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ORIGINAL ARTICLE

Effect of intense physical exercise on hepcidin levels and selected parameters of iron metabolism in two different trial of training

Effet d'un exercice physique intensif sur le niveau d'hepcidine et de certains paramètres du métabolisme du fer dans deux phases d'entraînement différentes

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KEYWORDS
Rowers;
Strenuous exercise;
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Inflammation;
Iron parameters

Summary
Research question. — The aim of the study was to analyse changes in selected pro-inflammatory cytokines and parameters of iron metabolism, observed after an intense ergometric test conducted during two different phases of a yearly training cycle.
Research methods. — The study included a group of elite rowers ($n = 15$). During each of the analysed training phases, the athletes were subjected to a 2000-m ergometric exercise test.

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Results and findings. — Irrespective of the training phase, similar levels of IL-6, hepcidin and sTfR were found in blood samples collected immediately after the ergometric tests. In contrast, post-exercise levels of Fe, TNF-α and CK were significantly higher in material collected after the test conducted during the competitive phase of a yearly training cycle. This study of elite rowers showed that physical exercise may stimulate changes in the pro-inflammatory profile of blood serum, via the influence on TNF-α levels. This effect is modulated by a number of training-related factors that acted prior to the ergometric test, such as exercise frequency and intensity.
Implications. — Therefore, post-training adaptation to physical exercise is not always sufficient, which may result in the impairment of the immune response and thus negatively affect iron metabolism.

MOTS CLÉS
Rameurs ;
Effort intense ;
Entraînement ;
Inflammation ;
Paramètres du métabolisme du fer

Résumé
But de la recherche. — Le but de l'étude était d'analyser l'impact des charges d'entraînement sur les changements dans le niveau de cytokines pro-inflammatoires et certains paramètres du métabolisme du fer chez les athlètes effectuant un test d'effort intense, ergométrique effectué dans deux périodes d'entraînement différentes.
Méthodes. — Les tests ont été réalisés parmi les athlètes s'entraînant à l'aviron ($n = 15$) en deux périodes d'entraînement (préparatoire et de démarrage). Au cours de chacune des périodes d'entraînement, les participants ont effectué un test d'effort ergométrique à 2000 m, surmontant cette distance dans les plus court temps. Avant le test ergométrique et dans la première minute après son achèvement et après 24 heures de repos, le sang de la veine octogonale a été prélevé sur les athlètes. Dans le sang prélevé pour les tests, on a indiqué: niveau d'IL-6, TNF-α, hepcidine, taux de Fe, ferritine, sTfR et CK.
Résultats. — Les échantillons sanguins prélevés après l'épreuve d'effort ont montré des niveaux similaires d'IL-6, d'hepcidine, de sTfR et de transferrine pendant la période de préparation et de démarrage. Cependant, au cours de la période de démarrage, les taux de TNFα et de CK étaient significativement plus élevés qu'au cours de la période préparatoire.
Conclusion. — Dans le groupe d'athlètes examinés, une augmentation du niveau de TNF n'a été observée qu'au cours de la période de démarrage. On suppose que ce résultat a été influencé par les lourdes charges d'entraînement typiques de la période de démarrage utilisée la semaine précédant l'essai ergométrique. Par conséquent, augmentée par l'entraînement, l'adaptation des athlètes à l'exercice physique n'est pas toujours suffisante, ce qui peut en conséquence conduire à une réponse immunologique altérée, et ainsi affecter négativement le métabolisme du fer.

1. Introduction

One prerequisite of health is to maintain the homeostasis of iron. Physiological levels of iron, providing normal functioning of all human tissues, including the immune system — a key component of infection control, are very narrow. Published data imply that both excess and deficiency of iron may contribute to impairment of immune function [1].

It is still unclear which mechanisms contribute primarily to the disturbances of iron metabolism observed in athletes. Available evidence suggests that some proportion of circulating erythrocytes may undergo haemolysis due to high training loads [2]. The resultant increase in serum concentration of ionised iron seems to contribute considerably to the enhancement of free radical-mediated reactions on the one hand, and activation of the immune response and inflammatory reactions on the other [3]. Ongoing inflammation interferes with intestinal absorption of iron and stimulates mobilisation of this element from its body stores, which eventually results in iron deficiency and even anaemia [4].

It needs to be stressed that the effects of physical exercise on the immune system may vary depending on the type of training loads. While moderate exercise is postulated to stimulate immunological mechanisms, repeated high-intensity exercise may impair the immune response and thus exerts unfavourable effects on iron metabolism parameters [5].

A retrospective observation of elite athletes (representatives of various disciplines) over a period of 1 calendar year demonstrated that in the large proportion of the study subjects, the levels of ferritin, iron and TIBC remained below

Table 1 Training schedules during the weeks preceding blood sample collections before Trial I and after Trial II.

	Days before Trial I						
	1	2	3	4	5	6	7
Total training time, min/day	—	200	110	200	120	190	110
Time rowed, min/day	—	80	100	80	100	70	90
Distance rowed, km/day	—	18	22	18	22	14	20
Training for force development, min/day	—	90	—	90	—	85	—
Extensive endurance rowing training time, min/day	—	80	100	55	100	50	60
High-intensity endurance rowing training time, min/day	—	—	—	25	—	20	30
Unspecific training (running, etc.), min/day	—	30	10	30	20	35	20
	Days before Trial II						
	1	2	3	4	5	6	7
Total training time, min/day	120	100	200	110	210	200	100
Time rowed, min/day	90	80	80	90	140	110	80
Distance rowed, km/day	12	18	18	20	30	24	18
Training for force development, min/day	—	—	90	—	50	50	—
Extensive endurance rowing training time, min/day	75	80	80	90	100	90	60
High-intensity endurance rowing training time, min/day	15	—	—	—	40	20	20
Unspecific training (running, etc.), min/day	30	20	30	20	20	40	20

the reference limits [6]. Abnormalities of iron status were also documented in a previous study [7] of 20 elite rowing athletes and 10 professional soccer players examined during a competitive season.

However, supplementation of iron in athletes exposed to high training loads does not seem to be an appropriate solution, since elevated concentrations of hepcidin prevent absorption of this element. Moreover, extra supplementation of iron may enhance ongoing inflammation, negatively affecting the general condition and performance of athletes [8,9].

As mentioned previously, iron is vital for normal functioning of the human body. An understanding of the relationship between iron status and inflammation in athletes during various periods of their yearly training cycle may provide better insight into the diagnostics of iron deficiency anaemia in this group. This may have important therapeutic implications, contributing to more effective treatment of anaemia without compromising the safety of iron preparations.

The aim of this study was to verify if the period of a yearly training cycle, during which an intense ergometric test was conducted, directly influences and, if so, the extent of changes in the levels of pro-inflammatory cytokines, hepcidin and selected parameters of iron metabolism in rowers.

2. Material and methods

2.1. Study population

The study included 15 male athletes (age = 21 ± 1 years, body mass = 90.3 ± 8.67 kg and height = 1.92 ± 0.04 m) who were members of the Polish Rowing Team (11 heavyweight and 4 lightweight rowers). The study was conducted between April and June, during an 8-week training camp between the preparatory and competitive phase of a yearly training cycle. The study participants did not report any health problems or dietary restrictions, and none of them took anti-inflammatory drugs, vitamins or medications with established effects on iron metabolism within 2 weeks preceding the tests. The protocol of the study was approved by the local Bioethics Committee at Poznań University of Medical Sciences, and written informed consent was sought from all the study participants.

2.2. Training program

Training volumes (expressed in minutes per day) during the week preceding the first and the second trial (referred to as Trial I and Trial II, respectively), separately for extensive rowing, intensive rowing, kilometres and extensive nonspecific training, are summarised in Table 1. Training volume during the load training phase (before Trial 1) amounted to 930 min-wk-1 week; approximately 48% of this time was allocated for extensive rowing and 16% for nonspecific training (e.g., power training and intensive rowing). Overall training volume before Trial II was 1040 min-wk-1; approximately 55% of this time was spent for extensive rowing, 9% for intensive rowing and the rest for land training.

2.3. Rowing performance test

The athletes were subjected to a controlled 2000-m time test twice: during the preparatory phase of their yearly training cycle (Trial I) and 8 weeks thereafter, during the competitive phase (Trial II). Each participant had to cover the 2000-m distance on a rowing ergometer (Concept II, USA) in as short a time as possible. Since the results of both trials were taken into consideration during selection to the championship team, the athletes were well motivated to perform

the tests with maximum effort. Each trial was preceded by a 5-minute individual warm-up.

2.4. Sample treatment

Blood samples were collected before the 2000-m test (in the morning, after an overnight fast), 1 minute after completing the trial and following a 24-hour recovery period. Blood was obtained from the antecubital vein and collected in dipotassium ethylene diamine tetra-acetic acid (K_2EDTA)-coated tubes. The samples were immediately centrifuged to separate erythrocytes from plasma and to determine the activity of creatine kinase (CK).

Blood samples for determination of serum iron (Fe), soluble transferrin receptor (sTfR), ferritin, interleukin (IL)-6, tumour necrosis factor (TNF)-α and hepcidin levels were placed in tubes without additives, frozen immediately after the centrifugation and stored at −80 °C until analysis. Additionally, capillary blood samples were obtained from an earlobe prior to and after each exercise test to assess the lactic acid levels of athletes.

2.5. Measurements

Serum concentrations of IL-6 were determined with a commercially available enzyme-linked immunosorbent assay (ELISA) (Quantikine HS, cat. No. HS600B, R&D Systems, Minneapolis, Minnesota, USA), with an assay range of 0.38—10 pg/mL^{-1}. The average interassay CV of the test was 8.7%. Serum concentrations of TNF-α (expressed in pg/mL) were quantified using a commercially available enzyme immunoassay (Quantikine Human Immunoassay, cat. No. DTA00C, R&D Systems Inc., Minneapolis, Minnesota, USA). The average interassay CV of the test was 8.7%. Serum levels of hepcidin were measured with a commercially available ELISA (Wuhan EIAab Science Co., China), with a 0.187—12 ng/mL^{-1} assay range. The average interassay CV of the test was 4.1%.

Concentrations of iron were determined using the colorimetric method with chromogens (cat. No. 1-418-01-50, BioMaxima, Poland); the results were expressed in μmol/L. Serum levels of ferritin were determined immunochemically, with a commercially available diagnostic kit (cat. No. DE4408, Demeditec Diagnostic, Germany); the results were expressed in μg/L. The average interassay CV of the test was 4.6%. Concentrations of sTfR were measured immunochemically with a commercially available kit (cat. No. RD194011100, BioVendor, Brno, Czech Republic); the results were expressed in nmol/L. The average interassay CV of the test was 3.4%. Plasma activity of CK was determined with a commercially available kit (cat. No. 1-233-0150, BioMaxima, Poland); the results were expressed in U/L. Concentrations of lactic acid were determined immediately after collecting capillary blood samples, using a commercially available kit (cat. No. LKM 140, HACH LANGE, Düsseldorf, Germany). The results were expressed in mmol/L; whenever necessary, they were adjusted for haemoconcentration using the exercise-induced changes in haematocrit as a covariate.

Table 2 Changes in 2000-m rowing ergometer performance and creatine kinase (CK) during Trial I and Trial II (mean ± sd).

Parameter	Trial I (n = 15)	Trial II (n = 15)
Power (Watt)	440 ± 30.18	445 ± 29.81
(W/kg-1)	(4.89 ± 0.29)	(4.95 ± 0.30)
LA_{min} (mmol/L^{-1})	1.24 ± 0.32	1.24 ± 0.41
LA_{max} (mmol/L^{-1})	14.73 ± 2.61	15.65 ± 1.77
Time (s)	370.7 ± 8.93	369.2 ± 9.07
CK (U/L^{-1})		
B	111.87 ± 46.70	131.47 ± 38.47
Ex	156.53 ± 51.48	178.87 ± 61.45[a]
R	127.47 ± 43.68[c]	179.60 ± 59.89[a,b]

Values represent means ± standard deviations. LA: lactic acid; CK: creatine kinase; B: baseline; Ex: immediately after the exercise; R: after a 1-day recovery.
[a] Significantly different compared to Trial I.
[b] Significantly different compared to baseline level.
[c] Significantly different compared to post-exercise level.

2.6. Statistical analysis

Statistical analysis was conducted with STATISTICA v. 12.0 software package (Stat-Soft, Cracow, Poland). Main effects of exercise and training phase (Trial I vs. Trial II), and the interaction effect, were assessed with two-way analysis of variance (ANOVA). If any significant changes were documented on ANOVA, Fisher post-hoc tests were conducted to identify the source of variance. Associations between pairs of continuous variables were analysed on the basis of Pearson's coefficients of linear correlation. All the results are presented as arithmetic means and their standard deviations. The results of all the tests were considered significant at $P < 0.05$.

3. Results

Power output, blood lactate levels and total run times are presented in Table 2. Training loads for the ergometer tests conducted during the preparatory (Trial I) and competitive phase of a yearly training cycle (Trial II) are shown in Table 1.

3.1. Inflammatory response

The inflammatory response was assessed on the basis of serum levels of IL-6 and TNF-α. The training phase was shown to exert a statistically significant effect on serum levels of TNF-α during Trial I and Trial II ($P = 0.0001$). While Trial I was not associated with any significant changes in TNF-α levels, this parameter increased significantly in response to the ergometric test conducted within the framework of Trial II and still remained elevated after a 24-hour restitution period (Fig. 1A). The serum levels of IL-6 were similar irrespective of the analysed period; a significant increase in this parameter immediately after the ergometric test was followed by its normalisation during the restitution period (Fig. 1B).

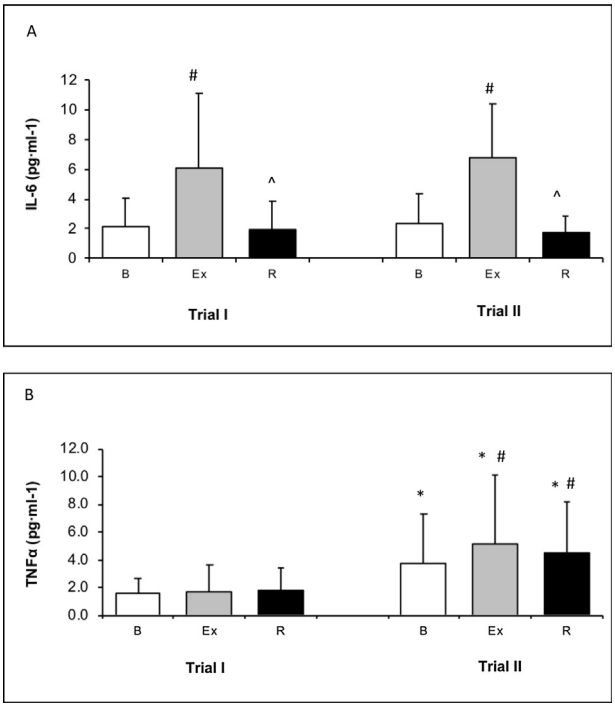

Figure 1 Changes in interleukin 6 (A) and tumour necrosis factor alpha (B) levels during the exercise tests performed within the framework of Trial I and Trial II (mean ± s). Note: IL-6: interleukin 6; TNF-α: tumour necrosis factor alpha; B: baseline; Ex: immediately after the exercise; R: after a 1-day recovery; *: significantly different compared to Trial I; #: significantly different compared to baseline level; ^: significantly different compared to post-exercise level.

3.2. Hepcidin and parameters of iron metabolism

ANOVA did not document a significant main effect of the training phase on serum hepcidin levels during Trial I and Trial II ($P = 0.253$). Irrespective of the training phase, serum concentrations of hepcidin increased significantly in response to the ergometric test and returned to their baseline values during the restitution period ($P = 0.0001$; Fig. 2A).

The ergometric test conducted during the preparatory phase of a yearly training cycle (Trial I) did not exert significant effect on serum concentrations of iron in our athletes. In contrast, the test conducted within the framework of Trial II stimulated a significant increase in serum concentrations of iron with subsequent normalisation of this parameter following a 24-hour period ($P = 0.0345$). No statistically significant differences were found between serum levels of iron determined during both the preparatory and competitive phases of a yearly training cycle ($P = 0.652$; Fig. 2B).

No significant differences in serum ferritin levels were documented, either between the measurements taken prior to and after each ergometric test ($P = 0.912$) or between Trial I and Trial II ($P = 0.581$; Fig. 2C).

ANOVA showed that the exercise test exerted significant effects on serum levels of sTfR ($P = 0.002$). Irrespective of

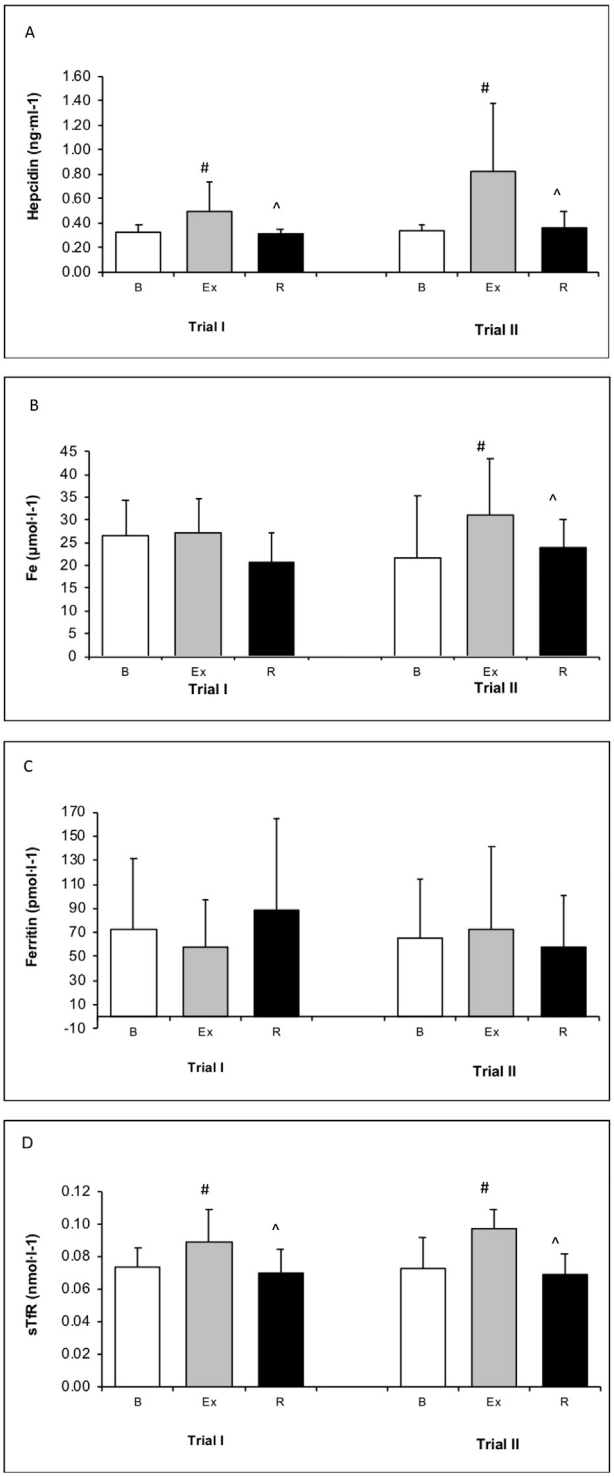

Figure 2 Changes in serum levels of hepcidin (A), iron (B), ferritin (C) and sTfR (D) during the exercise tests performed within the framework of Trial I and Trial II (mean ± s). Note: B: baseline; Ex: immediately after the exercise; R: after a 1-day recovery; #: significantly different compared to baseline level; ^: significantly different compared to post-exercise level.

the analysed training phase, the ergometric test stimulated a significant increase in sTfR concentration. Following a 24-hour restitution period, the serum concentration of sTfR either returned to its baseline level (Trial I) or at least was significantly lower than immediately after the ergometric test (Trial II; Fig. 2D).

3.3. Other parameters

Both the ergometric test ($P = 0.003$) and training phase ($P = 0.009$) exerted significant effects on the plasma activity of CK (Table 2). Irrespective of the training phase, we observed a significant increase in CK activity immediately after the exercise test. However, depending on the analysed training phase, the plasma activity of CK normalised to its baseline level following a 24-hour restitution period (Trial I) or still remained significantly elevated (Trial II).

4. Discussion

In this study, transition from preparatory to competitive phase of a yearly training cycle did not exert a significant effect on the dynamics of serum IL-6 after the intense ergometric test. Irrespective of the analysed phase, the test stimulated an increase in serum concentrations of IL-6 with subsequent normalisation of this parameter (Fig. 1A). These observations are consistent with the results of previous studies in humans [10—12] and experiments in animal models [13]. Available evidence suggests that the degree of exercise-induced changes in IL-6 levels may depend on the intensity and duration of exercise [14]. Also, an association between concentrations of IL-6 and dietary intake of carbohydrates was observed; an increase in pre-exercise intake of carbohydrates was associated with a decrease in IL-6 levels [11,15]. However, the post-exercise supply of carbohydrates does not exert a significant effect on the synthesis of this cytokine [16].

According to literature [13,17], the increase in IL-6 level is associated with a concomitant increase in hepcidin concentration. Hepcidin plays an important role in iron metabolism, regulating intestinal absorption of this element and its release from macrophages of the reticuloendothelial system. An increase in serum concentration of hepcidin results in a blockade of iron release from the reticuloendothelial system, which is reflected by a functional deficiency of this element, observed inter alia in chronic inflammation [18]. Post-exercise increases in hepcidin concentrations in our athletes, observed during Trial I and Trial II, seem to be primarily associated with an acute phase reaction and synthesis of IL-6, since a significant positive correlation was found between these two parameters (Fig. 3). According to Nemeth et al. [17], an increase in serum IL-6 is sufficient to stimulate expression of hepcidin during the course of the inflammatory response. This hypothesis was confirmed experimentally by Banzet et al. [13] who showed that administration of an immunosuppressant, cyclosporine A (CsA), to rats exposed to strenuous physical exercise was reflected by a significant decrease in IL-6 concentration, which in turn resulted in a 50% drop in hepcidin mRNA levels in the liver.

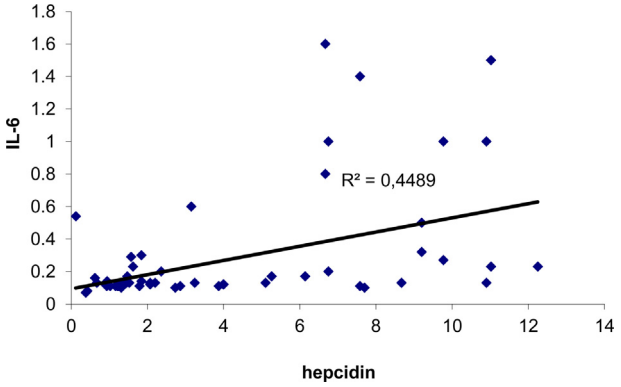

Figure 3 Relationship between interleukin (IL)-6 and hepcidin levels.

Irrespective of the analysed training phase, the ergometric test did not exert a significant effect on the level of ferritin in our rowers (Fig. 2C). Ferritin is a principal iron storage protein, and its serum concentration reflects systemic resources of this element. Sim et al. [14] demonstrated that changes in ferritin levels in well-trained male triathletes depends on the type of exercise performed. In their study, both high- and low-intensity running tests stimulated an increase in ferritin level, which still persisted after 3 hours of restitution. In contrast, cycling ergometer exercise of similar intensity did not induce any significant changes in this parameter. According to authors of this study, the level of ferritin, an acute phase protein, may reflect a haemolysis-induced inflammatory response, which is particularly exacerbated with the impact forces associated with running. According to Nikolaidis et al. [19], the serum level of sTfR is a more stable parameter than ferritin concentration. Irrespective of the analysed training phase, the ergometric test was reflected by a significant increase in serum sTfR levels in our athletes, with subsequent normalisation of this parameter following a 24-hour restitution period (Fig. 2D). Also, in the study conducted by Schumacher et al. [20], strenuous physical exercise was reflected by a post-exercise increase in serum sTfR levels; however, a similar effect was not observed in response to moderate physical exercise.

The ergometric test conducted within the framework of Trial I did not stimulate significant changes in serum iron levels in our athletes. However, a significant post-exercise increase in this parameter, with its subsequent normalisation to the baseline level after a 24-hour restitution period, was observed during Trial II (Fig. 2A). Other authors also reported an increase in serum iron in response to strenuous exercise [11,16,21,22]. However, depending on the study, serum levels of iron following a 24-hour restitution period after strenuous physical exercise either decreased markedly or returned to their baseline levels [23].

TNF-α and CK were the only parameters whose post-exercise values determined during Trial I and Trial II differed significantly. While the ergometric test conducted within the framework of Trial I did not induce significant changes in TNF-α concentrations, a significant post-exercise increase in this parameter, persisting up to 24 hour thereafter, was observed during Trial II (Fig. 1B). This phenomenon might be associated with different training loads used during

the preparatory and competitive phases of a yearly training cycle (Table 1). Trainings preceding Trial II included a greater proportion of high-intensity exercises, especially the so-called ''competitive exercises'', than prior to Trial I. Moreover, the ergometric test conducted within the framework of Trial II stimulated an evident increase in plasma activity of CK, a marker of muscle injury, and this parameter did not normalise following a 24-hour restitution period (Table 2). A 30-minute session of cycloergometer exercise with the intensity corresponding to 70% VO_{2max} was reflected by a significant increase in TNF-α concentration in male participants of the study conducted by Kimura et al. [24]. However, the same test at lower intensity (50% VO_{2max}) did not stimulate any significant changes in TNF-α levels.

An animal study [25] was performed to analyse the effects of moderate, prolonged and overtraining exercise. Only moderate exercise turned out to exert a beneficial effect on the immune response; other types of exercise stimulated an increase in the levels of pro-inflammatory cytokines, including TNF-α. Also, in another study, a 6-week trial of high-intensity interval training resulted in a significant increase in serum levels of TNF-α in rats. The post-exercise changes in the concentration of this cytokine were likely associated with a greater number of used training units (6 days per week) [26]. Our rowers participated in seven trainings per week, which corresponded to 100—210 minutes of exercise per day (Table 1). As mentioned previously, trainings conducted prior to Trial II included a greater proportion of high-intensity exercises, which might contribute to the unfavourable changes in TNF-α concentration. The effect of training load on post-exercise levels of TNF-α in rowing athletes was also reported by Ramson et al. [27].

Enhanced synthesis of TNF-α observed after intense physical exercise may contribute to a decrease in insulin-stimulated glucose uptake in skeletal muscles. Available evidence suggests that TNF-α may prevent insulin-induced glucose uptake and translocation of GLUT4 (glucose transporters), blocking activation of insulin receptors and phosphatidylinositol 3-kinase signalling [28]. In our present study, elevated levels of TNF-α were still observed 24 hours post-exercise (Fig. 1B). This implies that the application of additional training loads (especially high-intensity exercises) to subjects with depleted muscle glycogen may result in further damage to muscle fibres. The results published by Singh et al. [29] suggest that oxidative stress may also contribute to the blockade of insulin signal transduction in damaged muscles. Moreover, TNF-α is known to promote accumulation of neutrophils and macrophages in skeletal muscles and to induce the generation of reactive oxygen species in neutrophils [30]. A cell line study conducted by Antosiewicz et al. [31] showed that TNF-α stimulates the formation of reactive oxygen species, which contributes to an increase in the labile iron pool. The same study demonstrated that both the quantity of generated reactive oxygen species and the severity of oxidative stress induced by TNF-α are determined by the amount of iron released during the degradation of ferritin. Thus, it can be hypothesised that inflammation may indirectly contribute to regulation of free radical-mediated processes induced by strenuous exercise, whereas the role of iron during these processes needs to be addressed by further comprehensive studies.

Future studies should center around the regulatory mechanisms of iron metabolism during and after strenuous physical exercise, as well as around the identification of factors that may attenuate the unfavourable effects of maximal training loads.

5. Conclusions

This study of elite rowers showed that physical exercise may stimulate changes in the pro-inflammatory profile of blood serum; this effect is modulated by a number of training-related factors, such as exercise frequency and intensity. Our findings and observations of other authors regarding the exercise-induced increase in serum TNF-α levels justify further research on potential methods to attenuate the unfavourable consequences of using maximum loads during periods of intensive training.

Practical Message

An increase in training loads during the competitive period may contribute to impairment of the immune response and thus predispose to disorders of iron metabolism. Therefore, administration of immunostimulatory compounds should be considered in athletes, especially those exposed to high training loads, to neutralise the harmful effects of strenuous exercise.

Disclosure of interest

The authors declare that they have no competing interest.

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