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DETERMINATION OF PARAMETERS OF IRON STATUS IN EVALUATION OF ANEMIA IN ELITE YOUNG SERBIAN WATER POLO PLAYERS

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Abstract The aim of the research was to determine iron status parameters so as to allow the evaluation of anemic states in elite young water polo players. The study included 42 members of the Serbian national youth water polo team in the competitive seasons of 2004 and 2005. The subjects' iron status was assessed by assaying a venous sample for red blood cell count (RBC), hemoglobin (Hgb), hematocrit (Hct), mean cell volume (MCV), mean cell hemoglobin content (MCH), mean cell hemoglobin concentration (MCHC), red cell distribution width (RDW), serum ferritin, serum iron, serum transferrin, total iron binding capacity, and serum haptoglobin. K-mean Cluster Analysis was used to classify the players into 5 clusters as follows: C1 with 2 players (4.76 %) whose iron status was low, C2 with 16 players (or 38.10 %) whose iron status was under average, C3 with 14 players (33.33 %) whose iron status was average, C4 with 6 players (14.29%) whose iron status was above average, and C5 with 4 players (9.52 %) whose iron status was excellent. Besides, the results showed that junior water polo players' iron status screening should particularly consider the following parameters: RBC, Hgb, Hct, MCV, MCH, TIBC, ferritin and transferrin. Those parameters proved statistically significant in discriminating among the water polo players in the study as for their anemic state evaluation.

Key words: water polo, iron status, anemic state evaluation, training

INTRODUCTION

The prevalence of iron deficiency anemia in non-athletic population has been estimated to be 3-5%, whereas the prevalence of iron deficiency without anemia is much higher, ranging between 11-13%. In all cases of iron deficiency (ID), the underlying etiology is either due to blood loss or to nutritional deficits. Also, all athletes can develop iron deficiency because the absorption of iron cannot balance the losses incurred through training. The loss of iron is increased by numerous factors such as perspiration, gastrointestinal and urogenital bleeding during training, or inefficient iron intake. The prevalence of inadequate iron balance in male athletes has been reported to be as high as 10% and can reach up to 20% in female athletes [2].

ID, defined as diminished total body iron content, develops in sequential stages over a period of negative iron balance. The loss of iron can be divided into 3 stages [25]. **Stage 1** is ***"iron depletion"***, at which there is an isolated decrease in serum ferritin levels. ***Stage 2*** is ***"iron deficient erythropoiesis"***. At this stage, the supply of iron to the erythroid marrow is inadequate, the serum ferritin level is low (10-20 µg/L), transferrin saturation is decreased (<16%), and the total iron binding capacity is increased. The anemia of iron deficient erythropoiesis may be too mild to be detected by some arbitrary value for hemoglobin, which is used to distinguish normal from anemic states. **Stage 3** is ***"iron deficient anemia"*** **(IDA),** in which hemoglobin levels are subnormal. Thus, ID may range in severity from reduced iron stress with no functional effects (stages 1 and 2) to severe anemia. Anemia

is defined by 2 criteria: the decrease of iron stores and the decrease in hemoglobin levels. Thus, an isolated decrease in ferritin does not indicate anemia, but it does indicate a risk of immediate anemia if the iron stores continue to be depleted.

Anemia impairs blood gas transport and limits work capacity. Decreased work capacity due to iron deficiency anemia has been well documented [7, 17] and is attributed to insufficient O_2 transport by hemoglobin to peripheral tissues. In contrast, the effects of iron depletion without anemia on physical performance have not been well characterized. With moderate iron deficiency, iron stores are depleted but hemoglobin remains above a specified cut-off value for anemia, and neither O_2–carrying capacity of the blood nor VO_{2max} is compromised [28]. However, studies have shown that the activities of iron-containing muscle mitochondrial oxidative enzymes and respiratory proteins are decreased during iron deficiency without anemia [37]. Because of the role of these iron-dependent enzymes and proteins in oxidative metabolism, it is expected that iron deficiency without anemia would impair the ability to sustain physical performance at 65-85% of maximal capacity, i.e., endurance. In addition, aerobic training indices increase in iron-dependent mitochondrial enzyme and respiratory chain cytochrome activities [22]. Thus iron deficiency may also impair the adaptive response to aerobic training. Furthermore, iron deficient erythropoiesis without objective anemia (stage 2) may induce clinical manifestations, muscle and hormonal dysfunction, and altered resistance to infection [8].

The effects of iron depletion without anemia on adaptation to training have not been fully characterized but laboratory evaluation of iron status is necessary and helpful in defining anemic states. Iron-containing compounds in the body are one of three types: a) *functional* compounds that serve in metabolic or enzymatic functions and b) compounds that serve as *transport* and c) *storage* forms for iron [10]. There are a number of markers that describe these functional, transport and storage compartments for iron: serum iron, total iron binding capacity, red blood cell count, hemoglobin, hematocrit, red blood cell indices (MCV, MCH, MCHC, RDW), transferrin, transferrin saturation, and serum ferritin. These laboratory tests are essential to an accurate diagnosis of ID and the evaluation of therapy [10, 14].

Hemoglobin constitutes the major fraction of body iron (functional iron) with a concentration of about 0.5 mg iron/mL blood. Iron is distributed within the body via transferrin in the plasma, a transport protein that mediates iron exchange between tissues. Ferritin is the primary storage compound for the body's iron and serum ferritin concentration is a reliable index of iron stores (1 ng/mL of serum ferritin indicates about 8 mg of storage iron). Serum ferritin does not exhibit diurnal variations as are seen with serum iron levels [10]. The serum ferritin level is decreased in all stages of ID and may be the first indication of a developing ID. Serum ferritin is generally considered the single best test to detect iron deficiency. Although highly trained athletes usually have normal absolute levels of hemoglobin, they often have ID, generally latent, that implies no decrease in hemoglobin. Swimmers with low ferritin levels may not be anemic, but their performance at maximal intensities may be compromised. Anemia in swimmers, like in other athletes, has negative effects on physical exercise capacity and their ability to train from day to day [14].

Water polo, as a sport game, is an 'intermittent' sport comprised of intense bursts of activity of < 15 seconds in duration with intervening, lower intensity intervals averaging < 20 seconds in duration [35]. Physiological measurements obtained during game play indicate a cumulative effect of repeated sequences of activities in different technical and tactical situations, as well as in different body positions, and suggest there is a high metabolic demand on the athletes [11, 21, 35].

We evaluated hematological and biochemical parameters of the iron status in elite young Serbian water polo players to identify subgroups with iron depletion and subclinical iron deficit.
In this way we will be able to determine the main factors, i.e., parameters for the determination of iron deficiency, and to make a model based on a multivariable approach that can detect different stages of ID. Firstly, the application of such a statistical method will delineate the groups differing in relation to their iron status, which will enable the evaluation of iron deficiency in the water polo players tested, and, secondly, it will be established which hematological and biochemical parameters best describe such differences with statistical significance.

The results obtained will facilitate the definition of methods as well as of laboratory analysis and diagnostic procedures that will follow iron status in order to prevent the onset of ID not only in young water polo players, but also in individuals in the final phase of their biological development who are exposed to immense and intensive physical endeavors within a system of permanent training and competition.

MATERIALS AND METHODS

SUBJECTS SAMPLE

The study included 42 water polo players, the members of the Serbian national youth water polo team in the competitive seasons of 2004 and 2005. The basic chrono-morphological sample data were as follows /the results are expressed in Mean ± SD, and Min - Max/: age = 17.1 ± 0.6 yrs (15.9 – 18.2 yrs), BH = 187.94 ± 5.93 cm (173.0 – 197.3 cm), BM = 84.79 ± 10.07 kg (67.0 – 101.4 kg), BMI = 23.95 ± 2.07 kg/m^2 (19.63 – 28.12 kg/m^2), and training experience = 8.1 ± 1.0 yrs (6.5 – 10.2 yrs). All the water polo players gave informed consent to the participation in the study.

SAMPLING AND ANALYSIS

Blood samples were collected between the last week of May and the first week of June in the years mentioned. The subjects were the players who had been invited to the beginning of the preparations of the Serbian youth national team (the wider player list), i.e., of 18 years of age and younger. The testing period coincided with the first training micro-cycle, that is, the first week in which the national team started preparing for the main competitions in the given seasons (in 2004 – 7 training weeks and the participation in two international tournaments; in 2005 – ten training weeks, and the participation in two international tournaments as well as in the European youth championship in Sofia, Bulgaria).

BLOOD SAMPLING PROCEDURE

The blood samples were obtained from the antecubital vein into a BD vacutainer system under standardized conditions at 09 a.m., 24 hours after the last training bout. On the day prior to the examination, only recuperation training was performed by the tested water polo players. None of the subjects had used iron supplements regularly, and none had a dietary restriction.

The iron status of the subjects was assessed by assaying a venous sample for the red blood count (RBC, in 10^{12}/L), hemoglobin (Hb, in g/L), hematocrit (Hct, in L/L), mean cell volume (MCV, in fL), mean cell hemoglobin content (MCH, in pg), mean cell hemoglobin concentration (MCHC, in g/L), red cell distribution width (RDW, in %), serum ferritin (in µg/L), serum iron (Fe, in µmol/L), serum transferrin (Trf, in g/L), total iron binding capacity (TIBC, in µmol/L) and serum haptoglobin (Hpt, in g/L).

Red blood cells and red blood cell indices were measured with an automated hematology analyzer (Hmx, Beckman-Coulter Inc). Serum ferritin, transferrin and haptoblobin were measured using a Behring Nephelometer (Dade-Behring, Dade Behring BN II, Marburg, Germany). Serum iron and total iron binding capacity (TIBC) were measured using an Olympus analyzer and reagent kits (Olympus System Reagents, Olympus analyzer AU 2700, Hamburg, Germany).

STATISTICAL ANALYSIS

Data are presented as Mean, SD, Min and Max values. To determine a normal distribution of the data analyzed, Kolmogorov-Smirnov testing (K-S) was performed. Following the standard metrological procedures in sports [39] and applying K-mean Cluster Analysis, the entire sample was classified into 5 characteristic groups according to whether the level of their iron status was low, under average, average, above average, or excellent. An ANOVA enabled the determination of statistically significant differences among the variables in individual clusters. A student t-test was carried out on the variables in the clusters defined by the ANOVA [19]. The whole statistical procedure was performed using the SPSS software for Windows, Release 10.0.1. (Copyright © SPSS Inc., 1989-1999).

RESULTS

Table 1 presents the basic descriptive statistics for the hematological and biochemical variables that indicated iron status (functional, transport and storage departments of iron) as well as K-S data.

The mean values for red blood cells, red blood cell indices, serum iron, TIBC, ferritin, transferrin and haptoglobin were all within reference ranges [10, 25].

The results of the Kolmogorov-Smirnov test showed that all the variables were normally distributed and that there were no statistically significant differences in the data distribution of the individual variables in comparison with the hypothetical Gauss model (Table 1, K-S $p > 0.05$, Haptoglobin K-S $p = 0.127$ to MCH K-S $p = 0.984$).

By the application of K-mean Cluster Analysis, the iron status parameters were divided into 5 clusters (Table 2). The first group (Cluster 1 - C1) comprised 2 players (or 4.76 %) whose level of iron status was low; the second group (Cluster 2 - C2) comprised 16 players (or 38.10 %) whose level of iron

status was under average; the third group (Cluster 3 - C3) comprised 14 players (33.33 %) whose level of iron status was average; the fourth group (Cluster 4 - C4) comprised 6 players (14.29%) whose level of iron status was above average; and the fifth group (Cluster 5 - C5) comprised 4 players (9.52 %) whose level of iron status was excellent (Figure 1).

Table 1. Iron status in elite young Serbian water polo players (N=42)

	RBC (10^{12}/L)	Hb (g/L)	Hct (L/L)	MCV (fL)	MCH (pg)	MCHC (g/L)	RDW (%)	Fe (µmol/L)	*TIBC* (µmol/L)	Ferritin (µg/L)	Trf (g/L)	Hpt (g/L)
Mean	5.118	152	0.45	87.7	29.6	338	12.5	17.3	58.3	55.426	2.74	0.63
SD	0.285	8.43	0.02	3.55	1.09	7.8	0.4	6.91	5.54	37.86	0.29	0.32
Min	4.54	128	0.37	79.6	27.1	325	11.5	5.8	49	6.8	2.26	0.09
Max	5.79	165	0.5	93.3	31.8	353	13.3	35.2	75.6	169	3.45	1.39
K-S Z	0.697	0.749	1.052	0.644	0.461	0.916	0.741	0.695	0.569	1.023	0.631	1.174
K-S p	0.716	0.630	0.218	0.802	0.984	0.371	0.643	0.720	0.902	0.246	0.821	0.127

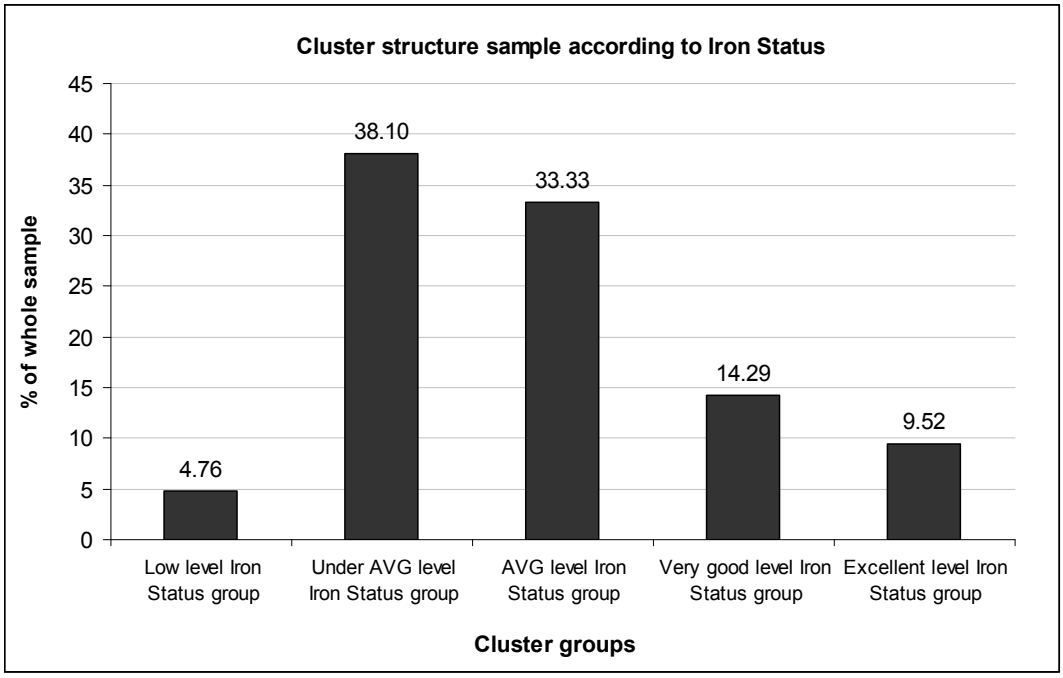

Figure 1. The sample breakdown into cluster groups

Table 2 shows the results of the descriptive analysis of iron status of elite young Serbian water polo players according to the defined clusters.

The results showed that the number of red blood cells differed significantly between C1 and C3, C1 and C4, C1 and C5, and C2 and C4. Those differences showed that the number of red blood cells, although within reference ranges, displays a statistically significant difference only between the groups below and above the average level of iron status. The hemoglobin concentration showed significant difference between C1 and all other groups (C2, C3, C4 and C5) while C2 was significantly different from C5 only. Hematocrit in Cluster 1 (0.385 ± 0.021) was significantly different from the hematocrit in all other groups (C2, C3, C4 and C5), and there was no statistically significant difference among other groups. MCV in Cluster 1 (81.20 ± 0.14 fL) differed significantly from C2, C3 and C4, as well as between C2 and C4. MCH in C1 (27.55 ± 0.64 pg) showed significant difference from MCH in C2, C3

and C4, while the other groups did not differ significantly. MCHC and RDW did not display statistical significance in the evaluation of iron status in the water polo players.

In contrast with the hematological parameters which describe the functional iron department in the evaluation of iron status, the transport department is defined by the serum iron, TIBC and transferrin. In the evaluation of the water polo players' iron status, TIBC and transferrin stood out as statistically significant parameters that described iron status in clusters, whereas serum Fe, however distinctly low in C1, did not show statistical significance.

Table 2. Iron status and hematological variables in the clusters

Cluster groups / Iron status parameters	CLUSTER 1 Low level of iron status group	CLUSTER 2 Under average level of iron status group	CLUSTER 3 Average level of iron status group	CLUSTER 4 Very good level of iron status group	CLUSTER 5 Excellent level of iron status group
RBC (10^{12}/L)	4.720 ± 0.198	5.045 ± 0.213	5.107 ± 0.281	5.302 ± 0.237	5.373 ± 0.382
Hgb (g/L)	130.00 ± 2.83	151.00 ± 5.72	151.64 ± 7.72	156.33 ± 8.64	157.25 ± 4.72
HCT (L/L)	0.385 ± 0.021	0.448 ± 0.014	0.451 ± 0.021	0.460 ± 0.021	0.455 ± 0.013
MCV (fL)	81.20 ± 0.14	88.86 ± 2.87	88.54 ± 3.00	86.60 ± 2.42	85.33 ± 5.79
MCH (pg)	27.55 ± 0.64	29.95 ± 0.95	29.73 ± 1.05	29.45 ± 0.48	29.38 ± 1.70
MCHC (g/L)	340.00 ± 8.49	337.19 ± 7.42	335.57 ± 7.20	340.17 ± 9.11	344.50 ± 8.39
RDW (%)	13.20 ± 0.01	12.51 ± 0.33	12.51 ± 0.42	12.33 ± 0.24	12.28 ± 0.56
Fe (μmol/L)	6.05 ± 0.35	18.86 ± 7.39	16.60 ± 5.80	19.30 ± 7.97	15.95 ± 4.31
TIBC (μmol/L)	72.55 ± 4.31	56.96 ± 4.25	58.62 ± 4.18	58.85 ± 6.78	54.33 ± 1.76
Ferritin (μg/L)	10.30 ± 4.95	27.69 ± 8.51	56.19 ± 5.02	78.92 ± 10.20	151.00 ± 14.79
Transferrin (g/L)	3.36 ± 0.13	2.74 ± 0.28	2.76 ± 0.26	2.63 ± 0.25	2.47 ± 0.08
Haptoglobin (g/L)	0.415 ± 0.092	0.593 ± 0.300	0.580 ± 0.349	0.682 ± 0.250	0.998 ± 0.297

Values are means ± SD

Transferrin in C1 (3.36 ± 0.13 g/L) had significantly higher values in comparison with all other clusters; transferrin from the second cluster (2.74 ± 0.28 g/L) displayed a statistically significant difference only in comparison to C5, while transferrin concentration in C3 showed a significant difference when compared with C4 and C5. The transferrin concentration in C3 was significantly different from C4 and C5. The ferritin concentration in C1 and C2 (10.30 ± 4.95 μg/L and 27.69 ± 8.51 μg/L) was significantly different from ferritin in all other clusters, while there was no significant difference between C3, C4 and C5.

The haptoglobin concentration was within the reference range and there were no statistically significant differences between clusters. In C1 haptoglobin was the lowest (0.415 ± 0.092 g/L), while its level was the highest in C5 (0.998 ± 0.297 g/L).

Table 3. ANOVA analysis of the clusters according to the iron status parameters

ANOVA						
	Cluster		*Error*		*F*	*p*
	Mean Square	*df*	*Mean Square*	*df*		
RBC	.216	4	0.067	37	**3.241**	***.022***
Hgb	300.247	4	46.305	37	**6.484**	***.000***
HCT	0.0023	4	0.0003	37	**7.073**	***.000***
MCV	36.463	4	10.023	37	**3.638**	***.013***
MCH	2.720	4	1.031	37	**2.639**	***.049***
MCHC	74.480	4	59.397	37	1.254	.305
RDW	.338	4	.140	37	2.410	.067
Fe	82.503	4	44.029	37	1.874	.136
TIBC	125.363	4	20.427	37	**6.137**	***.001***
Ferritin	14058.654	4	70.647	37	**198.998**	***.000***
Transferrin	.288	4	0.064	37	**4.467**	***.005***
Haptoglobin	.177	4	0.095	37	1.856	.139

The ANOVA and the student t-test for odd samples yielded the iron status parameters with a statistically significant difference among the clusters (Tables 3 and 4). A significant difference was found among the following variables of the iron status: ferritin ($F_{relation}$ = 198.99, p= 0.000), HCT ($F_{relation}$ = 7.073, p= 0.000), Hgb ($F_{relation}$ = 6.484, p= 0.000), TIBC ($F_{relation}$ = 6.137, p value = 0.001), transferrin ($F_{relation}$ = 4.467, p value = 0.005), MCV ($F_{relation}$ = 3.638, p value = 0.013), RBC ($F_{relation}$ = 3.241, p value = 0.022) and MCH ($F_{relation}$ = 2.639, p value = 0.049).

Table 4 presents the standardized values of the averaged iron status variables yielded by the ANOVA in relation to the clusters defined. All results were standardized according to C3, which was used to define the averages of the variables of the entire sample of the water players (the values of the given cluster represented the criterion, i.e., 100 %).

Table 4. Standardized values of the iron status variables in the study

Clusters / Variables	Low level (%)	Under AVG level (%)	AVG level (%)	Very good level (%)	Excellent level (%)
RBC	92.42	98.78	100	103.81	105.20
Hgb	85.73	99.58	100	103.09	103.70
Hct	85.28	99.27	100	101.90	100.79
MCV	91.71	100.36	100	97.81	96.37
MCH	92.67	100.74	100	99.06	98.81
TIBC	123.76	97.16	100	100.39	92.67
Ferritin	18.33	49.28	100	140.44	268.72
Transferrin	121.71	99.39	100	95.20	89.29

DISCUSSION

In this study, we aimed at displaying the laboratory profile of elite water polo players in order to improve the evaluation of these variables in detection of non-anemic iron deficiency. Several studies have described the values of hematological and biochemical variables in endurance-trained athletes [18, 23, 32]. There have been frequent reports of a sub-optimal hematological status observed in athletes involved in intensive physical activity [4]. There is still a great deal of controversy in finding the optimal laboratory parameters for screening iron status and non-anemic iron deficiency in the athletic population, while the crucial role of hemoglobin in aerobic exercise has been well accepted. Serum ferritin is well-known to undergo a characteristic sequence of changes as body iron stores decrease from normal iron-replete levels to those found in iron deficiency anemia. Such anemia develops as the hemoglobin concentration falls below the lower limit of normal as a result of progressive depletion of the functional iron compartment [20].There are a number of markers that collectively describe an individual's iron status, including serum iron, red blood cell count, hemoglobin, red blood cells indices, hematocrit, total iron binding capacity, transferrin, haptoglobin and serum ferritin [3]. The last applied definition for iron store depletion is: serum ferritin concentration of 15-20 µg/L plus two of the three variables of serum iron concentration<10 µmol/L, TIBC>68 µmol/L, or transferrin saturation <15% [9, 29].

Studying iron status on the entire population of 42 elite young Serbian water polo players it was found that the mean values of all hematological and biochemical parameters were within reference ranges (5.118 x 10^{12}/L for red blood cells, 152 g/L for hemoglobin, 0.45 for hematocrit, 87.7 fL for MCV, 29.6 pg for MCH, 338 g/L for MCHC, 17.3 µmol/L for serum iron, 58.3 µmol/L for TIBC, 55.426 µg/L for ferritin, 2.74 g/L for transferrin and 0.63 g/L for haptoglobin). Our data supports the results of other studies of athletes [12, 34]. Taking into account that a lack of iron in the body can reduce aerobic capacity and impair endurance workout, it was necessary to determine the extent to which iron deficiency without anemia and iron repletion affect the athletic performance. The first studies in rats suggested that iron deficiency, even in the absence of anemia, can lead to exercise dysfunction, via muscle disruption [16]. However, most studies in humans have demonstrated exercise deficits with non-anemic iron deficiency [7, 28]. Most experts feel that non-anemic iron deficiency does not measurably affect exercise performance, yet agree that mild iron deficiency anemia can be difficult to differentiate from dilutional pseudo-anemia coexisting with non-anemic iron deficiency [13, 33].

Although the values of the hematological and biochemical parameters of the entire sample of the elite water polo players fell within the reference range, we identified five groups with different iron status

(Table 2, Figure 1). The first two groups represented low and under-average levels of iron status (42.86 % in total), the third group represented an average level of iron status (33.33 %), while the fourth and the fifth groups represented very good and excellent levels of iron status (23.81 % in total). The first two groups, with a total of 18 elite water polo players were characterized by the changes in iron status that corresponded to non-anemic iron deficiency.

The lower limit for normal hemoglobin concentration in males according to the recommendations of the World Health Organization (WHO) is 140 g/L [25]. It is not unusual for athletes to be 10-15 g/L below these values and still be considered within the normal range. It is important to check these data before starting the sport term, because deficiency can develop in the athletes who are in the limit values [4, 26, 30]. According to these findings iron deficiency anemia coincides with hemoglobin values<130 g/L in males [26]. Although highly trained athletes usually have normal absolute levels of hemoglobin, they often experience iron deficiency, generally latent, that implies no decrease in hemoglobin. These results are reinforced by the hemoglobin concentration in 40 elite water polo players in our study (average value 154.05 ±6.70 g/L), while 2 water polo players had hemoglobin concentration of 130.00 ± 2.83 g/L. Those 2 players also had significant lower levels of red blood cells (4.720±0.198x10^{12}/L), hematocrit (0.385±0.021), MCV (81.20±0.14 fL) and MCH (27.55±0.64 pg), raised levels of TIBC (72.55 ± 4.31 μmol/L) and transferrin (3.36 ± 0.13 g/L), as well as very low levels of ferritin (10.30 ± 4.95μg/L) and transferrin saturation (8.33%) (Table 2). Although red blood cells, hematocrit and hemoglobin concentrations are the main hematological parameters which significantly differ among individual clusters, they are not sufficient to monitor non-anemic iron deficiency in athletes because of the hemodilution that occurs in training. Hemoglobin and hematocrit showed the same difference between C1 and other clusters, and since they reflect a relative concentration of hemoglobin, i.e., red blood cells in blood, they also vary simultaneously.

Red blood cell indices, MCV and MCH, give a closer description of red blood cell morphology so that their values in 2 elite water polo players in C1 correspond to normocyte-normochrome iron deficiency.

Serum iron, although lower in C1, did not show any statistical significance. Serum iron levels show a circadian variation with the morning peak and evening and considerable day-to-day variations due to the variable release of iron to the plasma by the reticuloendothelial system. The difference between the morning and the evening serum iron values can be up to 9 μmol/L. Stress also reduces the serum iron concentration considerably, which is of vital importance in athletes. Consequently, serum iron as an individual test can be claimed to have unpredictable variability and relative insensitivity so that only in combination with TIBC or transferrin it bears clinical significance [5, 36].

In 2 elite water polo players in C1 reduced serum iron (6.05 ± 0.35 μmol/L) coincides with a rise in TIBC (72.55 ± 4.31, or 123.76 % of C3 as an AVG level) and a drop in transferrin saturation (only 8.34 %), while a significantly higher concentration in transferrin in relation to the other clusters (3.36 ± 0.13, or 121.71 % of C3 as an AVG level), although within a reference range, can be caused by a stimulated synthesis in the liver due to a low serum iron concentration (Tables 2 and 4).

A great number of studies have shown that the most sensitive marker that describes an individual's iron status is serum ferritin, a marker of stored iron. This iron store is not tapped into until circulating levels of iron become too low to support demands. For example, if the body is using (via exercise) and excreting (via sweat, or menstrual cycle) more iron than it is receiving (via ingestion, absorption, storage), the ferritin level will slowly decline. For this reason, practitioners have historically used the ferritin value to identify cases of iron deficiency anemia (poor iron status). According to Nickerson et al [26] iron deficiency in athletes is established when the levels of ferritin are lower than 12 μg/L with a normal hemoglobin concentration. A lower ferritin concentration in C1 and C2 (10.30 ± 4.95 μg/L and 27.69 ± 8.51 μg/L) indicates depleted iron stores, which confirms previous results.

In our study, ferritin was proved to have the strongest and most dominant influence on iron status among the groups of the water polo players (Table 3, F = 198.99, p=0.000). The results showed that in comparison to ferritin, the hemoglobin concentration has a substantially lesser influence on the variability of the differences in iron status among the groups of the water polo players in the study (Table 3, F = 6.48, p=0.000). However, hemoglobin, together with hematocrit and TIBC, represents another statistically significant marker that describes the variability of the differences among the groups. The best evaluation of the iron store status is made possible upon the recommended cut-off values for the ferritin concentrations in athletes. Athletes at risk of iron deficiency include all female and male middle- and long- distance runners, as well as all female athletes in other disciplines (including team sports). About 50% of the sport institutions in Germany recommend beginning treatment at rather low serum ferritin levels (<25 μg/L), but based on experiences with long-distance runners it isrecommended to

institute controlled iron supplementation for athletes with serum ferritin <35 μg/L [27]. A level of less than 30 μg/L is used at the Australian Institute of Sport as an indication of a need for iron supplementation based on sensitivity and specificity data published by Mast et al [24] and Fallon [15].

Swimmers with ferritin levels below 35 μg/L may not be anemic, but their performance at maximal intensities may be compromised and can impact their ability to train from day to day [31]. So the traditional signs of poor iron status that a coach might observe are not evident and the effects may only be experienced during exercise. Bringing a low ferritin level back into the normal range can restore an athletes potential to work aerobically for a longer period of time.

The ferritin concentration and subnormal serum hemoglobin indicate that 2 elite water polo players in C1 were in stage 2 of iron deficiency, while 16 elite water polo players in C2 were in stage 1 of iron deficiency [25, 34, 38]. There have been studies of athletes which have demonstrated a high prevalence of iron deficiency with up to 80% of female athletes and 30% of elite male athletes who were iron deficient [8]. Rowland and Kelleher [31] found increased prevalence of low ferritin (27% in control individuals, 40% in runners and 47% in swimmers). In our study, 26.19% young elite water polo players had ferritin values below the recommended cut-off values of 30 μg/L (11 players out of 42 from the whole sample).

When all this is taken into consideration, there remains the question which athletes should be included in routine laboratory screening tests for iron status and how often, because there is little information in published pre-participation recommendations. American College of Sports Medicine (ACSM) recommends screening every year before the season begins, but, with variation in training regimens and menstrual cycles, this may be done more frequently, depending on individual athletes and their particular sports [1]. Follow-up tests on athletes who have a low iron status should be performed monthly to ensure that iron supplementation has sufficiently restored iron stores in the bone marrow.

Once the diagnosis has been established, the next diagnostic challenge is to determine the underlying etiology of iron deficiency, which is in all cases due to either blood loss or nutritional deficit. While the evaluation is directed towards the underlying etiology, primary treatment of iron deficiency consists of iron replacement therapy.

CONCLUSIONS

While the crucial role of hemoglobin in aerobic exercise has been well accepted, there is a great deal of controversy about the optimal hematological parameters in the athletic population. Further investigation of anemia that occurs in athletes needs to be done to clarify the epidemiology as well as the pathophysiology, especially in case of iron deficiency.

PRACTICAL APPLICATION

The results emphasized that there is a need for systematic iron status screening and evaluation in water polo players who have undergone year-long periods of serious training and competition.

Besides, the findings indicated that for iron status screening in junior water polo players there should be special attention paid to the following parameters: RBC, Hgb, HCT, MCV, MCH, TIBC, ferritin and transferrin. It was shown that those parameters significantly discriminated among the water polo players in the study as for their anemic state evaluation.

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