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# Effect of baseline ferritin levels on post-exercise iron metabolism in male elite youth rowers

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Iron metabolism plays a crucial role in athletic performance, and iron storage levels may influence its regulation in athletes. However, the potential mechanisms through which iron stores modify metabolic responses to intense exercise remain unclear. This study examined 16 trained male rowers from the Polish Youth National Team during the preparatory phase of training. Athletes were divided based on baseline serum ferritin (SF) levels: SF < 75 µg/L and SF > 75 µg/L. Participants performed a 2000-m exercise test on a rowing ergometer. Blood samples were collected before the test, after exercise, and after 1 h of restitution. The SF < 75 µg/L group showed a temporary post-exercise increase in serum iron, followed by a sharp decline after 1 h, while ferritin levels remained stable. In contrast, the SF > 75 µg/L group exhibited only a decrease in serum iron after 1 h, with a ferritin increase. No significant changes were observed in hepcidin and interleukin-6 levels. Differing post-exercise responses in iron and ferritin levels between groups suggest that baseline iron stores may influence the regulation of free iron. This could indicate the involvement of distinct iron chelation mechanisms depending on initial ferritin levels. Understanding these mechanisms could affect optimizing iron management strategies in elite endurance athletes.

As a functional component of hemoglobin and myoglobin, iron is involved in important physiological processes, among which are oxygen uptake and transport, energy production at the cellular level, and heme oxidative metabolism in skeletal muscle[1,2]. This micronutrient significantly impacts athlete's ability to train and their overall athletic performance, highlighting the importance of maintaining optimal iron levels to support these functions. However, assessing iron metabolism requires understanding its absorption, utilization, and storage mechanisms under exercise conditions[1].

Maintenance of iron homeostasis is closely linked to the role of a protein called ferritin. When iron levels are elevated, ferritin stores the excess iron, and when the stores decrease, it degrades, releasing the stored iron[3]. This reflects the body's total iron stores, and determining plasma iron concentrations has become a common practice for diagnosing iron deficiency, and monitoring athletes[4]. Iron deficiency affects more than 600 million people worldwide and is considered as one of the most common nutrient deficiencies[2]. Athletes are not exempt to this condition, on the contrary, it is not uncommon for them to be diagnosed with iron deficiency. However, the current literature lacks standardization of ferritin concentration values, representing the normal range for this protein among athletes[5]. Factors responsible for iron deficiency in athletes include haemolysis, increased demand, and heightened loss (through urine, sweat, and menstrual blood in female athletes). Moreover, cytokines and hormones influence iron levels in athletes. Elevated IL-6 levels increase liver production of hepcidin 3 to 6 h after exercise, negatively impacting iron transport and absorption and reducing iron uptake within the body[2]. For this reason, the effect of iron supplementation on athletes with iron deficiency has been studied for years. However, there are growing concerns about the side effects associated with iron supplementation[6]. Furthermore, the research results obtained so far are inconclusive, failing to support the conclusion that iron supplementation consistently enhances performance in individuals with iron-deficient endurance athletes. This can be explained by the various study protocols conducted (length and dosage), low effectiveness of iron absorption and different cut-off values for iron deficiency[4]. In addition, a meta-analysis by Rubeor (2018) suggests that iron supplementation is beneficial for athletes with ferritin < 20 µg/l more than those with higher protein levels[4].

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Furthermore, Galetti et al. (2021) showed that increased absorption of this element may be a sensitive and early indicator of a reduction in iron stores. Iron absorption increased in a group of young women with ferritin concentrations below 51.1 µg/l, which corresponded to a hepcidin level of 3.09 nmol/l. Above these values, iron absorption remained stable[7]. This could indicate that exercise-induced changes are modulated by iron levels in the body.

Analysis of the available literature has shown that resting serum ferritin levels can affect post-exercise changes in iron status in athletes. However, the underlying mechanism remains unclear due to the limited number of studies conducted to date[8,9]. According to Peeling et al. (2014), stored iron affects resting and post-exercise hepcidin concentrations. Authors observed that low iron stores suppressed the post-exercise hepcidin response, seemingly offsetting the exercise-induced increase. However, it is important to note that their study included male and female participants that took part in five different running sessions[8]. In contrast, Kortas et al. (2024) divided older participants (>60 years old) into two groups, taking the cut-off values of ferritin to be 75 ng/ml. A 12-week Nordic walking workout combined with 10 h of time-restricted eating resulted in significantly lower post-workout ferritin concentrations in the group with higher iron stores, with no significant changes in iron and transferrin concentrations[10]. Regardless, neither of the two studies[8,10] assessed iron metabolism in a homogeneous group of trained athletes.

This study aimed to compare the effects of acute exercise on the post-exercise response of iron regulatory markers in groups of athletes with suboptimal (SF < 75) and optimal (SF > 75) iron levels stores. Particular attention was given to analyzing the dynamics of post-exercise changes in the concentrations of key parameters of iron metabolism, including ferritin, iron, transferrin, hepcidin, IL-6 and hematological parameters, as well as their potential differences based on baseline ferritin levels. Hypothesis was proposed that the degree of exercise-induced changes in iron metabolism parameters differs between athletes with suboptimal iron status and those with optimal iron levels, which may indicate significant physiological implications in the context of the body's adaptation to exercise.

## Materials and methods
### Participants
A total of 16 trained men from the Polish Youth National Rowing Team were enrolled in this cohort study. Before exercise, the anthropometric parameters were assessed using an electronic scale to the nearest 0.05 kg (Tanita BC-980 MA, Tanita Corporation, Tokyo, Japan). The results are presented in Table 1. All athletes presented as medically fit, with no underlying health problems. Inclusion criteria included at least five years of training experience, absence of iron homeostasis disturbances (iron deficiency, anemia), and completion of the scheduled 2000-meter ergometer test in the study. Exclusion criteria consisted of: the presence of acute or chronic inflammation of pain disability, iron supplementation, and use of any anti-inflammatory drugs. In this group, baseline serum ferritin levels were considered, and the athletes were divided into two groups:

(1) Serum ferritin < 75 ng/ml (SF < 75): $n = 9$.
(2) Serum ferritin > 75 ng/ml (SF > 75): $n = 7$.

Various cut-off values appeared in the literature to determine an optimal ferritin level in athletes. Mielgo-Ayuso et al. (2018) have classified iron status using the following criteria: sufficient iron stores (ferritin > 100 µg/l), functional deficiency (ferritin 30–99 µg/l), and absolute deficiency (ferritin < 30 µg/l). Criteria also included transferrin saturation index and hemoglobin[11,12]. On the other hand, Kortas et al. (2024) have divided participants into two groups: baseline ferritin concentration low < 75 ng/ml and high level ≥ 75 ng/ml[10], and Garvican et al. (2014) have shown a value of 65 µg/l as a cut-off point[13]. Furthermore, Peeling et al. (2014) have determined athletes presenting with healthy serum ferritin levels (50–100 µg/L), and those which would be considered high in athletes (> 100 µg/l)[8]. Considering the study results of existing literature, the optimal ferritin level seems to range between 50 and 130 ng/ml. In addition, Galetti et al. (2021) showed that in healthy women there is increased iron absorption below the ferritin threshold value of 51,1 µg/l, indicating that higher cutoff values can be used for men with potential post-exercise inflammation[7]. Thus, 75 ng/ml ferritin seems appropriate as a cut-off value for observing differences in the post-exercise response between the groups studied.

### Study design
The rowing test was performed at the beginning of the preparatory phase of the annual training cycle (April), which is characterized by high volume and training load. After a 5-minute individual warm-up, the athletes performed a 2000-m exercise test on a rowing ergometer (Concept II, USA). Blood samples were taken from the antecubital vein at three-time points: before the exercise test (pre-exercise), directly after the test (post-exercise), and after a 1-hour recovery period (1 h recovery) [Fig. 1]. Athletes showed great motivation by completing the

| Parameters | SF < 75 ($n = 9$) | SF > 75 ($n = 7$) |
|---|---|---|
| Age (years) | 20,33 ± 0.94 | 19.67 ± 1.11 |
| Body height (cm) | 190.56 ± 3.74 | 187.71 ± 3.33 |
| Body mass (kg) | 88.27 ± 3.22 | 82.23 ± 8.62 |
| Training experience (years) | 6.50 ± 1.80 | 5.93 ± 1.08 |

**Table 1.** Characteristics of study participants ($N = 16$). Values are presented as the mean ± SD. $p > 0.05$.
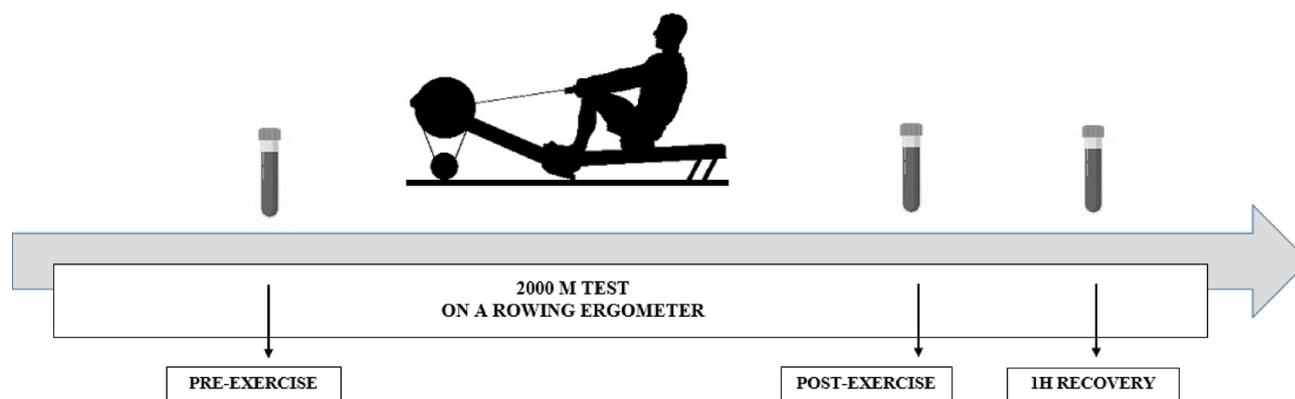
**Fig. 1**. Study design.

| Parameters | SF < 75 ($n = 9$) | SF > 75 ($n = 7$) |
|---|---|---|
| Test time (s) | 371.80 ± 5.50 | 379.41 ± 10.01 |
| Power output (W) | 436.33 ± 19.10 | 407.43 ± 36.13 |
| (W/kg) | 4.91 ± 0.33 | 5.05 ± 0.31 |
| $LA_{min}$ (mmol/L) | 2.26 ± 0.50 | 1.88 ± 0.59 |
| $LA_{max}$ (mmol/L) | 10.30 ± 1.37 | 10.76 ± 2.66 |

**Table 2**. Characteristics of the exercise test ($N = 16$). Values are presented as the mean ± SD. $p > 0.05$. LA-lactate.

| Days before the rowing test | | I | II | III | IV | V | VI | VII |
|---|---|---|---|---|---|---|---|---|
| Distance rowed | km/day | – | – | 30 | 20 | 18 | 20 | – |
| Time rowed | min/day | – | – | 150 | 90 | 80 | 100 | 40 |
| Training for force development | min/day | – | – | 70 | – | 80 | – | – |
| Extensive endurance rowing training time | min/day | 80 | – | 130 | 65 | 80 | 100 | 40 |
| High-intensity endurance rowing training time | min/day | 26 | – | 20 | 45 | – | – | - |
| Unspecific training (running, etc.) | min/day | 30 | 80 | 20 | 10 | 30 | 10 | 10 |
| Total training time | min/day | 136 | 80 | 240 | 120 | 190 | 110 | 50 |

**Table 3**. Training program 7 days before the rowing test.

test at maximum load in the shortest time possible, as the rowing test results were taken into account for selecting the championship team [Table 2]. The study was conducted by the principles of the Declaration of Helsinki and approved by the Bioethical Committee at the Poznan University of Medical Science, Poland (decision no. 685/23 in 2023), and was registered on clinicaltrials.gov under NCT06878040. All procedures and potential risks were discussed with the participants before the study. Informed consent was obtained from participants and their parents or legal guardians before participation in the study.

Daily records were kept documenting the characteristics of the training profile, including intensity, volume (measured in min.), and type. Training types were categorized as specific—such as rowing, which includes endurance, technical elements, and speed—or nonspecific, encompassing strength training and jogging. Intensity of the workouts was assessed to the lactate (LA) threshold of 4 mmol/L, which allowed for classification into extensive (below the LA threshold) and intensive (above the LA threshold) workloads, as detailed in Table 3. All national team members were required to adhere to the established training schedule as specified in the training plan.

### Food intake
Participants recorded their total dietary intake the day before and the day of the test. They were instructed to log their meals and beverages, including quantities, with assistance from a dietitian at all meal times. Then, various nutritional elements, such as energy, protein, fat, carbohydrates, fiber, and iron content, were measured using DietetykPro, a software developed by DietetykPro in Wrocław, Poland. Participants ate at the same canteen, where the dietitian had access to recipes for each dish, which helped assess the quality and quantity of their food consumption.

## Blood analysis

Polyethylene tubes containing dipotassium ethylenediaminetetraacetic acid (EDTAK2) anticoagulant were used to perform a complete blood count. Red blood cell indices, such as red blood cell count (RBC), hemoglobin (Hb), hematocrit (HCT), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), and red cell distribution width (RDW) were analyzed using the MYTHIC 18 hematology analyzer (Orphee Medical, Geneva, Switzerland).

Polyethylene clotting activator tubes were centrifuged to separate the morphotic elements from the serum using a centrifuge (3000 rpm for 10 min). Serum was pipetted into several Eppendorf tubes, which would then be frozen (temp. -80 °C). Biochemical parameters (transferrin, ferritin, hepcidin) were determined from the extracted serum using the ELISA (SunRed Biotechnology Company, Shanghai, China) method according to the test manufacturer's instructions. Assay range was 0,05–15 mg/ml for transferrin, 2,84–800 ng/ml for ferritin, and 7-1500 ng/ml for hepcidin, 1-300 ng/l for IL-6. Iron was determined using the colorimetric method on the SPECTROstar Nano reader. LA concentration was determined from the capillary blood immediately after collection using a commercially available kit (dr. Lange, Germany).

## Sample size

Representative study population: the sample size was calculated using a G-power program[14] for iron[15], assuming 0.05 as the alpha level, 0.62 as the effect size, and 0.9 power analyses. Minimum sample size was estimated at 14 participants. However, due to possible dropout, we decided to qualify all rowers who met the inclusion/exclusion criteria for the study.

## Statistical analyses

GraphPad Prism 10 software (GraphPad Software, Boston, USA) was used for statistical analyses and graphical representations. Descriptive statistics, including the mean and standard deviation (SD), were calculated to visualize trends and patterns at three different time points. Shapiro-Wilk test assessed the normal distribution of variables. Next, Spearman's test for heteroscedasticity evaluated the equality of variances between the two groups. To examine the differences among the groups and the measured variables across the three-time points (Baseline, Post, and 1-hour recovery), a two-way analysis of variance with repeated measures (ANOVA) was conducted, along with Tukey's post hoc analysis, with or without Greenhouse-Geisser correction as necessary. T-test was used for nutrition outcomes. For the effect size measure, Cohen`s d was calculated. Using Cohen`s criteria, the effect size was interpreted as small (0.2), moderate (0.5), and large (0.8)[16].

# Results

## Iron status

Serum iron levels, ferritin, transferrin, IL-6, and hepcidin are presented in Fig. 2. Iron serum concentration significantly increased after exercise ($p = 0.0026$, Cohen`s d = 2.67, pre-exercise vs. post-exercise) and decreased in the 1 h recovery ($p = 0.0013$, Cohen`s d = 1.68, pre-exercise vs. 1 h recovery; $p = 0.0002$, Cohen's d = 4.05, post-exercise vs. 1 h recovery) in SF < 75. Iron level decreased 1 h after exercise in SF > 75 ($p = 0.0074$, Cohen`s d = 2.54, post-exercise vs. 1 h recovery). Nevertheless, both groups exhibited the same general pattern of transient iron mobilization followed by a decline during recovery. There were no between-group differences for serum iron levels ($p=0.5160$, Cohen's d = 0.36, $p = 0.2612$, Cohen's d = 0.64 and $p=0.6385$, Cohen's d = 0.28, respectively) at either time point. Serum ferritin was significantly increased after exercise in the SF > 75 ($p = 0.0026$, Cohen`s d = 1.23, pre-exercise vs. post-exercise); in the following 1 h of restitution, the ferritin level decreased and reached values close to the baseline ($p = 0.0003$, Cohen`s d = 1.45, post-exercise vs. 1 h recovery). No significant changes in serum ferritin levels were observed in SF < 75, although a similar pattern of changes was noted as in the second group. Significant between-group differences were evident for baseline ($p<0.0001$, Cohen`s d = 4.31), post-exercise ($p<0.0001$, Cohen`s d = 3.28), and recovery ($p<0.0001$, Cohen`s d = 3.75) serum ferritin levels. Transferrin level decreased after 1 h recovery in both groups (SF < 75: $p = 0.0380$, Cohen`s d = 2.00, pre-exercise vs. 1 h recovery; SF > 75: $p = 0.0203$, Cohen`s d = 0.51, pre-exercise vs. 1 h recovery). There were no between-group differences for serum transferrin levels ($p=0.6626$, Cohen`s d = 0.25, $p = 0.3816$, Cohen`s d = 0.58, and $p=0.6065$, Cohen`s d = 0.52, respectively). However, there were between-group differences for baseline serum hepcidin levels ($p=0.0329$, Cohen`s d = 1.73, pre-exercise vs. pre-exercise). No significant changes in the serum concentration of serum IL-6 ($p=0.1336$, Cohen`s d = 1.04, $p = 0.1703$, Cohen`s d = 0.92, and $p=0.0852$, Cohen`s d = 1.2, respectively) at either time point. No significant changes in the serum concentration of hepcidin and IL-6 were observed in both groups. However both groups show the same pattern of changes.

## RBC indices

Among the blood parameters examined, a significant increase in RBC after exercise was observed compared to the baseline values ($p<0.0001$, Cohen`s d = 1.60, pre-exercise vs. post-exercise) and decreased in the 1 h recovery ($p = 0.0111$, Cohen`s d = 0.40, pre-exercise vs. 1 h recovery; $p<0.0001$, Cohen`s d = 1.67, post-exercise vs. 1 h recovery) in SF < 75. Similarly, RBC significantly increased after exercise ($p = 0.2607$, Cohen`s d = 0.00, pre-exercise vs. post-exercise) and decreased 1 h after exercise ($p = 0.0016$, Cohen`s d = 1.15, pre-exercise vs. 1 h recovery; $p<0.001$, Cohen`s d = 1.30, post-exercise vs. 1 h recovery) in SF > 75. HGB was significantly increased after exercise in the SF < 75 ($p<0.0001$, Cohen`s d = 1.33, pre-exercise vs. post-exercise) in the following 1 h of restitution, the HGB decreased ($p = 0.0022$, Cohen`s d = 0.77, pre-exercise vs. 1 h recovery; $p<0.001$, Cohen`s d = 1.88, post-exercise vs. 1 h recovery). Similarly, HGB significantly increased after exercise ($p = 0.2610$, Cohen`s d = 0.48, pre-exercise vs. post-exercise) and decreased 1 h after exercise ($p=0.0003$, Cohen`s d = 0.95, pre-exercise vs. 1 h recovery; $p<0.001$, Cohen`s d = 1.34, post-exercise vs. 1 h recovery) in SF > 75. HCT significantly increased after exercise ($p<0.0001$, Cohen`s d = 2.27, pre-exercise vs. post-exercise) and decreased in the 1 h
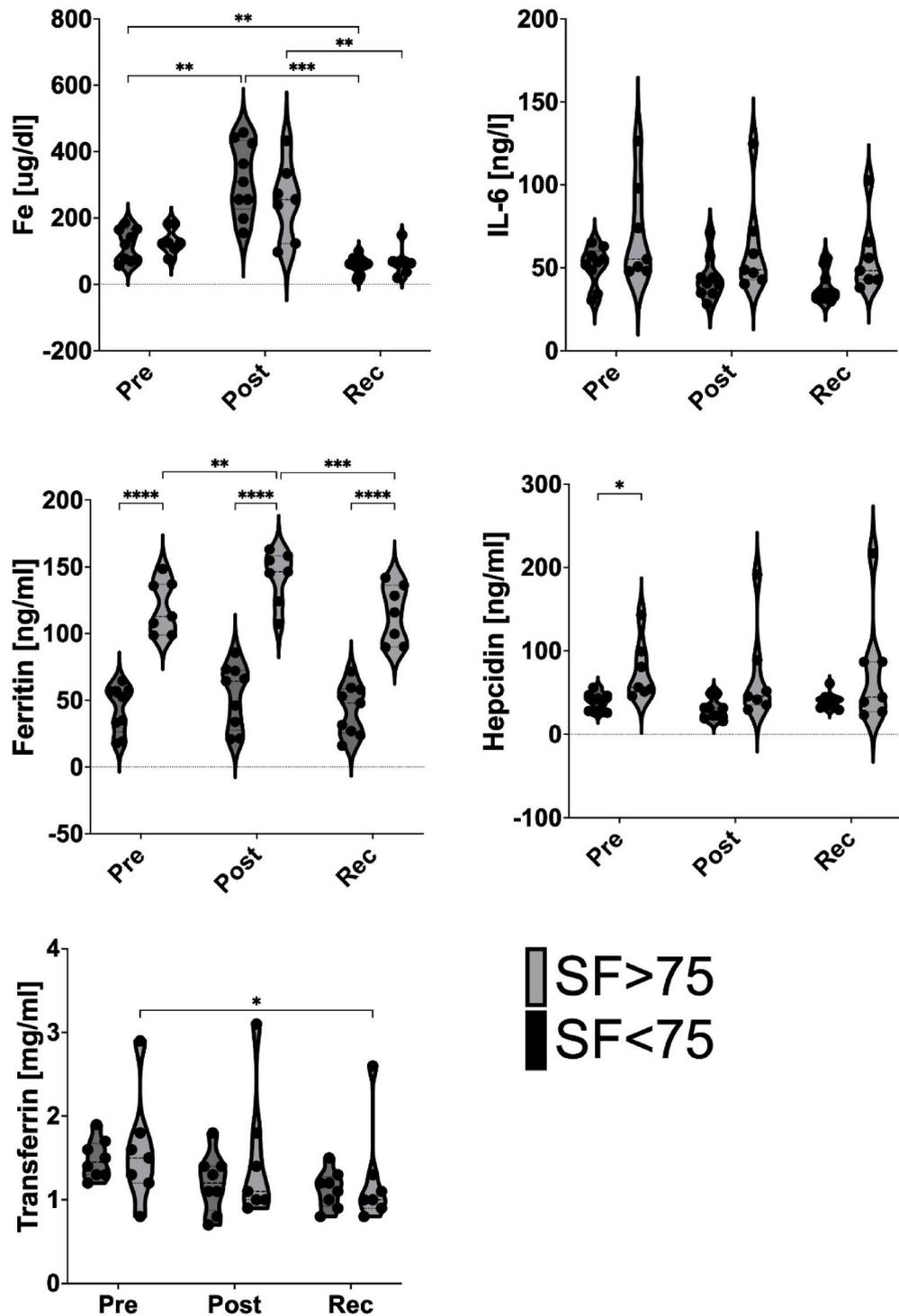
**Fig. 2**. The effect of intense exercise on iron, ferritin, transferrin, IL-6, and hepcidin serum concentration. *Note* Fe- iron, IL-6-interleukin-6, PRE-pre-exercise, POST-post-exercise, REC-1 h recovery. Significant differences: *<0.05, **<0.01,***$p < 0.001$, ****$p < 0.0001$.

recovery ($p = 0.0096$, Cohen`s d = 0.81, pre-exercise vs. 1 h recovery; $p < 0.001$, Cohen`s d = 2.38, post-exercise vs. 1 h recovery) in SF < 75. Similarly, HCT significantly increased after exercise ($p = 0.0099$, Cohen`s d = 1.11, pre-exercise vs. post-exercise) and decreased 1 h after exercise ($p = 0.0020$, Cohen`s d = 1.08, pre-exercise vs. 1 h recovery; $p < 0.001$, Cohen`s d = 2.32, post-exercise vs. 1 h-recovery) in SF > 75. MCV significantly increased

| Parameter | SF < 75 | | | SF > 75 | | |
|---|---|---|---|---|---|---|
| | Pre-exercise | Post-exercise | 1 h Recovery | Pre-exercise | Post-exercise | 1 h Recovery |
| RBC ($10^6 \times \mu L^{-1}$) | 5.04 ± 0.23 | 5.41 ± 0.31 [a] | 4.89 ± 0.28 [b, c] | 5.06 ± 0.26 | 5.15 ± 0.20 | 4.85 ± 0.26 [b, c] |
| HGB (g/dL) | 15.70 ± 0.58 | 16.68 ± 0.92 [a] | 15.17 ± 0.70 [b, c] | 16.10 ± 0.62 | 16.36 ± 0.64 | 15.37 ± 0.85 [b, c] |
| HCT (%) | 44.26 ± 1.53 | 48.46 ± 2.65 [a] | 42.79 ± 2.17 [b, c] | 45.37 ± 1.74 | 47.03 ± 1.13 [a] | 43.39 ± 1.98 [b, c] |
| MCV (fl) | 87.88 ± 3.34 | 89.67 ± 3.54 [a] | 87.60 ± 3.26 [c] | 89.79 ± 3.09 | 91.43 ± 3.11 [a] | 89.61 ± 3.03 [c] |
| MCH (pg) | 31.17 ± 1.18 | 30.86 ± 1.01 | 31.06 ± 1.17 | 31.86 ± 1.04 | 31.79 ± 1.11 | 31.74 ± 1.30 |
| RDW (%) | 12.13 ± 0.35 | 12.43 ± 0.55 | 12.68 ± 0.36 [b] | 11.59 ± 0.48 | 11.70 ± 0.45 | 11.96 ± 0.45 |

**Table 4**. Changes in RBC parameters during acute exercise. Values are presented in the mean ± SD; [a] pre-exercise vs. post-exercise; [b] pre-exercise vs. recovery; [c] post-exercise vs. recovery ($p < 0.05$). RBC, red blood cell count, HGB, hemoglobin, HCT, hematocrit; MCV, mean corpuscular volume, MCH, mean corpuscular hemoglobin, RDW, red cell distribution width.

| | Energy | Protein | Fat | Carbohydrates | Fiber | Iron content |
|---|---|---|---|---|---|---|
| | [kcal/kg] | [g/kg] | [g/kcal] | [g/kg] | [g] | [mg] |
| SF < 75 | 52.16 ± 13.25 | 2.48 ± 0.32 | 1.40 ± 0.47 | 7.12 ± 2.98 | 29.84 ± 16.83 | 18.65 ± 4.20 |
| SF > 75 | 44.08 ± 13.78 | 2.20 ± 0.60 | 1.20 ± 0.36 | 6.22 ± 2.89 | 24.82 ± 8.90 | 20.25 ± 8.36 |
| SF < 75 | 8.60 ± 5.59 | 0.52 ± 0.19 | 0.34 ± 0.14 | 1.48 ± 0.79 | 7.23 ± 4.52 | 6.80 ± 3.59 |
| SF > 75 | 14.63 ± 6.14 | 0.58 ± 0.18 | 0.35 ± 0.16 | 1.88 ± 0.94 | 7.24 ± 4.80 | 6.66 ± 1.63 |

**Table 5**. Food intake the day before the test and the morning before the test. Values are presented in the mean ± SD. $p > 0.05$.

after exercise and decreased after 1 h recovery ($p < 0.0001$, Cohen`s d = 0.53, pre-exercise vs. post-exercise; $p < 0.001$, Cohen`s d = 0.62, post-exercise vs. 1 h recovery) in SF < 75. Similarly, MCV significantly increased after exercise ($p < 0.0001$, Cohen`s d = 0.52, pre-exercise vs. post-exercise) and decreased 1 h after exercise ($p < 0.001$, Cohen`s d = 0.59, post-exercise vs. 1 h recovery) in SF > 75. No significant changes in the serum concentration of serum MCH in both group. RDW slightly decreased in the 1 h after exercise ($p = 0.0161$, Cohen`s d = 1.73, pre-exercise vs. 1 h recovery) in SF < 75. Significant between-group differences were evident for baseline ($p = 0.0271$, Cohen`s d = 1.28), post-exercise ($p = 0.0038$, Cohen`s d = 1.33), and recovery ($p = 0.0044$, Cohen`s d = 1.65) RDW; however, there were no between-group differences for other RBC parameters levels at either time point (Table 4).

### Food intake
There were no significant differences in the average intake of energy, protein, fat, carbohydrates, and fibre between SF < 75 and SF > 75 (Table 5).

### Discussion
Intense exercise can lead to an increase in iron levels after physical activity. This is thought to be mainly due to hemolysis and rhabdomyolysis, which are associated with coexisting inflammation[17]. The released iron is toxic, as it participates in the Fenton reaction, where ferrous iron ($Fe^{2+}$) reacts with $H_2O_2$, resulting in the formation of the hydroxyl radical, one of the most reactive oxygen radicals. By entering the fluids and tissues surrounding the inflammation, it causes significant cellular damage by reacting with cellular components[18]. For this reason, mechanisms aimed at chelating the free pool of iron from the plasma play a crucial role.

When comparing the obtained ferritin concentration results to the changes in iron levels, a different response to the stimulus, i.e., physical exercise, can be observed in both groups. In the case of SF < 75, an increase in iron concentration was observed immediately after the test, followed by a significant decrease after 1 h of recovery, while no significant changes in ferritin concentration were noted over time. In the SF > 75 group, only a significant decrease in iron concentration was observed after one h of restitution, which was accompanied by an increase in ferritin concentration in the post-exercise response. However both groups show the same pattern of changes [Fig. 2]. Considering that ferritin induction in macrophages is regarded as an essential element of the post-exercise response of the organism due to its role in the regulation of iron balance as scavengers of old and damaged red blood cells[19], and the fact that iron stored in ferritin does not participate in redox reactions, making it considered a safe source of this element[20]. It can be assumed that in the SF > 75 group, an increase in ferritin level occurred in response to physical exercise, which probably prevented a significant post-exercise increase in the pool of available iron. On the one hand, free iron ions can increase the production of iron-dependent free radicals, particularly in skeletal muscles during intense exercise. Consequently, ferritin-mediated iron binding may act as a protective mechanism against the redox toxicity associated with this element[21,22]. Conversely, research indicates that higher iron levels are linked to a greater release of pro-inflammatory cytokines, including follistatin-like protein 1 (FSTL-1) and IL-6, during exercise. This implies that iron chelation might also influence the inflammatory response following exercise[23]. In the present study, no significant differences in IL-6 levels were observed between the groups; however, it is possible that the timing of IL-6 release was missed due to the

study's sampling schedule. In the group with lower iron stores, however, the mechanisms aimed at the chelation of free plasma iron were activated more slowly, as the reduction in iron concentration was observed only after an hour of restitution. Furthermore, it can be assumed that this process took place without the participation of ferritin. The increase in iron levels in cells leads to both enhanced ferritin synthesis and reduced expression of the transferrin receptor (TfR), a protein responsible for iron transport into the cell[10]. The transferrin receptor (sTfr), anchored in the cell membrane, enables the endocytosis of iron from transferrin protein into erythroid cells. Thus, it serves as a sensitive marker for functional iron metabolism and erythropoietic activity, with levels rising during iron deficiency or increased iron turnover. Unlike ferritin, sTfr concentrations remain unaffected by inflammation, rendering them a dependable indicator of iron deficiency even in inflammatory states[24]. In both groups, a significant decrease in transferrin concentration was observed after one h of restitution compared to the resting values [Fig. 2]. In the SF > 75 group, this can be explained by the immediate increase in the level of ferritin, which was the first to chelate the free pool of iron, which resulted in a limitation of iron transport by transferrin and, consequently, a decrease in its concentration[25]. In the second group, similar changes in transferrin levels were observed; however, this may be the result of the action of other unstudied proteins involved in chelating this element (e.g., hemosiderin, lactoferrin, haptoglobin, or hemopexin)[26].

Ferritin and transferrin are acute-phase proteins, playing a crucial role in inflammatory processes. However, in this study, the increase in ferritin and decrease in transferrin do not seem to be part of the acute phase response, as no significant changes in IL-6 levels were observed in either group [Fig. 2][2]. Moreover, the athletes studied performed intense but short physical exercises. Literature data confirm that the IL-6 response is significantly less pronounced in the case of short, intense physical efforts compared to long-term activities such as marathons or triathlons[27,28]. It is worth emphasizing that the examined rowers are a highly trained group, characterized by high training adaptation, which probably contributed to the reduction of both resting and post-exercise IL-6 concentration[29]. Our study confirms previous reports that IL-6 magnitude depends on many factors, including the level of training, duration, and intensity of the physical exercise[30].

In addition, the study observed a higher concentration of hepcidin in the SF > 75 group compared to the SF < 75 group [Fig. 2]. Obtained result is consistent with previous reports that showed a strong positive correlation between baseline ferritin levels and serum hepcidin concentration in athletes[8]. Decrease in hepcidin activity is thought to result in enhanced iron absorption from the intestines and its recycling from macrophages involved in iron clearance, consequently increasing the pool of iron available for erythropoiesis[9]. Described protective effect seems to apply to athletes with low iron stores, in the case of this study, in the SF < 75 group.

However, the magnitude of the hepcidin response to exercise appears to depend on multiple factors, including resting iron status (i.e., ferritin levels), expression of the proinflammatory cytokine IL-6, as well as energy availability, and carbohydrate intake. Peeling et al. (2017) showed no post-exercise changes in hepcidin levels in athletes with low resting ferritin levels (< 30 μg/l), while they observed an increase in its level after exercise in those with higher ferritin levels at the beginning of the study[9]. This suggests that post-exercise changes in hepcidin levels may depend on ferritin levels and thus, on body iron stores. Therefore, normalization of these parameters seems necessary to further investigate the effect of physical exercise on iron metabolism via the iron-hepcidin relationship[31]. On the other hand, studies conducted so far have shown that the level of hepcidin expression increases under the influence of inflammation. This is described as a host defense mechanism that seeks to limit the availability of iron to pathogens that rely on this element. Available literature suggests that homeostatic regulation might be a more significant regulator of hepcidin expression than inflammation[32]. It should also be emphasized that iron status is strongly related to the diet of athletes. Compared to the group with normal energy intake, an increased post-exercise hepcidin response is observed in athletes with energy deficiency[33,34]. Furthermore, Solberg et al. (2023) demonstrated an increased hepcidin response with low energy availability (LEA) as well as with low carbohydrate intake[35].

Observed lack of significant changes in hepcidin concentration after exercise in both groups [Fig. 2], may suggest that in the studied athletes, the regulatory mechanism via the IL-6-hepcidin axis is more potent than homeostatic regulation. On the other hand, Dominquez et al. (2018), in their review, indicate that physical exercise causes an increase in serum hepcidin concentration mainly after three hours of restitution, but the response may be faster, as in several studies it occurred immediately after exercise[31]. This may indicate that in our study, we may not have captured the moment of the highest secretion of this protein because the blood was collected after an hour of restitution. Moreover, the analysis of daily energy intake and the amount of carbohydrates supplied with food (the whole day before the test, and in the morning before the test) did not reveal any significant differences between the groups, which allows to exclude their influence on the obtained results. Therefore, it is difficult to evaluate the hepcidin results obtained in this study unequivocally.

In the SF < 75 group, an increase in RBC and HGB was observed in response to physical exercise, followed by a significant decrease. In comparison, in the SF > 75 group, only a decrease in these parameters was observed after an hour of restitution. Changes in HCT levels were the same in both groups, first increasing and then decreasing after restitution [Table 5]. Kilic et al. (2004) observed similar results to ours, who explained the post-exercise increase in RBC levels as a consequence of hemoconcentration[36]. In contrast, Abbasciano et al. (1998) showed a reduction in red blood cell count after physical exercise[37], while Spiropoulos et al. (2003) did not report any changes in concentration after a marathon[38]. The state of knowledge regarding changes in RBC indices remains inconsistent, with variations observed depending on the type and duration of training[39]. On one hand, intense physical exercise can trigger an inflammatory response, often accompanied by a reduction in serum iron levels, which limits Iron availability to the bone marrow is restricted, reducing RBC production. This may result in lower RBC and HGB levels while maintaining normocytic and normochromic red blood cells — a condition described in the literature as anemia of inflammation[39]. On the other hand, it has been shown that intense exercise causes plasma volume shifts, which may result in pseudoanemia, also known as dilutional or sports anemia, a condition that does not negatively affect athletic performance. It is characterized by reduced

HGB, RBC, and HCT values (parameters influenced by plasma volume), without significant changes in other red cell indices[40]. The alterations in red blood cell parameters observed after exercise in this study may be attributed to hemoconcentration. However, a one-hour recovery period may be too short to detect post-exercise changes in erythropoiesis. This study focused on differences in post-exercise red blood cell indices between the groups. The effect of physical exertion on the recovery of erythropoiesis could be a promising subject for future research.

The two groups differed in their iron stores, suggesting potential differences in the intensity of erythropoiesis between them. However, the lack of significant differences in red blood cell indices such as RBC, HB, and HCT may stem from the higher resting hepcidin levels observed in the group with serum ferritin levels above 75 µg/L. The elevated concentration of this protein likely reduced the availability of iron for erythropoiesis, which may explain the lack of significant variation between the groups[9]. Moreover, a significant increase in MCV was observed in both groups, followed by a significant decrease [Table 5]. It seems significant that in all examined players MCV was > 83 fl. at three time points, which indicates the absence of microcytosis in both groups[37]. This may confirm that all rowers who entered the study had a balanced iron status that remained stable and within the appropriate range.

## Conclusion

We assumed that changes in iron status in response to physical exercise in an athlete with iron deficiency might differ from those in an athlete with optimal iron levels. However, in our study, we did not observe significant differences between the analyzed groups, except for baseline hepcidin levels. These results may be due to the use of an inappropriate ferritin value range as a classification criterion for the participants in observing adaptation. Additionally, the short duration of the protocol and the limited number of participants may have influenced the outcomes. Another limitation of this study is that sTfR levels were not measured. Future research could consider including sTfR as a parameter not influenced by inflammatory status to provide a more accurate and comprehensive assessment of iron status. Findings suggest that future studies should include a larger group of athletes and a greater number of blood sampling points. This would enable the division of the study population into more subgroups, which in turn could allow for a more precise assessment of changes within narrower ferritin concentration ranges, as well as the observation of a greater number of changes over a longer period of time. Although classical repeated measures ANOVA was applied in this study to assess the time-dependent changes in iron-related parameters, we acknowledge that more advanced modeling techniques, such as linear mixed-effects models, would offer greater flexibility and allow for the inclusion of relevant covariates (e.g., serum ferritin, IL-6) in the analysis. However, due to the limited sample size in our study and the risk of overfitting, we opted for a more conservative approach to ensure the robustness and interpretability of our results. Future studies with larger cohorts will allow for the implementation of more refined statistical models that can better account for individual variability and complex interdependencies among iron metabolism markers.

Although there are no significant differences in most of the analyzed parameters, the differing dynamics of iron and ferritin concentration changes between the groups suggest that the mechanisms regulating the chelation of the iron pool may depend on baseline iron status. This process seems to initiate more quickly in athletes with higher iron stores, likely due to ferritin. However, the mechanism in the group with lower iron status remains unclear. To achieve a more detailed characterization, it would be necessary to expand the study to include an analysis of other proteins involved in iron homeostasis, such as lactoferrin or ferroportin. A better understanding of these mechanisms could have implications for optimizing supplementation and dietary strategies among elite endurance athletes, particularly in preventing iron deficiencies and maximizing training effectiveness adaptations.

## Data availability

Due to ethical concerns, the datasets generated and/or analyzed during the current study supporting data cannot be made openly available; however, they are available from the corresponding author upon reasonable request.

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## Author contributions

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## Declarations

## Competing interests

## Ethical approval and consent to participate

The study was conducted following the Declaration of Helsinki, and its protocol was approved by the local Ethics Committee at Poznań University of Medical Sciences (decision no. 685/23 in 2023). All procedures and potential risks were discussed with the participants before the study. Informed consent was obtained from all parents or legal guardians and participants before participation in the study.

## Additional information

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