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## **High level of serum hepcidin in the group of juniors training canoeing - preliminary report**

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### **Abstract**

#### **Introduction**

The results of biological and clinical studies available in the literature indicate a relationship between physical effort and iron deficiency with coexisting microcytic anemia (iron deficiency anemia) or without anemia (IDNA; iron depletion without anemia). Currently, due to unfavourable clinical consequences for athletes, both IDA and IDNA, i.e. reduced endurance and reduced energy efficiency, intensive research is underway to determine the mechanism of these disorders. In the light of recent studies on biological activity of hepcidin, it is believed that hepcidin can play an important role in the pathogenesis of syderopenia in athletes. Therefore, the aim of the study was to assess the hepcidin concentration against the background of iron management parameters in young junior team members training canoeing in high-performance kayaking.

#### **Objective**

The aim of this study was to evaluate the concentration of serum hepcidin and selected blood morphology parameters in the group of junior canoeists.

## Material and methods

The study group consisted of 29 young athletes (20 boys and 9 girls) participating in a training camp at the Central Sports Centre in Wałcz. The average age of participants of both boys and girls was  $16.5 \pm 0.6$  years.

## Results

Results of this study showed that the average hepcidin concentration in the study group was higher than that observed by other authors for healthy peers. In addition, in 8 juniors (including 6 boys and 2 girls), the hepcidin concentration was above the adult reference range. There was no statistically significant difference in hepcidin concentration between boys and girls. However, a wide range of individual hepcidin values was observed in both groups. In addition, ferritin levels were below normal with 8 patients. A positive correlation between hepcidin and ferritin has been shown.

## Conclusion

The results obtained in the study indicate the hepcidin could be a major contributor to poor iron status observed in the athlete trains professionally. Our research suggests it is necessary to monitor the diet of young athletes with respect to iron content and its proper bioavailability.

**Key words: hepcidin, physical activity, juniors**

## Introduction

The results of biological and clinical studies available in the literature indicate a relationship between physical effort and iron deficiency with coexisting microcytic anaemia (iron deficiency anaemia) or without anaemia (IDNA; iron depletion without anaemia) [2,3,5,9,23,25,31]. The prevalence of iron deficiency (expressed as low ferritin concentration) among people practising competitive training is at a relatively high level of 25-50%. The problem of iron deficit is particularly present in the following disciplines: endurance, sports competitions requiring low body weight, athletes on a vegetarian diet, women, and athletes training in alpine conditions [2,9,23]. Etiopathogenesis of iron deficiency in athletes is most often multifactorial [2,9,23]. The influence of physical activity on systemic iron homeostasis is associated, among other things, with increased production of lactates during maximum physical activity, which in turn results in a decrease in serum iron concentration, limiting oxygen metabolism of muscles. In addition, exercise stress leads to an increase in the release of IL-1 associated with lactoferrin, increasing serum levels. On the other hand, lactoferrin, showing higher affinity, forms complexes with Fe ions reducing the concentration of this element in the functional pool. Another important factor (associated with intensive physical effort) contributing to the development of iron deficiency is post-workout hemolysis of red blood cells, which intensifies iron utilization [16,31,33]. Another factor that can affect the iron metabolism in athletes is the increased loss of iron ions from the body along with: 1). Afterwards (the loss of Fe with sweat is about 0.3mg/litre of sweat, while during intensive training the players lose about 2 litres of sweat per hour), 2). urine (post-workout haemoglobinuria) and 3). faeces (Fe is released into the gastrointestinal tract during exhausting physical activity).

Clinical consequences of iron deficiency in athletes are related to the reduction of oxygen supply to working muscles, which in turn results in lower tolerance of physical activity, loss

of breath, shortness of breath, shortness of breath and shortness of breath. Moreover, as a result of impaired erythropoiesis rate and decreased activity of enzymes of which Fe is a cofactor, the body's tolerance to exercise stress is reduced [5].

In the last few years, a group of unknown proteins involved in the regulation of systemic iron homeostasis has been identified, of which hepcidin, a small cysteine-rich peptide, arouses the greatest interest [7,14,20]. Recent studies have confirmed that hepcidin regulates iron homeostasis by interacting with one of the transport proteins - ferroportin 1 [15,19]. Hepcidin interacts directly with ferroportin to form a protein complex that is internalized and degraded in lysosomes. Since ferroportin occurs not only in the basal and lateral membrane of enterocytes, but also in macrophages and hepatocytes, both the process of iron absorption in the small intestine and the recirculation of the element in the cells of the reticulo-endothelial system is regulated [8,15,19,27,34,35].

Hepcidin concentration in the human body is significantly affected by many factors [17,18,21]. Hypoxia, anaemia and erythropoietin are among the strongest suppressors of this protein synthesis, while the inflammatory process and high iron supply induce hepcidin production [17,18,21].

Taking into account the specificity of training, including the intensity of physical activity, duration of physical activity, changes in body composition (expressed by reduction of body fat and increase in lean body mass) and metabolic changes taking place in the body during intensive physical activity, it can be assumed that hepcidin is a key mediator of systemic iron homeostasis disorders in people practicing endurance disciplines, which include canoeing and rowing [6,13].

## **Objective**

The aim of this study was evaluation of hepcidin serum level in the group of juniors training canoeing.

## **Material and methods**

The study group consisted of 29 young athletes (20 boys and 9 girls) participating in a training camp at the Central Sports Centre in Wałcz. The average age of participants of both boys and girls was  $16.5 \pm 0.6$  years.

The material for laboratory tests was blood serum. Blood was collected on the first day of the training camp, before the start of the training plan. The Bioethical Committee of the Nicolaus Copernicus University in Toruń at the Ludwik Rydygier Medical College in Bydgoszcz (consent no.: KB 388/2015) was obtained for the research. All persons participating in the study shall be acquainted with the test procedure and informed of the purpose of the study. After getting acquainted with the research procedure, parents or legal guardians gave conscious, written consent to the participation of children / guests in the research.

In all subjects, peripheral blood morphology was performed and hepcidin, ferritin and iron concentrations were determined. Blood morphology and concentration of iron and ferritin were performed in GUTLAB analytical laboratory in Wałcz. Hepcidin was determined by immunoenzymatic method (HS ELISA) in the Department and Department of Nutrition and Dietetics, Collegium Medicum in Bydgoszcz. A test by DRG Instruments GmbH with catalogue number EIA-5782 was used for the markings.

No reference values for serum hepcidin for either the adult population or children have been developed so far. Therefore, the results obtained in own work were compared with the values indicated by the manufacturer of the reagent and developed for adults (Mediana 8.30ng/ml; Q1;Q3 0.16; 43.84ng/ml).

The values of other biochemical parameters and blood count were compared with reference values indicated by the GUTLAB laboratory. The reference values used in the study are presented in Table 1.

**Table 1: Reference values**

Parameters	Reference values	
	Boys	Girls
WBC (*10 <sup>3</sup> /ul)	4.00 – 12.00	4.00 – 12.00
RBC (*10 <sup>6</sup> /ul)	4.5 – 5.5	4.0 – 5.0
HGB (g/dl)	13.7 – 17.5	11.2 – 15.7
HCT (%)	40.1 – 51.0	34.1 – 44.9
MCV (fL)	79.0 – 92.2	79.4 – 94.8
MCH (pg)	25.6 – 32.2	25.6 – 32.2
MCHC (g/dl)	32.3 – 36.5	32.2 – 35.5
PLT (10 <sup>3</sup> /ul)	140 – 370	140 – 370
Iron (ug/dl)	59 - 158	37 - 145
Ferrite (ng/ml)	30 - 400	20 - 200

All statistics were conducted using StatSoft STATISTICA software, version 13. The normal distribution of the variables was evaluated using the W-Shapiro-Wilk test. Parametric data was expressed as mean, standard deviation, minimum and maximum. Non-parametric parameters were presented by median and percentile values. An independent sample t test for parametric continuous variables. The following statistical tests were used in the study: one-way ANOVA and post-hoc tests. The relationships between the examined parameters were determined by Spearman's rank-order correlation. A value of  $p < 0.05$  was considered statistically significant.

## Results

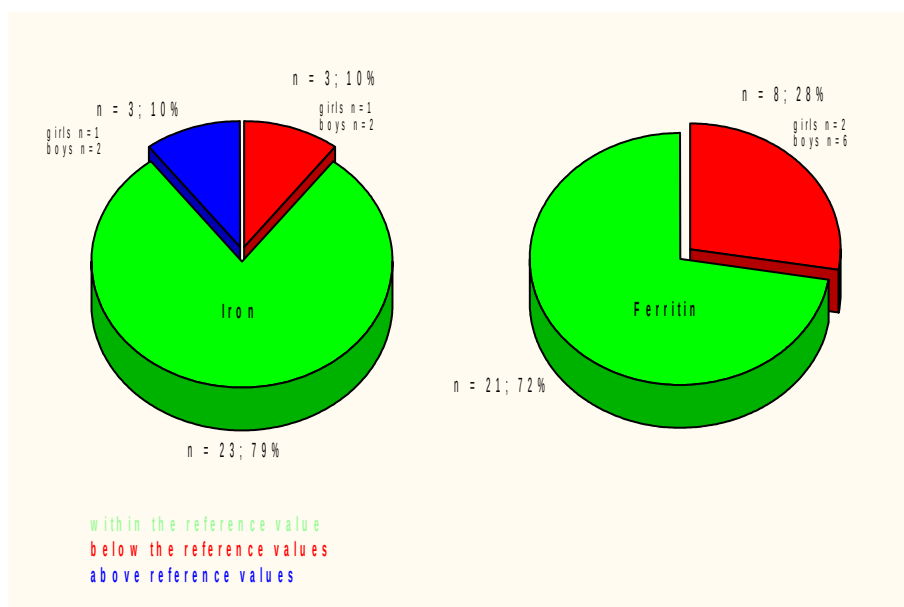
The analysis of mean values of basic blood morphology parameters in the group of examined juniors did not show any abnormalities (tab. 2). As expected, significantly higher values of red blood cell count (RBC), haemoglobin (HGB) and haematocrit (HCT) were observed in the group of boys (tab. 2).

**Table 2. Hematological parameters in the study group**

	All subjects		Boys		Girls		p
	Me x±SD	Q1,Q3 min-max	Me x±SD	Q1,Q3 min-max	Me x±SD	Q1,Q3 min-max	
WBC (*10 <sup>3</sup> /ul)	6.99	5.95;7.78	6.86	5.89;7.67	7.51	6.45;8.79	0.3171
RBC (*10 <sup>6</sup> /ul)	4.95±0.39	4.10-5.63	5.13±0.28	4.58-5.63	4.54±0.30	4.10-5.01	0.0001
HGB (g/dl)	14.81±1.15	12.50-17.50	15.36±0.85	14.30-17.5	13.58±0.69	12.50-14.70	0.0001
HCT (%)	42.09±2.81	34.60-46.80	43.41±1.95	40.30-46.80	39.17±2.16	34.60-41.40	0.0001
MCV (fL)	85.21±2.94	80.80-94.00	84.70±2.55	80.80-90.00	86.36±3.56	82.30 94.00	0.1644
MCH (pg)	29.74±1.29	25.80-32.10	29.66±1.46	25.80-32.10	29.92±0.83	28.70-31.50	0.6143
MCHC (g/dl)	35.02±0.83	33.50-37.40	35.18±0.83	33.60-37.40	34.67±0.76	33.50-36.10	0.1254
PLT (*10 <sup>3</sup> /ul)	276.97±47.79	176.00-392.00	271.35±54.81	176.00-392.00	289.44±24.59	257.00-322.00	0.3549

HGB – hemoglobin, HCT – hematocrit, MCV - mean corpuscular volume, RBC – erythrocyte count, WBC – leukocyte count, x – mean value, SD – standard deviation, Me – median, Q1 and Q3 – 25<sup>th</sup> and 75<sup>th</sup> percentiles, p - significance level

The analysis of blood biochemical parameters showed that the mean iron concentration in both boys and girls was within the range of the assumed reference values (tab. 3). The average iron concentration in the total group was 101.17  $\mu\text{g/dl}$ . The lowest observed value was 20 $\mu\text{g/dl}$  and the highest was 185 $\mu\text{g/dl}$ . (tab. 3). In the group of boys, the mean concentration of iron was 105.55 $\pm$ 36.70  $\mu\text{g/dl}$ . The lowest iron concentration in the group of boys was 51 $\mu\text{g/dl}$ , while the highest concentration was 171 $\mu\text{g/dl}$  (tab. 3). In the group of young male athletes, there were 2 cases of iron concentration below the norm (Fig. 1) and 2 cases of iron concentration above the norm (Fig. 1). In the group of girls the average iron concentration was 91.44  $\mu\text{g/dl}$  (tab.3). The range of individual values in the group of girls was wider than in the group of boys. The lowest iron concentration in girls was 20 $\mu\text{g/dl}$ , while the highest was 185 $\mu\text{g/dl}$ . One of the 9 girls participating in this study had below-standard iron levels and one hyperferremia (Fig. 1). There was no statistically significant difference in iron concentration between boys and girls ( $p=0.3967$ ) (tab. 3). The analysis of ferritin concentration allowed to observe that its average concentration in the total group was within the range of the standard (tab.3) and amounted to 44.23ng/ml (tab. 3). On the other hand, the analysis of individual values allowed us to observe that in 8 competitors the concentration of ferritin was below the range of the norm (Fig. 1). In the group of boys the mean concentration of ferritin was 46.6ng/ml, the lowest recorded value was 17.40ng/ml, while the highest was 79.80ng/ml higher and was 97.20ng/ml. In the studied group of boys the analysis of individual values allowed to observe 6 cases of ferritin concentration below the norm (Fig. 1). In the group of girls the mean concentration of ferritin was slightly lower than in the group of boys ( $p=0.3833$ ) at the level of 38.87ng/ml (tab. 3). In the group of girls ferritin deficiency was significant for 2 female athletes (Fig. 1).



**Figure 1. Values of iron and ferritin in the study group**

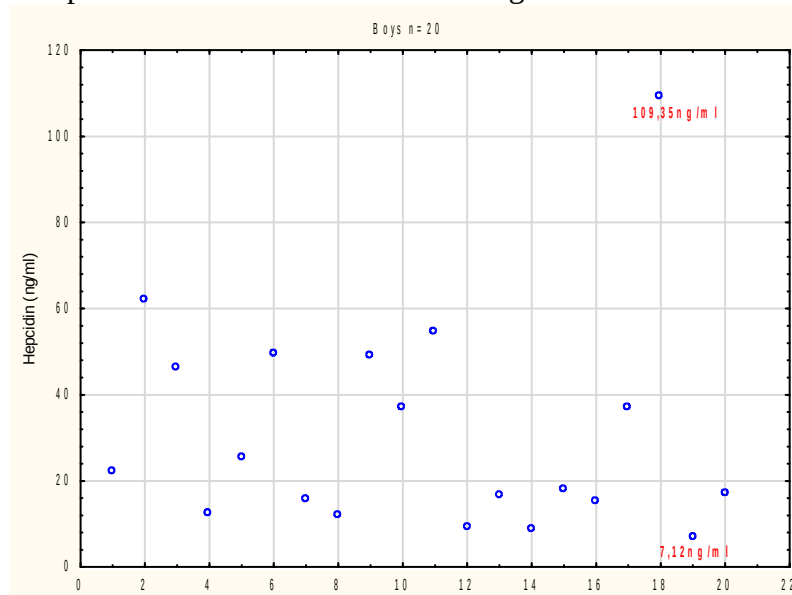
The analysis of hepcidin concentration showed that hepcidin median in the group of juniors was 19.19ng/ml and was about twice as high as the median value indicated by the manufacturer of the hepcidin concentration kit (8.30ng/ml) (tab. 3). In the group of boys the median value for hepcidin concentration was 20.09ng/ml, the lower quartile value was 13.93ng/ml, while the upper quartile value was over 30ng/ml higher and amounted to 47.77ng/ml (tab. 3).

**Table 3. Biochemical parameters in the study group**

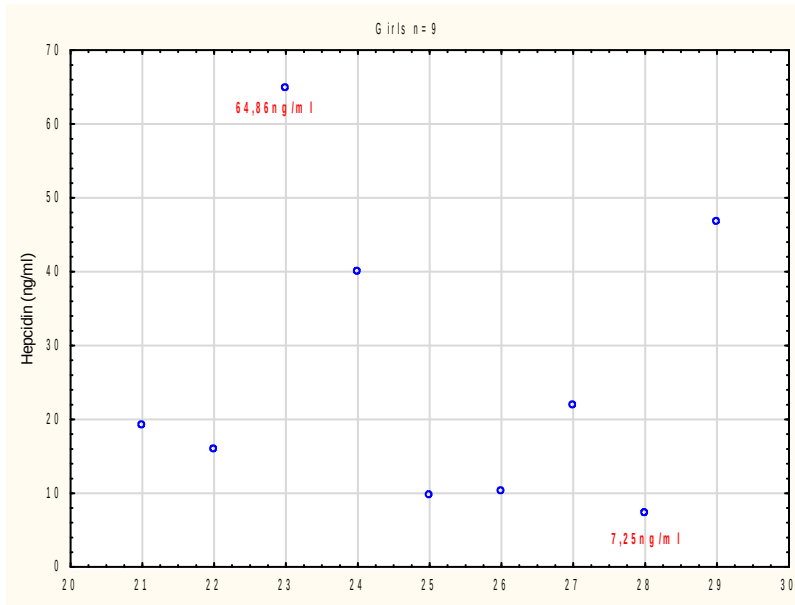
	All subjects		Boys		Girls		p
	Me x±SD	Q1,Q3 min-max	Me x±SD	Q1,Q3 min-max	Me x±SD	Q1,Q3 min-max	
Iron (ug/dl)	101.17±40.62	20.00- 185.00	105.55±36.70	51.00-171.00	91.44±49.20	20.00-185.00	0.3967
Ferritin (ng/ml)	44.23±21.77	12.20-97.20	46.64±21.40	17.40-97.20	38.87±22.88	12.20-79.50	0.3833
Hepcidin (ng/ml)	19.19	12.43;46.21	20.09	13.93;47.77	19.19	10.30;40.13	0.7285

x – mean value, SD – standard deviation, Me – median, Q1 and Q3 – 25<sup>th</sup> and 75<sup>th</sup> percentiles, p - significance level

The analysis of individual results allowed us to observe an even wider range of values. The lowest hepcidin concentration in the group of boys was 7.12ng/ml, while the highest recorded value was 102.23ng/ml higher and was 109.35ng/ml (Fig. 2). Comparing own results with reference values for adults it was observed that for 10 out of 20 examined boys relatively low values of hepcidin concentration within the lower limits of reference values from 0.16-20ng/ml were observed (Fig. 2). In 4 boys hepcidin concentration values within the upper limit of the adult reference values of 43.84ng/ml were observed (Fig. 2). On the other hand, 6 boys had hepcidin levels above the normal range indicated for adults (Fig. 2).

**Figure 2. Values of individual hepcidin levels in the group of boys**

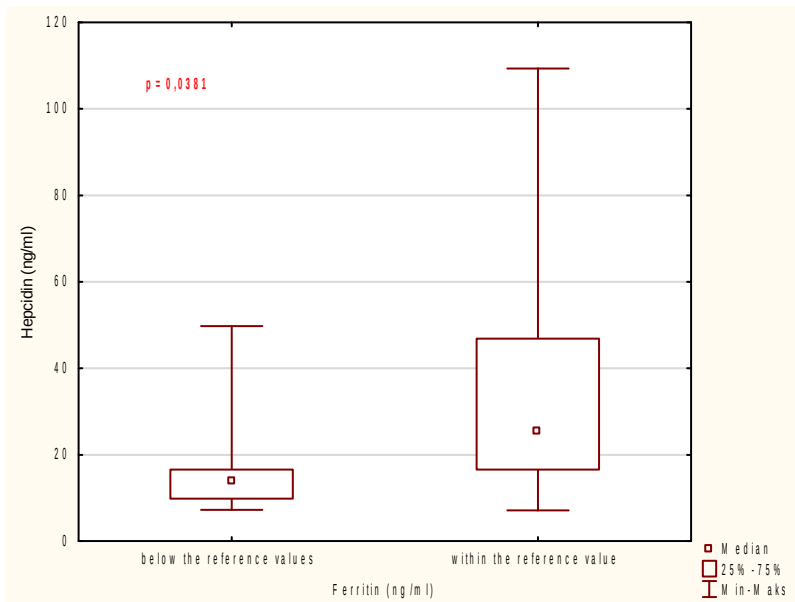
In the group of girls the median hepcidin concentration was 19.18ng/ml and the range between lower and upper quartile was slightly narrower than in the group of boys (Fig. 2, Fig. 3). The value of the lower quartile in the group of girls was 10.30ng/ml, while the value of the upper quartile was about 29ng/ml higher and amounted to 40.13ng/ml. Girls had the lowest hepcidin concentration of 7.25ng/ml, while the highest value obtained in the group of girls was 64.86ng/ml (Fig. 3) and was almost half lower than the maximum values determined in the group of boys. Comparing own results in the group of girls with reference values for adults, it was observed that for 5 out of 9 girls examined, relatively low (<20ng/ml) hepcidin levels were significant (Fig. 3). In two girls hepcidin concentration values within the upper reference values for adults of 20.00-43.84ng/ml were observed (Fig. 3). The remaining 2 girls had very high hepcidin values >43.84ng/ml (Fig. 3).



**Figure 3. Values of individual hepcidin levels in the group of girls**

There was no statistically significant difference in hepcidin values between a group of boys and a group of girls ( $p=0.7285$ ) (tab. 3).

In our study, the values of hepcidin concentration in the total study group were also evaluated, depending on the concentration of ferritin. The analysis made it possible to observe that in 8 athletes with below-standard ferritin concentration, hepcidin concentration was statistically significantly lower as well (Fig. 4). A positive correlation between ferritin and hepcidin was found ( $r=0.56$ ;  $p=0.01$ ).



**Figure 4. Hepcidin levels in juniors with serum ferritin level below standards and within the reference values**

## Discussion

The level of hepcidin in 29 juniors training canoeing was examined in our own study. Due to the fact that no reference values for hepcidin concentration have been developed so far, there is also no scope of the standard for training adolescents, the reference values indicated by the manufacturer of the hepcidin determination kit (Me 8.30ng/ml, Q1; Q3 0.16; 43.84ng/ml) were used in the description and discussion of own results. When comparing own

results with the indicated reference values for adults, it can be noted that young athletes were characterized by significantly higher hepcidin levels in comparison with adults. Median hepcidin concentration in the group of juniors (19.19ng/ml) was twice as high as the median hepcidin concentration in the group of adults (8.30ng/ml). The analysis of individual results in the group of juniors allowed us to observe that as many as 8 out of 29 competitors (28%) had high hepcidin levels, above the range of reference values for adults. For 6 juniors the hepcidin concentration was in the range of upper values of the adult standard (20-44ng/ml). Normal hepcidin concentration within the lower reference limits (0.2-20.0ng/ml) was significant for only one in two young athletes taking part in this study. The results obtained in our own study, hepcidin concentrations in the group of training juniors were also significantly higher than the values observed by Aeberli et al. In the group of 33 non-training, properly nourished children aged 6-14 years [1]. The median hepcidin concentration in the cited study was 3.9ng/ml, i.e. about 15ng/ml lower than the value recorded in our study [1]. High concentrations of hepcidin in the blood of training juniors, observed in our own studies, may be the result of an inflammatory reaction characteristic of intensive physical activity. Scientific research indicate that IL-6, leptin and other inflammatory mediators may increase hepcidin concentration in human serum through the JAK2/STAT3 signaling pathway [4,20]. So, intense physical effort, related inflammation followed by increasing hepcidin concentration may reduce iron bioavailability [10,14]. Similar observations concerning the increased serum value of hepcidin in the training group of adolescents were made by Sandström et al [30]. The quoted authors compared, in a study published in 2017, hepcidin levels in the blood of regularly training girls (mean age  $16.8 \pm 0.9$  years), in relation to girls who do not train professionally. The authors showed a significantly higher concentration of hepcidin ( $13.12 \pm 8.38$ ng/ml) in the group of girls practicing at high performance compared to untrained persons ( $9.21 \pm 5.3$ ng/ml). Moreover, the authors of the study cited above noted, similarly as in their own studies, a wide range of hepcidin values obtained in the group of training adolescents [30]. In the group of female athletes, hepcidin concentrations ranged from 5.58 to 64.24ng/ml. In the group of girls not training the range of individual values was much smaller (1.95-39.10ng/ml) [30]. Also Ziemann et al. observed high serum concentrations of hepcidin in the group of 58 young (mean age  $16 \pm 0.9$  years) athletes practicing tennis [36]. The authors observed that the average value of hepcidin in the group of adolescents training tennis was 71ng/ml (range 48-100ng/ml) and was 41ng/ml higher than the average value in the group of adolescents not training [36]. In the study cited above, it is worth noting that the mean value of hepcidin in the group of young tennis players (71ng/ml) was significantly higher than the median calculated for the examined in their own work canoeists (19.19ng/ml) and the mean value indicated by Sandström et al. for girls training in high performance (13.12ng/ml). Such significant differences between the results described above may be due to different blood collection times for testing and different sports discipline. At work, blood was collected before the start of the training season, while at work Ziemann et al. blood was collected after training. Ishibashi et al. observed that elevated serum hepcidin levels were associated with increased training intensity [11]. Moreover, significantly higher hepcidin concentrations observed by Ziemann et al. may result from the fact that tennis is more traumatic than canoeing. According to literature data, intensive training and microinjuries or injuries associated with it stimulate inflammatory development and intensify the secretion of inflammatory cytokines [22,24,26]. One of them is interleukin-6, which, as it has already been written, indirectly contributes to the intensification of hepcidin expression [[18]. Many researchers observed an increase in IL-6 concentration in response to intensive effort [24,25,29,32,36]. High concentrations of hepcidin in groups of professional training adolescents, observed in own work and in studies by other authors, should arouse particular anxiety. Because the major action of hepcidin is to internalize and degrade the iron efflux transporter ferroportin expressed on all iron-exporting



cells [19]. So, high hepcidin level leads to the suppression of intestinal iron absorption and iron release from macrophages and hepatocytes, whereas a low concentration of hepcidin leads to acceleration of iron release from these cells [12,19,27,34,35]. As a result, the high levels of hepcidin observed in young athletes may cause sideropenia [28,34,35].

In our work ferritin deficiency, indicating latent anemia, was noted in about 30% of the examined athletes. An even higher percentage of juniors (about 50%) with ferritin concentration below the reference values was recorded by Ziemann et al. in the group of tennis players [36]. A lower percentage (about 22%) of athletes with ferritin deficiency was recorded by Peeling et al. in the studied group of 54 athletes practising competitive running or triathletes (38 men and 16 women, mean age 25.8 years) [25].

In our study, a positive correlation between ferritin and hepcidin was found ( $r=0.56$ ;  $p=0.01$ ). A significant, strong correlation between ferritin and hepcidin was also observed in Peeling et al. What is more, the quoted authors demonstrated the occurrence of correlation both before ( $r=0.50$ ;  $p=0.01$ ) and after physical effort ( $r=0.52$ ;  $p=0.01$ ) [25]. The role of hepcidin in the development of latent anaemia in athletes is still little known. In order to learn more about the role of hepcidin in the development of anaemia, further research is necessary.

### Conclusion

Results of this study showed that the average hepcidin concentration in the study group was higher than that observed by other authors for healthy peers. In addition, in 8 juniors (including 6 boys and 2 girls), the hepcidin concentration was above the adult reference range. There was no statistically significant difference in hepcidin concentration between boys and girls. However, a wide range of individual hepcidin values was observed in both groups. In addition, ferritin levels were below normal with 8 patients. A positive correlation between hepcidin and ferritin has been shown. The results obtained in the study indicate the hepcidin could be a major contributor to poor iron status observed in the athlete trains professionally. Our research suggests it is necessary to monitor the diet of young athletes with respect to iron content and its proper bioavailability.

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