Science & Sports (2019) 34, 328.e1—328.e8

Disponible en ligne sur
ScienceDirect
www.sciencedirect.com

Elsevier Masson France
EM|consulte
www.em-consulte.com

ORIGINAL ARTICLE

Plasma iron status in elite weightlifters after four weeks of intensive training

Statut en fer chez des haltérophiles élites après quatre semaines d'entraînement intense

R. Khlif[a,*], **R. Marrakchi**[b], **K. Jamoussi**[b], **Z. Sahnoun**[a], **H. Chtourou**[c,d], **N. Souissi**[d]

[a] *Laboratory of Pharmacology, Faculty of Medicine, Sfax, Tunisia*
[b] *Biochemistry Laboratory, Hedi-Chaker Hospital, Sfax, Tunisia*
[c] *Institut supérieur du sport et de l'éducation physique de Sfax, université de Sfax, Tunisia*
[d] *Activité physique: sport et santé, UR18JS01, observatoire national du sport, Tunis, Tunisia*

Received 22 September 2018; accepted 18 February 2019
Available online 15 May 2019

KEYWORDS
Weightlifting;
Overreaching;
Iron Deficiency;
Anemia;
Inflammation

Summary With or without anemia, iron deficiency, is a common disorder among athletes and it can decrease both physical and mental performances. Actually, no previous study has investigated the effects of weightlifting training on iron status responses. Therefore, the purpose of the present study was to examine the effects of four weeks of intensive training on plasma iron status of elite weightlifters. Sixteen participants (a group of eight men and a group of eight women) from the elite Tunisian team took part in the present study. Blood levels of some hematological, iron, anemia and inflammation parameters were assessed before (pre) and after four weeks (Post) of intensive training during a precompetitive phase. For both groups, ferritin concentrations and plasma Creatine kinase activity increased significantly at post-compared to pre-training ($P < 0.05$). Plasma Creatine kinase activity displayed a significant increase after training ($P < 0.01$) for both men and women lifters. However, transferrin levels decreased for both gender at post-compared to pre-training ($P < 0.05$). For C-reactive protein levels and plasma iron concentrations, no-significant pre- to post-training changes have been reported. The hemoglobin concentration was lower in the women group than the men group only before training ($P < 0.05$). Red blood cells, hematocrit and mean corpuscular volume decreased and mean corpuscular hemoglobin concentration increased significantly from pre- to post-training only in men ($P < 0.05$). For women, only red blood cells and hematocrit increased from pre- to post-training ($P < 0.05$). In conclusion, the present study showed changes in the majority of iron status balance parameters, which seems to be close to the lowest

* Corresponding author.
E-mail address: Rochdi.khlif@yahoo.com (R. Khlif).

https://doi.org/10.1016/j.scispo.2019.02.003

reference limit, with a considerable depletion of iron stores and low serum ferritin concentrations especially in woman weightlifters.

MOTS CLÉS
Haltérophilie ;
Surmenage ;
Carence en fer ;
Anémie ;
Inflammation

Résumé Avec ou sans anémie, la carence en fer est un trouble fréquent chez les athlètes et peut diminuer les performances physiques et mentales. Actuellement, aucune étude n'a examiné les effets de l'entraînement en haltérophilie sur le statut en fer. Ainsi, l'objectif de la présente étude était d'examiner les effets de quatre semaines d'entraînement intense sur le statut en fer chez des haltérophiles élites. Seize participants (un groupe de huit hommes et un groupe de huit femmes) de l'équipe nationale tunisienne d'haltérophilie ont participé à cette étude. Les taux sanguins de marqueurs biochimiques et hématologiques du statut en fer, d'anémie et d'inflammation ont été évalués avant et après 4 semaines d'entraînement intense lors de la phase précompétitive. Pour les deux groupes, la concentration de ferritine et l'activité plasmatique de la créatine kinase ont augmenté significativement après en comparaison avec avant le programme d'entraînement ($p < 0{,}05$). Cependant, les taux de transferrine ont diminué pour les deux groupes après en comparaison avec avant le programme d'entraînement ($p < 0{,}05$). Les résultats n'ont révélé aucun changement significatif de la protéine C-réactive et de la concentration plasmatique en fer entre les deux périodes de mesures chez les deux groupes. La concentration en hémoglobine était plus faible chez les femmes que chez les hommes seulement avant l'entraînement ($p < 0{,}05$). Les globules rouges, l'hématocrite et le volume corpusculaire ont diminué et la concentration corpusculaire en hémoglobine a augmenté significativement seulement chez les hommes entre avant et après l'entraînement ($p < 0{,}05$). Pour les femmes, seulement les globules rouges et l'hématocrite ont augmenté significativement entre avant et après l'entraînement ($p < 0{,}05$). En conclusion, la présente étude a montré des changements pour la majorité des paramètres du statut en fer qui semble être proche de la limite inférieure des valeurs de références, avec un épuisement considérable des réserves en fer et de faibles concentrations en ferritine en particulier chez les femmes.

1. Introduction

Iron is an essential mineral which plays a key role in a number of specific biochemical — physiologic processes including mitochondrial electron transport and protein synthesis [1,2].

Iron status is the result of the balance between iron intake and iron loss. Several factors can induce poor iron status such as a reduced iron intake [3], hemolysis, hematuria, menstrual blood loss and gastrointestinal blood loss [4]. In the absence of inflammation, serum ferritin (sFer) measurement is commonly used to evaluate iron storage [5,6]. Latent iron deficiency (LID), non-anemic iron deficiency (NAID) and iron deficiency anemia (IDA) have been the most common alterations in iron status disorder. Over the last few decades, an accurate detection of LID and IDA has been frequent among athletes [7—10]. According to several review articles detailing iron status in physically active females, ferritin from 12 to 35 μg/L [7—10] associated with low transferrin saturation (STrf) (<20%) and with low threshold hematocrit (<36%) [4] were indicators of early functional iron deficiency (ID) without anemia in athletes [11,12]. ID with microcytosis and/or hypochromia (IDMH) is defined by the drop of mean cellular volume (MCV) and mean cellular hemoglobin (MCH) concentrations below the lowest limit of the normal range (80 fl and 28 pg). When sFer is less than 30 μg/L, the red cell indices are mostly affected and the hemoglobin (Hb) concentration remains normal (men > 14 g/dL, women > 12 g/dL). Actually, these stages of iron deficiency are called ''non-anemic iron deficiency'' [13]. IDA arises when the balance of iron intake, iron stores, and the body's loss of iron are insufficient to fully support the production of erythrocytes. This last stage is characterized by a reduced hemoglobin concentration for adult women (<12 g/dL) and for adult men (<13 g/dL) [14], by a sFer level below 12 μg/L, STrf below 16% and reduced or maintained red cell indices [15].

Actually, conflicting results still exists as to whether poor iron status leads to physical performance impairment [16—18]. Recent reports have demonstrated that iron status disorders may decrease physical and cognitive performances in female athletes with ID and IDA [19,20]. Moreover, both iron deficiency and iron excess might impair immune functions and cause irritability and increased risk of injury [16]. On the other side, recent reports have highlighted the risk of iron metabolism disorders as a consequence of intensive training [21] or long period of increased training load [18,19]. However, Di Santolo [10] reported no-significant difference in the frequency of anemia, IDA or LID between physically active and inactive females. Furthermore, while the biochemical and the hematological indices related to

Table 1 Age, body-mass, training experience and training volume of the participants (mean ± standard deviation).

Variables	Age (Years)	Body-mass (kg)	Training experience (Years)	Training volume (Hours/week)
Men, (n = 8)	19.46 ± 1.2	87.88 ± 21.5	5	21
Women, (n = 8)	18.25 ± 1.2	64.5 ± 21.5	4	18

iron status have extensively been studied [22], especially in endurance athletes, swimmers, skiers and athletes of team sports, information about the cumulative effect of daily weightlifting training on iron status responses has not been examined yet.

In a review study, Bricout et al. [23] suggested that the measurement of biological markers could prevent to reach an overtraining status. Therefore, the aim of the present study was to examine the effects of a precompetitive weightlifting intensive training on the iron and the inflammatory status in both men and women elite weightlifters.

2. Methods

2.1. Participants

Sixteen elite Tunisian weightlifters (men [n = 8] and women [n = 8]) from the elite Tunisian team took part in the present study. They were all informed about the purposes of the study and they agreed to participate by a written consent. The age, body-mass, training experience and training volume of the participants are presented in Table 1. All participants were involved in a regular training program. Women weightlifters were with regular menstrual cycles (30 ± 2 days). During the study, none of the weightlifters experienced any kind of infections or injuries and none of them used anti-inflammatory medication. Iron supplementation was a criterion of exclusion in the present study. Weightlifters were asked to maintain their normal diet during all periods of the study. The protocol of this study was approved by the ethical committee of the university.

2.2. Training program

The current study was carried out in the summer (August, 2016). The weightlifting training program was realized by the coaches of the Tunisian national team. It consisted of 3 intensive weeks (INT) followed by one moderate intensity week (RED) aiming at inducing a variety of training adaptations, and improving both strength and power of the weightlifters. The INT period consisted of ten weekly training sessions, with two sessions per day, lasting between 75-min and 105-min. The number of sessions in the RED period was reduced to six. In each week, Sunday was a rest day. The intensity ranged from 70% to 100% of the one maximum repetition (1RM). The rest intervals between sets and exercises were between two and five minutes. Each training session consisted of technical lifts (i.e., snatch, clean, jerk, clean and jerk), related lifts (i.e., power snatch, power clean and power jerk) and assistance lifts (i.e., front and back squat, snatch and clean pulls and shoulders press exercises).

2.3. Blood samples collection and analysis

Fasting blood samples (8 mL) was taken using standard venipuncture between 07h00 and 08h30 (to eliminate the diurnal variation of performance [24,25] and biochemical parameters [26,27]), before and after the four training weeks and 36 hours after the last training session. Samples were centrifuged at 3000 rpm for 10 minutes within 30 minutes of the collection. Serum was stored at −80 °C. Samples were thawed only once before the analysis. All laboratory analyses were carried out at the biochemistry laboratory of Hedi-Chaker University Hospital of Sfax. Hematological parameters (i.e., red blood cells [RBC], hemoglobin [Hb], Hematocrit [HCT], mean corpuscular volume [MCV], mean corpuscular hemoglobin [MCH], mean corpuscular hemoglobin concentration [MCHC], erythrocyte distribution width [RDW], mean platelets volume [MPV] and platelets [PLT]) were measured within 2 hours after blood collection using a multichannel automated hematology analyzer (Beckman Coulter Gen system-2 instrument [Coulter T540]). Intra- and inter-assay coefficients of variation were less than 3%. Iron, transferrin (Trf), Creatine kinase activity (CK) and C-reactive protein (CRP) were assessed on Synchron®-DXC800 analyser (Beckman Coulter Inc, CA, USA). Also, plasma ferritin concentration was assessed by Access-2 Immunoassay System Analyzer (Beckman Coulter Inc, CA, USA) and percent transferrin saturation (STrf) was calculated using the following equation: Plasma iron (μg/dL)/plasma transferrin (μg/dL) × 71 [28].

2.4. Statistical analysis

Statistical analyses were carried out using STATISTICA® 10.0. The Shapiro-Wilk test confirmed that all variables were normally distributed. All data is expressed as means ± standard deviation (Mean ± SD) and as percent difference (delta %) [{(post-value−pre-value)/pre-value} × 100]. Besides, variability coefficients (CV) for all parameters were also computed for each athlete. An alpha (α) critical level of < 0.05 was used to indicate statistical significance. In each group, a paired simple t-test was used in order to analyze the effects of the training program (post vs. pre). In order to assess the practical significance of our finding, the Cohen's d was calculated. Values of 0.1, 0.3 and > 0.5 were considered small, medium and large, respectively. The exact P-values were reported in the results' section.

Table 2 Iron parameters, CK and CRP levels recorded at pre- and post-training.

Parameters	RR	Minimum—maximum	Pre	Post	Δ (Δ %)	*P*	EZ
Men							
sFer, (ng/mL)	30.0—200.0	3.9—103.1	23.4 ± 23.44	54.29 ± 33.1	23.3 (99.8)	0.03[a]	0.474
Iron, (μmol/L)	12.0—32.0	6.1—30.3	10.7 ± 3.14	15.06 ± 6.8	3.8 (37.7)	0.17	ns
Trf, (g/L)	2.0—35.0	1.85—2.95	2.59 ± 0.24	2.2 ± 0.22	−0.39 (−15)	0.016[b]	0.642
STrf, (%)	20.0—50.0	9.5—65.4	16.64 ± 5.2	28.34 ± 16.08	10 (65.4)	0.11	ns
CRP, (mg/L)	<6	2.0—30.0	3.13 ± 3.18	9.5 ± 11.45	6.4 (204)	0.096	ns
CK, (U/L)	25.0—350.0	92.0—745.0	250(166)	396 (195)	146 (58.5)	0.020[a]	0.374
Women							
sFer, (ng/mL)	16.0—150.0	7.4—119.6	24.7 (25.3)	58.7 (42.7)	34 (137.7)	0.016[b]	0.436
Iron, (μmol/L)	9.0—30.0	7.8—24.8	16.1 (4.6)	13.9 (5.6)	−2.2 (−13.7)	0.15	ns
Trf, (g/L)	2.0—4.0	1.94—2.81	2.59 (0.18)	2.26 (0.32)	−0.33 (−12.7)	0.03[b]	0.539
STrf, (%)	16.0—40.0	13.0—35.0	25 (7)	24 (7)	−0.81 (−3.22)	0.67	ns
CRP, (mg/L)	<6	2.0—35.0	2 (0)	10.5 (12.6)	8.5 (425)	0.097	ns
CK, (U/L)	30.0—300.0	57—1171	218 (133)	438 (330)	220 (101)	0.032[a]	0.4

RR: reference range for healthy subjects; Δ: difference between two consecutive analyses; Δ %: percent difference between two consecutive analyses; sFer: ferritin; Trf: transferrin; STrf: percent transferrin saturation; CK: Creatine kinase; ns: no-significant difference.

[a] $P < 0.05$ in comparison to pre-training values respectively.

[b] $P < 0.01$ in comparison to pre-training values respectively.

3. Results

3.1. Iron status, CK and CRP responses in men and women weightlifters

Iron parameters, CK and CRP levels are presented in Table 2. A significant increase pre- to post-training of plasma ferritin concentrations was observed for both men (+100%, $P = 0.03$, d = 0.474) and women (+138%, $P = 0.016$, d = 0.436). Transferrin levels decreased significantly from pre- to post-training for both men (—39%, $P = 0.016$, d = 0.642) and women (—33%, $P = 0.03$, d = 0.539). Moreover, plasma CK activity increased significantly from pre- to post-training for both men (+58%, $P = 0.02$, d = 0.374) and women (+101%, $P = 0.032$; d = 0.400). However, no-significant difference was observed between pre- and post-training for CRP, plasma iron and percent STrf levels for the two groups.

3.2. Erytrogram responses in men and women weightlifters

As indicated in Table 3, all hematological parameters were within the normal range of healthy peoples. RBC, HTC and MCV were significantly lower at post-compared to pre-training for the men group (—4.5%, $P = 0.042$, d = 0.497; —6.7%, $P = 0.002$, d = 0.629; —3.4%, $P = 0.00001$, d = 0.586 respectively). However, for men, MCHC demonstrated a significant increase pre- to post-training (3.7%, $P = 0.0001$, d = 0.753). For women lifters, only RBC, Hb and HTC demonstrated an increase from pre- to post-training (+3.5%, $P = 0.046$, d = 0.154; +3.4%, $P = 0.02$, d = 0.305; +3.3%, $P = 0.048$, d = 0.342 respectively). However, in women, PLT count decreased significantly from pre- to post-training (+12%, $P = 0.047$; d = 0.439).

3.3. Magnitude of iron deficiency and/or iron deficiency anemia in weightlifters

As indicated in Table 4 and using references values for healthy peoples, the results of the present study prove that two male and two female weightlifters at pre-training and 5 weightlifters for both groups at the post-training have an elevated plasma ferritin (i.e., >50 ug/L) associated with a lower transferrin saturation (i.e., STrf < 20%) paralleled by threshold HTC < 35% and < 40% for male and female respectively. Additionally, NAID was found in three men and two women at pre-training and one woman at post-training. Concerning the prevalence of iron depletion with microcytosis and/or hypochromia, only one woman showed an occasional microcytosis at pre- and post-training indicated by a reduction in mean red cell volume (MCV < 80 fL); but without any evidence of anemia symptoms. Moreover, IDA, existed only in one woman at pre- and post-training.

4. Discussion

The main findings of the present study was a change in the majority of iron status balance parameters (i.e., plasma iron, TSAT, HTC, MCV, MCH, RBC and Hg concentration), which seems to be close to the lowest reference limit, with a considerable depletion of iron stores and low serum ferritin concentrations especially in woman weightlifters. Such results were not found in male weightlifters. Also, higher levels of ferritin, CK activity and plasma CRP were observed in three men and three women athletes. These results, were mostly relevant to the presence of an inflammatory state. The present study findings confirm those of previous studies [28—31]. Indeed, these studies show that training load induces change in iron metabolism and contributes to a disorder in the iron status with and/or without affecting erythropoiesis [28—32]. On elite athletes competing in different

Table 3 Change in the erytrogram recorded at pre- and post-training.

Parameters	RR	Minimum—maximum	Pre	Post	Δ (Δ%)	*P*	EZ
Men							
RBC, (M/μL)	4.4—5.9	4.6—5.43	5.08 (0.23)	4.85 (0.16)	−0.23 (−4.5)	0.042[a]	0.497
PLT, (×10^3/μL)	155—450	146—343	231 (49)	255 (56)	24 (10.4)	0.065	ns
Hb, (g/dL)	13—16	13.5—16.4	15 (0.8)	14.7 (0.7)	−0.4 (−2.4)	0.16	ns
HTC, (%)	40—52	40—50	47.1 (1.9)	43.9 (2)	−3.2 (−6.7)	0.002[b]	0.629
MCHC, (g/dL)	32—36	31.4—33.7	32.16 (0.46)	33.25 (0.4)	1.2 (3.7)	0.0001[b]	0.753
MCV, (fL)	80—98	68.8—96.5	92.78 (2.2)	89.68 (1.2)	−3.1 (−3.4)	0.00001[c]	0.589
MCH, (pg)	26—34	29.3—37	30.84 (2.4)	30.9 (2.5)	0.11 (0.37)	0.567	ns
Women							
RBC, (×10^{12}/L)	4.2—5.4	4.18—6.06	4.54 (0.44)	4.7 (0.58)	0.16 (3.5)	0.046[a]	0.154
PLT, (×10^3/μL)	155—450	228—350	304 (40)	268 (31)	−36 (−12)	0.047[b]	0.439
Hb, (g/dL)	12—15	11.3—13.4	12.24 (0.7)	12.65 (0.6)	0.4 (3.4)	0.020[a]	0.305
HTC, (%)	36—46	35.1—40.8	37.5 (1.8)	38.8 (1.6)	1.2 (3.3)	0.048[a]	0.342
MCHC, (g/dL)	32—36	31—33.7	32.61 (0.67)	32.66 (0.77)	0.05 (0.15)	0.77	ns
MCV, (fL)	80—98	63.9—88.9	83.43 (8.4)	83.62 (7.7)	0.19 (0.2)	0.67	ns
MCH, (pg)	26—34	20.2—36.7	30.21 (5.7)	30.38 (5.7)	0.16 (0.5)	0.18	ns

RR: reference range for healthy subjects; Δ: difference between two consecutive analyses; Δ %: percent difference between two consecutive analyses; M: million; MPV: mean platelets volume; RBC: red blood cells; PLT: platelets; Hb: hemoglobin; HTC: hematocrit; MCHC: mean corpuscular hemoglobin concentration; MCV: mean corpuscular volume; MCH: mean corpuscular hemoglobin; ns: no-significant difference.

[a] $P < 0.05$ in comparison to pre-training values respectively.

[b] $P < 0.01$ in comparison to pre-training values respectively.

[c] $P < 0.001$ in comparison to pre-training values respectively.

Table 4 Individual changes in indices of different stages of iron deficiency throughout the training period.

Parameters	Stages of iron deficiency			
	Pre-LID	NAID	IDMH	IDA
Erytrocytes, (M/μL)			↓<4.4 or normal	↓<4.2 or normal
Hb, (g/dL)	Normal	Normal	≥12 W; ≥13 M	W: <12; M <13
HTC, (%)	↓ (35 W; 40 M)			↓
PLT, (×10^3/μL)				↑>450
MCV, (fl)		Normal	↓<80	↓<80
MCH, (pg)		Normal	↓<28	↓<28
MCHC, (g/dL)	<32	Normal	↓<32	
Ferritin, (ng/mL)	>30	↓<30	↓<20 W; <30 M	↓<12
STrf, (%)	↓<20	↓<20	↓<16	↓<16
Transferrin, (g/L)	Normal	↑	↑	
MPV			↑	↑
Pre-camp	2 M + 2 W	3 M + 2 W	1 W	1 W
Post-camp	5 M + 5 W	1 W	1 W	1 W

↓: decrease; ↑: increase; > superior; < inferior; W: women; M: men; Pre-LID; pre-latent iron deficiency; NAID: non-anemic iron deficiency; IDMH: iron deficiency with microcytosis and/or hypochromia; IDA: mild iron deficiency anemia.

sports, it has been suggested that about 25% and 35% of them were iron depleted without any signs of anemia in both genders; but, a higher prevalence was found among woman athletes [28,29]. It has been shown that premenopausal athletes were mostly at risk of iron status disorders [30] which might lead to iron deficiency (with or without anemia) due to negative iron status balance. Furthermore, Malczewska et al. [28] have previously noted that 26% of 126 female athletes whose iron intake was sufficient (14.6 mg/day) were LID without anemia symptoms. The authors concluded that the principal cause of this disorders was the high iron losses through menses. Additionally, in the study made by Constantini et al. [31], none of 43 male athletes involved in different sports had anemia (defined as Hb lower than 13 g/dL); but iron deficiency was found in 33% of female gymnasts, swimmers, and tennis players and in 36% of male gymnasts, and approximately in 20% of male swimmers and tennis players. Similarly, a study on 181 male athletes in various sports revealed that about 10% had ferritin levels below 20 μg/L [33]. Recently, a significantly high total dietary iron intake has been reported in highly active females when compared to inactive subjects; but low iron storage indices in physically active women was observed and this indicates the negative effect of exercise on iron status [34]. Woolf et al. [12] showed iron depletion in 21% of active female athletes after regular physical activity (>12 h per week). Spodaryk et al. [7] suggested that although the mean iron intake was similar in 40 female athletes (12.2 mg/d) and 40 inactive women (10.8 mg/d), iron depletion, defined by a ferritin level below 20 μg/L, was noted in 20% of runners compared to 10% of a control group. Also, hematological indices were considerably lower in athletes than sedentary women. Several studies investigated the effect of endurance training on premenopausal endurance athletes [35—37]. These studies highlighted the increase in tissue iron storage and the decrease in iron levels, transferrin and transferrin percent saturation, which reflects a change in iron metabolism and causes latent iron deficiency [35—37].

Recently, Ostojicet al. [37] have reported that female athletes who took part in sports that require mixed sources of energy supply (i.e., anaerobic and aerobic), such as rowing, volleyball, water-polo, track and field sports, had the highest risk of iron deficiency compared to predominantly aerobic (running, triathlon, tennis, cross-country skiing, road cycling) and anaerobic (sprinting, swimming, alpine skiing) sports.

Since Ferritin is an acute phase reactant, its elevated levels were a response to the rise in inflammatory and muscle damage markers [38] and it may persist for several days following heavy exercise load [39]. Besides, transferrin, as a negative acute phase reactant, has a tendency to decrease with various types of exercises [11]. Yet, these proteins do not reflect the adequate iron stores and, so, they do not present reliable markers to identify LID.

For these reasons, the iron deficiency diagnosis, particularly in overreached athletes, is somewhat delicate and it should be taken with much caution in order to make a firm conclusion. This issue is clearly complex since findings are likely to represent the combined effect of iron balance, inflammation, and physiological changes in water balance (i.e., hemodilution or hemoconcentration).

In the present study, we have utilized assays such as MCH and MCV that might be reliable parameters to estimate the rate of erythropoiesis and to distinguish the diagnosis of LID from anemia disorders. The moderate prevalence of anemia, as presented by low hemoglobin concentration found in three female athletes with normal iron status, could probably be attributed to the expansion of the plasma volume [40]. The hemodilution process takes place during the 48 h following each training session and it can persist throughout a week [41]. Indeed, the reduction in haemoglobin, haematocit or both, in association with normochromic and normocytic red cells and normal iron status in athletes was generally thought to be related to the dilutional anemia consequence of training [42]. In this context, Dubnov et al. [43] found that 7% (3% in males vs. 4% in females) of 108 basketball players had LID. A survey done on 114 Olympic level athletes in different individual sports showed that 3.5% had LID [44]. However, Varlet-Marie et al. [45] indicated that there is a biphasic effect of training status on this hemodilution, with a decrease in hematocrit after training when it is well adapted and efficient; but a reversal effect could be observed during overrreaching and overtraining periods. In addition, iron-deficient sportsmen have been shown to have a higher blood viscosity explained by a higher plasma viscosity [46] and this can play a role in the effects of overreaching on blood structure [45].

The tendency of having lower concentrations of Hb, HTC, MCV and MCHC observed in athletes was a result of heavy training [11]. In the present study, the results indicated that red blood cell count decreased in men and increased in women weightlifters. Contrary to other studies that reported no-changes in RBC [37,44], it has been shown that strenuous exercise may elevate the permeability for intracellular acute phase proteins like CK, CRP and ferritin [11] indicating that markers of muscle damage remain elevated for at least seven days after a weightlifting intense training [47].

The difference between pre- and post-training periods values in inflammatory markers have been especially due to the accumulation of more hard days in the third week. This could explain the higher production of post-training CK, ferritin (sFer > 50 μg/L) and CRP levels in three male and three female athletes, reflecting a developed trauma and greater muscle damage incidence. Malczewska et al. [29] demonstrated that plasma mean concentration of ferritin was 32 μg/L (range: 6—89 μg/L) and this correlated to CK activities in elite men judoists (10 days and 2 sessions daily for two hours). Recently, Ammar et al. [47] demonstrated a considerable rise of CRP concentrations after one weightlifting training session. Moreover, some authors deducted that this phenomenon might be the result to generalized muscle damage due to overreaching or trauma. Accordingly, since CRP is a direct indicator of inflammation and CK might be a consequence of both metabolic and mechanical stress [48], it was suggested that in post-exercise these metabolites efflux might reflect different reactions including muscle damage, repair, and regeneration and/or this could be probably proportional to the severity of muscle soreness [49].

Therefore, repetitive weightlifting exercises (e.g., snatch and clean, pulls from the floor or full bottom position in squat) are multi-joints exercises that involve great muscle mass and highly produced mechanical forces especially in the first phase characterized by greater negative acceleration, could be the major cause of pain as they could initiate a repetitive micro-trauma in tendons, ligaments and muscles. Also, they are likely to induce muscle inflammation and increased muscle damage incidence.

5. Limitations

Monitoring high level athletes is so difficult especially during a real preparation season. However, we assume that one limitation of the present study is lacks in a baseline data and/or in a resting data to reach a more firm conclusion. Also, the small sample size that was insufficient to show potentially relevant changes in selected variables and to yield an adequate statistical power.

Also, iron loss and dietary intake assessments could be beneficial to make a more firm conclusion.

6. Conclusion

The findings of present study reported changes in iron and erythrocyte metabolism associated with a marked increase in inflammatory indices. Nonetheless, athletes with high change values outside the reference range did not seem to be necessarily pathologic and did not allow us to make a firm conclusion about the real cause of LID and whether this relationship is caused by strenuous training load and/or to other factors such as poor dietary iron intake, blood losses due to menses, and/or to other agent contributors to compromised iron status values.

Most of acute proteins were affected reflecting an inflammatory state. Additionally, non-anemic iron depletion existed among elite weightlifters especially female athletes in the course of successive weightlifting training stages. Iron loss through menses might be possibly explained by gender differences responsiveness to experience iron deficiency compared with male's athletes. In fact, athletes were put under a physical and a psychological stress caused by training, which could be associated with decrements in exercise performance.

7. Practical considerations

The revealed tendency for decline in iron store and hemoglobin could be balanced by the consumption of food rich in iron available in fruits and vegetables that contain high levels of vitamin C and/or, if needed, by an appropriate iron supplementation (10 mg to 18 mg depending on sex and age) [50]. Also, to deal with the increasing tendencies of inflammation and muscle damage in athletes, coaches should relook for the nature of periodized training in the precompetitive phase to minimize the risk of inflammation.

Disclosure of interest

The authors declare that they have no competing interest.

Acknowledgments

First, we should thank the Tunisian Weightlifting Federation (TWF) for its great collaboration. Also, we are very grateful to all weightlifters and coaches who participated in the present study.

References

[1] Schumacher YO, Schmid A, König D, Berg A. Effects of exercise on soluble transferrin receptor and other variables of the iron status. Br J Sports Med 2002;36:195—200.
[2] Schumacher YO, Schmid A, Grathwohl D, Bültermann D, Berg A. Indices and iron status in athletes of various sports and performances. Med Sci Sports Exerc 2002;34(5):869—75.
[3] Lukaski HC. Vitamin and mineral status: effects on physical performance. Nut 2004;20:632—44.
[4] Peeling P, Dawson B, Goodman C, Landers G, Trinder D. Athletic induced iron deficiency: new insights into the role of inflammation, cytokines and hormones. Eur J Appl Physiol 2008;103:381—91.
[5] Guyatt GH, Oxman AD, Ali M, Willan A, McIlroy W, Patterson C. Laboratory diagnosis of iron-deficiency anemia: an overview. J General Internal Med 1992;7:145—53.
[6] Beard J, Han O. Systemic ion status. Biochim Biophys Acta 2009;1790:284—8.
[7] Spodaryk K, Czekaj J, Sowa W. Relationship among reduced level of stored iron and dietary iron in trained women. Physiol Res 1996;45:393—7.
[8] Sinclair LM, Hinton PS. Prevalence of iron deficiency with and without anemia in recreationally active men and women. J Am Diet Assoc 2005;105:975—8.
[9] Rodriguez NR, DiMarco NM, Langley S. Position of the American Dietetic Association, Dietitians of Canada, and the American College of Sports Medicine: nutrition and athletic performance. J Am Diet Assoc 2009;109:509—27.
[10] Di Santolo M, Stel G, Banfi G, Gonano F, Cauci S. Anemia and iron status in young fertile non-professional female athletes. Eur J Appl Physiol 2008;102:703—9.
[11] Fallon KE. Utility of haematological and iron-related screening in elite athletes. Clin J Sports Med 2004;14:145—52.
[12] Woolf K, St Thomas MM, Hahn N, Vaughan LA, Carlson AG, Hinton P. Iron status in highly active and sedentary young women. Int J Sport Nut Exerc Metab 2009;19:519—35.
[13] Nemeth E. Iron regulation and erythropoiesis. Curr Opin Hematol 2008;15:169—75.
[14] WHO. Haemoglobin concentrations for the diagnosis of anemia and assessment of severity. Vitamin and mineral nutrition information system. Geneva: World Health Organization; 2011.
[15] Herklotz R, Huber A. Labordiagnose von Eisenstoffwechselstörungen. In Schweiz Med Forum 2010;10:500—7.
[16] Beard J, Tobin B. Iron status and exercise. Am J Clin Nut 2000;72:594—7.
[17] Della Valle DM, Haas JD. Impact of iron depletion without anemia on performance in trained endurance athletes at the beginning of a training season: a study of female collegiate rowers. Int J Sport Nutr Exerc Metab 2011;21:501—6.
[18] Newlin MK, Williams S, McNamara T, Tjalsma H, Swinkels DW, Haymes EM. The effects of acute exercise bouts on hepcidin in women. Int J Sport Nut Exerc Metab 2012;22:79—88.
[19] McClung JP, Gaffney-Stomberg E, Lee JJ. Female athletes: a population at risk of vitamin and mineral deficiencies affecting health and performance. J Trace Element Med Biol 2014;28:388—92.
[20] Haas JD, Brownlie T. Iron deficiency and reduced work capacity: a critical review of the research to determine a causal relationship. J Nut 2001;131:676—90.
[21] Alaunyte SV, Plunkett A. Iron and the female athlete: a review of dietary treatment methods for improving iron status and exercise performance. J Int Soci Sports Nut 2015;12:38.
[22] Ascensão A, Rebelo A, Oliveira E, Marques F, Pereira L, Magalhães J. Biochemical impact of a soccer match-analysis of oxidative stress and muscle damage markers throughout recovery. Clin Biochem 2008;41:841—51.
[23] Bricout VA, Guinot M, Duclos M, Koulmann N, Serrurier B, Brun JF, et al. Position statement: contribution of the biologic analyses in the diagnosis of overtraining syndrome. Sci Sports 2006;21(6):319—50.
[24] Chtourou H, Aloui A, Hammouda O, Chaouachi A, Chamari K, Souissi N. The effect of time-of-day and judo match on short-term maximal performances in judokas. Biol Rhythm Res 2013;44(5):797—806.
[25] Chtourou H, Engel FA, Fakhfakh H, Fakhfakh H, Hammouda O, Souissi N, et al. Diurnal variation of short-term repetitive maximal performance and psychological variables in elite judo athletes. Front Physiol 2018;9:1499.
[26] Ammar A, Chtourou H, Hammouda O, Turki M, Ayedi F, Kallel C, et al. Relationship between biomarkers of muscle damage and redox status in response to a weightlifting training session: effect of time-of-day. Acta Physiol Hung 2016;103(2): 243—61.
[27] Ammar A, Chtourou H, Hammouda O, Trabelsi K, Chiboub J, Turki M, et al. Acute and delayed responses of C-reactive protein, malondialdehyde and antioxidant markers after

resistance training session in elite weightlifters: effect of time of day. Chronobiol Int 2015;32(9):1211—22.
[28] Malczewska J, Raczynski G, Stupnicki R. Iron status in female endurance athletes and in non-athletes. Int J Sport Nut Exerc Metab 2000;10:260—76.
[29] Malczewska J, Stupnicki R, Blach W, Turek-lepa E. The effects of physical exercise on the concentrations of ferritin and transferrin receptor in plasma in male judoists. Int J Sport Med 2000;25:516—21.
[30] Wang W, Bourgeois T, Klima J, Berlan ED, Fischer AN, O'Brien SH. Iron deficiency and fatigue in adolescent females with heavy menstrual bleeding. Haemophilia 2013;19:225—30.
[31] Constantini NA, Eliakim L, Zigel M, Yaaron B, Falk. Iron status of highly active adolescents: evidence of depleted iron stores in gymnasts. Int J Sport Nut Exerc Metab 2000;10:62—70.
[32] Biancotti PP, Caropreso A, Di Vincenzo GC, Ganzit GP, Gribaudo CG. Hematological status in a group of male athletes of different sports. J Sports Med Physical Fit 1992;32:70—5.
[33] Voss SC, Alsayrafi M, Bourdon PC, Klodt F, Nonis D, Hopkins WG, et al. Variability of serum markers of erythropoiesis during 6 days of racing in highly trained cyclists. Int J Sport Med 2014;35:89—94.
[34] Konijn AM. Iron metabolism in inflammation. Bailliere's Clinl Rheumatol 1994;8:829—49.
[35] Baynes R, Bezwoda W, Bothwell T. The non-immune inflammatory response: serial changes in plasma iron, iron binding capacity, lactoferrin, ferritin and C-reactive protein. Scand J Clin Laboratory Investig 1986;46:695—704.
[36] Blum SM, Sherman AR, Boileau RA. The effects of fitness-type exercise on iron status in adult women. Am J Clin Nut 1986;43:456—63.
[37] Ostojic SM, Ahmetovic Z. Weekly training volume and hematological status in female top-level athletes of different sports. J Sports Med Phys Fitness 2008;48:398—403.
[38] Kłapcińska B, Waśkiewicz Z, Chrapusta SJ, Sadowska-Krępa E, Czuba M, Langfort J. Metabolic responses to a 48-h ultramarathon run in middle-aged male amateur runners. Eur J Appl Physiol 2013;113:2781—93.
[39] Suedekum NA, Dimeff RJ. Iron athlete. Curr Sports Med Reports 2005;4:199—202.
[40] Eichner ER. Sports anemia, iron supplements, and blood doping. Med Sci Sports Exerc 1992;24:315—8.
[41] Chatard JC, Mujika I, Guy C, Lacour JR. Anemia and iron deficiency in athletes. Sports Med 1999;27:229—40.
[42] Shaskey DJ, Green GA. Sports haematology. Sports Med 2000;29:27—38.
[43] Dubnov G, Naama W, Constantini N. Prevalence of iron depletion and anemia in top-level basketball players. Int J Sport Nut Exerc Metab 2004;14:30—7.
[44] Eliakim A, Nemet D, Constantini N. Screening blood tests in members of the Israeli National Olympic team. J Sports Med Phys Fitness 2002;42:250—5.
[45] Varlet-Marie E, Maso F, Lac G, Brun JF. Hemorheological disturbances in the overtraining syndrome. Clin Hemorheol Microcircul 2004;30:211—8.
[46] Khaled S, Brun JF, Wagner A, Mercier J, Bringer J, Préfaut C. Increased blood viscosity in iron-depleted elite athletes. Clin Hemorheol Microcircul 1998;18(4):309—18.
[47] Ammar A, Chtourou H, Trabelsi K, Padulo J, Turki M, El Abed K, et al. Temporal specificity of training: intra-day effects on biochemical responses and Olympic weightlifting performances. J Sports Sci 2015;33:358—68.
[48] Brancaccio P, Giuseppe L, Nicola M. Biochemical markers of muscular damage. Clin Chemistry Laboratory Med 2010;48:757—67.
[49] Flann KL, LaStayo PC, McClain DA, Hazel M, Lindstedt SL. Muscle damage and muscle remodeling: no pain, no gain? J Exp Biol 2011;214:674—9.
[50] Karl JP, Lieberman HR, Cable SJ, Williams KW, Young AJ, McClung JP. Randomized, double-blind, placebo-controlled trial of an iron-fortified food product in female soldiers during military training: relations between iron status, serum hepcidin, and inflammation. Am J Clin Nut 2010;92(1):93—100.