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SCREENING FOR IRON DEFICIENCY IN ELITE ATHLETES OF DIFFERENT SPORTING DISCIPLINES PERFORMING CARDIOPULMONARY EXERCISE STRESS TEST

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SKRINING NA DEFICIT GVOŽĐA KOD VRHUNSKIH SPORTISTA KOJI SE TAKMIČE U RAZLIČITIM SPORTSKIM DISCIPLINAMA I KOJI SU OBAVILI ERGOSPIROMETRIJSKI TEST FIZIČKIM OPTEREĆENJEM

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ABSTRACT

Objective. Given the inconsistent data on the prevalence of iron deficiency (ID) and its effects on athletic performance, this research aims to investigate ID and iron deficiency anemia (IDA) parameters in athletes across different sport disciplines and to examine their correlation with maximal oxygen uptake (VO_2max).

Methods. A total of 1,373 elite athletes (303 female) underwent a sports medical exam, including body composition analysis, laboratory analyses, and a treadmill cardiopulmonary stress test (CPET).

Results. Significantly higher values in males are observed in hemoglobin (147.77 vs 127.46 g/L, p<0.001) and serum ferritin (95.56 vs 57.67, p<0.033301). Prevalence of ID varies by cutoff value, with ID more prevalent in females (10.9-40.9%) than in males (1.7-10.2%). Mixed sporting disciplines show the highest prevalence of ID and IDA irrespective of gender. Males show higher VO2max levels than females (53.28±6.67 vs 46.48±5.65; p<0.001). Ferritin levels in males and hemoglobin in females can suggest better CPET performance. In male athletes, significant positive predictors of ferritin levels include age, training history,

SAŽETAK

Cilj. S obzirom na nekonzistentne podatke o prevalenciji deficita gvožđa (DG) i posledicama na sportske performanse, ovo istraživanje ima za cilj da istraži parametre DG i anemije kao posledice deficita gvožđa kod sportista koji se takmiče u različitim sportskim disciplinama i povezanost s maksimalnom potrošnjom kiseonika (VO_2max).

Metode. Ukupno 1.373 vrhunskih sportista (303 žena) obavilo je sportskomedicinski pregled, koji je uključivao i određivanje telesne kompozicije i ergospirometrijski test fizičkim opterećenjem (EST) na traci.

Rezultati. U koncentraciji hemoglobina (147,77 vs 127,46 g/L, p < 0,001) i serumskog feritina (95,56 vs 57,67, p < 0,001) primećene su značajno više vrednosti kod muškaraca. Prevalencija DG varira u odnosu na referentnu vrednost, a DG je prevalentnija kod žena (10,9–40,9%) nego kod muškaraca (1,7–10,2%). U mešovitim sportskim disciplinama pokazuje se najveća prevalencija DG i anemije, nezavisno od pola. Muškarci imaju veće vrednosti VO_2max od žena (53,28 ± 6,67 vs 46,48 ± 5,65; p < 0,001). Vrednosti feritina kod muškaraca i hemoglobina kod žena mogu da ukažu na bolje performanse na EST. Kod muškaraca godine života, godine bavljenja sportom, indeks telesne mase i

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body mass index, and body fat percentage (BF), whereas in female athletes no single variable is a significant predictor. Positive predictors of Hb values in male athletes are BF, resting heart rate, and diastolic blood pressure, while VO_2max is the sole significant positive predictor in females.

Conclusion. Female athletes and those participating in mixed sports are more frequently affected by ID and IDA, highlighting the importance of regular blood iron screenings. More research is needed to establish thresholds for ID and assess its impact on performance.

Key words: *iron deficiencies; anemia; athletes; sports.*

procenat telesnih masti (PTM) značajni su pozitivni prediktori nivoa feritina, dok kod žena nijedna varijabla nije značajan prediktor. Pozitivni prediktori vrednosti hemoglobina kod muškaraca jesu PTM, srčana frekvencija u miru i dijastolni krvni pritisak, dok je VO_2max jedini pozitivan prediktor kod žena.

Zaključak. Sportisti ženskog pola, kao i oni u mešovitim sportovima, pokazuju veću prevalenciju DG i anemije, što ističe značaj redovnog skrininga parametara gvožđa. Da bi se ispitala tačna referentna vrednost DG i utvrdio efekat na performanse neophodna su dodatna istraživanja.

Ključne reči: *deficit gvožđa; anemija; sportisti; sport.*

INTRODUCTION

Iron is a critical micronutrient that plays a central role in oxygen transport, energy metabolism, and numerous biochemical processes (1). The role of iron in hemoglobin (Hb) production and oxygen transport is well studied. However, iron also plays important roles in DNA synthesis, cellular energy production, immune function, and oxidative stress regulation (2). Total iron content in the body is about 4g in men and 2.5g in women. This iron is distributed among three active sites: Hb, myoglobin, and various enzymes. The remaining 20% of total iron is stored in an inactive form as depot iron, in the form of ferritin and hemosiderin. Additionally, 0.2% of total iron is in the form of transferrin, which acts as an iron transport carrier (3). Dietary iron comes in two forms: heme and non-heme. Non-heme iron is mostly found in plant-based foods and is less easily absorbed, while heme iron from animal flesh (e.g. red meat) is more bioavailable, although animal sources also contain non-heme iron. Additionally, some dietary components can either enhance (e.g. ascorbic acid) or inhibit (e.g. calcium, phytate) iron absorption (4). Iron homeostasis is regulated by hepcidin, a liver-derived peptide that controls the distribution of iron among cells and tissues and promotes its internalization and degradation. Hepcidin itself is regulated by iron intake and demand (5), as well as physical exercise (6). Iron deficiency in athletes can result from various mechanisms, including increased iron losses during training caused by microischemia, hemolysis, sweating, altitude training, and more. Female athletes are at additional risk because of menstrual bleeding. Having adequate iron stores is crucial for athletes, as iron deficiency (ID) and particularly iron deficiency anemia (IDA) can decrease energy production, cause fatigue, and impair physical performance (7).

The prevalence of ID is notably higher among athletes compared to the general population. While the rates vary, they are often reported as 10-11% in males and about 35% in females, reaching up to 52% among female adolescent athletes. It also occurs more frequently in endurance sports and in disciplines with a higher rate of eating disorders (8).

Iron status can be assessed by using a number of indicators, with common basic biomarkers reflecting iron metabolism, including Hb, hematocrit, red blood cell count (RBC), mean corpuscular volume (MCV) of RBC, and mean corpuscular hemoglobin (MCH) in RBC (9). Ferritin is the most commonly used parameter for assessing ID. Ferritin levels indicate iron stores, but for actual iron availability for erythropoiesis, other parameters, such as transferrin saturation and soluble transferrin receptor, can be used. Since ferritin also functions as an acute-phase protein, it is important to consider the athlete's history and to measure inflammatory markers, such as the erythrocyte sedimentation rate (ESR) or C-reactive protein (9, 10).

The threshold for defining ID, iron-deficiency non-anemia (IDNA), and IDA in athletes varies across studies. Cutoff values proposed by Sim et al. in a 2019 review are set at ferritin <35µg/L (or ng/mL) (ID), <20ng/mL (IDNA), and Hb<115g/L (IDA), also taking into account transferrin saturation. Additionally, the frequency of iron screening for athletes varies based on numerous factors, as presented in the review (11).

The aim of this research is to investigate the ID and IDA parameters in athletes from different sporting disciplines and to examine their correlation with aerobic capacity.

SUBJECTS AND METHODS

This was an observational, cross-sectional study. Retrospective data from 1373 Caucasian athletes (1,070 male and 303 female) were analyzed. Athletes were included if they performed a sports medical screening exam that included a cardiopulmonary exercise test (CPET) in a five-year period (01/12/2021-31/12/2025) at the Serbian Institute of Sport and Sports Medicine.

This study was conducted in accordance with the Declaration of Helsinki and the principles of Good Clinical Practice. It was approved by the Institutional Ethical Committee of the Serbian Institute of Sports and Sports Medicine (no. 641/1 on 13/03/2026). Informed consent was obtained from all subjects involved in the study as part of the standard procedure. Written informed

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consent was obtained from the participants to publish this paper (and parent's consent for participants younger than 18 years).

The athletes underwent a comprehensive sports medical screening exam that included a physical exam, body composition assessment using the bioelectrical impedance analysis (BIA) method (InBody 370, Republic of Korea), routine laboratory analyses, and a maximal CPET (Quark CPET metabolic cart and h/p/cosmos pulsar treadmill, Cosmed, Rome, Italy).

A sample for the basic laboratory analyses was collected in the morning (8 to 10 a.m.) after an overnight fast. The laboratory analyses conducted on the Mythic 18 analyzer (Orphee, Plan-les-Ouates, Switzerland) included a complete blood count of leukocytes (WBC), erythrocytes (RBC), platelets (PLT), hemoglobin (Hb), hematocrit (HCT), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), and ESR. The biochemical parameters of glucose, total cholesterol, triglycerides, urea, serum creatinine, aspartate aminotransferase (AST), alanine aminotransferase (ALT), total proteins, iron, total bilirubin, direct bilirubin, and ferritin were determined using commercial reagents on the Aries analyzer (Instrumentation Laboratory, Milan, Italy).

Body height (HT) was measured on a stadiometer, and BIA was used to obtain body weight (BW), body fat percentage (BF), and skeletal muscle mass (SMM). Body mass index (BMI) was calculated. Measurements were made after an overnight fast.

The functional parameters examined during CPET (Quark CPET metabolic cart and h/p/cosmos pulsar treadmill, Cosmed, Rome, Italy) included maximal heart rate (HR), percentage of maximal achieved HR, HR recovery (HRR) at minutes 1 and 3 of recovery test stage, maximal systolic blood pressure (SBP) and diastolic blood pressure (DBP), maximal oxygen consumption (VO_2max), respiratory exchange ratio (RER), HR at aerobic threshold (or ventilatory threshold 1, (VT1)), and HR at anaerobic threshold (or ventilatory threshold 2 (VT2)). The percentage of achieved VO_2max relative to the predicted value was calculated using the formula proposed by Myers et al. (12).

The sample size was determined by data availability and is comparable to a previous study that included CPET and VO_2max analyses in 1,190 athletes in relation to ID (13). Given the large sample size in our study (1,373 participants), it is considered to have sufficient statistical power to detect small-to-moderate associations.

Data were initially screened for extreme values using the Interquartile Range (IQR) method and evaluated for normality using the Shapiro–Wilk test. Continuous variables are expressed as Mean ± Standard Deviation. For gender-based comparisons, the Independent-Samples T Test was used for normally distributed variables, while the Mann–Whitney U test was used for non-normally distributed variables. Ferritin distribution was analyzed through the calculation of cumulative prevalence across specified thresholds (<12, <20, <35, and <50 ng/mL), where each successive category was inclusive of all lower-tier observations; a standalone category was utilized for athletes with adequate iron stores (≥50 ng/mL). To identify factors associated with Ferritin and Hemoglobin levels, a two-step linear regression analysis was conducted. Initially, univariate regression models were utilized to screen all potential predictors. Variables that reached statistical significance ($p < 0.05$) were then included in a multivariate regression model to determine independent associations. For all statistical procedures, a p-value < 0.05 was defined as the threshold for statistical significance, with $p < 0.001$ denoting strong statistical significance. Statistical analysis was performed using SPSS Statistics 24 software (IBM Corporation, Armonk, NY, USA).

RESULTS

General characteristics

Gender differences are presented in Table 1. Males and females do not differ in age or weekly training volume, with a small, but statistically significant difference in the number of years of training history (males 13.41 ± 5.80, females 11.69 ± 5.09; $p<0.01$). Body composition parameters are all larger in males ($p<0.01$ for all). Laboratory parameters that reflect iron metabolism and show statistically significantly higher values in males are RBC (5.01 vs 4.39), Hb (147.77 vs 127.46 g/L), HCT (0.44 vs 0.39), MCH (29.56 vs 29.10), MCHC (335.96 vs 329.93), serum ferritin (95.56 vs 57.67), and serum iron (18.87 vs 16.59), $p<0.01$ for all parameters. No statistically significant difference is observed in MCV (87.98 vs 88.18). Females show higher ESR levels (6.27 vs 3.49, $p < 0.001$).

Functional cardiovascular parameters at rest and during CPET, compared between genders, are shown in Table 2. Resting HR is slightly, but statistically significantly higher in females (60.48 vs 58.02 bpm, $p < 0.001$), and resting SBP (109.84 vs 101.06 mmHg, $p < 0.001$) and DBP (67.55 vs 62.59 mmHg, $p < 0.001$) were higher in males. Males show higher VO_2max (ml/kg/min) levels than females (53.28 ± 6.67 vs 46.48 ± 5.65; $p < 0.001$). Maximal effort during CPET was achieved, as evidenced by RER, which was slightly higher in males (1.09 vs 1.08, $p < 0.001$), with no difference in achieved maximal HR or the percentage predicted HR between genders. HR recovery (HRR) was similar in the first minute of recovery between males and females (25.60 vs 26.29 bpm), with a small difference in HRR in the third recovery minute (80.58 vs 78.65 bpm, $p = 0.018$). Slight, but statistically significant differences were observed in

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Table 1. Gender differences in general parameters.

Parameter	Male (n=1070)	Female (n=303)	Statistics
General characteristics			
Age (years)	22.71 ± 7.25	21.76 ± 6.31	NS
Training history (years)	13.41 ± 5.80	11.69 ± 5.09	$p < 0.01$
Average weekly training (Hours)	15.99 ± 6.06	16.02 ± 5.47	NS
Body composition			
Body height (cm)	188.69 ± 10.48	177.93 ± 8.84	$p < 0.01$
Body mass (kg)	85.55 ± 13.88	70.25 ± 11.26	$p < 0.01$
BMI (kg/m²)	23.93 ± 2.59	22.11 ± 2.55	$p < 0.01$
Body fat (%)	10.79 ± 4.60	18.60 ± 4.56	$p < 0.01$
Skeletal muscle mass (kg)	43.86 ± 7.39	31.96 ± 4.80	$p < 0.01$
Laboratory analyses			
Leukocytes (n)	5.65 ± 1.23	5.67 ± 1.31	NS
Platelets (n)	250.52 ± 50.64	271.19 ± 58.64	$p < 0.01$
Erythrocytes (n)	5.01 ± 0.33	4.39 ± 0.28	$p < 0.01$
Hemoglobin (g/L)	147.77 ± 9.36	127.46 ± 8.72	$p < 0.01$
HCT (L/L)	0.44 ± 0.03	0.39 ± 0.02	$p < 0.01$
MCV (fL)	87.98 ± 3.77	88.18 ± 4.14	NS
MCH (pg)	29.56 ± 1.51	29.10 ± 1.63	$p < 0.01$
MCHC (g/L)	335.96 ± 9.55	329.93 ± 9.51	$p < 0.01$
Serum ferritin (ng/mL)	95.56 ± 44.57	57.67 ± 23.36	$p < 0.01$
ESR (mm/h)	3.49 ± 2.15	6.27 ± 3.67	$p < 0.01$
Iron (µmol/L)	18.87 ± 6.97	16.59 ± 7.00	$p < 0.01$
Glucose (mmol/L)	5.12 ± 0.56	4.91 ± 0.45	$p < 0.01$
Total cholesterol (mmol/L)	4.35 ± 0.86	4.50 ± 0.83	$p < 0.01$
Triglycerides (mmol/L)	0.75 ± 0.39	0.63 ± 0.34	$p < 0.01$
Urea (mmol/L)	6.14 ± 1.44	5.33 ± 1.40	$p < 0.01$
Serum creatinine (µmol/L)	95.49 ± 14.46	80.49 ± 11.75	$p < 0.01$
AST (U/L)	32.01 ± 15.38	25.64 ± 6.74	$p < 0.01$
ALT (U/L)	25.97 ± 14.80	18.52 ± 7.10	$p < 0.01$
Total proteins (g/L)	70.52 ± 3.55	69.59 ± 3.48	$p < 0.01$
Total bilirubin (µmol/L)	15.83 ± 9.03	12.49 ± 6.88	$p < 0.01$
Direct bilirubin (µmol/L)	3.32 ± 1.41	2.88 ± 1.17	$p < 0.01$

The results are presented as mean±SD; NS, not significant; BMI, body mass index; HCT, hematocrit; MCV, mean corpuscular volume; MCH, mean corpuscular hemoglobin; MCHC, mean corpuscular hemoglobin concentration; ESR, erythrocyte sedimentation rate; AST, aspartate transaminase; ALT, alanine aminotransferase.

Table 2. Gender differences in functional cardiovascular parameters obtained at rest and during CPET.

Parameter	Male (n=1070)	Female (n=303)	Statistics
Resting HR (bpm)	58.02 ± 10.26	60.48 ± 10.51	$p < 0.001$
Resting SBP (mmHg)	109.84 ± 10.06	101.06 ± 8.63	$p < 0.001$
Resting DBP (mmHg)	67.55 ± 7.79	62.59 ± 6.44	$p < 0.001$
Maximal HR (bpm)	181.49 ± 10.53	182.14 ± 9.06	NS
Achieved max. HR (%)	92.41 ± 4.60	92.40 ± 4.24	NS
HRR min 1 (bpm)	25.60 ± 11.19	26.29 ± 10.89	NS
HRR min 3 (bpm)	78.65 ± 11.40	80.58 ± 11.15	$p < 0.05$
HR at AT 1 (bpm)	158.82 ± 11.63	160.92 ± 9.97	$p < 0.05$
HR at AT 2 (bpm)	173.36 ± 11.29	174.98 ± 9.50	$p < 0.05$
VO_2max (ml/kg/min)	53.28 ± 6.67	46.48 ± 5.65	$p < 0.001$
RER	1.08 ± 0.05	1.09 ± 0.05	$p < 0.001$
Maximal SBP (mmHg)	178.21 ± 17.75	165.10 ± 15.53	$p < 0.001$
Maximal DBP (mmHg)	53.52 ± 12.01	53.17 ± 10.64	NS

The results are presented as mean±SD; CPET, cardiopulmonary exercise test; NS, not significant; HR, heart rate; SBP, systolic blood pressure; DBP, diastolic blood pressure; HRR min 1(3), heart rate recovery at the first (third) minute of recovery stage; VT1, ventilatory threshold 1 (aerobic threshold); VT1, ventilatory threshold 2 (anaerobic threshold); VO_2max, maximal oxygen consumption; RER, respiratory exchange ratio.

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Table 3. The study cohort distributed by different ferritin levels proposed thresholds for diagnosing ID.

Serum ferritin level threshold for ID	Male, N (%)	Female, N (%)
< 12 ng/mL	0	3 (1%)
< 20 ng/mL	1 (0.1%)	8 (2.6%)
<35 ng/mL	18 (1.7%)	33 (10.9%)
<50 ng/mL	109 (10.2%)	124 (40.9%)

ID, iron deficiency

Table 4. Hemoglobin concentration percentile distribution by gender.

Gender	P3	P25	P50	P75	P97
Male	130	141.25	148	154	165
Female	113	121	127	133	144

P, percentile (g/L)

Table 5. Hemoglobin and ferritin levels according to CPET performance.

Parameter	VO_2max <120% PV	VO_2max ≥120% PV	Statistics
Males	n=766	n=304	
VO_2max (ml/kg/min)	51.83 ± 6.23	56.92 ± 6.35	$p < 0.001$
Hemoglobin (g/L)	147.88 ± 9.48	147.51 ± 9.07	NS
Serum ferritin (ng/mL)	92.53 ± 41.30	103.18 ± 51.17	$p < 0.05$
Females	n=139	n=164	
VO_2max (ml/kg/min)	43.68 ± 3.97	48.85 ± 5.79	$p < 0.001$
Hemoglobin (g/L)	126.22 ± 8.78	128.50 ± 8.56	$p < 0.05$
Serum ferritin (ng/mL)	57.29 ± 21.16	57.99 ± 25.14	NS

The results are presented as mean±SD; CPET, cardiopulmonary exercise test; NS, not significant; VO_2max, maximal oxygen consumption; RER, respiratory exchange ratio; PV, predicted value.

Table 6. Univariate regression analysis for the association of gender and VO2max with ferritin and hemoglobin levels.

Predictor	β	SE	Statistics
Male gender as a predictor			
Ferritin	37.89	2.66	$p < 0.001$
Hgb	20.32	0.60	$p < 0.001$
VO_2max as a predictor			
Ferritin	-0.17	0.17	NS
Hgb	0.47	0.05	$p < 0.001$

SE, standard error; NS, not significant; VO_2max, maximal oxygen consumption.

HR at two thresholds, with females showing higher HR values at VT1 (160.92 vs 158.82 bpm; p = 0.012) and VT2 (174.98 vs 173.36 bpm, p = 0.027).

Serum ferritin and hemoglobin distribution

The distribution of ferritin values is shown in Figure 1. When displayed across percentiles, the male group shows higher values at each percentile. Various thresholds are proposed to define ID in various populations. Table 3. presents different ferritin thresholds for defining ID and the number of male and female athletes in our cohort. Using the highest value of 50 ng/mL, 89.8% of male athletes and 59.1% of female athletes do not meet the criteria for ID. The percentile distribution of Hb is shown in Table 4, and the male group shows higher values at each percentile.

Ferritin and hemoglobin distribution across sporting disciplines

Four sport disciplines, in relation to predominant component, are classified as: skill, power, mixed or endurance, as outlined in the European Society of Cardiology (ESC) 2020 Guidelines on sports cardiology (14). We added a fifth category for recreational athletes, given that no single sport type is predominant. The distribution of ferritin and Hb values according to the

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Table 7. Univariate and multivariate regression analysis: association of different variables with ferritin levels.

Univariate				Multivariate			
Predictor	β	SE	Statistics	Predictor	β	SE	Statistics
Male athletes							
Age (years)	2.29	0.18	$p < 0.001$	Age (years)	0.72	0.27	$p < 0.001$
Training history (years)	2.78	0.22	$p < 0.001$	Training history (years)	1.72	0.29	$p < 0.001$
Average weekly training (hours)	0.2	0.23	NS	BMI (kg/m²)	1.84	0.91	$p < 0.05$
BMI (kg/m²)	5.7	0.51	$p < 0.001$	Body Fat (%)	1.23	0.45	$p < 0.05$
Body fat (%)	2.38	0.3	$p < 0.001$	Skeletal Muscle Mass (kg)	0.53	0.28	NS
Skeletal muscle mass (kg)	1.01	0.19	$p < 0.001$	VO_2max (ml/kg/min)	-0.1	0.24	NS
VO_2max (ml/kg/min)	-1.57	0.21	$p < 0.001$	Resting DBP (mmHg)	-0.33	0.17	NS

SE, standard error; NS, not significant; BMI, body mass index; VO_2max, maximal oxygen consumption; HR, heart rate; SBP, systolic blood pressure; DBP, diastolic blood pressure.

Table 7. Cont'd.

Univariate			
Predictor	β	SE	Statistics
Male athletes			
Resting HR (bpm)	-0.21	0.14	NS
Resting SBP (mmHg)	0.26	0.14	NS
Resting DBP (mmHg)	0.47	0.18	$p < 0.001$
Female athletes			
Age (years)	0.32	0.22	NS
Training history (years)	0.4	0.27	NS
Average weekly training (Hours)	-0.23	0.26	NS
BMI (kg/m²)	0.82	0.57	NS
Body fat (%)	0.24	0.3	NS
Skeletal muscle mass (kg)	0.16	0.28	NS
VO_2max (ml/kg/min)	-0.09	0.24	NS
Resting HR (bpm)	-0.07	0.13	NS
Resting SBP (mmHg)	-0.18	0.16	NS
Resting DBP (mmHg)	0.003	0.21	NS

aforementioned categories is shown in Figures 2 and 3. Mixed sporting disciplines show the highest prevalence of ID and IDA irrespective of gender.

Iron deficiency and iron deficiency anemia

The criterion for defining IDA, as proposed by Sim et al. (11), is a hemoglobin level below 115 g/L. 19 (6.3%) female athletes, and no male athletes, can be classified as having IDA. Using the World Health Organization (WHO) criteria for anemia (Hb <130 g/L in males and <120 g/L in females) (15), 31 (2.9%) of males, and 58 (25.4%) of females could be diagnosed with IDA. Among female athletes, 15 in the mixed sporting category and 4 in the power category can be classified as those with IDA. According to Sim et al., the definition of ID is a serum ferritin level < 35 ng/mL. The distribution of male and female athletes with ID across sporting disciplines is shown in Figure 4. Male athletes in mixed and endurance categories, and female athletes in mixed, power, and skill categories, show ID.

Cardiopulmonary stress test performance

The percentages of athletes achieving ≥120% of predicted VO_2max values on CPET, along with their Hb and ferritin levels, are presented in Table 5. In both groups, VO_2max levels are greater in the group achieving ≥120%. In male athletes, serum ferritin is lower in the <120% group (92.53 ± 41.30 vs 103.18 ± 51.17; $p < 0.05$), and Hb does not differ. In females, Hb is lower in the <120% group (126.22 ± 8.78 vs 128.50 ± 8.56; $p < 0.05$), whereas ferritin levels do not differ significantly.

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Table 8. Univariate and multivariate regression analysis: association of different variables with hemoglobin levels.

Predictor	β	SE	Statistics
Male athletes			
Age (years)	0.02	0.04	NS
Training history (years)	-0.05	0.05	NS
Average Weekly Training (Hours)	-0.03	0.05	NS
BMI (kg/m²)	0.4	0.11	$p < 0.001$
Body Fat (%)	0.41	0.06	$p < 0.001$
Skeletal Muscle Mass (kg)	0.01	0.04	NS
VO_2max (ml/kg/min)	-0.06	0.04	NS
Resting HR (bpm)	0.15	0.03	$p < 0.001$
Resting SBP (mmHg)	0.13	0.03	$p < 0.001$
Resting DBP (mmHg)	0.22	0.04	$p < 0.001$
Female athletes			
Age (years)	0.14	0.08	NS
Training history (years)	0.11	0.1	NS
Average Weekly Training (Hours)	-0.16	0.1	NS
BMI (kg/m²)	0.03	0.21	NS
Body Fat (%)	-0.01	0.11	NS
Skeletal Muscle Mass (kg)	-0.02	0.11	NS
VO_2max (ml/kg/min)	0.23	0.09	$p < 0.05$
Resting HR (bpm)	0.01	0.05	NS
Resting SBP (mmHg)	0.11	0.06	NS
Resting DBP (mmHg)	0.16	0.08	$p < 0.05$

SE, standard error; NS, not significant; BMI, body mass index; VO_2max, maximal oxygen consumption; HR, heart rate; SBP, systolic blood pressure; DBP, diastolic blood pressure.

Table 9. Multivariate regression analysis: association of different variables with hemoglobin levels.

Predictor	β	SE	Statistics
Male athletes			
Body Fat (%)	0.34	0.07	$p < 0.001$
Resting DBP (mmHg)	0.16	0.05	$p < 0.001$
Resting HR (bpm)	0.12	0.03	$p < 0.001$
Resting SBP (mmHg)	0.02	0.04	NS
BMI (kg/m²)	-0.1	0.14	NS
Female athletes			
VO_2max (ml/kg/min)	0.22	0.09	$p < 0.05$
Resting DBP (mmHg)	0.15	0.08	NS

SE, standard error; NS, not significant; BMI, body mass index; VO_2max, maximal oxygen consumption; HR, heart rate; SBP, systolic blood pressure; DBP, diastolic blood pressure.

Regression analysis

Investigating gender and VO_2max in correlation with ferritin and Hb values, the results show that male gender and VO_2 values are positively correlated with Hb levels, and male gender is also positively correlated with ferritin values (Table 6). Next, we performed univariate and multivariate regression for ferritin and Hb levels for each gender separately. Table 7 shows the association of different variables with ferritin levels. In male athletes, age (β = 0.72, SE = 0.27, p <0.001), training history (β = 1.72, SE = 0.29, p <0.001), BMI (β = 1.84, SE = 0.91, p <0.05), and BF (β = 1.23, SE = 0.45, p <0.05) are significant positive predictors of ferritin levels, whereas in female athletes, no single variable is a significant predictor of serum ferritin.

Association of Hb levels is presented in Tables 8 and 9. In male athletes, BF (β = 0.34, SE = 0.07, p <0.001), resting HR (β = 0.12, SE = 0.03, p <0.001), and DBP (β = 0.16, SE = 0.05, p <0.001) are positive predictors of Hb values. VO_2max (β = 0.22, SE = 0.09, p <0.05) is the sole significant positive predictor of Hb values in multivariate regression among female athletes. Serum iron was not a significant predictor of either ferritin or Hb values.

DISCUSSION

Our study presents data on markers of ID and IDA in 1373 athletes. The results show that females have lower Hb and ferritin values than males and are at greater risk of ID and IDA, particularly in mixed sports disciplines. VO_2max correlates with Hb levels, but the gender sub-

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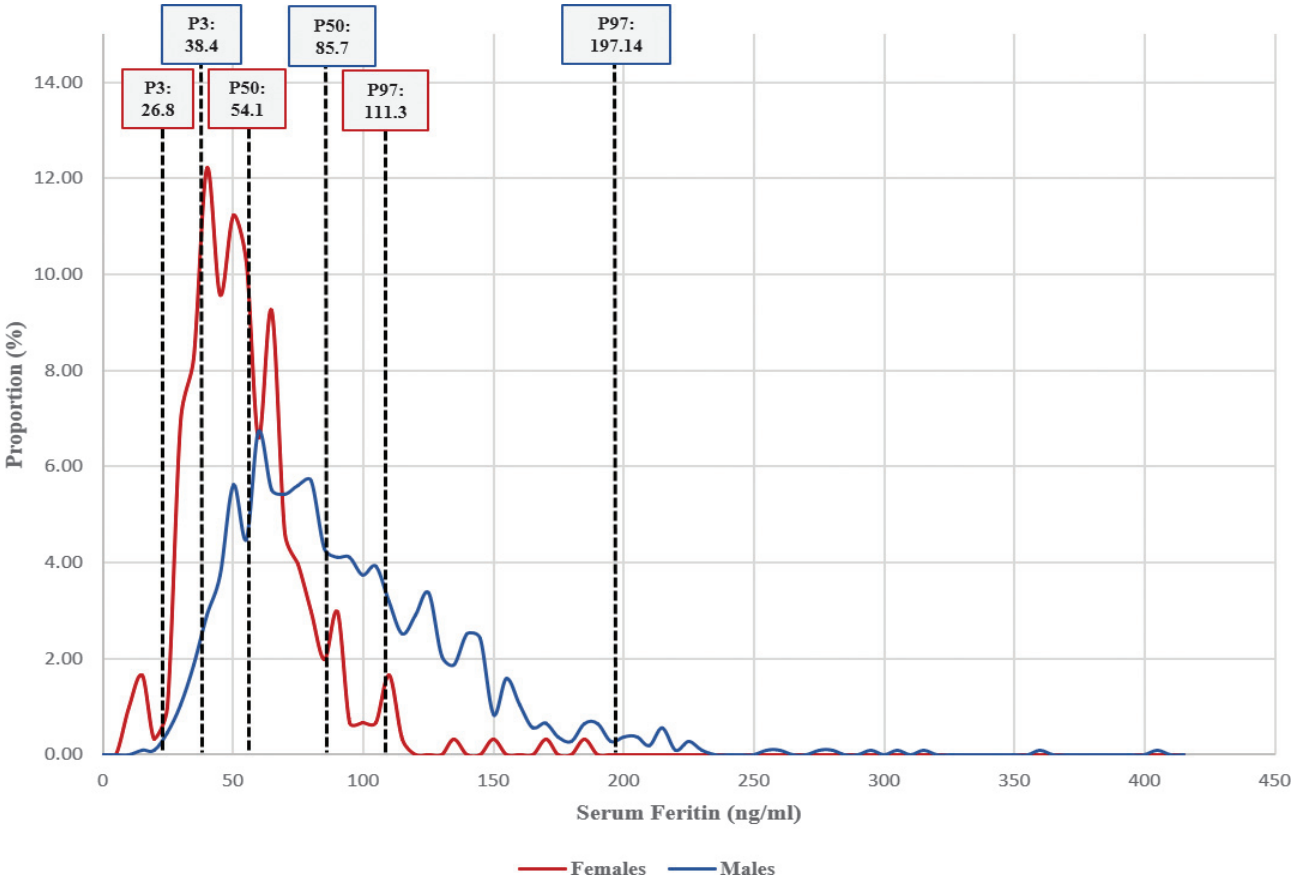
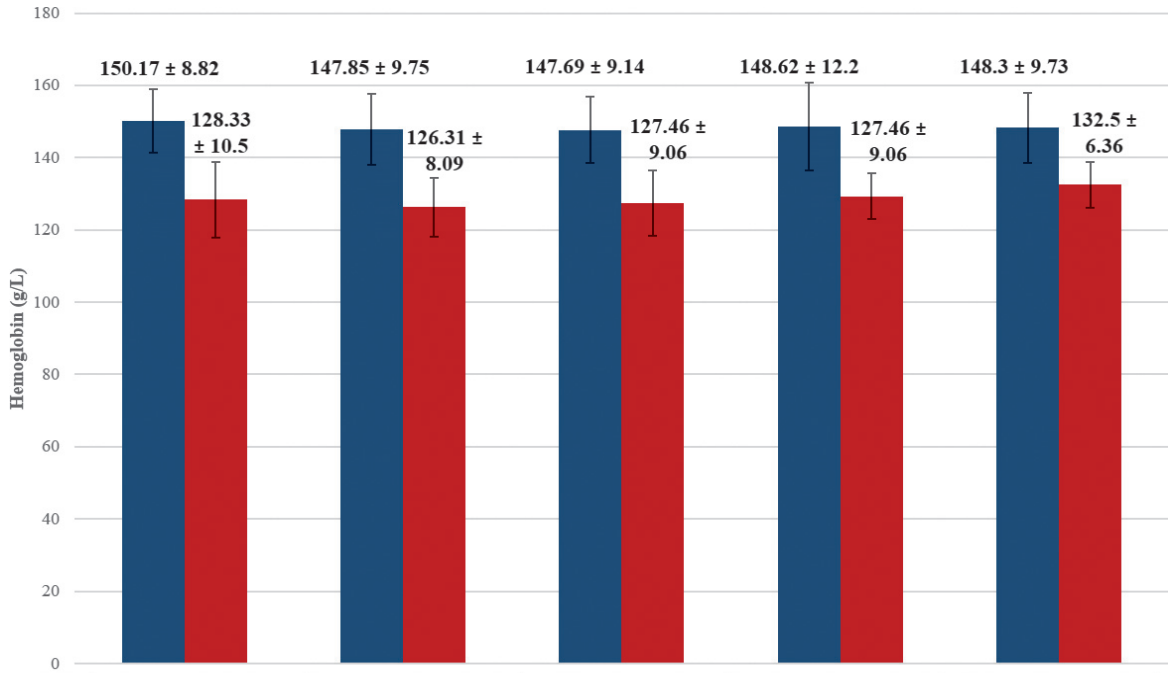


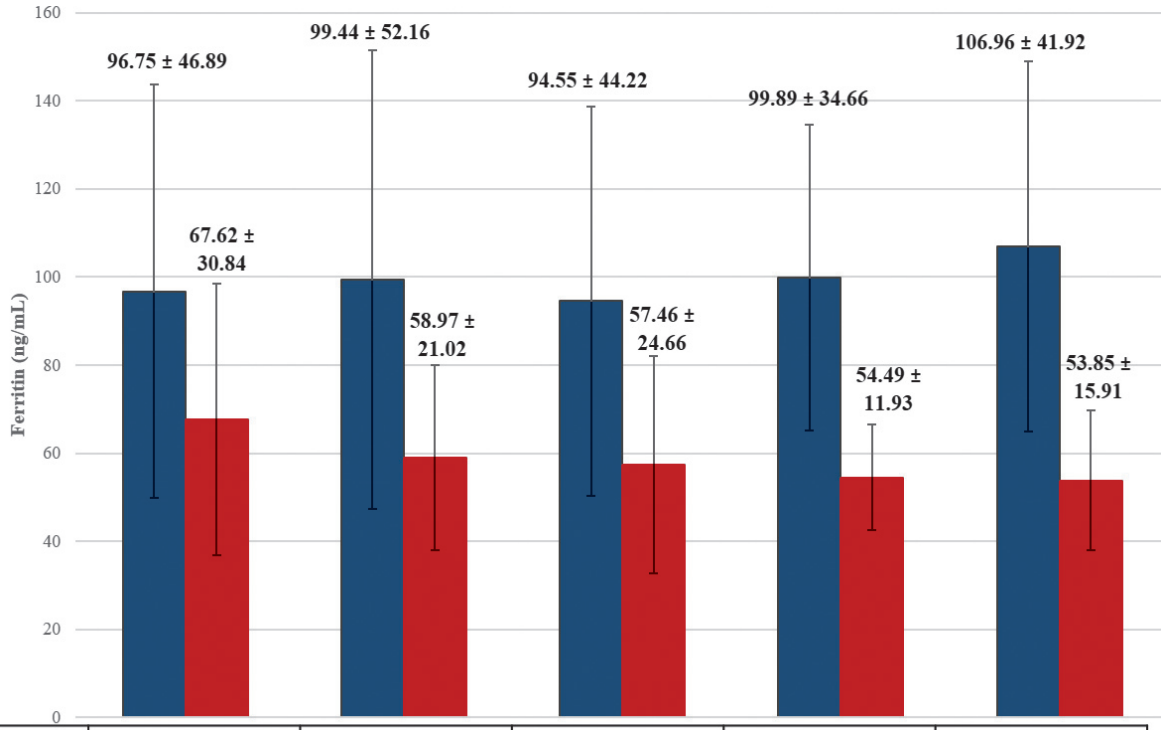
Figure 1. Ferritin distribution across genders. P3, 3. percentile; P50, 50. percentile; P97, 97. percentile.



	Skill (n)	Power (n)	Mixed (n)	Endurance (n)	Recreational (n)
Male	6	101	887	53	23
Female	6	52	220	23	2

Figure 2. Hemoglobin values across sporting disciplines.

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	Skill (n)	Power (n)	Mixed (n)	Endurance (n)	Recreational (n)
Male	6	101	887	53	23
Female	6	52	220	23	2

Figure 3. Serum ferritin values across sporting disciplines.

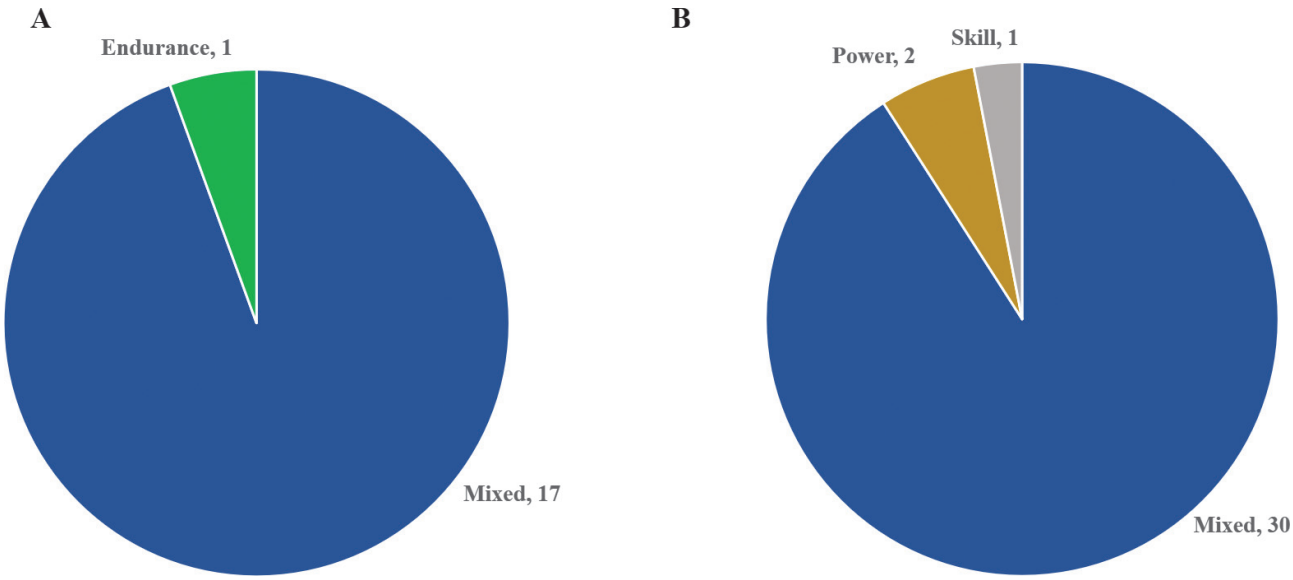


Figure 4. Sports discipline distribution showing the number of athletes with serum ferritin levels <35 ng/mL across sporting disciplines in male (A) and female (B) athletes.

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analysis shows this correlation occurs only in female athletes, whereas BF, resting HR, and DBP correlate with Hb levels in male athletes. In male athletes, age, training history, BMI, and BF predict ferritin levels, whereas in females, no single parameter correlates with serum ferritin.

It is worth noting that only a few athletes in our study reported ever taking iron supplements, and none reported taking them for longer than a month consecutively. Generally, it is recommended to take iron supplements for 4 to 12 weeks to assess their effectiveness (16), with a duration of more than 6 weeks needed to significantly improve serum ferritin concentration (17). Additionally, athletes show low ESR values, which is important to consider when interpreting serum ferritin because they function as acute-phase proteins (9).

The 2020 guidelines document on sports cardiology (14), classifies athletes with ≥10 hours per week as "elite". The population in our study meets that criterion with an average of 15-16 training hours per week. Also, athletes have an average of 11-13 years of experience in their sport. A study on 2525 Italian Olympic (elite) athletes (18) reported the presence of ID in 9.7% of athletes, mostly in skill (12.1%) and mixed (11.9%) sporting disciplines, and IDA in 0.9% of athletes, the largest number in skill sport disciplines (2.7%). In this study, ID was defined as serum ferritin <30 ng/mL with normal Hb levels, and anemia was defined as microcytic, with Hb <136 g/L in males and <117 g/L in females, and MCV <80 fL. The authors report that microcytic anemia was observed more often in males (15 athletes, 1.1%) than in females (9 athletes, 0.8%), but ID was more frequent in females (16.5%) than in males (4.2%). The lower reported ID frequency may have been due to the cohort being Olympic athletes, as a pinnacle of sport, who are suggested to have greater knowledge and team support. The higher number of anemic male athletes was not explored further in the paper. Generally, one of the main issues in estimating the prevalence of ID is the criteria used to define ID relative to a serum ferritin cutoff value.

Another study on Olympic (elite) athletes from the United States was published in 2020 (19). Authors presented the serum ferritin distribution in 1,085 athletes based on four proposed threshold values (12<, 20<, <35<, 50< ng/mL), which are the same as those we analyzed in our study. The percentages of male athletes with ID in these categories were 0.8%, 3.5%, 14.8%, and 28.9%, while for female athletes they were 6.8%, 23.3%, 52.1%, and 72.8%. These percentages are higher across all categories compared to our study. Serum ferritin levels and participant characteristics were not reported in this study. Therefore, we cannot discuss the reasons for these discrepancies further. The authors also compared athletes to the non-athletic population in two age groups, 20-23 and 24-28 years old. This analysis showed a higher percentage of ID in male athletes than in controls, but results for females varied. We did not use the same criteria, as all athletes are past puberty, and female athletes are of reproductive age.

One of the few studies that used serum ferritin levels <100 ng/mL investigated iron supplementation in young basketball players in a placebo-controlled study (20). After 3 months of supplementation (ferrous sulfate containing 100mg elemental iron and 60mg vitamin C), ferritin levels were higher in the experimental group, but no improvement in performance (measured as VO_2max and peak lactate) was observed.

A study of 56 kendo participants used the WHO criteria for defining anemia, and various serum ferritin thresholds (21). The participants were aged 18-23 years, with 15 (13-17) years of sport experience, but weekly training volume was not reported. No athlete was diagnosed with IDA according to WHO criteria. ID, based on ferritin levels <30 ng/mL, was present in 41% (n=7) of females and none in males, while ferritin levels <50 ng/mL were observed in 4 males and 11 females. In female participants, higher BMI was reported in the ferritin <30 ng/mL group. In our study, BMI or other body composition parameters were not predictive of ferritin or Hb levels.

Data from a German Olympic Training Center on 629 competitive athletes was published in 2022 (22). Athletes were divided into three age groups, with different ferritin thresholds used to define iron deficiency (ID): <30 ng/mL for adults (>18 years old), <20 ng/mL for ages 15-18, and <15 ng/mL for those under 15. The criteria for anemia classification were not clearly specified. The authors did not report the mean training volume. Four hundred thirty-one athletes also underwent functional bicycle ergometry testing with blood lactate measurement. ID was observed in 10.9% of male athletes and 35.9% of female athletes, mainly during adolescence. In our study, older age was associated with higher ferritin values, but we did not include adolescent athletes, and no age-related correlation was observed in female athletes. In the adult group, ID was found in 0.9% of males and 11.7% of females. No significant difference in ID and IDA prevalence was noted across sport categories (skill, power, mixed, endurance), consistent with our study; the group with ID was more common. No difference in performance was observed across different ID groups.

A study by Parks et al. (23) investigated ID and IDA in collegiate athletes. ID was defined as serum ferritin <20 ng/mL, and was observed in 30.9% of female and 2.9% of male athletes. IDA was diagnosed in 2.2% of females (Hb < 116g/L) and 1.2% of males (Hb < 136 g/L). In a similar category of collegiate athletes, Thomson et al. (24)

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investigated serum ferritin levels. 253 athletes had ferritin levels tested (mean age 18.4), 50% were female. Low ferritin levels (ID) were present in 24.5% (<20 ng/mL), 41.4% (<30 ng/mL), and 66.7% were classified as hypoferritinemia (<50 ng/mL), with no gender analysis performed.

The study by Keller et al. (13) included 1,190 athletes (407 female) who underwent CPET. Training volume or sport status was not reported, and the testing modality was not specified (e.g., treadmill, ergocycle). ID was defined as serum ferritin < 20 ng/mL, and athletes with Hb < 110 g/L were excluded. Most athletes with ID were female (64.5%), which is a significantly higher proportion than in our study (2.6% females and 0.1% males with ferritin < 20 ng/mL). The multivariate regression model performed in the study revealed age, male sex, BMI, and BF as significant ID predictors, while the VO_2max value (adjusted for age and BMI) was independently associated with ID. The authors did not perform a subanalysis by gender. Our results showed that VO_2max levels were not associated with ferritin levels (i.e., ID), and they correlated to Hb levels only in females. Furthermore, the authors analyzed athletes' CPET performance, with mean VO_2max values in the non-ID and ID groups of 45.6 and 43.4 ml/kg/min, respectively. Also, 16.1% of athletes in the non-ID group and 8.5% in the ID group achieved a VO_2max exceeding 50 ml/kg/min. We used a cutoff of 120% of predicted VO_2max as optimal CPET performance, given that athletes generally exceed predicted values on CPET compared with non-athletes, and optimal VO_2max levels differ by gender and age (25). High-intensity interval training at 120% of an athlete's VO_2max is suggested to improve aerobic capacity (26), suggesting its practical application. In our cohort of elite athletes, ferritin levels were significantly higher in males achieving ≥120% of predicted VO_2max, and Hb levels were higher in females in the ≥120% group. The difference and significance of this finding should be explored further, given that VO_2max is influenced by many factors beyond Hb and oxygen transport, and that athletic performance analysis is complex (27).

The correlation in male athletes between BF (Hb and ferritin) and BMI (ferritin) may be related to dietary habits beyond iron intake alone and could suggest low energy availability (LEA). In the 2023 International Olympic Committee consensus statement on relative energy deficiency in sport (REDs) (28) the details on screening for LEA and REDs are outlined. Serum ferritin and Hb are proposed as emerging potential indicators for REDs, warranting further research. The correlation between HB and DBP observed in the regression analysis may also be explained by the fact that lower SBP and DBP are markers of REDs. Also, the frequency of iron blood screening is proposed at least biannually for female athletes and quarterly for athletes with <24 months of any ID, irrespective of gender (11).

However, the effect is small, and the correlation in male athletes with higher Hb, BF, and DBP warrants further investigation, not necessarily suggesting a positive effect on health and performance. Studies vary in how they report ID related to serum ferritin thresholds, athlete categorization, and training volume. Additionally, a small number of studies correlate these findings with both objective and subjective measures of performance. It should be noted that different sports categories—mainly power-based, endurance-based, or mixed—require appropriate testing methods to determine performance benefits within each sport (e.g., marathon runners vs. Olympic weightlifters). Dietary intake is also sometimes not reported, which is a limitation in our study.

In conclusion, the results from this study of elite athletes show that female athletes have lower Hb and ferritin levels, and the mixed sporting category has the highest number of athletes with ID. Higher ferritin levels in males and Hb in females are suggestive of better CPET performance. VO_2max values correlate with Hb levels only in females, while BF, resting HR, and DBP correlate with Hb in male athletes. Age, training history, BMI, and BF predict ferritin levels in males, but no single parameter predicts ferritin in female athletes. These results suggest that female athletes, as well as younger male athletes, should be screened more frequently for ID and IDA, especially in mixed sports disciplines. More studies are needed to clearly define thresholds for serum ferritin and Hb to diagnose ID and IDA, to correlate findings with dietary intake patterns. Also, the impact of Hb and ferritin on performance is complex and should be investigated in multiple ways, with adequate measurement across different sport types.

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ABBREVIATIONS

ALT, alanine aminotransferase ALT
AST, aspartate aminotransferase
BF, body fat percentage
BIA, bioelectrical impedance analysis
BMI, body mass index
BW, body weight
CPET, cardiopulmonary exercise test
DBP, diastolic blood pressure

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ESC, European Society of Cardiology
ESR, erythrocyte sedimentation rate
FRIEND, Fitness Registry and the Importance of Exercise National Database
Hb, hemoglobin
HCT, hematocrit
HR, heart rate
HRR, heart rate recovery
ID, iron deficiency
IDA, iron deficiency anemia
IDNA, iron-deficiency non-anemia
HT, body height
LEA, low energy availability
MCH, mean corpuscular hemoglobin
MCHC, mean corpuscular hemoglobin concentration
MCV, mean corpuscular volume
PLT, platelets
RBC, red blood cell
REDs, relative energy deficiency in sport
RER, respiratory exchange ratio
SBP, systolic blood pressure
SMM, skeletal muscle mass
VO_2max, maximal oxygen consumption
VT1, ventilatory threshold 1
VT2, ventilatory threshold 2
WBC, leukocytes
WHO, World Health Organization

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