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Research article

Do High Blood Hepcidin Concentrations Contribute to Low Ferritin Levels in Young Tennis Players at the End of Tournament Season?

Ewa Ziemann [1]✉, Katarzyna Kasprowicz [2], Anna Kasperska [3], Agnieszka Zembroń-Lacny [3], Jedrzej Antosiewicz [4] and Radosław Laskowski [1]

[1] Gdansk University of Physical Education and Sport, Department of Physiology, Poland; [2] Gdansk University of Physical Education and Sport, Department of Biology, Poland; [3] Academy of Physical Education Poznan, Faculty of Physical Culture Gorzow Wlkp, Poland; [4] Warsaw School of Social Sciences and Humanities, Department of Sport Psychology, Poland

Abstract

The purpose of the present study was to verify whether impaired iron metabolism in young athletes is a consequence of an excessive workload during the tournament season. Low levels of ferritin (under 25 $\mu g \cdot L^{-1}$) have been frequently observed in young tennis players. We considered this finding to be related to the high-intensity workload or to insufficient rest, which both trigger a strong immune response. Groups of male, well-trained young tennis players (16 ± 0.9 years old, average of 10-year training experience) and a control peer group participated in this study. The research consisted of two examination sessions (March and September 2010). Blood samples were collected to determine haematological and immunological parameters. Additionally, body composition and physical capacity were assessed. In both periods of the study, the trained groups were characterised by low levels of ferritin, but also elevated levels of pro-inflammatory cytokine IL-1β. Moreover, an inverse correlation between IL-1β and blood ferritin was observed. Additionally, an increased concentration of the iron homeostasis regulator hepcidin was found in blood samples (mean 71 $ng \cdot ml^{-1}$; range from 48 to 100 $ng \cdot ml^{-1}$). We concluded that the pro-inflammatory cytokine IL-1β, most likely induced by an extensive workload during the tournament season, was responsible for the low level of ferritin in young, professional athletes.

Key words: Pro-inflammatory cytokine, overreaching, hepcidin.

Introduction

Professional tennis players are regularly exposed to a high-intensity workload as well as emotional stress throughout the tournament season. These stresses, both physical and psychological, may directly stimulate the production of pro-inflammatory cytokines (Kiecolt-Glaser et al., 2002). At the same time, acute exercise triggers the production of anti-inflammatory cytokines, which leads to progressing adaptive changes. Consequently, it is an issue of the highest importance to adjust physical workloads in training in such a way to maintain balance between the pro- and anti-inflammatory responses (Cooper et al., 2007).

The sustained and elevated inflammatory response to stress conditions may lead to overreaching or even overtraining among competitive athletes (Smith, 2000; 2004). One of the symptoms of overtraining is a low ferritin blood level (Smith, 2000). Ferritin itself is an iron storage protein. Its level increases when tissue iron stores expand, yet its biosynthesis is inhibited when iron levels are low. Therefore, low ferritin serum concentrations are a reliable indicator of poor iron stores.

On the one hand iron, like oxygen, is essential for a living organism. Proper iron homeostasis is crucial for effective energy metabolism and the transport of oxygen, which both require several iron-containing proteins, such as cytochromes in the mitochondria, myoglobin, and haemoglobin. On the other hand, excess iron has several deleterious properties, including an ability to stimulate the formation of reactive oxygen species (ROS). Iron can react with hydrogen peroxide to form the hydroxyl radical (HO), yet it can also react with lipid peroxides to form alkoxyl and peroxyl radicals. Additionally, the reaction of Fe^{2+} with an oxygen can lead to the formation of a very strong oxidants, such as the perferyl and ferryl ions, with a reactivity approaching that of $HO^{\cdot}$ (Qian and Buettner, 1999).

Because of the toxicity of free iron, its cellular level must be strictly controlled. Most stored iron is located in ferritin, yet ferritin iron does not stimulate ROS formation. Nevertheless, certain stressors such as UV light, TNF-α or hydrogen peroxide, which can cause cell and tissue damage, are known to induce iron release from ferritin and hence, stimulate iron-dependent ROS formation (Antosiewicz et al., 2007; Pourzand et al., 1999). Thus, an inflammatory process is associated with increased ROS formation (Zager et al., 2005). Enhanced cellular chelatable iron and ROS production result in increased nuclear factor-κB (NF- κB) DNA binding, which leads to intensified gene expression and protein synthesis (Pham et al., 2004). Among others, pro-inflammatory mediators such as IL-1β, IL-6 and TNF-α are up-regulated by NF-κB. Thus, the interrelations, described above, summarize the mechanism of pro-inflammatory iron activity.

In addition, pro-inflammatory cytokines stimulate the synthesis of hepcidin, which is an iron-regulating hormone (Ganz and Nemeth, 2011). Increased levels of blood hepcidin inhibit iron transport from the duodenum. On the one hand, long-term elevation of hepcidin leads to a decrease in the iron available for erythropoiesis; on the other hand, iron depletion may reduce inflammatory processes and limit iron availability to invading microorganisms and thus contribute to host defence (Ganz, 2006;

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Nemeth and Ganz, 2006). Hence, paradoxically, chronic low-grade inflammation may lead to iron deficiency anaemia. In such a case, iron supplementation would be an inappropriate treatment strategy because administered iron may potentiate the inflammation process.

Low levels of haemoglobin that remain within the normal range and iron deficiency are commonly recorded conditions among some groups of professional athletes, both female and male (Landahl et al., 2005). These types of deficiency may result from an unbalanced diet, gastrointestinal (GI) bleeding during and after long distance running and, possibly, chronic low-grade systemic inflammation (Peeling et al., 2008). In professional sports, despite the different causes of iron deficiency, iron supplements or injections are commonly used, in many cases unnecessarily (McClung et al., 2009). Moreover, misused iron therapy, which randomly results in iron overload, is not free from metabolic risks (Lippi et al., 2005). There is a great deal of evidence suggesting that excessive sport activity may lead to systemic inflammation (Smith, 2000). Nonetheless, the data confirming that exercise-induced low grade inflammation leads to iron deficiency, especially in groups of young athletes starting professional careers very early, are scarce.

Consequently, the aim of this study was to determine whether iron deficiency observed in young tennis players after the tournament season, manifested by a low level of ferritin, results from a low-grade systemic inflammation and elevated blood hepcidin levels. We hypothesised that changes in diet as well as modifications introduced to applied workloads in physical training might be a crucial step in approaching iron deficiency, which brings more effective results than iron supplementation.

Methods

Data collection

To monitor the development of the best national-level young tennis players, special examinations are organised twice a year by the Polish Tennis Association. These examinations are held before the conditioning camp and between the end of a tournament season and the start of the preparatory period for the next season. Tournament season involves all indoor and outdoor, national and international tournaments, in which players participate both individually as well as in teams. Participants are selected by the national coach according to their annual achievements and rankings. Since many athletes manage to maintain high professional performance, they very often participate in consecutive examinations.

The schedule of the examinations remains constant. For the baseline, following a 3-day break, each examination includes a medical check-up, blood collection and assessment of body composition. Next, an exercise test is conducted. The examination is officially approved by the Bioethical Committee of the Regional Medical Society in Gdansk NKEBN/39/2009 according to the Helsinki Declaration. Participation must be approved by written consent from the tennis players' parents.

The examinations included in this study were held in March and September of 2009 and 2010 (the examinations are chronologically named A, B, C and D; Figure 1). Throughout these four observations, low levels of blood ferritin were recorded. Overall, 30 out of the 58 young, male, young athletes were found to present this condition (Figure 2). Because of the sustained ferritin deficiency, we increased the number of assessed blood parameters with each consecutive examination. During observations A and B, only haematological parameters were assessed, whereas in period C, both pro- and anti-inflammatory cytokine levels were also measured. As a result, examination D (September 2010) included the evaluation of haematological parameters, iron status and levels of ferritin, pro- and anti-inflammatory cytokines and hepcidin. Moreover, the data were compared and contrasted

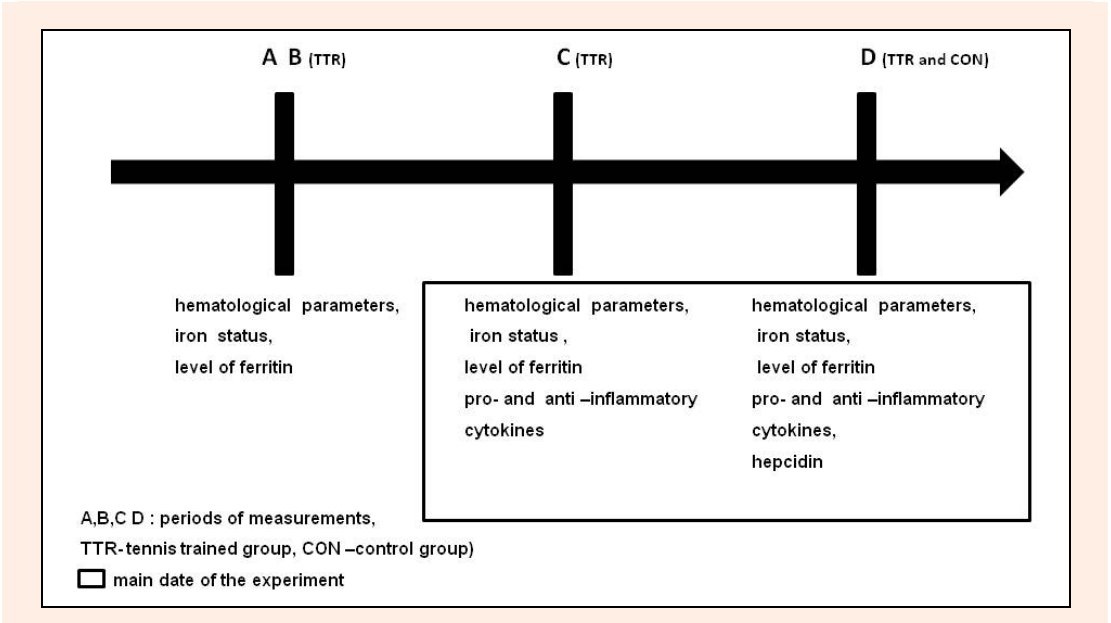

Figure 1 . The schedule of examinations and the blood parameters measured in the subsequent periods of measurements.

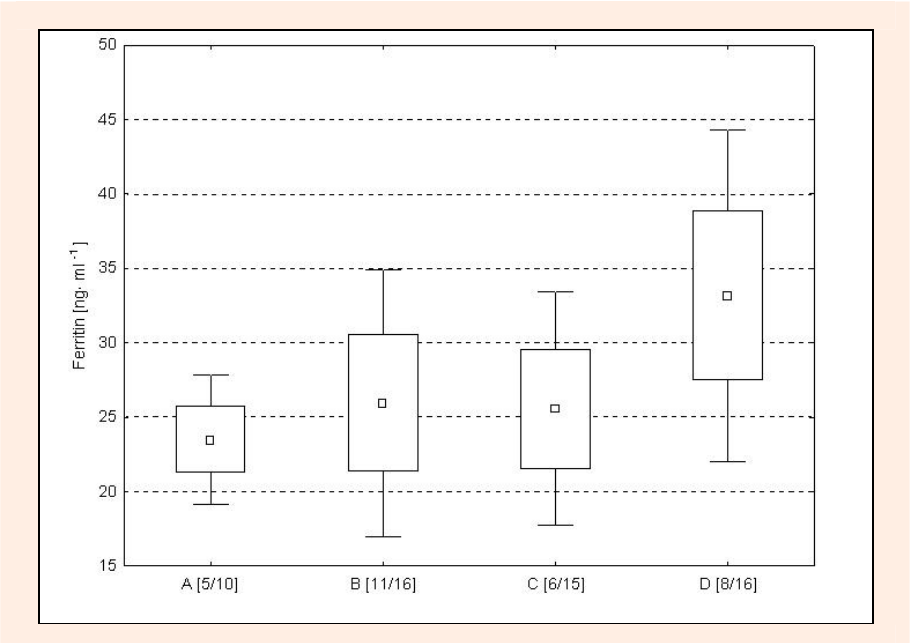

Figure 2. The average level of serum ferritin in tennis players in the subsequent rounds of regular examination. The values in brackets are the number of subjects out of the total group whose ferritin level was lower than 25 ng·ml $^{-1}$.

between the tennis player groups and a control group of untrained peers.

The present paper covers the analysis of the observations from examinations C and D (March and September 2010).

Subjects

In examination C (March 2010), only a Tennis Trained Group (TTR) (C) of young, male tennis players participated, whereas examination D (September 2010) involved two groups: TTR (D) and CON for tennis players and untrained peers, respectively. All participants were 16 ± 0.9 years old. On average, the tennis players had 10 years of training experience, and their national rankings ranged from 2-39. Their typical week's workload consists of 7-9 tennis training units, 2-3 resistance exercise units, 2-3 conditioning training units and 7-10 stretching units. Nine of the participants who participated in examination C, participated in examination D as well. Both 2nd and 39th (best and worst ranked respectively) player in the national ranking were in this group of nine. Hence, when evaluating the induced inflammatory response in TTR, we took into consideration the overall workload performed by these two players during the whole tournament season, expressed in numbers of singles and doubles played. For the 2nd player the total workload constituted 39 singles and 17 doubles, whereas for the 39th player it consisted of 28 singles and 21 doubles. The percentage of matches won was equal 72% and 59%for 2nd for singles and doubles and 57% and 55% for 39th player respectively.

Body composition assessment

Body mass (BM) and body composition were estimated using a multi-frequency impedance plethysmograph body composition analyser (InBody 720, Biospace Analyzer, Korea). Using a diverse range of frequencies from 1 kHz to 1 MHz, the InBody 720 accurately measured the amount of body water and the body composition including fat mass, free fat mass and skeletal muscle mass. The precision of the repeated measurements was expressed as the coefficient of variation, which was, on average, 0.6% for fat mass percentage (Lim et al., 2009; Volgyi et al., 2008). The measurements were taken one hour before breakfast. The participants emptied their bladders and bowels prior to the assessment. During the measurement, the participants wore only briefs and remained barefoot.

Blood sampling and cytokine analysis

Blood samples were taken from an antecubital vein into single-use containers with an anticoagulant ($EDTAK_2$). After collection, the samples were immediately stored at a temperature of 4°C. Within 10 minutes, they were centrifuged at 3000 g and 4°C for 10 min. Aliquots of the plasma were stored at -80°C. The blood was collected at rest.

Red blood cells counts [10^6 µL] (RBC), haematocrit [%] (Hct) and blood haemoglobin concentration ($g \cdot dl^{-1}$) (Hb) were determined from the venous blood samples by conventional methods using a COULTER® LH 750 Hematology Analyzer, (Beckman-Coulter, USA).

Plasma creatine kinase (CK) activity was used as a marker of muscle damage and was evaluated using an Emapol kit (Poland). The CK detection limit of the applied kit was 6 $U \cdot l^{-1}$. The intra-assay CV for the CK kit was 1.85%.

Plasma interleukin-1β (IL-1β), interleukin-6 (IL-6) and interleukin-10 (IL-10) levels were determined via enzyme immunoassay methods using commercial kits (R&D Systems, USA). The detection limits for IL-1β, IL-6 and IL-10 were 0.023, 0.039 and 0.500 $pg \cdot ml^{-1}$, respectively. The average intra-assay CV was <8.0% for all cytokines.

Serum hepcidin was determined using a DRG Elisa kit (DRG Instruments, GmbH, Marburg, Germany) according to the manufacturer's protocol. This assay detects

the 25 amino-acid form of hepcidin, a biological active form of the hormone. The dynamic range of the assay is between 0.9 and 140 ng·ml^{-1}. The average CV precisions intra assay and inter assay are 4.41% and 9.70% respectively. The normal 5–95% range in healthy adults described by the manufacturer is 13.3–54.4 ng·ml^{-1}. The analytical sensitivity of the hepcidin kit was calculated by subtracting two standard deviations from the mean of 20 replicate analyses of the Zero Standard (SO). Similar hepcidin measurements (Elisa kit) has been used in previous study (Ganz et al., 2008) and at the moment it seems to be the most time and cost effective method.

Aerobic capacity

Aerobic capacity was determined during a VO_2 max test. Breath-by-breath pulmonary gas exchange was measured (Meta-Max 3B, Cortex, Germany) throughout the test. The participants performed a continuously graded multistage field tennis test according to the protocol suggested by Smekal (Smekal et al., 2000). The series of 3-minute exercise stages were separated by 1-minute breaks for machine adjustments and were based on typical tennis movements when reaching for a stroke. The participants alternated between forehand and backhand strokes with balls thrown by the HOT SHOT DXSR-1594 ball machine. The participants were allowed a 3-min warm up period before the test. Immediately following the warm up, the VO_2 max testing began and continued until the participant reached the point of volitional exhaustion (oxygen uptake did not increase any more or the frequency of the ball was so high that completing strokes became impossible). Before subsequent players began the test, the O_2 and CO_2 analysers were calibrated using standard gases at known concentrations in accordance with manufacturer guidelines.

The subjects in the control group performed a graded cycle ergometry test on an electromagnetically braked cycle ergometer (ER 900 Jaeger, Germany/Viasys Health Care). Breath-by-breath pulmonary gas exchange was measured (MetaMax 3B, Cortex Biophysik, Germany) throughout the exercise. The ergometer seat height was individually adjusted to attain a 5° bend in the knee at the lowest point in the pedal revolution. The participants were allowed a 5-min warm up period at an intensity of 1.5 W·kg^{-1} and a pedalling cadence of 60 rpm. Immediately following the warm up, the participants began VO_2max testing by cycling at increasingly difficult workloads in which the resistance was increased by 25 W·min^{-1} until the participant reached the point of volitional exhaustion. The O_2 and CO_2 analysers were calibrated prior to each test using standard gases at known concentrations in accordance with manufacturer guidelines. The heart rates were monitored continuously by telemetry (S-625, Polar Electro-Oy, Finland) during each test session and during the first 5 min of passive recovery in a seated position.

Statistical analysis

Statistical calculations were performed using STATISTICA 9.0. The results are expressed as the mean (± standard deviation). To establish differences between the trained and control groups, a comparison analysis (Student's t-test) was performed. Some of the trained athletes (65%) participated in subsequent examination sessions repeatedly; hence, their comparison data were excluded. The associations among measured parameters were analysed using Pearson's linear regression (coefficient, r). Statistical significance was set at $p < 0.05$.

Results

The main purpose of this study was to evaluate whether impaired iron metabolism is associated with an elevated level of pro-inflammatory cytokines induced by an extensive workload throughout the tournament season. This hypothesis was based on the low level of blood ferritin observed during examinations A and B (March and September 2009, not presented in this study), which preceded our main research using data gathered during examinations C and D (Figure 2). An analysis of the A and B data showed that five out of ten and eleven out of sixteen players, respectively, experienced low ferritin levels (<25 µg·L^{-1}). Ferritin levels in blood should reflect the intracellular ferritin concentration. Additionally, cellular ferritin synthesis changes in parallel to cellular iron levels. Hence, low blood ferritin is considered to be the best indicator of an iron deficiency.

Baseline characteristics of study participants

Table 1 summarises the anthropometric and physiological characteristics of the subjects from the TTR (examinations C and D) and CON groups (examination D only).

Table 1. Characteristics of three groups of participants. Data are means (±SD).

Variables	TTR (C) Group 1	TTR (D) Group 2	CON Group 3
Height (m)	1.79 (.08)	1.79 (.05)	177.4 (4.1)
Weight (kg)	67.9 (.6)	64.2 (7.0)	66.9 (8.7)
FFM (kg)	61.4 (11.0)	58.6 (5.2)	58.8 (6.0)
SMM (kg)	34.8 (6.6)	33.2 (3.0)	33.0 (3.5)
Fat (kg)	6.5 (2.4)	5.6 (2.4) [3]	8.1 (3.0) [2]
Fat %	9.4 (2.5)	8.6 (2.8) [3]	11.8 (3.3) [2]
TBW (kg)	45.0 (8.0)	43.0 (3.8)	43.1 (4.4)
BMI (kg·m^{-2})	21.1 (2.3)	20.2 (1.8)	21.2 (2.5)
$VO_{2\ max}$ (mL·kg^{-1}·min^{-1})	64.8 (8.3) [3]	67.9 (5.1) [3]	52.3 (6.4) [1, 2]

FFM - free fat mass, SMM - skeletal muscle mass, Fat - fat mass, Fat% - percentage of body fat, BMI - body mass index, VO_2max-maximal oxygen consumption. TTR(C) – tennis trained group in period March 2010; TTR(D) – tennis trained group in period September 2010; CON – control group in September 2010. Superscripts denote $p < 0.05$ between the groups.

Table 2. Hematological parameters of young tennis players and control group. Data are means (±SD).

Variables	TTR (C) Group 1	TTR (D) Group 2	CON Group 3
White blood cells (10^3 µL)	5.4 (1.0)	5.7 (1.1)	5.8 (.9)
Thrombocytes (10^3 µL)	240.1 (37.3)	238.6 (46.6)	235.8 (36.6)
Red blood cells 10^6 µL)	5.0 (.2)	4.9 (.3)	4.9 (.4)
Hemoglobin (g·dL^{-1})	14.5 (.9)	14.6 (.8)	14.4 (1.0)
Hematocrit (%)	43.4 (2.2)	43.4 (2.3)	43.6 (2.3)
MCV (fl)	86.6 (2.6) $^{3\#}$	87.9 (2.2) $^{3\#}$	88.7 (3.6) $^{1\#,2\#}$
MCH (pg)	29.0 (1.4)	29.5 (1.0)	29.4 (2.5)
MCHC (g ·dl $^{-1}$)	33.5 (.2)	33.5 (.5)	33.0 (2.1)
Iron (µg ·dl $^{-1}$)	123.0 (44.2)	99.7 (39.1) ^{3*}	145.4 (60.2) ^{2*}
Ferritin (ng ·ml $^{-1}$)	38.5 (25.7) $^{3\#}$	33.2 (22.7) $^{3\#}$	93.2 (67.2) $^{1\#,2\#}$

MCV-mean corpuscular volume, MCH-mean corpuscular hemoglobin, MCHC-mean corpuscular hemoglobin concentration, TTR(C) – tennis trained group in period March 2010; TTR(D) – tennis trained group in period September 2010; CON – control group in September 2010.
Superscript# and superscript* denote $p < 0.01$ and $p < 0.05$ respectively between the groups.

Although the participants were all of the same age, the physical and physiological parameters were, in some cases, quite distinct within as well as between groups. Statistically significant differences were observed for fat tissue (expressed in percentage and kilograms) between the TTR (D) group and the CON group; however, the range of skeletal muscle mass was similar between the groups. In agreement with our expectations, both training groups were characterised by considerably higher aerobic capacity, expressed as maximal oxygen consumption (VO_2 max), than the CON group.

Haematological and immunological measurements

Haematological and immunological parameters are presented in Tables 2 and 3. Most of the blood parameters were in a standard range; however, analyses of the individual data revealed that in some players, the amount of haemoglobin in red blood cells (MCH and MCHC) was at the low end of the standard value range**.** To verify whether low Hb values were caused by the low level of stored iron, blood ferritin was measured. Our study revealed that ferritin concentrations were below 25 µg·L^{-1} in six and eight athletes during examinations C and D, respectively. Therefore, it may be deduced that low iron stores, which are not able to sustain erythropoiesis completely, are the main cause of the low haemoglobin concentration. Comparison of the haematological parameters indicated that there were no statistically significant differences were observed between the groups, except with regard to the MCV as well as iron and ferritin concentrations (Table 2). In the TTR (C and D) groups, the ferritin levels were 38.5 and 33.2 ng·ml^{-1} and were 2.4 and 2.8-fold lower, respectively, than in the CON group. Moreover, the concentrations of the pro-inflammatory cytokines IL-6 and IL-1β were elevated. Consequently, statistically significant inverse correlations were observed between cytokine IL-1β levels and blood haemoglobin ($r = -0.60$), IL-1β levels and mean corpuscular haemoglobin ($r = -0.64$) and IL-1β levels and mean corpuscular haemoglobin concentration ($r = -0.69$) in the TTR in period C (not shown). Thus, we performed an even more detailed analysis during examination D. The data from TTR (D) was also compared with data from untrained CON group. Furthermore, in both trained groups, significant inverse correlations were noted between the pro-inflammatory cytokine IL-1β and ferritin levels ($r = -0.49$ and $r = -0.61$, respectively, for periods C and D; Figure 3).

The levels of pro-inflammatory cytokine IL-1β differed considerably between the TTR and CON groups. The concentrations of IL-1β for both TTR groups were 8.7-fold higher than for the CON group (Table 3). In contrast, the concentrations of IL-6 for the TTR group differed in comparison with the control group only in period C. During the last examination, significant differences were not observed. At the same time, the anti-inflammatory cytokine IL-10 was lower in the CON group with 10.3- and 3.2-fold differences in comparison with the TTR group from examinations C and D, respectively.

These data suggested that the inflammation may have contributed to impaired iron metabolism in young tennis players. To verify whether the impact of inflammation on iron metabolism was related to impaired hormonal regulation, the blood hepcidin level was measured during examination D. As shown in Table 3, the hepcidin concentration was elevated in all of the tennis players; even the lowest value exceeds 20 ng·ml^{-1}, which is the normal value (Kaneko et al., 2010). On average, the hepcidin

Table 3. Concentration of pro- and anti-inflammatory cytokines in three groups of subjects. Data are means (±SD).

Variables	TTR (C) Group 1	TTR (D) Group 2	CON Group 3
Hepcidin (ng· ml $^{-1}$)	nd	72.0 (15.0) ^{3†}	29.9 (7.5) ^{2†}
CK (U· l^{-1})	248.0 (221.0)	201.2 (106.4) ^{3*}	108.6 (47.2) ^{2*}
IL-1β (pg· ml^{-1})	3.0 (2.5) $^{3\#}$	3.0 (1.1) ^{3†}	.4 (.5) $^{1\#,2\dagger}$
IL- 6 (pg· ml^{-1})	1.3 (.5) ^{3*}	1.0 (.4)	.9 (.5) ^{1}
IL -10 (pg· ml^{-1})	9.3 (.9) ^{3†}	2.9 (1.5) ^{3†}	.9 (.2) ^{1†,2†}

TTR(C) – tennis trained group in period March 2010; TTR(D) – tennis trained group in period September 2010; CON – control group in September 2010. Superscript*, superscript# and superscript† denote $p < 0.05$, 0.01 and 0.001 respectively between the groups.

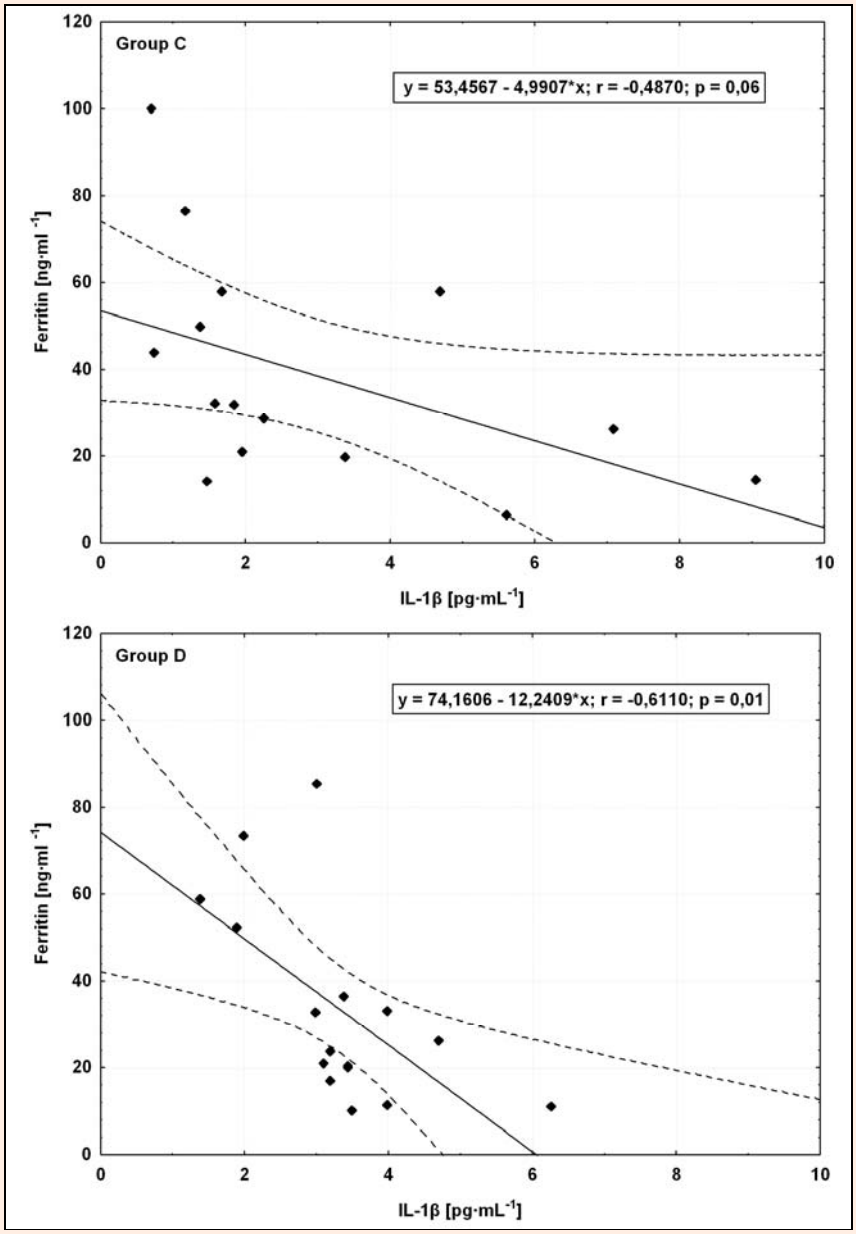

Figure 3. The correlation between the concentration of the pro-inflammatory cytokine IL-1β and the ferritin level in the two periods of examination (trained group in period C 03 2010 and trained group in period D 09 2010).

concentration in the TTR group was 72.0 ng·ml^{-1}, whereas it was in 29.9 ng·ml^{-1} in the CON group. Nonetheless, we did not observe any significant correlation between the hepcidin and ferritin concentration in any of the groups.

Discussion

The present study reveals that increased levels of pro-inflammatory cytokines and elevated blood hepcidin concentrations are associated with iron deficiency in young tennis players after completion of the tournament season. We observed that almost 50% of the tennis players had ferritin concentrations below the reference range. Furthermore, a statistically significant inverse correlation between the pro-inflammatory cytokine IL-1β and ferritin was observed in both groups of tennis players. The TTR athletes had an 8.7-fold higher concentration of IL-1β than the CON group. All of the participants rested for 3 days after the previous strenuous exercise before commencing the examination. Thus, the direct effect of exercise on blood cytokine levels can be excluded e.g., it has been shown that an increased TNF α after a soccer game returned to the resting value after 24-45 h (Andersson et al., 2010). There are several reports that skeletal muscle could be a source of cytokines, however cells like lymphocytes, monocytes, mast cells, epithelial cells and fibroblasts as well as fat cells might be release cytokines, which are involved in regulation immune response (Berger et al., 2003; Pedersen et al., 2007; Pedersen, 2012; Schaffler et al., 2006; Steensberg et al., 2002).

Ferritin is an acute-phase inflammatory response protein, and its concentration increases in the presence of

inflammation (Thurnham et al., 2010). In overweight and obese people with low-grade systemic inflammation, a positive association has been found between CRP (C-reactive protein) and ferritin (Ausk and Ioannou, 2008). In contrast, both low ferritin levels (Smith, 2000) and elevated levels of pro-inflammatory cytokines (Zembron-Lacny et al., 2010) have been reported in overreached athletes. Our data show that most of the athletes experienced elevated levels of the pro-inflammatory cytokine IL-1β at the end of the tournament season, indicating an enhanced immune response after their extended workloads. Exercise has an anti-inflammatory effect (Petersen and Pedersen, 2005); however, excessive exercise may contribute to the deregulation of the balance between pro- and anti-inflammatory cytokines (Cooper et al., 2007). In this study, we also observed an increased level of the anti-inflammatory cytokine IL-10 in the TTR groups; this increase may not have been sufficient to compensate for the pro-inflammatory effects of the physical workload during tournaments. To establish whether hepcidin mediates inflammation-induced iron deficiency, blood hepcidin levels were measured. Hepcidin is the main regulator of iron homeostasis in humans (Means, 2004). Elevated amounts of iron stores or pro-inflammatory cytokines have been shown to induce the biosynthesis of hepcidin (Nemeth and Ganz, 2006). Previous and recently, cell culture and animals experiments demonstrated that IL-6 stimulated hepcidin biosynthesis (Banzet et al., 2012; Kemna et al., 2008; (Nemeth et al., 2004a). Possibly, IL-6 may also stimulate hepcidin biosynthesis in response to exercise (Banzet et al., 2012; Liu et al., 2011). However, some human studies indicate that changes in IL-6 concentration did not directly correlate with hepcidin biosynthesis (Robson-Ansley et al., 2011). Our data do not permit us to discuss the role of IL-6 in hepcidin biosynthesis regulation because we assessed IL-6 only at rest.

Hepcidin controls iron absorption from the intestines via blocking of the iron transporter ferroportin (Nemeth et al., 2004b). Thus, reduced iron availability is most likely an adaptive response to the inflammation process. Iron induces an increase in ROS formation, potentiating inflammation by increasing pro-inflammatory cytokines synthesis as well as tissue injury (Kell, 2009). A normal serum hepcidin concentration is approximately 20 $ng \cdot ml^{-1}$ (Kaneko et al., 2010). Moreover, exercise may modified both blood (Sim et al., 2012) and urinary hepcidin concentration (Borrione et al., 2011; Peeling et al., 2009). Studies performed on human endotoxemia model indicated for relatively fast clearness of blood hepcidin into urine where its concentration peaked at 6 h (Kemna et al., 2005). Thus elevated hepcidin in tennis players despite of 3 days of rest indicate for continues stimulation of hepcidin biosynthesis. Furthermore, the response of hepcidin to exercise is not equivocal. For example Roecker and co-workers observed that in 8 out of 14 marathon runners urine hepcidin was elevated while in six of them remained constant (Roecker et al., 2005). Interestingly, higher urine hepcidin concentration was observed in young athletes with blood ferritin above 30 $\mu \cdot L^{-1}$ compared to those with ferritin lower than 30 $\mu \cdot L^{-1}$ (Borrione et al., 2011). These data suggest that changes of hepcidin induced by exercise are dependent on intracellular iron stores. Our data of hepcidin levels in the CON group were within recommended range, whereas the levels among all of the examined athletes were considerably greater than this value (mean 72 $ng \cdot ml^{-1}$; variable 48 to 100 $ng \cdot ml^{-1}$), indicating that they were elevated. These data strongly suggest that in young tennis players, systemic inflammation leads to increased blood hepcidin levels, which could be the main cause of both impaired iron metabolism and low haemoglobin content.

Iron deficiency and low haemoglobin concentration are conditions observed in both female and male professional athletes (Eliakim et al., 2002). A recent study indicated that 57% of tested female soccer players at the top international level were iron deficient, and 29% of them experienced iron deficiency anaemia (Landahl et al., 2005). Because of a commonly occurring 'demand', iron supplementation therapy is often given to athletes to counterbalance physiological or pathologic anaemia and to prevent physiologic dysfunction (Dascombe et al., 2010; McClung et al., 2009). Athletes with serum levels of ferritin below 25–30 $ng \cdot ml^{-1}$ are considered by the Australian Institute of Sport (AIS) to require iron supplementation. These ferritin concentrations indicate that iron stores are below the level sufficient to sustain proper erythropoiesis. Unfortunately, iron supplementation is often prescribed without identifying the cause of iron deficiency (Handelman and Levin, 2008). Accordingly, several athletes from our study would have been classified as iron deficient, requiring iron supplementation (Figure 2). However, it has been reported that in most of the cases, iron supplementation was ineffective. The treatment was also observed to cause deleterious effects in particular cases (Lippi et al., 2005; Peeling et al., 2007) or to have no impact whatsoever in others (Peeling, 2007). It has been shown that intramuscular iron injections administered to 16 iron-deficient non-anaemic female runners induced a significant increase in serum ferritin levels (from 19 to 65 $ng \cdot mL^{-1}$), which had no beneficial impact on aerobic capacity (Peeling et al., 2007). In another study, oral iron supplementation (100 mg of ferrous sulphate/day) enhanced serum ferritin levels in 18 iron-deficient female runners, but only to 23.44 $ng \cdot mL^{-1}$ and without increasing endurance capacity (Klingshirn et al., 1992). The low efficiency of iron supplementation in athletes may be explained by the presence of systemic inflammation and elevated blood hepcidin levels. Interestingly, an elevated level of pro-inflammatory cytokines was shown to be associated with deteriorated physical and mental performance (Cunningham et al., 2009; Hamer and Molloy, 2009).

Conclusion

Our investigation incorporated examinations performed after the tournament seasons, which may suggest that the length or quality of the recovery period between tournaments was not sufficient for the young athletes. Given the small number of participants engaged in our research, we classified this evaluation as a pilot study. Observations

gave us reasons to conclude that young tennis players experience low-grade systemic inflammation and impaired iron metabolism. On the one hand, the elevated hepcidin concentration observed in young tennis players can lead to iron deficiency, on the other hand, it may constitute an adaptive response protecting from inflammation. All in all data collected suggest that changes in both training and diet are vital and likely more important than iron supplementation. However, no definitive conclusion can be reached at this time and further investigations are necessary to establish, whether iron supplementation should be applied or not, especially among young athletes.

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Key points

- The first research demonstrating low grade inflammation-induced iron deficiency to be associated with elevated blood hepcidin levels in young tennis athletes.
- Evaluation of immunological response after the complete tournament season in young male tennis players.
- Conclusion to introduce the assessment of hepcidin to monitor trainings as well as symptoms of over-reaching more effectively.
- Research providing practical information for coaches that changes in diet and modifications in workloads applied in physical training could be more effective than iron supplementation in iron deficient athletes.

AUTHORS BIOGRAPHY

Ewa ZIEMANN
Employment
Gdańsk University of Physical Education and Sport, Department of Physiology
Degree
PhD
Research interests
Physiological aspects of muscle regeneration and methods supporting recovery, complementary to physical training..
E-mail: ewann@awf.gda.pl

Katarzyna KASPROWICZ
Employment
Gdańsk University of Physical Education and Sport, Department of Biology and Ecology.
Degree
MSc
Research interests
Molecular biology of exercise, especially mechanisms connecting exercise and health benefits and epigenetic determinants of physical activity.

Anna KASPERSKA
Employment
University of Physical Education Poznan and Faculty of Physical Culture Gorzow
Degree
MA
Research interests
Biochemical examinations in skeletal muscle regeneration; inflammatory response to exercise-induced muscle damage; health benefits of physical activity.

Agnieszka ZEMBROŃ-LACNY
Employment
University School of Physical Education Poznan in Department of Medical Sciences. **Degree**
Bachelor
Research interests
Hypoxia, nitric oxide and skeletal muscles regeneration.

Jedrzej ANTOSIEWICZ
Employment
Professor, Warsaw School of Social Sciences and Humanities, Department of Sport Psychology, Poland
Degree
PhD
Research interests
Iron metabolism and mechanism of ROS formation.

Radoslaw LASKOWSKI
Employment
Associate professor at Gdańsk University of Physical Education and Sport, Department of Physiology.
Degree
PhD
Research interests
Physiological aspects of judo discipline and athletes.

✉ **Ewa Ziemann, PhD**
Gdansk University of Physical Education and Sport, Department of Physiology, Kazimierza Górskiego 1, 80-336 Gdansk, Poland