

# EFFECTS OF EXERCISE ON THE MARKERS OF IRON STATUS IN SERUM OF CROSS-COUNTRY SKIERS

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**AUTHORS:** Malczewska-Lenczowska J.<sup>1</sup>, Stupnicki R.<sup>2</sup>, Gabrys T.<sup>3</sup>

<sup>1</sup> Department of Nutrition Physiology, Institute of Sport, Warsaw, Poland

<sup>2</sup> University of Physical Education, Warsaw, Poland

<sup>3</sup> Department of Theory and Methodology of Sports Training, University of Physical Education, Kraków, Poland

Reprint request to:

Jadwiga Malczewska-Lenczowska  
Institute of Sport,  
ul. Trylogii 2/16, 01-982 Warsaw  
jadwiga.malczewska@insp.waw.pl  
tel. +48 22 834 08 12 int. 248  
fax +48 22 835 09 77

**ABSTRACT:** The study aim was to assess the within-subject, day-to-day variability for ferritin and soluble transferrin receptor (sTfR) concentrations in serum of 6 female and 8 male cross-country skiers aged 16-18 years under a regular training regimen throughout 8 consecutive days. The concentrations of iron status variables and creatine kinase (CK) activities were adjusted to plasma volume changes. Mean ferritin concentrations were  $30.6 \cdot 1.142^{\pm 1}$  and  $22.6 \cdot 1.167^{\pm 1}$   $\mu\text{g/l}$  for men and women, respectively, the average within-subject, mean day-to-day variability coefficients (CV) being 13.4% in men and 15.2% in women. Mean sTfR was  $2.14 \cdot 1.050^{\pm 1}$  and  $2.62 \cdot 1.047^{\pm 1}$   $\text{mg/l}$ , respectively, and mean day-to-day CV 6.5% and 4.6%. Mean CV for sTfR/logFerr were 6.0% and 7.4%, respectively. Neither index correlated with training loads or CK activities. Thus, the training performed once daily had no significant effect on ferritin concentrations on the following morning, so ferritin alone may prove insufficient in detecting iron deficiency in endurance athletes. The low variability of sTfR under endurance loads made it useful in detecting iron deficiency together with ferritin and the sTfR/logFerr index. Adjusting the concentrations of ferritin and sTfR by changes in plasma volume might be recommendable for endurance athletes.

**KEY WORDS:** Exercise, soluble transferrin receptor, ferritin, sTfR/logFerr index

## INTRODUCTION

Iron deficiency is the most prevalent micronutrient deficiency world-wide [9,13] but its detection poses difficulties as the assessment of iron status requires the use of appropriate diagnostic indices as well as those of health status. This is because some indices of iron metabolism, e.g. ferritin or transferrin, are acute-phase proteins and may undergo marked fluctuations in various diseases while other ones, like the soluble transferrin receptor (sTfR), may rise not only in iron deficiency but also at an increased rate of erythropoiesis [9,32]. Thus, many authors emphasize the need to apply at least two iron-related parameters simultaneously [5,9].

Athletes, like women in reproductive age, children and youths, belong to the category of increased risk of iron deficiency. However, detecting that deficiency in physically active individuals is difficult due to possible exercise-induced changes in iron metabolism indices, especially ferritin. Increased ferritin levels were reported immediately post-exercise [21,23] and even several days following strenuous exertions [12,27]. In most studies on the effects of exercise on iron metabolism, its indices were determined before

and immediately post-exercise [20,21,23,26]. Such studies are, however, of inconclusive value since during the hours following an exercise bout, the concentrations of many blood constituents may change. Moreover, when iron status is to be assessed, blood is usually withdrawn in the morning, in the preprandial state; therefore, iron metabolism indices ought to be determined on consecutive training days, following a night rest.

Earlier studies on male [16] and female [14] judoists revealed a marked day-to-day variability of ferritin levels under high physical loads and demonstrated that the soluble transferrin receptor (sTfR) was a much more stable indicator of iron metabolism in speed-strength athletes under such conditions compared with ferritin alone. The lack of similar studies on endurance athletes prompted us to determine the within-subject, day-to-day variability under conditions of varying training loads for the following iron status indices: concentrations of ferritin and of soluble transferrin receptor (sTfR), and for the ratio of those expressed as the sTfR/log ferr index in male and female cross-country skiers during a training camp.

**TABLE 1.** BASIC CHARACTERISTICS (MEAN VALUES  $\pm$ SD AND RANGES) OF CROSS-COUNTRY SKIERS STUDIED

Variable	Male (n=8)	Female (n=6)
Age (years)	16.8 $\pm$ 0.8 (16-18)	16.8 $\pm$ 0.75 (16-18)
Body mass (kg)	70.8 $\pm$ 4.7 (63-76.5)	54.8 $\pm$ 4.64 (49-61)
Body height (cm)	178.7 $\pm$ 6.6 (171-191)	167.2 $\pm$ 4.36 (163-175)
VO2 max (ml/kg $\cdot$ min)	61.9 $\pm$ 2.53 (58.5-64.6)	53.3 $\pm$ 2.11 (49.8-57.8)
Training experience (years)	5.5 $\pm$ 1.2 (5-7)	5.6 $\pm$ 1.3 (5-7)
Training volume (h/day)	3.0 (1.5-3.5)	2.5 (1.0-3.5)
Training volume (h/week)	21.0	17.5

## MATERIALS AND METHODS

**Subjects:** A group of 11 male and 7 female cross-country skiers, aged 16 - 18 years, volunteered to participate in the study after having been informed about the study objective and protocol. They attended a competitive sport-oriented school, some of them being members the National junior team. Their parents submitted informed consents to participate and the study was approved by the Institute's of Sport Committee of Ethics. All subjects were in good health condition, in none of them symptoms of infections or injuries were detected upon entering the study. None of them had iron supplementation for at least 3 weeks prior to the study and during the study. All subjects were accommodated in a boarding house with school canteen. During the study, three subjects were excluded for health reasons (infection signs, supported by increased CRP – above 8 mg/l, and increased erythrocyte sedimentation rate – >20), and another one due to gastro-intestinal disorders combined with abstaining from training for 3 days. Eventually, 8 male and 6 female subjects completed the study. Their basic data are presented in Table 1.

Maximum oxygen uptake (VO<sub>2</sub> max) was determined by applying a ski running test (adapted laboratory protocol of A.M. Jones) [10]. The initial velocity was equal to 8 or 6 km/h (for men and women, respectively) and increased every 3 min by 2 km/h until exhaustion, i.e. usually 5 – 7 stages. The test was conducted on the road, a cyclist serving as the pacesetter.

**Training:** All skiers trained together and were subjected to nearly the same training loads. Their daily schedules remained unchanged when

taken for study examinations, except that the preceding day was training-free. All trainings took place at altitudes of 500 – 1000 m. The training consisted of one morning session daily with the exception of one day with an additional afternoon session. Mean duration of training sessions and mean weekly training volumes are shown in Table 1. The training had two objectives: shaping the aerobic endurance by applying continuous runs at varying intensities (FARTLEK) lasting 60 – 210 min in an uneven country profile, and by applying interval workouts (short runs lasting 2 – 3 min); the other objective was shaping strength endurance by applying loads equal to 60 – 75% of the maximal ones determined in a gym. Training loads were established from heart rate monitor (Polar, Finland) records; HR intervals corresponding to various intensity zones were computed individually from the results of work capacity tests conducted 5 days earlier. Then, the total heart work in given intensity zone was determined as related to HRmax and multiplied by the duration of that zone and by arbitrary weights. The weights amounted to 0.5, 1, 2, 3 for Zones I (recovery – 68-74% HRmax), II (extensive aerobic – 75-80% HRmax), III (intensive aerobic – 81-90% HRmax) and IV (threshold – 85-90% HR max), respectively. The sums of those values for individual intensity zones were used in estimating the training load on that day in a way similar to that presented by Banister et al. [1]. Mean values and ranges of training loads applied to individual subjects are presented in Table 2.

**Methods:** During the study period, blood (about 2.5 ml) for determining whole blood indices and for biochemical assays in serum was withdrawn from the antecubital vein in the morning (7<sup>00</sup>-7<sup>30</sup>) after overnight fasting for 8 consecutive days, always after staying at least 15 min in sitting position. Hemoglobin concentration (Hb), hematocrit (Hct), red blood cell count (RBC), leucocyte count (WBC), mean corpuscular hemoglobin concentration (MCHC), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH) were determined in whole blood in duplicates, not later than 3 h after withdrawal, by using a hematological analyzer (Sysmex, 4500, Kobe, Japan). Additionally, blood sedimentation rate (ESR) was determined every day.

The following assays were conducted in blood serum: soluble transferrin receptor (sTfR) concentration by using immunoenzymatic commercial kits (Orion Diagnostica, Finland); ferritin concentration by using immunoenzymatic commercial kits (BioSource, Belgium), C-reactive protein (CRP) by using immunoturbidimetric test (Orion

**TABLE 2.** MEAN VALUES ( $\pm$ SD) AND RANGES OF TRAINING LOADS EXPRESSED IN ARBITRARY POINTS COMPUTED FOR INDIVIDUAL SUBJECTS

Subject No.	Male (n=8)	Female (n=6)
I	3.4 $\pm$ 1.11 (1.7-5.5)	3.2 $\pm$ 1.32 (1.4-5.5)
II	2.9 $\pm$ 0.94 (1.3-4.6)	2.5 $\pm$ 0.98 (1.6-4.5)
III	2.9 $\pm$ 1.14 (1.2-5.3)	3.2 $\pm$ 1.37 (1.6-5.5)
IV	2.9 $\pm$ 1.00 (1.4-4.9)	3.1 $\pm$ 1.22 (1.8-5.4)
V	2.8 $\pm$ 0.84 (1.3-4.2)	2.6 $\pm$ 1.04 (1.2-4.6)
VI	2.9 $\pm$ 0.99 (1.4-4.9)	2.7 $\pm$ 1.06 (1.5-4.8)
VII	2.9 $\pm$ 0.99 (1.5-4.9)	
VIII	3.0 $\pm$ 1.06 (1.3-5.1)	

Diagnostica, Finland), creatine kinase (CK) activity by spectrophotometric method (Analco, France) and erythropoietin (EPO) concentrations were determined by using immunoenzymatic commercial kits (Medac, Germany). C-reactive protein concentrations and CK activities were determined immediately upon blood withdrawal while serum for other assays (ferritin, sTfR, EPO) was stored frozen throughout the study; EPO was assayed only once, on the first day.

In order to eliminate the between-assay error, ferritin and sTfR assays for given subject were run in one batch. Intra-assay coefficients of variation for ferritin and sTfR were computed from all duplicate measurements. The intra-assay coefficients of variability (CV) for sTfR and ferritin were 2.5% (2.1-3.3) and 5.4% (4.1-6.7), respectively. Concentrations of ferritin and sTfR, as well as CK activities, were adjusted for changes in plasma volume according to Dill and Costill [7]. Values of the sTfR/logFerr index exceeding 1.8 were considered a criterion of iron deficiency [31].

*Statistical analysis:* The values of those variables which exhibited skewed distributions (ferritin and sTfR) were subjected to logarithmic transformation prior to data processing. Residual (within-subject) standard deviations were computed from two-way ANOVA for three variables: logarithm of ferritin concentration (logFerr), logarithm of soluble transferrin receptor concentration (log sTfR), and the sTfR/logFerr ratio. Within-subject correlations between training load

scores and CK activities or concentrations of ferritin, soluble transferrin receptor (sTfR) and sTfR/logFerr index were computed. The differences between raw and plasma-volume-adjusted log values of ferritin and sTfR and the gender-related differences in plasma volume were assessed by applying Student's t-test for dependent and independent data, respectively, the level of  $p \leq 0.05$  being considered significant.

**RESULTS**

Mean values of hematological and iron-related indices and of CK activities in serum are presented in Table 3. The values of hematological indices were within normal limits with the exception of two female and one male athletes in whom decreased hemoglobin was noted on some days.

Mean concentration of ferritin in the female skiers in the entire study period amounted to  $22.6 \cdot 1.167^{\pm 1} \mu\text{g/l}$  (range: 7 – 45  $\mu\text{g/l}$ ). Values indicative of iron deficiency, i.e. below 20  $\mu\text{g/l}$ , were detected in two subjects. The corresponding values in male skiers were  $30.6 \cdot 1.142^{\pm 1} \mu\text{g/l}$  (13 – 101  $\mu\text{g/l}$ ), and values indicating deficiency, i.e. below 25  $\mu\text{g/l}$ , were found in 5 subjects.

Mean concentrations of the soluble transferrin receptor in serum of male and female cross-country skiers amounted to  $2.62 \cdot 1.047^{\pm 1} \text{mg/l}$  (range: 1.7 – 3.7  $\text{mg/l}$ ) and  $2.14 \cdot 1.050^{\pm 1} \text{mg/l}$  (range: 1.4 – 3.5  $\text{mg/l}$ ), respectively.

**TABLE 3. HEMATOLOGICAL AND IRON METABOLISM INDICES (MEAN VALUES  $\pm$ SD AND RANGES) IN FEMALE AND MALE SKI RUNNERS**

Variable	Mean, SD and (range)		Normal range
	Male	Female	
Ferritin (mg/l) #	$30.6 \cdot 1.142^{\pm 1}$ (13 – 101)	$22.6 \cdot 1.167^{\pm 1}$ (7 – 45)	F 20 - 140 @ <sup>§</sup> M 25- 250
sTfR (mg/l) #	$2.62 \cdot 1.047^{\pm 1}$ (1.7 – 3.7)	$2.14 \cdot 1.050^{\pm 1}$ (1.4 - 3.5)	1.3 – 2.75@
sTfR/logFerr	$1.86 \pm 0.58$ (1.3-3.0)	$1.77 \pm 0.97$ (1.1-3.6)	<1.8 <sup>^</sup>
Hemoglobin (g/l)	$146.6 \pm 5.9$ (134.0-157.0)	$129.2 \pm 0.8$ (117.0-144.0)	F 120 -160 M 130 -180
Erythrocyte count ( $\times 10^{12}/\text{l}$ )	$4.88 \pm 0.21$ (4.5 – 5.3)	$4.26 \pm 0.24$ (3.85 – 4.4)	3.8 - 5.2 4.4 - 5.9
Hematocrit (l/l)	$0.43 \pm 0.01$ (0.40-0.45)	$0.39 \pm 0.02$ (0.36-0.43)	F 0.35 - 0.47 M 0.40 - 0.52
MCV (fl)	$87.4 \pm 2.6$ (83.0 – 91.8)	$90.8 \pm 2.4$ (86.8 – 96.0)	80 - 100
MCH (pg)	$30.1 \pm 0.9$ (28.4 – 32.3)	$30.3 \pm 1.0$ (28.7 – 32.1)	26 - 34
MCHC (g/l)	$343.9 \pm 6.0$ (334 - 356)	$333.5 \pm 6.9$ (321 - 345)	320 - 360
Leukocyte count ( $\times 10^9/\text{l}$ )	$5.7 \pm 0.9$ (3.9 – 8.0)	$5.7 \pm 0.9$ (3.7 – 7.5)	F 3.6 -11.0 M 3.8 - 10.6
EPO (mIU/ml) <sup>&amp;</sup>	$9.1 \pm 3.5$ (5.4 – 15.4)	$9.0 \pm 2.9$ (6.0 – 12.2)	4.0 - 24.0@
CK (U/l)	$334.1 \pm 129.4$ (153 -854)	$182.0 \pm 86.1$ (68 -488)	up to 190@
ESR (mm/hr)	$3.7 \pm 1.5$ (2 – 8)	$4.4 \pm 2.3$ (2 - 14)	0-20

**Legend:**

@ - Normal ranges as given by kit manufacturers; for other variables the normal ranges were taken after Wintrobe et al. [31]

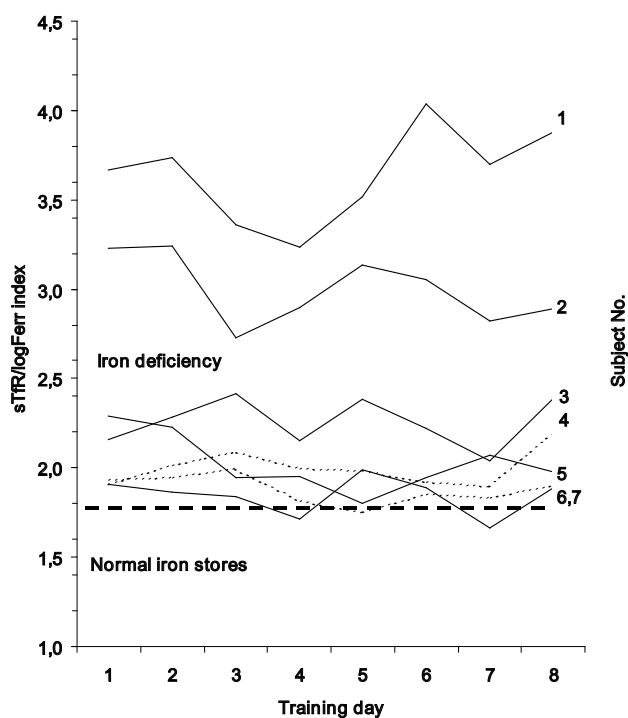
§ - Lower normal limit after Malczewska et al. [15], upper one – after kit manufacturer

^ - after Suominen et al. [30]

# - Geometric mean  $\times$  SD  $\pm 1$ ; for other variables – arithmetic mean  $\pm$ SD for the 8 day study period

& - arithmetic mean  $\pm$ SD recorded on the first day of study

ESR – Erythrocyte sedimentation rate



**FIG. 1.** INDIVIDUAL CHANGES IN VALUES OF sTfR/logFerr INDEX THROUGHOUT 8 CONSECUTIVE DAYS OF TRAINING CAMP IN SUBJECT WITH IRON DEFICIENCY

**TABLE 4.** POOLED WITHIN-SUBJECT CORRELATIONS BETWEEN TRAINING LOAD SCORES AND CONCENTRATIONS OF FERRITIN, SOLUBLE TRANSFERRIN RECEPTOR (sTfR), sTfR/logFerr INDEX AND CREATINE KINASE (CK) ACTIVITY RECORDED DAILY FOR 8 DAYS IN MALE (n =8) AND FEMALE (n =6) CROSS-COUNTRY SKIERS

	Ferritin	sTfR	sTfR/ log Ferr	CK
<b>Men (df = 48)</b>				
Training load score	-0.265 °	-0.156	0.069	0.342*
Ferritin		0.066		-0.107
sTfR				-0.149
sTfR/log Ferr				-0.001
<b>Women (df = 36)</b>				
Training load score	0.039	0.235	0.176	0.389*
Ferritin		-0.234		0.148
sTfR				0.111
sTfR/log Ferr				0.121

Legend: ° p = 0.06; \* p.<0.05;

Values exceeding the normal limit (above 2.75 mg/l) were noted in 4 male and two female subjects, while in only three of them (Nos. 1, 2 and 3, Fig. 1) the increased sTfR was accompanied by very low ferritin levels throughout the study period and, in consequence, the values of the sTfR/logFerr index were elevated. In another 3 subjects (Nos. 4, 5 and 6, Fig. 1), increased sTfR and decreased ferritin values were observed only on some days; yet, the values of the sTfR/log Ferr index were elevated (above 1.8) on almost all days of the study. In one subject with elevated sTfR/log Ferr index (No. 7), low concentration of ferritin was observed on some days only, while the levels of sTfR were slightly elevated but within normal limits throughout the study period.

Ferritin concentrations showed much higher relative day-to-day, within-subject variability than sTfR levels or sTfR/log Ferr index values. The respective CV% values were 15.2% (range: 8.8 – 27.1%) in women and 13.4% (range: 6.1 – 18.7%) in men for ferritin, 6.5% (range: 2.1 – 8.1%) in women and 4.6% (range: 2.2 – 8.8%) in men for sTfR, and 7.4% (range: 5.0 – 8.7%) in women and 6.0% (range: 4.2 – 9.7%) in men for sTfR/logFerr index. The gender-related differences in the day-to-day CV% between male and female athletes proved non-significant.

Coefficients of correlation between ferritin, sTfR, sTfR/logFerr index and creatine kinase (CK) activity or training loads, based on residual sums, are presented in Table 4. None of the iron status indices was significantly correlated with either CK or training loads. The only weak but significant (p<0.05) correlation was that between CK activity and training loads (r=0.342 and 0.389 in male and female groups, respectively) and a nearly significant one (r=-0.265; p=0.06) between ferritin and training loads in male skiers.

Changes in plasma volume (ΔPV), mainly hemodilution, were in both groups alike throughout the study period and averaged +4.4% (range: -4.7 to +20.9%) and +4.3% (range: -5.0 to +15.5%) in female and male skiers, respectively. In Table 5 are presented mean values of ferritin, sTfR and sTfR/logFerr index, raw and adjusted for changes in plasma volume. The adjusted values were significantly higher from the raw ones in male but not in the female cross-country skiers.

**DISCUSSION**

Iron deficiency is fairly common in athletes, especially in the endurance ones [4,23]. It appears mostly in latent form, the iron

**TABLE 5.** MEAN VALUES (±SD) OF IRON METABOLISM INDICES, RAW AND CORRECTED FOR CHANGES IN PLASMA VOLUME

	Male (n=8)		Female (n=6)	
	Raw values	Corrected for plasma volume	Raw values	Corrected for plasma volume
Ferritin (µg/l)	29.4 · 1.153 ±1	30.6 · 1.142 ±1 *	21.7 · 1.152 ±1	22.6 · 1.167 ±1
STfR (mg/l)	2.52 · 1.071 ±1	2.62 · 1.047 ±1 *	2.06 · 1.064 ±1	2.14 · 1.050 ±1
sTfR/log Ferr	1.81 ± 0.57	1.86 ± 0.58 **	1.72 ± 0.90	1.77 ± 0.97

Legend: Significantly different from the respective raw value by Student's t-test for dependent data: \* p<0.05; \*\* p<0.01

deficiency-induced anemia being, however, infrequent [4,15,17,19,25]. The results of this study confirm that iron deficiency is present mostly in its early, latent stage in both, male and female cross-country skiers. The observed isolated cases of decreased hemoglobin and hematocrit were due to hemodilution on those occasions. The increases in plasma volume ( $\Delta$ PV) in these subjects ranged from 3.4 to 11.3% and from 5 to 20.9% in male and female subjects, respectively. This is in accordance with other authors who reported increased blood or plasma volume in endurance athletes [11,20,26,33].

The detection of latent iron deficiency in athletes is not easy and requires the use of appropriate indices [5,9]. Although ferritin is the basic indicator of iron stores, its use as the sole index of iron status may not be sufficient, especially in health disorders, due to ferritin is an acute-phase protein [4,9,22]. Therefore, all results recorded in subjects exhibiting signs of infection or inflammatory states (concentration of CRP exceeding 8 mg/l and/or increased blood sedimentation rate) were omitted.

Mean concentration of ferritin recorded in this study was rather low in both male and female cross-country skiers ( $30.6 \cdot 1.142^{\pm 1}$  and  $22.6 \cdot 1.167^{\pm 1}$   $\mu$ g/l, respectively). Those low values were due to the fact that in the male group as many as 5 out of 8 subjects had ferritin levels below 25  $\mu$ g/l, and in the female ones all subjects had relatively low ferritin (mean values from 8 consecutive days for individual subjects ranged from 8.5 to 32.7  $\mu$ g/l, values below 20  $\mu$ g/l being noted in two subjects only). The low ferritin values could have resulted from decreased iron stores in the pubertal period, especially in male skiers, as reported by Samuelson *et al.* [24] for untrained subjects, as well as from the specificity of given sport. Also other authors [12,23] reported low ferritin in female endurance athletes.

The day-to-day variability of the levels of iron status variables is an important indicator of their diagnostic value [2,3,6]. In case of ferritin, that variability is quite high, especially in women. As reported by Cooper *et al.* [6] and Borel *et al.* [3], that variability in untrained men ranged from 12.5 to 13.9%, and was much higher in women (25.5 – 26.7%). In our earlier study [29] and in that of Gallagher *et al.* [8] lower values for untrained women were reported, ranging from 15 to 19%. A very low variability (8.2%) was reported by Belza *et al.* [2] for iron-depleted women. Physical exercise may considerably increase ferritin levels as reported for cross-country skiers [21] or female triathletes [23] and those exercise-induced increases may persist for several days [12,27], which may affect the day-to-day variability. Similar observations were made by us in male and female judoists in whom the within-subject, day-to-day variability averaged 27.4 and 46%, respectively, highest individual values amounting to 44 and 75%, respectively [14,16]. Thus, the day-to-day variabilities in ferritin concentrations found in this study in cross-country skiers were much lower than the abovementioned values for other athletes.

The low variability of ferritin levels in male and female cross-country skiers, close to that observed in untrained subjects [3,6,8,29],

suggests that the training loads applied to skiers had no serious effect on ferritin. This was further supported by a lack of significant correlations between these variables and suggested that the kind of physical exertion might have played an important role. The cross-country skiers performed predominantly aerobic work, the anaerobic threshold being rarely exceeded. Endurance exercises, in which the concentric work prevails, may cause less muscle damage and inflammation than the eccentric ones [18,22]; therefore, in sports like judo, the concentric/eccentric and isometric work, as well as multiple, short anaerobic bouts, may enhance muscle damage including a progressing inflammatory process, and an increased generation of ferritin as an acute-phase protein.

An alternative explanation would be that the loads applied to cross-country skiers were too small to induce a response like that observed in judoists [16]. That view might be supported by a rather low correlation between CK and training loads in this study ( $r < 0.4$ ) and by very high increases in ferritin concentrations observed in cross-country skiers following exhausting runs at distances of 70 – 130 km [21]; unfortunately, in that latter study ferritin was determined immediately post-exercise, and not on the following morning. Nevertheless, despite a relatively low CK/training loads correlation, the CK activities measured on the following morning were quite pronounced – up to 854 and 444 U/l in men and women, respectively.

Significant correlations between ferritin concentrations and training loads, as well as CK activities observed in male judoists [16], and a lack of such correlations in this study, support the view that the loads applied to cross-country skiers brought about only minor muscle damages which increased the permeability of cell membranes but without inducing an inflammatory process. It suggests that the exercise-induced ferritin increase would be due to stimulation of ferritin synthesis as a response to an acute-phase stimulus and not to increased liberation of ferritin from myocytes due to increased membrane permeability.

The fairly low variability of ferritin observed in this study could have been due also to the elimination of results recorded in subjects with symptoms of cold, in whom elevated levels of CRP and/or elevated ESR were detected, as emphasized by Belza *et al.* [2].

In spite of a low variability of ferritin levels in male and female cross-country skiers, ferritin concentration may prove insufficient in detecting iron deficiency in that sport as only 3 out of 7 subjects in whom iron deficiency was detected had decreased ferritin throughout the study period lasting 8 days. Thus, our results suggest the necessity of taking into account at least two indices of iron status simultaneously, not only ferritin, like recommended also by others [5,9].

The levels of soluble transferrin receptor (sTfR), the other studied indicator of the iron status, were higher in male than in the female skiers due to a high number of subjects with iron deficiency in that group. Namely, increased sTfR ( $> 2.75$  mg/ml) combined with decreased ferritin levels were found in 4 male subjects and in only two female ones. Normal levels of EPO in those subjects ruled out an augmented rate of erythropoiesis as the cause of possible sTfR



increases and confirmed the presence of latent iron deficiency in them.

Mean day-to-day variability for sTfR (4.5 and 5.0% for men and women, respectively) was slightly lower than reported by us earlier for male and female judoists [14,16] and considerably lower than in untrained subjects: 10.3 and 12.2% for men and women, respectively [6], 8.1% for iron-depleted women [2] and 8.3% for untrained women [29]. That lower sTfR variability may have resulted from adjusting the results for changes in plasma volume, the raw/adjusted values amounting to 6.9 and 4.5%, respectively, in men, and to 6.5 and 5.0% in women (Table 4). Nikolaidis et al. [20] reported sTfR variability amounting to about 5% throughout a 24-h period of post-exercise recovery, the adjustments for plasma volume changes being non-significant. That low variability of sTfR and lack of significant correlations with training loads or CK activities observed in this study confirm the conclusions from earlier studies [14,16,20,23,26] and are indicative of a high stability of sTfR levels under conditions of intense exertions also in cross-country skiers.

As follows from the reports of studies on untrained subjects [30] and on physically active ones [17,28], the sTfR/logFerr index proved very useful in detecting iron deficiency, especially when elevated concentrations of sTfR were accompanied by relatively high variability

of ferritin levels, like in case of subject No. 5 (19.5 – 45 µg/l). This, combined with normal EPO concentration, facilitated detecting latent iron deficiency. The sTfR/log Ferr index is also valuable in cases when ferritin is close to the lower and sTfR to the upper physiologic limits, like in subjects 4, 6 and 7; in these cases index values exceeding 1.8 enable detecting iron deficiency. In addition, the adjustment of results by changes in plasma volume mentioned above is of importance especially in endurance athletes as recommended also by Kargotich et al. [11].

## CONCLUSIONS

Summing up, endurance training lasting 2.5 – 3 h a day had no pronounced effect on ferritin concentrations measured on the following morning in male and female cross-country skiers. Despite the lack of relationship between ferritin levels and training loads, the use of ferritin alone in detecting iron deficiency may prove insufficient. Moreover, the results confirmed a low variability of sTfR under various endurance loads which made sTfR useful in detecting iron deficiency together with ferritin and the sTfR/logFerr index. In addition, when assessing the iron status in endurance athletes, especially the male ones, changes in plasma volume ought to be considered in order not to misestimate the magnitudes of iron-related variables.

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