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Blood Essential Trace Elements and Vitamins in Students with Different Physical Activity

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Abstract: The primary objective of the current investigation is to estimate the effect of different levels of physical activity on blood trace elements and vitamins concentration. A total of 97 students (55 male and 42 female) of P.G. Demidov Yaroslavl State University (Yaroslavl, Russia) took part in the current investigation. All the examinees gave their informed consent prior to the inclusion into the study. The female and male students were divided into the respective high, medium and low physical activity groups. Whole blood essential trace elements were assessed by inductively coupled plasma mass spectrometry using NexION 300D+NWR213 spectrometer. Quantitative estimation of water-and lipid-soluble vitamins was held using high-performance liquid chromatography at PerkinElmer S200. The results obtained indicate that blood trace elements' levels do not change in response to physical activity in females. At the same time, blood copper, iron, magnesium and selenium concentrations in males are decreased along with elevated physical activity. Increased physical activity in females is associated with a non-significant decrease in blood ascorbic acid level, whereas a significant decrease in blood retinol concentrations was observed in males. It is notable that the maximal gender differences in blood vitamin and trace element values were observed in the high physical activity groups. The results indicate gender difference in trace element and vitamin balance in response to different levels of physical activity. The obtained data underline the necessity of trace element and vitamin homeostasis monitoring before mineral and vitamin supplementation.

Key words: Vitamins, trace elements, sport, physical activity, gender

INTRODUCTION

Trace elements and vitamins are widely used by sportsmen as non-nutritional supplements (Volpe and Nguyen, 2013). These compounds may improve recovery, endurance and general health (Louis *et al.*, 2010). The actuality of this supplementation is increased due to the high risk of vitamin and trace element deficiencies in sportsmen (McClung *et al.*, 2014). At the same time, the uncontrolled supplementation may lead to a number of adverse health effects (Sirota, 1994). In particular, excessive consumption of lipid-soluble vitamins in the case of their normal content in the body may potentially lead to intoxication (Koutkia *et al.*, 2001; Hathcock *et al.*, 2007). The same effects may be observed in the case of essential trace elements (Goldhaber, 2003; Maret and Sandstead, 2006). Consequently, the estimation of the most characteristic changes in vitamin and mineral balance may help to improve prophylaxis and treatment of deficient and/or excessive disorders. Despite a large number of works devoted to the study of trace element homeostasis in exercise, the existing data are contradictory (Speich *et al.*, 2001). For example, there is still no consent between the studies regarding iron homeostasis in physical activity. One group on investigators postulates that sportsmen are characterized by lower iron stores and are more susceptible to anemia (Lombardi *et al.*, 2014). At the same time, the results of other studies indicate no

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significant effect of exercise on body iron content (Sandstrom *et al*., 2012).

Moreover, a high number of investigations indicating the change in vitamin D content in the organism exist (Close *et al*., 2013; Cooke, 2014; Villacis *et al*., 2014) whereas the influence of physical activity on other lipid-and water-soluble vitamins' balance is insufficiently studied (Shibata and Fukuwatari, 2013). Other studies regarding the role of vitamins in physical activity mainly investigate the influence of vitamin supplementation on sport performance (Chatterjee *et al*., 2011; Czaja *et al*., 2011; Taghiyar *et al*., 2013).

Therefore, the primary objective of the current investigation is to estimate the effect of different levels of physical activity on blood trace elements and vitamins concentration.

MATERIALS AND METHODS

A total of 97 students (55 male and 42 female) of P.G. Demidov Yaroslavl State University (Yaroslavl, Russia) took part in the current investigation. All procedures followed were in accordance with the ethical standards of the responsible committee on human experimentation (institutional and national) and with the Helsinki Declaration of 1975, as revised in 2000. Informed consent was obtained from all examinees for being included in the study. The research was approved by the Local Ethics Committee.

The exclusion criteria used in the study were: (1) acute traumas and posttraumatic period; (2) metabolic diseases; (3) menstruation in females; (4) specific dietary habits (vegetarians); (5) supplementation of sport nutrition supplements, vitamin and/or mineral complexes.

All examined students were divided into three groups according to the level of physical activity. 21 female students visiting a gym 4 and more times a week were classified as the high physical activity group. The second group (medium physical activity) consisted of 9 females which visit a gym 2-3 times a week. At the same time, 12 female students with low physical activity ($\leq$1 per week) presented the third group.

The general male sample was divided into similar groups. Respectively, the first (high physical activity) group consisted of 27 male students which visit a gym 4 and more times per week. 13 students who go in for sport 2-3 times a week presented the medium physical activity group. The third group consisted of 15 male students with low physical activity ($\leq$1 per week).

Fasting blood was collected from cubital vein into lithium heparin tubes "S-Monovette" collection tubes (Sarstedt, Nümbrecht, Germany). Whole blood samples were used for subsequent chemical analysis.

Whole blood essential trace elements were assessed by inductively coupled plasma mass spectrometry using NexION 300D+NWR213 spectrometer (Perkin-Elmer, Waltham, MA, USA). Standard samples (ClinChek Plasma Control lot 129, Recipe, Germany) were used for laboratory quality control.

Blood vitamins' analysis was performed after protein precipitation using acetonitrile and extraction of lipid-(A, D, E, K) and water-soluble-vitamins (B_1, B_5, B_6, B_{12}, C). Quantitative estimation of vitamins was held using high-performance liquid chromatography PerkinElmer S200 (Perkin-Elmer, Waltham, MA, USA).

All analyses were performed in accredited clinic-diagnostic laboratory of ANO "Centre for Biotic Medicine", Moscow (ISO 9001:2000 certificate 4017 from 05.04.2006, BM TRADA Certification Limited Incorporating CQA).

All data were stored in MS Excel calculation sheet (Microsoft Corp, USA). Subsequent statistical analyses were performed with Statistica 11.0 (StatSoft Inc., Tulsa, Oklahoma, USA). In order to evaluate the obtained data distribution Shapiro-Wilk test was used. Since the distribution was not Gaussian, the obtained data were reported as median and the respective 25 and 75% quartiles (Me(Q25-Q75)). Non-parametric Mann-Whitney U-test was used for paired-group comparison. Global comparison of three groups was performed by Kruskall-Wallis test (KWP). The level of significance was set as $p<0.05$ for all analyses.

RESULTS

Chemical and statistical analysis failed to reveal any significant changes in blood trace elements in response to physical activity in females (Table 1).

In contrast to the female samples, the level of physical activity had a significant impact on blood trace element content in males (Table 2). Blood copper levels were negatively associated with activity. Particularly, blood Cu concentration in the examinees from high and medium-activity groups was significantly decreased by 27 and 12% in comparison to the low physical activity group values, respectively. Blood iron levels in the medium and low physical activity groups exceeded the values obtained for the high activity group by 27 and 9%, respectively. Kruskall-Wallis test also revealed a significant tendency to decreased blood iron along with increasing physical activity. Similar changes were observed in the case of manganese. In particular, blood Mn levels in the medium and low activity groups were characterized by more than twofold increase in comparison to the high-activity group. Blood Se levels in the examinees of the 2nd and 3rd groups exceeded the respective values observed for the 1st group by 79 and 70%, respectively. The statistical significance of the observed tendency was also confirmed by Kruskall-Wallis test. At the same time, there were no significant differences in blood cobalt and zinc concentrations between the male groups analyzed.

Table 1: Blood essential trace element content (µg/ml) in female students in relation to physical activity levels

Metal	High activity (n = 21)	Medium activity (n = 9)	Low activity (n = 12)	KW P
Co	0.0014 (0.0011-0.0014)	0.0008 (0.0005-0.0014)	0.0012 (0.0008-0.0020)	0.294
Cu	0.845 (0.802-0.904)	0.832 (0.760-0.985)	0.790 (0.771-0.949)	0.930
Fe	419.99 (401.72-441.46)	409.71 (382.48-426.42)	451.354 (417.12-462.36)	0.213
Mn	0.013 (0.011-0.018)	0.017 (0.013-0.020)	0.016 (0.014-0.023)	0.456
Se	0.139 (0.123-0.163)	0.146 (0.127-0.202)	0.148 (0.136-0.162)	0.835
Zn	6.59 (6.12-6.81)	6.44 (5.32-6.78)	6.42 (6.12-6.87)	0.736

KW P-indicates p-values by Kruskal-Wallis test

Table 2: Blood essential trace element (µg/ml) content in male students in relation to physical activity levels

Metal	High activity (n = 27)	Medium activity (n = 13)	Low activity (n = 15)	KW P
Co	0.0005 (0.0005-0.0007)	0.0006 (0.0005-0.0016)	0.0005 (0.0005-0.0009)	0.910
Cu	0.661 (0.63-0.742)	0.8 (0.778-0.848)	0.904 (0.764-0.921)*	0.018
Fe	456.979 (431.734-495.454)	581.725 (556.3-618.4)*	495.3 (476.6-547.925)	0.023
Mn	0.009 (0.007-0.011)	0.023 (0.016-0.027)*	0.019 (0.013-0.029)*	0.002
Se	0.112 (0.098-0.118)	0.201 (0.184-0.229)*	0.191 (0.158-0.194)*	0.000
Zn	6.83 (5.81-7.19)	5.9 (5.49-6.42)	6.31 (5.83-7.02)	0.197

*Significant difference in comparison to the high physical activity group. KW P-indicates p values by Kruskal-Wallis test

Table 3: Comparison of the male and female blood essential trace element levels (µg/ml)

Metal	High activity	Medium activity	Low activity
Co	<0.001*	1.000	0.026*
Cu	<0.001*	0.689	0.916
Fe	0.007*	0.020*	0.008*
Mn	0.001*	0.093	0.916
Se	0.007*	0.173	0.072
Zn	0.593	0.810	0.916

p-values by Mann-Whitney U-test are presented
*p<0.05; the difference is significant

Data presented in the Table 3 indicate that maximal gender differences in blood trace element content were observed in the high physical activity group. In particular, blood cobalt, copper, iron, manganese and selenium levels in the female examinees from the high activity group differed significantly from the respective male values. It is notable that only blood zinc concentrations were nearly similar in males and females with high activity. At the same time, only blood iron concentration was different between men and women from the medium activity groups. As for low physical activity, blood cobalt and iron levels were characterized by a significant difference between males and females.

As observed for blood trace elements, the level of vitamins in females was stable and did not respond significantly to physical activity (Table 4). At the same time, a tendency to decreased blood ascorbic acid concentration was observed in the examinees with high physical activity. In particular, blood vitamin C levels in the low activity group significantly exceeded the respective values observed for the high physical activity group by 42%. Similar situation was observed in the case of pyridoxine. Females with low physical activity were characterized by a nearly twofold increase in blood vitamin B_6 when compared to the ones with high physical activity. However, the observed changes were not significant. A tendency to exercise-associated reduction in vitamin A was also observed.

The obtained data indicate that blood vitamins' levels in the male sample were also relatively stable (Table 5). However, a significant 20% decrease in blood retinol concentration was observed in the high physical activity group in comparison to the medium activity group values. Moreover, Kruskall-Wallis test also revealed a significant tendency to decreased blood vitamin A concentrations in association with increased physical activity.

Statistical analysis (Table 6) has indicated that significant difference in blood vitamin levels between the respective male and female groups were observed in the case of retinol in the medium activity group. It is notable that in the high activity group the difference between male and female blood retinol, pantothenic acid and pyridoxine were nearly significant. At the same time, in the low physical activity groups no gender differences in blood vitamin concentration were observed.

DISCUSSION

The obtained data indicate that blood trace element concentration is relatively stable in female students in relation to physical activity. This observation is in contrast to earlier data indicating female athletes as a high risk group for mineral deficiency development (McClung *et al*., 2014). Moreover, our previous data also indicated mineral deficiency in the professional female rugby players in the pre-competitive period (Fesenko and Skalny, 2011). However, it is important to note that the current study analyzed students, but not professional endurance athletes. This circumstance may have an impact on the divergence of our research and literature data.

At the same time, trace element balance seems to be more valuable in males. In particular, the level of physical activity significantly affected blood copper, iron, manganese and selenium concentrations.

Table 4: Blood vitamin concentration (μg/ml) in female students in relation to physical activity levels

Vitamin	High activity (n = 21)	Medium activity (n = 9)	Low activity (n = 12)	^{KW}P
B_1	0.446 (0.268-0.608)	0.331 (0.195-0.67)	0.334 (0.155-0.513)	0.536
B_{12}	0.007 (0.0039-0.0082)	0.007 (0.004-0.009)	0.006 (0.004-0.009)	0.962
B_5	1.95 (0.92-2.61)	2.80 (0.79-3.08)	1.56 (0.62-3.21)	0.637
B_6	0.074 (0.041-0.122)	0.100 (0.072-0.137)	0.138 (0.078-0.288)	0.182
C	8.94 (7.32-13.87)	10.91 (8.68-14.06)	12.66 (10.19-16.97)*	0.148
A	0.248 (0.234-0.322)	0.279 (0.221-0.313)	0.358 (0.229-0.401)	0.505
D	0.004 (0.003-0.005)	0.003 (0.002-0.005)	0.004 (0.003-0.005)	0.345
E	0.179 (0.137-0.253)	0.13 (0.125-0.165)*	0.151 (0.109-0.226)	0.177
K	0.003 (0.002-0.003)	0.003 (0.002-0.004)	0.002 (0.001-0.003)	0.377

*Significant difference in comparison to the high physical activity group. ^{KW}P-indicates p-values by Kruskal-Wallis test

Table 5: Blood vitamin concentration (μg/ml) in male students in relation to physical activity levels

Vitamin	High activity (n = 27)	Medium activity (n = 13)	Low activity (n = 15)	^{KW}P
B_1	0.331 (0.232-0.516)	0.291 (0.172-0.431)	0.281 (0.187-0.577)	0.792
B_{12}	0.007 (0.005-0.008)	0.006 (0.005-0.007)	0.007 (0.006-0.008)	0.536
B_5	1.05 (0.77-1.63)	1.45 (0.73-1.7)	1.26 (0.65-1.76)	0.988
B_6	0.119 (0.068-0.195)	0.085 (0.066-0.136)	0.156 (0.069-0.242)	0.363
C	10.96 (7.57-14.95)	11.06 (8.26-12.78)	9.59 (8.67-14.64)	0.877
A	0.329 (0.251-0.405)	0.394 (0.363-0.473)*	0.339 (0.31-0.477)	0.035
D	0.003 (0.002-0.004)	0.004 (0.002-0.005)	0.003 (0.002-0.005)	0.619
E	0.193 (0.145-0.265)	0.143 (0.127-0.216)	0.147 (0.104-0.236)	0.328
K	0.002 (0.002-0.003)	0.002 (0.002-0.004)	0.003 (0.002-0.003)	0.860

*Significant difference in comparison to the high physical activity group. ^{KW}P-indicates p-values by Kruskal-Wallis test

Table 6: Comparison of the male and female blood vitamin levels

Vitamin	High activity	Medium activity	Low activity
B_1	0.418	0.841	0.542
B_{12}	0.596	0.841	0.591
B_5	0.067	0.083	0.575
B_6	0.053	0.947	0.826
C	0.633	1.000	0.232
A	0.055	0.023*	0.341
D	0.112	0.640	0.317
E	0.716	0.350	0.961
K	0.513	0.404	0.495

p-values by Mann-Whitney U-test are presented
*p<0.05; the difference is significant

The observed exercise-associated decrease in blood copper levels is partially in accordance with earlier study indicating lower serum levels of copper in athletes when compared to the non-athletes (Lee *et al*., 2012). Another study by Koury and coauthors has revealed lowered serum copper concentrations in 32% of the examined athletes (Koury *et al*., 2007). Acute exercise was also shown to induce a decrease in plasma copper (Bordin *et al*., 1993). At the same time, the examination of swimmers has indicated that physical training does not affect plasma copper concentration when dietary intakes are adequate (Lukaski *et al*., 1990). Oppositely, an excellent study by Rodriguez Tuya *et al*. (1996) has shown an increase in plasma copper levels in professional sportsmen (Rodriguez Tuya *et al*., 1996).

The observed decrease in blood iron concentration in males with higher activity partially confirms the earlier data (Koehler *et al*., 2012). At the same time, this study has indicated higher prevalence of iron deficiency in females than in males. Our results have failed to reveal a significant impairment of blood iron levels in female students. However, the respective results indicating the absence of significant decrease in serum iron levels in college-age women were obtained earlier (Wirth *et al*., 1978). At the same time, one recent study involving 16 athletes has demonstrated that 16-week aerobic training had a positive effect on body iron status (Soslu *et al*., 2014). Experimental study has shown a significant increase in liver iron, whereas spleen iron was decreased, being indicative of a possible iron redistribution in response to exercise (Kaptanoglu *et al*., 2003).

The present data are in contrast to earlier studies where the increased blood cell copper content in the beginner sportswomen at the start of training has been revealed (Rusin *et al*., 1979). At the same time, evidence regarding this question seems to be insufficient.

Our research data have indicated that blood vitamin concentration is relatively stable in women. Only ascorbic acid levels tended to decrease along with elevated physical activity. This effect may result from higher ascorbic acid requirements with exercise (Keith and Pomerance, 1995). However, previous human study failed to detect significant differences in serum and urine ascorbic acid levels between athletics and non-athletics (Rokitzki *et al*., 1994).

The obtained data indicate that blood selenium levels were inversely related to physical activity. These data corresponds to earlier indication of decrease in serum selenium after maximal physical exercise in high physical activity group of males undertaking a soccer-training regimen (Emre *et al*., 2004).

Oppositely, blood ascorbic acid values remained stable in males, whereas blood retinol content seemed to be decreased with physical activity. These observations are in agreement with earlier data indicating lowered vitamin A concentrations in athletes (Borisov, 1970). Experimental studies also demonstrate a significant exercise-induced reduction in liver retinol content (Kobylinski *et al*., 1990; Valcarcel *et al*., 1990).
Moreover, the male examinees undergo more intensive physical training (wrestling) in relation to females (volleyball, fitness). This condition may hypothetically result in more variable physiological parameters in males than in females.
Statistical analysis indicated that maximal gender differences in blood trace element and vitamin levels were observed in the high physical activity groups. It is notable, that vitamins were less variable in comparison to trace elements. Hypothetically, higher gender difference in the parameters analyzed in the high activity is due to intensified metabolism of the organism.
The current study has a number of limitations. First, the observed tendencies should be verified on more wide samples of the examinees. Second, despite the similar frequency of physical training, males undergo more intensive physical training in relation to females that may at least partially influence the variability of the parameters analyzed. Consequently, further studies with similar controlled physical activity in men and women are required to specify the effect of physical activity on vitamin and trace element homeostasis. Finally, more indicators of mineral and vitamin balance should be estimated to obtain the complex state of the examinees.

Conclusions: Generally, the results obtained indicate that:

1: Blood trace elements' levels do not change in response to physical activity in females
2: Blood copper, iron, magnesium and selenium concentrations in males are decreased along with elevated physical activity
3: Increased physical activity in females is associated with a non-significant decrease in blood ascorbic acid level, whereas a significant decrease in blood retinol concentrations was observed in males
4: The maximal gender differences in blood vitamin and trace element values were observed in the high physical activity groups

The results indicate gender difference in trace element and vitamin balance in response to different levels of physical activity. The obtained data underline the necessity of trace element and vitamin homeostasis monitoring before mineral and vitamin supplementation.

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