



Hematological Predictors of Endurance Performance in Ethiopian Distance Runners Measured at High Altitude

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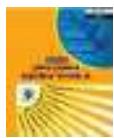
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Abstract

This study investigated associations between hematological parameters and endurance performance indicators ($VO_{2\max}$, running economy, and IAAF scores) in elite Ethiopian distance runners of both sexes. Fifty-two elite Ethiopian distance runners (29 males: weight 58.6 ± 5.8 kg, height 1.72 ± 0.07 m, BMI $19.7 \pm 1.8 \text{ kg}\cdot\text{m}^{-2}$, and 23 females: weight 49.49 ± 5.5 kg, height 1.59 ± 0.05 m, BMI $19.5 \pm 1.8 \text{ kg}\cdot\text{m}^{-2}$) residing at $\sim 2,400$ m underwent hematological profiling, including hemoglobin (HGB), mean corpuscular volume (MCV), platelet count (PLT), mean platelet volume (MPV), lymphocytes (LYM#), neutrophils (NEU#), and red cell distribution width (RDW-CV). Endurance performance was assessed using $VO_{2\max}$, RE at 14 and $16 \text{ km}\cdot\text{h}^{-1}$, and IAAF scores. We employed a multivariate multiple regression to simultaneously evaluate the associations between hematological parameters and multiple endurance performance indicators ($VO_{2\max}$, RE14, RE16, and IAAF scores). The analyses revealed that higher HGB was significantly associated with greater $VO_{2\max}$ and improved RE at 16 kmh^{-1} . MPV and PLT showed positive relationships with RE at 14 and 16 kmh^{-1} . RDW-CV was positively associated with $VO_{2\max}$ but inversely related to IAAF performance scores. Sex-specific analyses revealed that HGB predicted $VO_{2\max}$ more strongly in females, while RDW-CV and platelet indices were more relevant to performance in males. Baseline lymphocyte and neutrophil counts showed no significant relationships with endurance outcomes. Hemoglobin remains a key predictor of aerobic capacity in altitude-adapted elite Ethiopian distance runners. Novel associations of RDW-CV and platelet indices with $VO_{2\max}$ and running economy, most evident in males, suggest additional hematological pathways that may contribute to endurance performance. Immune cell counts demonstrated limited predictive value. These findings support the integration of hematological profiling into athlete monitoring and provide new insights into sex-specific physiological determinants of elite endurance performance.

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Introduction

Distance running performance in East African athletes, particularly those from Ethiopia and Kenya, has long attracted global attention due to their repeated dominance in international competitions. This success is widely recognized as a result of a multifactorial interplay rather than a single defining trait. Wilber & Pitsiladis (2012) argue that environmental exposure to altitude, favorable anthropometry, efficient metabolic function, lifelong high levels of physical activity, and strong socio-economic motivation combine to produce this advantage. These attributes, along with psychological resilience and culturally ingrained endurance traditions, contribute to their dominance in long-distance events.

Within this framework, aerobic capacity, typically expressed as maximal oxygen uptake ($\text{VO}_{2\text{max}}$), and running economy (RE) remain among the most extensively studied physiological determinants of elite performance (Brandon, 1995; Jones & Carter, 2000; Grivas et al., 2024). Foster & Lucia (2007) highlighted that $\text{VO}_{2\text{max}}$, lactate threshold, and RE together provide a comprehensive view of an athlete's endurance potential, emphasizing that no single metric fully predicts performance. Running Economy reflects the energy cost of maintaining a given submaximal running speed, while $\text{VO}_{2\text{max}}$ indicates the upper limit of oxygen utilization. Fletcher et al. (2009) proposed that RE, when expressed as caloric cost ($\text{kcal}\cdot\text{kg}^{-1}\cdot\text{km}^{-1}$), offers a more sensitive and functionally relevant measure than oxygen cost ($\text{ml}\cdot\text{kg}^{-1}\cdot\text{km}^{-1}$), particularly across varying speeds. Building on earlier observations (Brandon, 1995; Jones & Carter, 2000), these measures

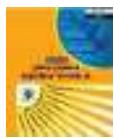
together capture both central cardiovascular capacity and peripheral muscular efficiency. Barnes & Kilding (2015) further highlighted that RE is influenced by multiple factors, including biomechanics, muscle fiber composition, and neuromuscular coordination, emphasizing the importance of comprehensive assessment in elite endurance athletes. Recent research on Ethiopian elite runners (Bayissa et al., 2025) reported consistently high RE and $\text{VO}_{2\text{max}}$ values in both sexes, with significant correlations between the two. This finding supports the concept that superior RE can partly offset a comparatively lower $\text{VO}_{2\text{max}}$, thereby sustaining elite performance. Weston et al. (2000) specifically compared African and Caucasian runners, showing that African runners utilize a higher fraction of their $\text{VO}_{2\text{max}}$ and demonstrate greater efficiency despite lower absolute $\text{VO}_{2\text{peak}}$ values. In contrast, Foster & Lucia (2007) reviewed key physiological determinants of endurance performance, highlighting the combined roles of $\text{VO}_{2\text{max}}$, lactate threshold, and running economy in elite athletes.

However, RE alone does not universally predict competitive success. Mooses et al., (2015) found no association between RE and race outcomes among elite Kenyan runners, despite their high $\text{VO}_{2\text{max}}$ values. This suggests that biomechanical characteristics, limb proportions, and psychological or tactical factors may compensate for less efficient RE. Conversely, Lucia et al., (2006) reported that elite Eritrean runners exhibited superior RE compared with equally matched Spanish counterparts despite similar $\text{VO}_{2\text{max}}$ values. These differences were potentially linked to anthropometric features such as longer shank length, reduced calf

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circumference, and lower body mass index, which may confer biomechanical advantages that improve efficiency over distance. Complementing these findings, Saltin et al.

(1995) demonstrated that Kenyan boys, juniors, and senior runners possessed markedly higher aerobic exercise capacity than Scandinavian counterparts both at sea level and at altitude, indicating that East African athletes exhibit superior physiological adaptations from an early age that persist into elite competition. Such traits may offer biomechanical advantages that translate into improved efficiency over distance.

Beyond $\text{VO}_{2\text{max}}$ and RE, other metrics, particularly velocity at the anaerobic threshold (vAT), fractional utilization of $\text{VO}_{2\text{max}}$ (% $\text{VO}_{2\text{max}}$), and velocity at maximal oxygen uptake (v $\text{VO}_{2\text{max}}$), provide additional predictive value for endurance performance. According to Tjelta & Shalfawi (2016), these indicators reveal how well athletes can sustain a high proportion of their aerobic power. In contrast, Bragada et al. (2010) found v $\text{VO}_{2\text{max}}$ to be a stronger long-term predictor of middle-distance results than either $\text{VO}_{2\text{max}}$ or RE alone. Aerobic performance is critically dependent on the efficiency of oxygen transport, making hematological characteristics a central focus in endurance research. Key parameters such as hemoglobin concentration (HGB), hematocrit (HCT), and red cell indices such as mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), and Red Cell Distribution Width (RDW) reflect the blood's oxygen-carrying capacity. They are regarded as stable indicators of long-term physiological adaptation, persisting even after strenuous altitude training or competition (Banfi et al., 2004). Prommer et al. (2010) demonstrated that elite Kenyan runners have significantly higher tHb and BV

compared with non-African athletes, highlighting the role of hematological adaptation in supporting exceptional $\text{VO}_{2\text{max}}$. Schmidt & Prommer (2008) further demonstrated that alterations in tHb directly influence $\text{VO}_{2\text{max}}$, with training-induced expansion of blood volume and hypoxia-driven increases in red cell volume enhancing oxygen transport capacity. Consistently,

Schmidt & Prommer (2008) demonstrated that training at altitudes above 2500 m for 3–4 weeks can increase tHb by approximately 6.5%. However, genetic predisposition remains a significant factor, and athletes who are native to high altitudes may have a lasting advantage. Notably, both total hemoglobin mass (tHb) and blood volume (BV) have a significant impact. Heinicke et al. (2001) documented that tHb and BV values were 35–40% higher in elite endurance athletes compared to untrained individuals, highlighting their significance in distinguishing top performers. Similarly, Mancera-Soto et al. (2022) found that adolescent endurance athletes living at approximately 2640 m had increased HCT, HGB, tHb, and erythrocyte volume compared to those at around 966 m, along with higher $\text{VO}_{2\text{peak}}$ values. Additionally, male athletes exhibited higher hematological measures and $\text{VO}_{2\text{peak}}$ than females, highlighting the significance of sex-specific hematological profiles.

Not all hematological changes are chronic adaptations. Moulongo et al., (2019) documented substantial acute shifts in red blood cell (RBC), white blood cell (WBC), HGB, and HCT, following a half-marathon, with some

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persisting up to 24 hours post-race. These findings underscore the need to interpret post-race hematological data with caution. Within Ethiopia, altitude-linked variation is also evident. Muche et al., (2021) reported that runners training at 3100 m had significantly higher HGB, MCH, and mean corpuscular hemoglobin concentration (MCHC) values than those at 2400 m, pointing to altitude-specific hematological adaptation. Similarly, Saunders et al., (2009) emphasized that elevated tHb and red cell volume, shaped by genetic predisposition, altitude residence, and chronic training, underpin the exceptional aerobic capacity of East African runners.

While red cell parameters have long dominated endurance profiling, evidence indicates that platelet characteristics are relevant predictors. Lippi et al., (2014) found that a higher mean platelet volume (MPV)

is associated with improved middle-distance performance, regardless of VO₂max and training load. This indicates a potential connection between platelet function, vascular health, and endurance capacity, an area that needs further research.

Despite the extensive literature on East African endurance dominance, relatively few studies have simultaneously examined the relationship between hematological profiles and multiple performance indicators in Ethiopian distance runners at high altitude. This study fills this gap by examining the associations of VO₂max, RE, and IAAF scores with hematological markers in elite Ethiopian male and female athletes. We hypothesize that higher hemoglobin concentration and hematocrit will be positively associated with VO₂max and IAAF scores, while higher RDW-CV will be negatively associated with performance indicators.



Methods

Participants

Fifty-two competitive Ethiopian distance runners (29 male, 23 female), ranging from 800 meters to the marathon, participated in the study. Seasonal best times were converted to IAAF performance scores (Spiriev, 2017) to enable comparison across events (Mooses et al., 2015).

A priori sample size estimation for multivariate multiple regression was performed using G*Power 3.1.5.1 (Düsseldorf, Germany). Assuming a medium effect size ($f^2 = 0.15$), an α -level of 0.05, power $(1 - \beta)$ of 0.80, and 7 predictors, at least 49 participants were needed. The current study included 52 elite Ethiopian distance runners (29 males, 23 females), exceeding the required sample size. A post-hoc sensitivity analysis showed that, with this sample size, the study could detect a minimum effect size of $f^2 = 0.14$ for multivariate outcomes, confirming sufficient power to detect moderate associations between hematological parameters and performance indicators.

Participants were recruited through Ethiopian athletics clubs and the Ethiopian Sports Academy. Club administrators provided permission, and coaches assisted in identifying eligible athletes. The study's goals, procedures, benefits, and risks were explained in consultation with coaches, and written informed consent was obtained. Testing took place at the Ethiopian Sports Academy laboratory after all athletes had been familiarized with the process.

Inclusion Criteria

Participants were competitive Ethiopian middle- and long-distance runners (800 m to marathon) affiliated with clubs recognized by the Ethiopian Athletics Federation or the Ethiopian Sports Academy. All athletes had to be healthy, free from injury, and actively involved in structured training programs with at least five sessions per week. Only athletes who voluntarily agreed to participate in all testing procedures, including venous blood sampling, were included.

Exclusion Criteria

Athletes were excluded if they had any known chronic cardiovascular, respiratory, or metabolic disorders, a history of using performance-enhancing substances, or incomplete training or competition records. Furthermore, individuals who were unwilling or unable to give informed consent, follow testing protocols, or undergo blood sampling were excluded from the study.

Procedures

A cross-sectional study was conducted from October 2017 to May 2018 involving male and female middle- and long-distance runners. During the first visit, athletes were familiarized with the treadmill protocol and respiratory face masks to reduce learning effects during testing. On the second visit, anthropometric measurements were collected before the treadmill testing. Body height (Power Tape, Lidu Hardware, China) and body mass (Seca Robusta 813, Seca GmbH & Co, Hamburg, Germany) were measured to the nearest 0.01 m and 0.1 kg, respectively. Participants



were instructed to refrain from intense training for at least 24 hours, avoid caffeine and alcohol, and maintain their usual diet to reduce acute influences on physiological responses.

After a 10-minute familiarization at self-selected speeds, participants performed an incremental treadmill test (Life Fitness 4HP, Canning Vale, Australia) to voluntary exhaustion. Before the test, athletes stood still on the treadmill for three minutes to collect baseline cardiorespiratory data. The test began at $8 \text{ km}\cdot\text{h}^{-1}$ for females and $10 \text{ km}\cdot\text{h}^{-1}$ for males with a 1% gradient (Jones & Doust, 1996; Mooses et al., 2015), increasing by $2 \text{ km}\cdot\text{h}^{-1}$ every three minutes until reaching $16 \text{ km}\cdot\text{h}^{-1}$ for females and $18 \text{ km}\cdot\text{h}^{-1}$ for males. Thereafter, speed was held constant while the incline increased by 1% each minute until voluntary exhaustion (Mooses et al., 2015).

Maximum running time (tmax) and heart rate (Polar RS400, Polar Electro Oy, Kempele, Finland) were recorded. Expired gases were measured using a calibrated MetaLyzer 3B system (Cortex Biophysic GMBH, Leipzig, Germany). VO_2max was defined as the highest 30-second average of VO_2 with no further increase despite rising workload. If no plateau was observed, the highest value was considered VO_2peak ; however, VO_2max is used throughout for simplicity (Billat et al., 2003).

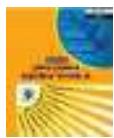
hematology analyzer (DIRUI BCC-3000B, Changchun, China) within one hour of sampling to minimize pre-analytical variability and maintain sample integrity.

RE was calculated from the final two minutes of submaximal VO_2 at 12 , 14 , and $16 \text{ km}\cdot\text{h}^{-1}$ for females and 14 , 16 , and $18 \text{ km}\cdot\text{h}^{-1}$ for males. RE was expressed as oxygen cost ($\text{ml}\cdot\text{kg}^{-1}\cdot\text{km}^{-1}$) and computed as: $RE = 1000 \cdot \text{VO}_2 \cdot v^{-1}$; where VO_2 is steady-state oxygen uptake ($\text{ml}\cdot\text{kg}^{-1}\cdot\text{min}^{-1}$) and v is running speed ($\text{m}\cdot\text{min}^{-1}$) (Bragada et al., 2010). Steady state was defined as a change of less than 100 ml O_2 over the last two minutes of the respective running stage (Fletcher et al., 2009).

On the third visit, venous blood samples were collected from each participant following an overnight fast of approximately 12 hours. To standardize conditions and minimize postural effects, participants rested in a supine position for at least 5 minutes before sampling. Blood was drawn aseptically from the antecubital vein by a trained and certified laboratory professional between 07:00 and 09:00 a.m., a time window corresponding to minimal diurnal variation in hematological values. For complete blood count (CBC) analysis, 4 mL of whole blood was collected into BD Vacutainer K₂EDTA lavender-top plastic tubes (Becton, Dickinson and Company, Franklin Lakes, NJ, USA), which contain ethylenediaminetetraacetic acid (EDTA) as an anticoagulant to preserve cellular morphology and prevent clotting. Tubes were gently inverted 8 to 10 times immediately after collection to ensure proper mixing. All analyses were conducted at the Ethiopian Sports Academy Laboratory using an automated

Statistical Analysis

Before performing the multivariate multiple regression, key assumptions were assessed. Independence of observations was ensured by the cross-sectional design.



Linearity was deemed reasonable based on graphical inspection. Levene's tests indicated no violation of homogeneity of variances for IAAF ($p = 0.858$), $\text{VO}_{2\text{max}}$ ($p = 0.999$), RE14 ($p = 0.668$), and RE16 ($p = 0.326$). Residual and Q–Q plots suggested no substantial departure from multivariate normality, and multicollinearity diagnostics showed tolerance > 0.1 and VIF < 5 for all predictors. The Pillai's Trace test indicated equality of covariance matrices. The assumptions were therefore considered adequately met for further analysis.

Table 1: Characteristics of the participants (mean \pm SD)

Variables	M (n=29)	F (n=23)
Age (Y)	24.03 ± 4.08	21.2 ± 3.9
Height (m)	1.72 ± 0.07	1.59 ± 0.05
Weight (Kg)	58.60 ± 5.83	49.49 ± 5.53
BMI ($\text{kg} \cdot \text{m}^{-2}$)	19.69 ± 1.77	19.49 ± 1.75

**Table 2:** Multivariate multiple regression results predicting endurance performance indicators from hematological parameters in elite Ethiopian distance runners.

	VO ₂ max	RE14	RE16	IAAF Score
	(mL·kg ⁻¹ ·min ⁻¹)	(mL·kg ⁻¹ ·km ⁻¹)	(mL·kg ⁻¹ ·km ⁻¹)	
HGB	**2.67 (0.73)	-0.43 (1.53)	*5.07 (2.12)	22.30 (17.13)
MCV	0.02 (0.28)	0.65 (0.59)	0.69 (0.82)	-4.55 (6.63)
PLT	0.02 (0.22)	*0.10 (0.05)	*0.13 (0.07)	-0.71 (0.52)
LYM#	-2.30 (2.24)	3.54 (4.69)	-2.33 (6.51)	90.05 (52.59)
NEU#	0.04 (0.08)	0.25 (0.18)	-0.21 (0.25)	2.39 (1.98)
RDW-CV	*4.22 (1.96)	4.81 (4.10)	6.83 (5.70)	*-104.75 (46.07)
MPV	0.69 (0.57)	**3.59 (1.19)	*3.38 (1.65)	-7.33 (13.32)

Note: Values are unstandardized regression coefficients (B) with standard errors (SE) in parentheses. VO₂max = maximal oxygen uptake; RE14 = running economy at 14 km.h⁻¹; RE16 = running economy at 16 km.h⁻¹; IAAF = International Association of Athletics Federations performance score. HGB = hemoglobin; MCV = mean corpuscular volume; PLT = platelet count; LYM# = Lymphocyte Count; NEU# Neutrophil Count; RDW-CV = red cell distribution width-coefficient of variation; MPV = Mean Platelet Volume.

*Significant at $p < 0.05$. **Significant at $p < 0.01$.

A comprehensive set of hematological parameters was initially collected to assess their potential impact on endurance performance in elite Ethiopian distance runners. These included red blood cell indices (RBC, HGB, HCT, MCV, MCH, MCHC, RDW-CV, RDW-SD), white blood cell counts and differentials (WBC, LYM#, MXD#, NEU#, LYM%, MXD%, NEU%), and platelet indices (PLT, PDW, MPV, P_LCR, PGT). Before regression modeling, multicollinearity diagnostics were performed to identify and exclude highly correlated variables, ensuring model stability and interpretability. Based on the variance inflation factor (VIF) and physiological relevance related to oxygen transport, immune function, and hemostasis, seven hematological variables were selected for the final analysis. HGB and MCV represent oxygen-carrying capacity and RBC morphology; PLT and MPV represent platelet function; LYM# and NEU# represent immune status; and RDW-CV serves as a marker of erythrocyte

size heterogeneity and inflammation.

The multivariate multiple regression revealed significant associations between several hematological parameters and endurance performance indicators (Table 2). Hemoglobin positively predicted VO₂max (B = 2.67, SE = 0.73, $p < 0.01$) and RE16 (B = 5.07, SE = 2.12, $p < 0.05$), indicating that higher HGB levels contribute to greater aerobic capacity and improved oxygen utilization efficiency at higher running speeds. Platelet count (PLT) was positively associated with RE14 (B = 0.10, SE = 0.05, $p < 0.05$) and RE16 (B = 0.13, SE = 0.07, $p < 0.05$), suggesting platelet involvement in metabolic efficiency during submaximal running. Mean platelet volume (MPV) showed a strong positive effect on RE14 (B = 3.59, SE = 1.19, $p < 0.01$) and a moderate positive effect on RE16 (B = 3.38, SE = 1.65, $p < 0.05$), implying that larger, more active platelets may enhance endurance performance.

Red cell distribution width (RDW-CV) was positively

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associated with $VO_{2\text{max}}$ ($B = 4.22$, $SE = 1.96$, $p < 0.05$),

indicating that greater variability in red blood cell size may correlate with improved oxygen transport capacity.

Conversely, RDW-CV was negatively associated with IAAF performance scores ($B = -104.75$, $SE = 46.07$, $p < 0.05$), suggesting that excessive anisocytosis may detrimentally affect competitive outcomes. No significant relationships were found for MCV, lymphocyte count (LYM#), or neutrophil count (NEU#) with any performance variables (all $p > 0.05$), although observed trends warrant further investigation.

Table 3: Multivariate regression coefficients (B) and standard errors (SE) for hematological predictors of endurance performance indicators in elite Ethiopian distance runners, presented separately for male and female athletes.

Predictor	Sex	$VO_{2\text{max}}$ B (SE)	RE14 B (SE)	RE16 B (SE)	IAAF B (SE)
HGB	Male	0.03 (1.27)	1.24 (2.67)	3.52 (3.96)	4.85 (35.55)
	Female	*2.94 (1.02)	-2.14 (3.22)	-0.27 (4.46)	55.09 (34.77)
MCV	Male	0.19 (0.41)	0.55 (0.92)	0.51 (1.27)	-1.59 (11.38)
	Female	0.32 (0.39)	-0.07 (1.23)	-0.24 (1.70)	-7.43 (13.29)-
PLT	Male	*-0.03 (0.03)	0.07 (0.07)	0.18 (0.10)	-0.81 (0.90)
	Female	0.07 (0.23)	0.14 (0.09)	0.15 (0.09)	0.49 (0.94)
LYM#	Male	-2.09 (2.99)	1.19 (6.75)	-1.15 (9.32)	122.99 (83.58)
	Female	1.62 (2.46)	7.08 (7.76)	4.44 (10.76)	33.01 (83.94)
NEU#	Male	0.08 (0.09)	0.27 (0.22)	-0.05 (0.31)	2.76 (2.75)
	Female	-0.03 (0.13)	0.03 (0.41)	-0.51 (0.57)	4.21 (4.42)
RDW-CV	Male	*6.01 (2.87)	8.17 (6.48)	13.22 (8.95)	-82.48 (80.29)
	Female	0.38 (2.21)	4.65 (6.99)	-0.45 (9.69)	-94.49 (75.58)
MPV	Male	1.29 (0.99)	*4.86 (2.24)	6.34 (3.09)	-2.76 (27.72)
	Female	1.10 (0.75)	1.51 (2.35)	1.86 (3.26)	-13.00 (25.44)

Note: Values are unstandardized regression coefficients (B) with standard errors (SE) in parentheses. $VO_{2\text{max}}$ = maximal oxygen uptake; RE14 = running economy at 14 km.h^{-1} ; RE16 = running economy at 16 km.h^{-1} ; IAAF = International Association of Athletics Federations performance score. HGB = hemoglobin; MCV = mean corpuscular volume; PLT = platelet count; LYM# = Lymphocyte Count; NEU# Neutrophil Count; RDW-CV = red cell distribution width-coefficient of variation; MPV = Mean Platelet Volume.

*Statistical significance: * $p < 0.05$; ** $p < 0.01$*



Table 3 presents the results of multivariate regression analyses examining the predictive value of selected hematological parameters on endurance performance indicators, VO₂max, RE 14, RE 16, and IAAF performance scores, separately for male and female elite Ethiopian distance runners. The regression coefficients (B) and standard errors (SE) are reported for each sex to highlight potential sex-specific physiological relationships.

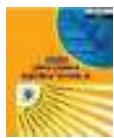
For male athletes, RDW-CV emerged as a significant positive predictor of VO₂max (B = 6.01, SE = 2.87, p < 0.05), indicating that greater variability in erythrocyte size may be associated with better aerobic capacity. MPV was also significantly associated with running economy at RE14 (B = 4.86, SE = 2.24, p < 0.05), indicating that platelet activation and function could contribute to submaximal exercise efficiency. PLT was negatively related to VO₂max (B = -0.03, SE = 0.03, p < 0.05), although this association was relatively modest. Other hematological variables did not reach statistical significance but showed varying directional trends.

In female athletes, HGB was the only significant

predictor, positively associated with VO₂max (B = 2.94, SE = 1.02, p < 0.05), reinforcing the critical role of oxygen-carrying capacity in aerobic performance among females. No other hematological parameters demonstrated statistically significant relationships with the endurance outcomes measured.

Notably, none of the evaluated hematological variables significantly predicted the IAAF performance score in either sex, indicating that competitive performance is likely influenced by a complex interplay of factors beyond the hematological markers assessed in this study.

Overall, these findings suggest sex-specific hematological influences on physiological determinants of endurance, with erythrocyte heterogeneity and platelet characteristics playing a more pronounced role in males. In contrast, HGB concentration remains a key predictor in females. Further research is warranted to elucidate the underlying mechanisms and their implications for performance optimization in elite distance runners.



Discussion

This study is the first to comprehensively investigate hematological predictors of $\text{VO}_{2\text{max}}$, RE, and competitive performance (IAAF score) in elite Ethiopian male and female distance runners living and training at high altitude (2400 m). Across the cohort, hemoglobin concentration showed consistent positive associations with aerobic capacity, particularly among female athletes, highlighting its importance for oxygen transport and endurance performance at altitude. In male athletes, variability in erythrocyte size (RDW-CV) and platelet-related indices such as MPV were associated with RE; however, these relationships should be interpreted cautiously and viewed as exploratory rather than indicative of direct performance effects. The contrasting associations observed for RDW-CV, positive with $\text{VO}_{2\text{max}}$ but negative with IAAF score, may reflect the influence of potential confounders, including iron status, inflammation, training load, erythrocyte turnover, and altitude exposure, rather than a causal relationship with competitive performance. Similarly, the positive association between platelet count and RE may relate to broader physiological processes such as vascular regulation and metabolic adaptation during submaximal exercise, but the role

of platelet indices in endurance performance remains preliminary. Overall, these findings highlight sex-specific and complex hematological influences on endurance physiology, while emphasizing the need for longitudinal studies that integrate iron status, inflammatory markers, and detailed training variables to clarify underlying mechanisms.

Red Blood Cell Parameters and Aerobic Capacity

The strong positive correlation between HGB and $\text{VO}_{2\text{max}}$ observed in our study is consistent with well-documented altitude adaptations, where athletes who were native to elevations (2,600–3,100 m) develop increased tHb to enhance oxygen transport (Schmidt & Prommer, 2008; Muche et al., 2021). Supporting this, Heinicke et al. (2001) evaluated blood volume (BV) and total hemoglobin (tHb) in elite athletes across various disciplines, reporting that endurance athletes have 35–40% higher BV and tHb compared to untrained controls. This elevated blood volume and hemoglobin mass underpin improved oxygen delivery capacity, a fundamental determinant of endurance performance. In our sample of elite Ethiopian distance runners living and training at ~2,400 m, HGB levels were not only strongly associated with $\text{VO}_{2\text{max}}$ but also with running economy at both 14 km/h and 16 km/h, suggesting that the oxygen transport benefits of higher hemoglobin concentration extend beyond maximal aerobic power to submaximal efficiency.

Rietjens et al., (2002) demonstrated that long-term altitude exposure increases HGB, HCT, and MCV in elite triathletes, whereas long-term endurance training alone has limited hematological effects. Our results mirror this altitude-dependent pattern for HGB and HCT but not altitude increased hemoglobin mass and red cell volume, improving $\text{VO}_{2\text{max}}$ and 5,000-m performance. Our current data align with the physiological premise of the

MCV, likely reflecting differences in duration or frequency of altitude exposure, iron status, or erythrocyte turnover. Similarly, Wehrlein et al. (2006) showed that living at high

"live high–train low" (LHTL) paradigm, although our athletes train and reside continuously at altitude rather than alternating environments. This chronic exposure

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may contribute to the persistently high hemoglobin concentrations and the observed association with both VO₂max and RE.

Brugniaux et al. (2006) confirmed that an 18-day live high train low (LHTL) intervention stimulated erythropoiesis, increased transferrin receptor and total hemoglobin mass, and improved aerobic performance in elite runners. Together, these studies confirm the potent effect of altitude exposure on hematological variables crucial for endurance performance. Our data provide additional evidence that these benefits are sustained in altitude-adapted athletes and are relevant for both maximal and submaximal aerobic performance indicators.

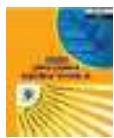
In our study, the lack of a significant association between MCV and performance contrasts with the findings of Rietjens et al. (2002) on altitude training, who reported that MCV increases during high-altitude exposure. This suggests that MCV may be more responsive to acute or block-periodized altitude interventions than to long-term altitude residency, or that it is influenced by other factors such as micronutrient status or erythrocyte turnover rate. Seasonal monitoring of hematological parameters is crucial, as HGB and HCT can fluctuate by 3–8% during intense competition periods (Banfi et al., 2011). In this context, regular assessment may help optimize training and altitude exposure strategies to sustain aerobic capacity while avoiding anemia or overtraining.

Hematological Behavior in Endurance Races and Altitude Settings

Longitudinal studies provide important insights into hematological responses during and after endurance competitions. Banfi et al. (2004) observed stable

erythrocyte counts, HGB, HCT, and red blood cell indices before and after marathon and ultramarathon races at altitude, indicating that well-adapted endurance athletes maintain hematological stability despite strenuous efforts, while post-race leukocyte elevations reflected immune activation. In contrast, Moulongo et al. (2019) reported significant alterations in RBC, HGB, HCT, PLT, and white blood cell differentials following half-marathon competition in Sub-Saharan Africa, highlighting the acute hematological adjustments that may accompany shorter but high-intensity endurance stress. Reid et al. (2004) also documented pronounced post-race leukocytosis, particularly neutrophilia, in marathon runners, confirming the inflammatory response triggered by prolonged endurance exercise. Collectively, these studies suggest that while red cell parameters may remain relatively stable in certain contexts, immune cell and platelet dynamics provide sensitive markers of systemic stress and recovery demands in endurance athletes. Complementing these observations, (Žákovská et al., 2017) examined hematological and immunological changes after a 100-km ultramarathon in cold conditions, reporting significant increases in leukocytes, immature neutrophils, monocytes, and platelets, without changes in RBC counts or HGB. These findings emphasize that hematological responses are event-specific and modulated by exercise duration, intensity, and environmental factors.

In the context of our study, the observed associations between platelet indices (PLT, MPV) and RE suggest that platelets



may contribute to optimizing oxygen delivery, microvascular function, and metabolic efficiency during submaximal endurance exercise, linking immune and hemostatic regulation with performance outcomes.

Platelet Indices and Their Role in Endurance Performance

Our study highlights platelet indices, particularly MPV and PLT, as predictors of running economy. Lippi et al., (2014) reported that baseline MPV predicts half-marathon performance in recreational runners, while Alis et al., (2016) observed that MPV responses vary with exercise duration. In our cohort, associations between MPV, PLT, and RE at $16 \text{ km} \cdot \text{h}^{-1}$ suggest that platelet-mediated vascular regulation, repair, and inflammatory modulation contribute to sustaining efficiency during submaximal endurance exercise. This emphasizes platelet indices as potential biomarkers of endurance adaptation, extending the traditional focus beyond red blood cells.

Immune Cell Counts and Endurance Performance

In this study, baseline lymphocyte (LYM#) and neutrophil (NEU#) counts showed no significant association with $\text{VO}_{2\text{max}}$, running economy (RE14, RE16), or IAAF scores in elite Ethiopian distance runners. One explanation is that these athletes are physiologically adapted to high training loads and altitude, keeping immune cell counts within a narrow, homeostatic range that limits their variability as performance predictors.

Previous research has reported links between immune parameters and endurance outcomes, mainly in ultra-endurance events. Amatori et al. (2024) found that lower pre-competition monocyte and eosinophil counts were

associated with superior results, possibly reflecting reduced systemic inflammation. Similarly, transient leukocytosis, especially neutrophilia, is common after marathon and ultramarathon races (Banfi et al., 2004; Reid et al., 2004; Žákovská et al., 2017; Moulongo et al., 2019).

However, these studies describe acute immune responses or different immune cell types, not baseline lymphocyte and neutrophil levels. Our findings support that oxygen transport (HGB, RDW-CV) and hemostatic markers (PLT, MPV) remain stronger determinants of endurance performance. Jung et al. (2020) further noted that hypoxic training can improve performance without adverse immune effects, reinforcing their limited predictive value in well-adapted athletes.

Sex Differences

In this study, hematological predictors of endurance performance showed clear sex-specific patterns. Hemoglobin (HGB) positively predicted $\text{VO}_{2\text{max}}$ in females, aligning with evidence that higher hemoglobin mass enhances oxygen transport and aerobic capacity (Heinicke et al., 2001; Schmidt & Prommer, 2008; Wilber & Pitsiladis, 2012). No effect was seen in males, possibly reflecting limited variability in HGB levels within this group, as also suggested by Saunders et al., (2009).

For males, RDW-CV predicted $\text{VO}_{2\text{max}}$, indicating that greater erythrocyte size heterogeneity may enhance oxygen delivery and utilization at altitude. It is also noted that RDW-CV reflects subclinical inflammation or micronutrient deficiencies, which were not directly assessed in this study, and therefore, caution is



warranted when interpreting these associations as purely performance-related. Platelet indices (PLT, MPV) were also positively associated with running economy (RE14, RE16) in males, suggesting a potential role for platelet function in sustaining submaximal endurance efficiency (Lippi et al., 2014).

Other hematological variables, including lymphocyte and neutrophil counts, showed no significant associations, consistent with previous findings that these parameters exert limited influence on performance when within physiological ranges.

Overall, these findings highlight sex-specific hematological influences, with oxygen-carrying capacity (HGB) predominating in females, erythrocyte heterogeneity (RDW-CV) in males, and platelet characteristics supporting submaximal running efficiency in males, largely aligning with prior studies while providing novel insight into RBC and platelet contributions in elite distance runners.

Conclusion

This study confirms the established role of hemoglobin

in supporting aerobic capacity while providing new evidence of sex-specific hematological associations in elite Ethiopian distance runners. Hemoglobin was a key determinant of VO₂max in females, whereas red cell distribution width and platelet indices were more strongly linked to performance in males. These findings suggest that, beyond oxygen transport, erythrocyte heterogeneity and platelet activity may influence running economy and overall endurance in a sex-dependent manner. Immune cell counts, by contrast, showed limited predictive value. Integrating hematological profiling with molecular and physiological markers may enhance individualized training strategies for altitude-adapted athletes.



Limitations

The cross-sectional design enables the identification of associations between hematological parameters and endurance performance, but does not permit causality or allow evaluation of longitudinal adaptations. The modest sample size may limit statistical power for sex-specific or subgroup analyses. The training load was not quantitatively assessed, limiting the ability to distinguish training-induced effects from chronic altitude adaptation. In addition, iron status markers (serum ferritin and transferrin saturation) were not assessed. Only standard hematological markers, including hemoglobin concentration, red blood cell indices, and platelet parameters, were evaluated. Hormonal and molecular regulators of erythropoiesis and hypoxic adaptation, such as erythropoietin and hypoxia-inducible factors, as well as indicators of iron status, were not measured. Future studies should include molecular markers, such as erythropoietin, hypoxia-inducible factors, and iron-regulation proteins, along with physiological markers, including lactate kinetics, muscle oxygenation, and mitochondrial capacity, to provide a more comprehensive understanding of endurance adaptations at high altitude.

Abbreviations

AAU	Addis Ababa University
AHRI	Armauer Hansen Research Institute
BV	Blood Volume
CBC	Complete Blood Count
HCT	Hematocrit
HGB	Hemoglobin
IAAF	International Association of Athletics Federations performance score
IAF	International Athletics Foundation
LHTL	Live High Train Low
LYM#	Lymphocyte Count
MCH	Mean Corpuscular Hemoglobin
MCHC	Mean Corpuscular Hemoglobin Concentration
MCV	Mean Corpuscular Volume
MPV	Mean Platelet Volume
NEU#	Neutrophil Count
RBC	Red Blood Cell
RDW	Red Cell Distribution Width
RDW-CV	Red Cell Distribution Width-Coefficient of Variation
RE	Running Economy
tHb	total hemoglobin mass
vAT	Velocity at anaerobic threshold
VIF	Variance Inflation Factor
VO ₂ max	Maximal Oxygen Uptake
vVO ₂ max	Velocity at maximal oxygen uptake
WBC	White Blood Cell



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Author contributions

D.H., M.M., and M.B. conceptualized the study and prepared the manuscript. D.A., M.B., and G.J. conducted data collection during treadmill exercise testing and contributed to drafting the manuscript. D.H., M.M., K.M., T.A., and M.B. performed data analysis. All authors reviewed and approved the final version of the manuscript and met the criteria for authorship.

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Data availability

To protect participant confidentiality, the datasets generated and analyzed during this study are not

publicly accessible. Access may be granted by the corresponding author following a reasonable request.

Declarations

Ethics approval and consent to participate

All ethical standards were adhered to in accordance with the World Medical Association's Declaration of Helsinki. Study protocols received approval from the Institutional Review Board of the College of Health Sciences, Addis Ababa University (Ref. No. 097/18/Phys), the National Research Ethics Review Committee, Addis Ababa, Ethiopia (Ref. No. 31/166/2018), and the Research Ethics Committee of the University of Tartu, Estonia. Participants were contacted at their training sites or at other convenient times, in coordination with their coaches, to discuss study details. The purpose, procedures, potential risks, and anticipated benefits were fully explained, and written and oral informed consent was obtained before participation.

Consent for publication

Not applicable.

Competing interests

The authors declare no competing interests.



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Result

The study included 52 elite Ethiopian distance runners, comprising 29 males and 23 females, all of whom were measured at high altitude, as indicated in Table 1. The mean age of the male athletes was 24.03 ± 4.08 years, while the female athletes were slightly younger, with a mean age of 21.39 ± 3.76 years. Male runners were taller (1.72 ± 0.07 m) than their female counterparts (1.59 ± 0.05 m) and had higher body mass (58.60 ± 5.83 kg vs. 49.49 ± 5.53 kg). Despite these anthropometric

differences, the body mass index (BMI) values were similar between groups, averaging $19.69 \pm 1.77 \text{ kg}\cdot\text{m}^{-2}$ for males and $19.49 \pm 1.75 \text{ kg}\cdot\text{m}^{-2}$ for females, both of which fall within the normal range for endurance athletes. These findings reflect typical sex-related anthropometric variations observed in elite long-distance runners who maintain comparable BMI levels, which are indicative of optimal body composition for endurance performance at high altitudes.