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Original Research

Sex-Specific Iron, Muscle Enzyme, and Hormonal Profiles Across Sport Energy Demands in Indian Athletes

***Dr. S. Srividhya[1], Dr. S. Jeyakumar[2]**

[1]*HOD & Associate Professor, Department of Sports & Exercise Science, REVA University, Bengaluru, Karnataka, India*

[2]*Professor & Research Coordinator, School of Health Sciences, Garden City University, Karnataka, India*

*Corresponding author: **Dr. S. Srividhya**
Email: srividhyasivaprakasam@gmail.com

Abstract

Sport training imposes distinct hematological, muscular, and endocrine demands, yet integrated biochemical comparisons across predominant energy-system categories remain limited in Indian athletes. This cross-sectional study compared iron status, muscle enzymes, and anabolic–catabolic hormones in 429 athletes (246 male and 183 female) classified as aerobic, anaerobic, or mixed aerobic–anaerobic. Hemoglobin, ferritin, serum iron, total iron-binding capacity, transferrin saturation, creatine kinase, lactate dehydrogenase, testosterone, cortisol, and the testosterone-to-cortisol ratio were evaluated. One-way analysis of variance and exploratory least significant difference post-hoc tests were performed separately by sex. In males, hemoglobin, ferritin, serum iron, transferrin saturation, lactate dehydrogenase, testosterone, and the testosterone-to-cortisol ratio differed significantly among sport groups ($p < .05$), whereas total iron-binding capacity, creatine kinase, and cortisol did not. In females, hemoglobin, ferritin, transferrin saturation, lactate dehydrogenase, testosterone, cortisol, and the testosterone-to-cortisol ratio differed significantly, while serum iron, total iron-binding capacity, and creatine kinase did not. Aerobic athletes generally had lower ferritin and hemoglobin, higher lactate dehydrogenase, and a less anabolic hormonal profile than anaerobic athletes. Low ferritin was most prevalent in female aerobic athletes (36.4%). These findings support sex- and sport-contextualized biochemical interpretation and multimarker monitoring rather than reliance on sedentary reference limits or isolated measurements.

Keywords: athletes; ferritin; creatine kinase; lactate dehydrogenase; testosterone; cortisol; training adaptation

1. Introduction

Biochemical monitoring is widely used in high-performance sport to evaluate health, adaptation, recovery, and potential maladaptation. Interpretation is challenging because habitual training, recent exercise, sex, sport type, diet, hydration, and analytical variation can shift biomarker distributions away from those observed in sedentary populations (Fallon, 2008; Mougios, 2007). Athlete-specific context is therefore essential when a laboratory result is used to guide training or clinical referral.

Iron is central to oxygen transport, mitochondrial metabolism, and erythropoiesis. Athletes may experience low iron stores through inadequate intake, gastrointestinal or menstrual losses, exercise-associated hemolysis, sweat loss, and hepcidin-mediated reductions in iron absorption after exercise (Badenhorst et al., 2021; Peeling et al., 2014; Sim et al., 2019). Endurance training can also expand plasma volume, lowering measured hemoglobin concentration without a proportional reduction in red-cell mass.

Female athletes have a particularly high prevalence of suboptimal iron stores, although iron depletion is also documented in male endurance and team-sport athletes (Coates et al., 2017; Dubnov & Constantini, 2004; Sims et al., 2022). Ferritin is useful for estimating iron stores, but it is an acute-phase reactant and should be interpreted with inflammation and training status in mind.

Creatine kinase (CK, historically termed CPK) and lactate dehydrogenase (LDH) are commonly measured as indirect indicators of muscle membrane disruption and metabolic stress. Their circulating concentrations can rise after unfamiliar, prolonged, or eccentric exercise; however, CK responses show marked interindividual variability and athlete reference limits are substantially higher than sedentary limits (Baird et al., 2012; Brancaccio et al., 2010; Mougios, 2007). Consequently, isolated enzyme elevations should not automatically be interpreted as pathological muscle damage.

Testosterone and cortisol reflect, respectively, important anabolic and stress-related endocrine processes. The testosterone-to-cortisol (T/C) ratio has been used to describe anabolic–catabolic balance, but its diagnostic specificity for overtraining is limited. Serial values collected under standardized conditions are more informative than a single measurement (Cadegiani & Kater, 2017; Urhausen et al., 1995; Urhausen & Kindermann, 2002). Earlier work in Indian swimmers demonstrated that training phase and load can influence testosterone, cortisol, and the T/C ratio (Majumdar & Srividhya, 2010).

Most studies examine iron status, muscle enzymes, or hormones separately, and many focus on one sex or one sport. Few investigations integrate these domains in a large athlete cohort stratified by the predominant aerobic or anaerobic demands of the sport. The present study therefore aimed to compare iron-profile variables, CK and LDH, testosterone, cortisol, and the T/C ratio among aerobic, anaerobic, and mixed-sport athletes, separately in males and females. It was hypothesized that aerobic athletes would demonstrate lower iron stores and a more catabolic resting profile, whereas anaerobic athletes would demonstrate higher CK and testosterone concentrations.

2. Methodology

2.1 Study design and participants

A cross-sectional comparative design was used. The analytical sample comprised 429 Indian athletes: 246 males and 183 females. Athletes were grouped according to the predominant energy-system demands of their sport. The male sample included 130 aerobic, 25 anaerobic, and 91 mixed aerobic–anaerobic athletes. The female sample included 66 aerobic, 10 anaerobic, and 107 mixed athletes. Sports with predominantly sustained oxidative demands were classified as aerobic; sprint/power-dominant sports were classified as anaerobic; and intermittent team or combat sports requiring substantial contributions from both systems were classified as mixed. The original study grouping included endurance disciplines such as distance events, cycling, rowing, swimming, and race walking; sprint events represented the anaerobic category; and sports such as hockey, water polo, and wushu were included in the mixed category.

Athletes included in the analysis were actively training and underwent laboratory evaluation as part of the study protocol. Individuals meeting the predefined exclusion criteria were excluded from the analysis. Ferritin below 12 ng/mL was one of the exclusion criteria.

2.2 Blood collection and biochemical analysis

Venous blood samples were collected and processed according to the laboratory's standardized protocol. Hemoglobin was determined using an automated hematology method. Serum ferritin, testosterone, and cortisol were measured using immunoassay procedures, whereas serum iron, total iron-binding capacity (TIBC), CK/CPK, and LDH were determined using routine automated clinical-chemistry methods. Transferrin saturation was calculated from serum iron and TIBC. All assays were performed under the laboratory's internal quality-control procedures

The iron profile comprised hemoglobin, ferritin, serum iron, TIBC, and transferrin saturation. The enzyme profile comprised CK/CPK and LDH. The hormone profile comprised testosterone, cortisol, and the T/C ratio. Values outside the laboratory limits applied in the original study were summarized as abnormal; low values were used for hemoglobin, ferritin, serum iron, transferrin saturation, testosterone, and the T/C ratio, whereas high values were used for TIBC, CK, LDH, and cortisol.

2.3 Statistical analysis

Results are presented as mean ± standard deviation and percentages. Separate one-way analyses of variance (ANOVA) were conducted in male and female athletes to test differences among aerobic, anaerobic, and mixed groups. When the omnibus ANOVA was significant, least significant difference

(LSD) post-hoc comparisons were performed. Statistical significance was set at $p < .05$; values of $p < .001$ are reported as such. Eta squared (η^2) was calculated from the reported sums of squares as SSbetween/SStotal to describe the proportion of variance associated with sport group.

2.4 Ethical considerations

The study protocol was reviewed and approved by the Institutional Review Board. Participant confidentiality was maintained by analysing de-identified data.

3. Results

3.1 Participant distribution

Table 1. Distribution of athletes by sex and sport-energy category

Sex	Aerobic	Anaerobic	Mixed	Total
Male	130	25	91	246
Female	66	10	107	183
Total	196	35	198	429

The cohort was weighted toward aerobic and mixed sports, with a comparatively small anaerobic subgroup, particularly among females.

3.2 Iron profile

Table 2. Iron profile in male athletes by sport-energy category

Parameter	Aerobic	Anaerobic	Mixed	F	p	η^2
Hemoglobin (g/dL)	15.19 ± 0.96	15.81 ± 0.98	15.49 ± 1.12	4.913	.008	.039
Ferritin (ng/mL)	36.33 ± 24.44	57.83 ± 32.80	55.89 ± 46.90	9.905	<.001	.075
Serum iron (µg/dL)	112.55 ± 29.69	126.22 ± 39.74	122.25 ± 34.55	3.345	.037	.027
TIBC (µg/dL)	362.85 ± 58.73	357.50 ± 61.10	360.04 ± 88.90	0.079	.924	.001
Transferrin saturation (%)	31.49 ± 8.65	35.12 ± 19.17	34.63 ± 18.64	4.303	.015	.034

In males, hemoglobin, ferritin, serum iron, and transferrin saturation differed significantly among groups. Aerobic athletes had the lowest mean values for all four markers. TIBC did not differ. The largest sport-group effect was observed for ferritin ($\eta^2 = .075$). Exploratory LSD comparisons indicated lower hemoglobin and ferritin in aerobic athletes than in both anaerobic and mixed athletes; serum iron and transferrin saturation were also lower in the aerobic group.

Table 3. Iron profile in female athletes by sport-energy category

Parameter	Aerobic	Anaerobic	Mixed	F	p	η^2
Hemoglobin (g/dL)	13.00 ± 0.84	13.74 ± 0.82	13.31 ± 0.92	4.247	.016	.045
Ferritin (ng/mL)	23.93 ± 20.60	39.80 ± 22.76	30.82 ± 23.98	3.103	.047	.033
Serum iron (µg/dL)	99.85 ± 35.49	119.95 ± 23.72	110.65 ± 42.88	2.064	.130	.022
TIBC (µg/dL)	374.97 ± 79.61	343.10 ± 57.90	357.89 ± 79.14	1.319	.270	.014
Transferrin saturation (%)	27.33 ± 9.66	35.20 ± 5.39	31.07 ± 9.93	4.614	.011	.049

In females, hemoglobin, ferritin, and transferrin saturation differed significantly among groups, with the aerobic group again showing the lowest means. Serum iron and TIBC did not differ significantly. Transferrin saturation had the largest sport-group effect among the female iron measures ($\eta^2 = .049$). Low ferritin was the most frequent iron-profile abnormality and occurred in 36.4% of aerobic, 20.0% of anaerobic, and 29.9% of mixed-sport females, compared with 15.4%, 4.0%, and 14.3%, respectively, in males.

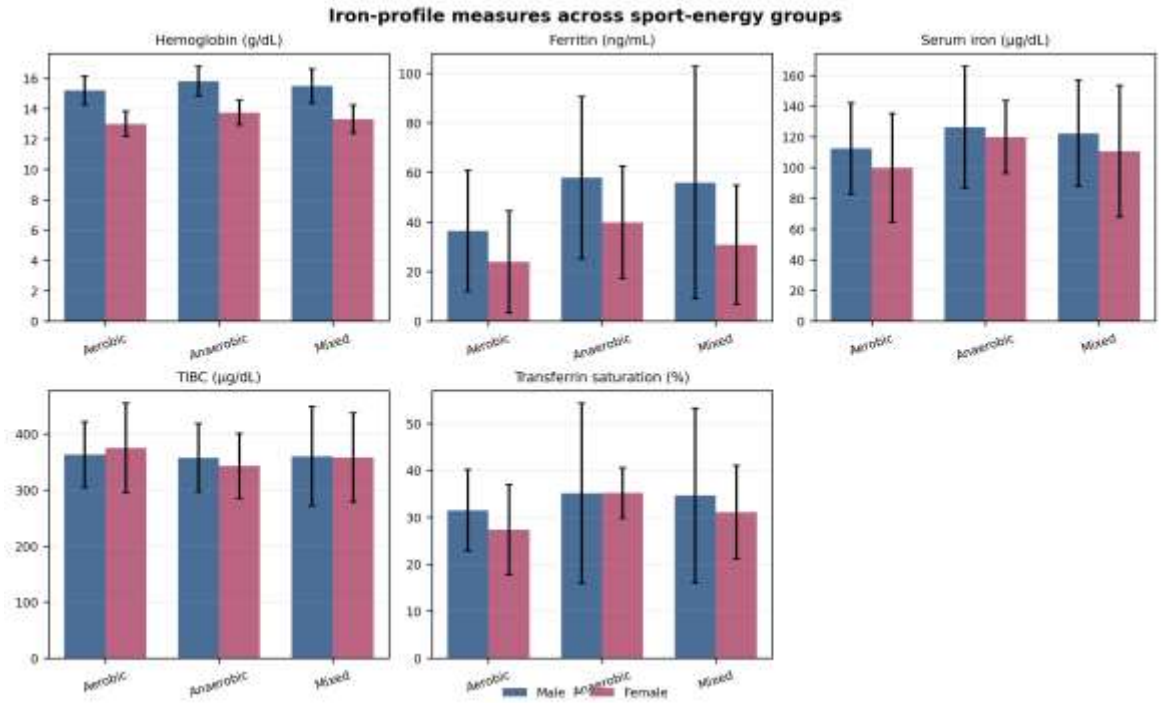

Fig 1: Mean ± SD iron-profile measures by sex and sport-energy category.

3.3 Muscle-enzyme profile

Table 4: Muscle-enzyme profile (IU/L) by sex and sport-energy category

Sex	Marker	Aerobic	Anaerobic	Mixed	F	p	η^2
Male	CK/CPK	267.0 ± 305.4	367.7 ± 197.5	320.2 ± 344.1	1.497	.226	.012
Male	LDH	435.3 ± 116.0	375.2 ± 139.1	387.4 ± 131.4	5.162	.006	.041
Female	CK/CPK	155.2 ± 110.9	262.2 ± 207.8	173.5 ± 183.4	1.896	.153	.021
Female	LDH	383.5 ± 141.6	236.2 ± 107.8	341.7 ± 118.1	6.565	.002	.068

CK was highest in anaerobic athletes of both sexes, but the between-group differences were not statistically significant. LDH differed significantly in both males and females and was highest in aerobic athletes. LSD comparisons showed that male aerobic athletes had higher LDH than anaerobic and mixed athletes. In females, all three pairwise LDH comparisons were significant. The female LDH effect (η^2 = .068) was larger than the male effect (η^2 = .041).

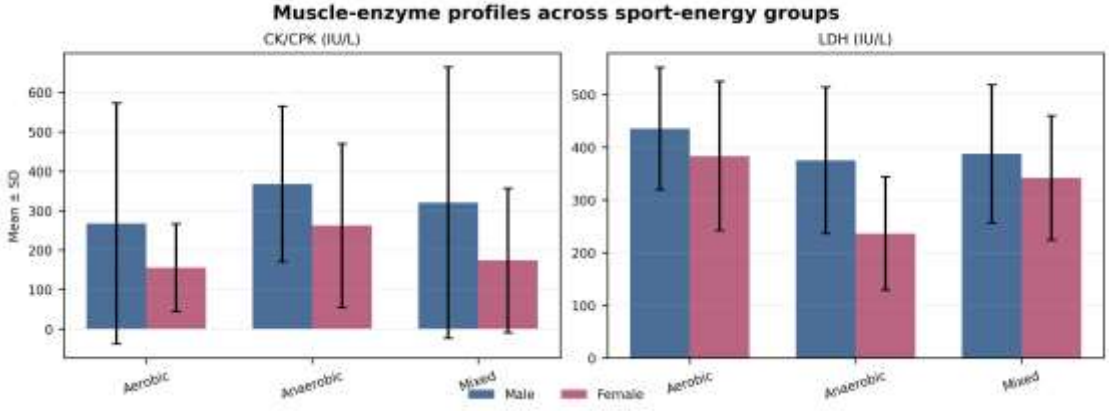

Fig 2: Mean ± SD CK/CPK and LDH concentrations by sex and sport-energy category.

3.4 Hormonal profile

Table 5. Hormonal profile by sex and sport-energy category

Sex	Marker	Aerobic	Anaerobic	Mixed	F	p	η²
Male	Testosterone	5.91 ± 4.31	8.38 ± 4.10	6.54 ± 4.20	3.620	.028	.029
Male	Cortisol	215.5 ± 178.9	181.8 ± 179.5	197.7 ± 153.6	0.563	.570	.005
Male	T/C ratio	0.0471 ± 0.050	0.0831 ± 0.060	0.0547 ± 0.050	4.941	.008	.038
Female	Testosterone	0.449 ± 0.620	0.913 ± 0.680	0.619 ± 0.550	3.519	.032	.038
Female	Cortisol	174.8 ± 116.4	87.22 ± 24.28	136.6 ± 113.5	3.930	.021	.042
Female	T/C ratio	0.0035 ± 0.006	0.0121 ± 0.012	0.0063 ± 0.007	8.339	<.001	.111

Male testosterone and the T/C ratio differed significantly among groups, whereas cortisol did not. In females, testosterone, cortisol, and the T/C ratio all differed significantly. Anaerobic athletes had the highest testosterone and T/C ratio in both sexes; aerobic athletes had the highest cortisol. The largest hormonal effect was observed for the female T/C ratio ($\eta^2 = .111$). Exploratory post-hoc results showed that female aerobic athletes had lower testosterone and T/C ratios than anaerobic athletes and higher cortisol than both anaerobic and mixed athletes.

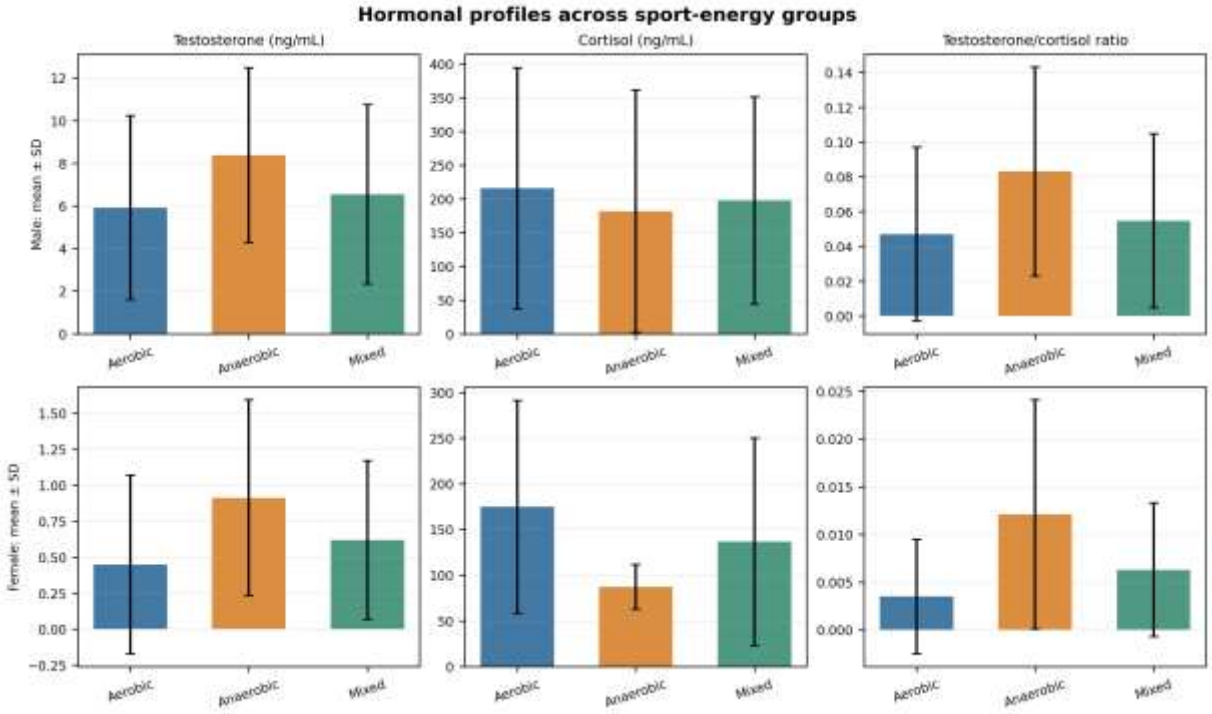

Fig 3: Mean ± SD testosterone, cortisol, and testosterone-to-cortisol ratio by sex and sport-energy category. Sex-specific panels are shown because the scales differ markedly.

3.5 Prevalence of values outside laboratory limits

Table 6. Percentage of athletes with selected values outside the laboratory limits

Sex	Classification	Aerobic (%)	Anaerobic (%)	Mixed (%)
Male	Low ferritin	15.4	4.0	14.3
Female	Low ferritin	36.4	20.0	29.9
Male	High CK/CPK	1.5	0	0.9

Female	High CK/CPK	3.1	0	2.2
Male	High LDH	9.15	0	0
Female	High LDH	0.8	0	0
Male	Low testosterone	13.1	12.0	12.1
Female	Low testosterone	6.2	0	5.6
Male	High cortisol	10.0	8.0	8.8
Female	High cortisol	3.0	0	2.8
Male	Low T/C ratio	16.9	12.0	8.8
Female	Low T/C ratio	13.6	0	6.5

The highest prevalence in the selected markers was low ferritin among female aerobic athletes (36.4%), followed by female mixed-sport athletes (29.9%). Aerobic groups also showed the greatest prevalence of high LDH and low T/C ratios. These percentages describe screening classifications and should not be treated as diagnoses without clinical assessment and repeat testing.

4. Discussion

The principal finding was a coherent sport-specific pattern across three biochemical domains. Compared with anaerobic athletes, aerobic athletes generally showed lower hemoglobin and ferritin, higher LDH, lower testosterone, and a lower T/C ratio. These differences were detectable in both sexes, although the statistical pattern varied. The results support the interpretation that habitual sport demands are associated with distinct biochemical distributions, but the cross-sectional design does not establish that training type caused the observed differences.

4.1 Iron status and sport demands

Aerobic athletes had the lowest mean hemoglobin, ferritin, serum iron, and transferrin saturation in both sexes. The result is consistent with reports that endurance athletes face multiple pathways of iron loss or restricted availability, including gastrointestinal loss, sweating, mechanical hemolysis, greater erythropoietic demand, and transient post-exercise increases in hepcidin (Peeling et al., 2014; Sim et al., 2019; Telford et al., 2003). Plasma-volume expansion may also lower measured hemoglobin concentration in trained endurance athletes without true loss of red-cell mass, reinforcing the need to interpret hemoglobin alongside ferritin, transferrin saturation, symptoms, inflammatory markers, and longitudinal trends.

Female athletes had lower mean ferritin than their male counterparts across all sport categories, and low ferritin was common in female aerobic and mixed-sport groups. This pattern agrees with previous studies reporting frequent iron depletion among female endurance and team-sport athletes (Badenhorst et al., 2021; DellaValle & Haas, 2011; Dubnov & Constantini, 2004; Sims et al., 2022). Menstrual blood loss, lower energy or iron intake, and low energy availability may contribute, but these factors were not assessed in the present study. Importantly, low ferritin alone does not establish iron-deficiency anemia. Hemoglobin, ferritin, transferrin saturation, inflammation, and clinical symptoms should be evaluated together, and supplementation should follow medical assessment rather than screening alone.

The sport-group effect was largest for ferritin in males and transferrin saturation in females. TIBC did not differ significantly in either sex, suggesting that stored and circulating iron indicators were more discriminating than binding capacity in this cohort. The relatively high variability in ferritin and transferrin saturation further indicates substantial between-athlete heterogeneity and supports individualized longitudinal monitoring.

4.2 Muscle enzymes

CK was numerically highest in anaerobic athletes but did not differ significantly among groups. High-force and eccentric contractions can increase sarcolemmal permeability and CK release, making a higher mean in power-oriented athletes physiologically plausible. Nevertheless, CK has wide biological and interindividual variation, and training status, ethnicity, muscle mass, recent exercise, and genetic factors

can strongly influence the response (Baird et al., 2012; Brancaccio et al., 2007; Mougios, 2007). The large standard deviations observed here reinforce the limited value of a single universal CK cutoff.

LDH was significantly higher in aerobic athletes of both sexes. Prolonged training can increase metabolic stress and alter membrane permeability, but total serum LDH is not muscle-specific. Exercise duration, recent workload, tissue distribution, and sampling time can all affect its concentration (Brancaccio et al., 2010). Thus, the observed LDH pattern is compatible with sustained endurance demand, but it should not be interpreted as direct evidence of muscle injury or overtraining.

4.3 Testosterone, cortisol, and the T/C ratio

Anaerobic athletes had higher testosterone and T/C ratios in both sexes, whereas aerobic athletes had higher mean cortisol. Resistance and high-intensity exercise can stimulate acute anabolic endocrine responses, while prolonged endurance work can alter hypothalamic–pituitary–gonadal and hypothalamic–pituitary–adrenal activity (Kraemer & Ratamess, 2005; Wheeler et al., 1991). The present cross-sectional findings are also consistent with longitudinal observations in Indian swimmers in which higher training load was associated with lower testosterone and T/C ratio and higher cortisol (Majumdar & Srividhya, 2010).

The female T/C ratio showed the largest effect in the hormone analysis. However, testosterone and cortisol have strong circadian, menstrual, nutritional, psychological, and training-phase influences. Assay sensitivity is particularly important for the lower testosterone concentrations typical in females.

Although the T/C ratio is often described as an anabolic–catabolic index, evidence does not support its use as a stand-alone diagnostic test for overtraining syndrome. Urhausen et al. (1995) proposed that it may better reflect current training strain, while later reviews emphasized the need to combine endocrine markers with performance change, symptoms, wellness, and longitudinal load data (Cadegiani & Kater, 2017; Urhausen & Kindermann, 2002). Accordingly, the present prevalence of low T/C values should be interpreted as a screening signal, not as the prevalence of overtraining.

4.4 Practical implications

The findings support a multimarker, context-specific monitoring model. For aerobic athletes, periodic assessment of hemoglobin, ferritin, transferrin saturation, and relevant clinical or dietary factors may help identify declining iron availability. For power athletes, CK should be interpreted against the athlete's own baseline and the timing of recent high-force training. Testosterone, cortisol, and the T/C ratio are most informative when collected repeatedly at the same time of day and interpreted with training load, wellness, performance, sleep, and nutritional status. Athlete-specific reference distributions may reduce false classification when sedentary limits are applied to well-trained populations (Fallon, 2008; Mougios, 2007; Nabhan et al., 2020).

4.5 Strengths and limitations

A major strength is the relatively large total cohort and simultaneous examination of iron, muscle-enzyme, and endocrine domains in both male and female athletes. Stratification by predominant sport energy demand provides a practical framework for sport-science interpretation. The use of effect sizes alongside p values also clarifies that statistically significant group differences were generally small to moderate, with the female T/C ratio showing the largest observed sport-group effect.

Several limitations require emphasis. The design was cross-sectional and group sizes were markedly unequal; the female anaerobic group contained only 10 athletes. Sport categories are broad proxies and cannot capture individual training content. LSD post-hoc tests provide limited protection against type I error when many outcomes are tested. Sex differences were not tested inferentially. Finally, the available summary-level data did not permit reassessment of assumptions, robust modelling, covariate adjustment, confidence intervals for effects, or independent verification of the abnormal-value classifications.

5. Conclusion

Iron, muscle-enzyme, and hormonal profiles differed according to the predominant energy demands of sport. Aerobic athletes generally displayed lower iron stores, higher LDH, and a less anabolic hormonal profile, whereas anaerobic athletes showed higher testosterone, T/C ratios, and numerically higher CK. Female aerobic athletes had the greatest prevalence of low ferritin. These findings support sex- and sport-contextualized interpretation of athlete blood tests and favour serial, multimarker monitoring over isolated measurements or uncritical use of sedentary reference limits. Future studies should use prospective

designs, standardized preanalytical conditions, balanced sport groups, inflammation-adjusted iron assessment, detailed menstrual and nutritional information, and concurrent performance and training-load measures.

Declarations

Ethics approval: The study protocol was reviewed and approved by the Institutional Review Board.
Conflict of interest: The authors declare no conflict of interest.

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