EPR Study of Iron Status in Human Body during Intensive Physical Activity

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Abstract—The iron metabolism was studied in serum blood samples collected from 26 professional sportsmen undergoing intensive physical exercises using EPR combined with hematological and biochemical laboratory tests. Only 23% of EPR spectra (n = 6) were practically normal while in the rest spectra additional abnormal absorption lines were detected. Presumably, the significant portion of new signals may be caused by different cytochromes. Moreover, the anisotropic signals with \( g_1 \approx 2.02; g_2 \approx 1.94 \) and \( g_3 \approx 1.86 \) registered in some spectra pointed to the sulfur-iron centers. There was nearly linear correlation between the concentration of \( \text{Fe}^{3+} \) in transferrin (\( \text{Fe}^{3+}\)-Tf) obtained from the EPR spectra and the serum iron concentration measured by absorption photometry both for sportsmen and controls (healthy individuals and patients with different diseases). At equal serum iron concentrations the \( \text{Fe}^{3+}\)-Tf level was higher in sportsmen than that in controls. The Pearson correlation coefficient \( r \) for \( \text{Fe}^{3+}\)-Tf and serum iron values was equal to 0.89 in sportsmen versus \( r = 0.97 \) in controls. Additional new lines in serum EPR spectra of professional sportsmen prove the suitability of EPR assay for scheduled medical exams since routine biochemical and hematological tests are insufficient to discover all abnormalities in iron metabolism under intensive physical exercises.

Keywords: sports medicine, iron metabolism, electron paramagnetic resonance, \( \text{Fe}^{3+}\)-transferrin, paramagnetic cytochromes

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INTRODUCTION

In modern professional sports, coupled with intense physical exercise, checking the state of health of sportsmen demands a special approach. The primary step of examination usually includes measurement of hematological indices of blood, among which important are the parameters determining the metabolism of iron (see, for example, \[1–6\]). The topicality of this problem is stipulated by that in professional sportsmen, often revealed are disturbances of iron metabolism: by data of work \[6\], conditions with iron deficit in males are observed in 51%, while with its excess, in 3.3%. In sports medical practice, screening of iron metabolism is usually restricted to determining the concentrations of serum iron (SI), hemoglobin (Hb), ferritin, and the total iron-binding capacity of blood serum (TIBC). Along with routine methods included in the list of protocol examinations of sportsmen, iron metabolism can be also studied by the method of electron paramagnetic resonance (EPR). This method allows for (without using any reagents) determination of not only the integral concentration of \( \text{Fe}^{3+} \) ions in transferrin molecules (Fe-Tf) and of \( \text{Cu}^{2+} \) ions in ceruloplasmin (Cu-CP), but also detection of weak dysmetabolic deviations by appearance in blood spectra of new signals presenting diagnostic markers of various disturbances of the metabolism. However, in the available literature we have not found works on investigating the iron metabolism in sportsmen by EPR.

Therefore, we aimed to study blood serum of professional sportsmen by the EPR method in combination with general and biochemical analyses of blood for revealing disturbances of iron metabolism, and also examine the possibilities of applying the EPR method in sports medical practice.

EXPERIMENTAL

In all experiments the EPR spectra were recorded from an identical volume of blood serum (0.5 mL) in a Varian E-12 spectrometer at a frequency of 9.38 GHz, microwave power of 50 mW and sample temperature of 77 K. For quantitative processing of the data, a spectrum was concurrently recorded from a reference specimen of anthracite with known \( g \)-factor and number of paramagnetic centers. It is well known that if the width and shape of lines in EPR spectra do not change, quantitative analysis of spectra may be performed by measuring the intensity of a derivative of the absorption line normalized to the reference signal.
Meanwhile, in some of our recorded spectra we disclosed a change of shape of the Fe-Tf line. The validity of estimating the Fe$^{3+}$ concentration in Tf by the “peak to peak” signal derivative in the given case is substantiated in the Discussion section.

The main group consisted of 26 professional sportsmen of the Junior team of the Continental hockey league aged 19 ± 1. Peripheral blood counts were performed with an automated hematological analyzer “SYSMEX pocH-100i” (Japan), while the biochemical status (SI, TIBC, AST, ALT, ferritin, creatinine, fibrinogen, myoglobin) was determined on a “Cobas integra 400 plus” analyzer (Roche, Switzerland) by manufacturers’ protocols. The reference group of 15 persons (age interval from 20 to 80) included both volunteers (clinically healthy persons) and patients with various pathologies: iron-deficient anemia, hypertensive disease, ischemic heart disease, liver cirrhosis.

**RESULTS**

An EPR spectrum of normal human blood serum usually contains signals from Fe$^{3+}$ in Tf with $g_\perp = 4.31$, Cu-CP with $g_\perp = 2.05$ and free radicals R with $g = 2.003$; 2 – signals from paramagnetic centers in the norm, and also lines from Cu$^{2+}$ ions in cytochrome c oxidase with $g_\parallel = 2.17$ and $g_\perp = 2.03$ (overlaps with signal from Cu-CP); Fe-S centers with $g_1 = 2.02$ (overlaps with signal from Cu-CP), $g_2 = 1.94$ and $g_3 = 1.86$; signal with $g = 4.9$ and broad line with $g = 2.78$ – not identified; 3 – signals from paramagnetic centers in the norm, and also lines from Cu$^{2+}$ ions in c-COx with $g_0 = 2.17$ and $g_\perp = 2.03$ (overlaps with signal from Cu-CP); lines from oxygenated high-spin state ($S = 5/2$) of Fe$^{3+}$ ion in heme $\alpha$ of c-COx with $g_1 = 4.5$; $g_2 = 1.78$; $g_3 = 1.69$; signal with $g = 2.39$ and broad line – not identified. Encircled dashed is the part of spectrum where change of line shape is observed.

Such spectra, practically corresponding to the norm, have been obtained by us only for 23% ($n = 6$) sportsmen (spectrum 1 in Fig. 1). However, in these sportsmen we have revealed deviations from the norm of some biochemical and hematological indices of blood, which is indicative of disturbances in the process of iron metabolism: the TIBC value below normal range was registered in four sportsmen, mean erythrocyte volume in two, ferritin concentration in one; while in another one the SI level was upregulated. In the rest of sportsmen (77%), in EPR spectra of blood serum we have disclosed a series of additional signals

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1 According to work [8], an EPR spectrum from Fe-Tf has parameters $g_\perp = 4.31$, $g_3 = 9.3$; and from Cu-CP, $g_\perp = 2.056$, $g_1 = 2.209$ ($A_\parallel = 155–200$ Oe).