hbmass more than 22 g/kg^{LBM} is likely pathological and warrants further investigation; such a finding would justify the request of a *JAK2* mutation assay to rule out polycythemia vera. At higher elevations, the criteria likely need upwards adjustments, but the magnitude hereof is at present unknown. Employing this Hbmass-based criterion could significantly decrease unnecessary evaluation of people with normal altitude adaptations. Hbmass quantification also has important use in the crucial consideration of differentiating essential thrombocytosis from masked polycythemia vera.[28] Moreover, blood volume and Hbmass measurements can be helpful to identify normal Hbmass in patients with volume expansion and dilutional anemia, for example, in individuals with heart failure, kidney failure, or chronic liver disease.

Conclusions

Residents living at moderate altitude (2250 meters) have elevated BV, RBCV, and Hbmass but normal PV when compared with recently arrived sea level controls. Lean mass assessment is more accurate especially with people with extreme weight and when comparing two groups with different body compositions. A local reference range is suggested based on the 5th and 97.5th percentiles. Direct assessment of blood volume will aid in managing a wide range of disorders impacting blood volume, such as liver, heart, and kidney diseases, pharmacologic effects (eg, SGLT-2 inhibitors). Meanwhile, evaluating total hemoglobin mass is crucial for selecting patients needing workup for polycythemia, especially to differentiate between masked polycythemia, essential thrombocytosis, and for dilutional anemia.

Data Sharing Statement

Supplementary Tables S1–S4 accompany this article (S1: 2.5–97.5% RIs; S2: 5–95% RIs; S3: CLSI gate for LBM-normalized endpoints; S4: Model summary). The datasets used and/or analyzed during the current study are available from the corresponding author.

Acknowledgment

The authors thank all the participants of this study for their commitment and the staff at the outpatient department of King Khalid University Hospital for facilitating this project. The authors extend their appreciation to University Higher Education Fund for funding this research under Research Support Program for Central Labs at King Khalid University through the project number CL/CO/C/7.

Disclosure

The authors report no conflicts of interest in this work.

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