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RISKY HEALTH BEHAVIOURS VS. LEVELS OF BIOELEMENTS IN SERUM OF SECONDARY SCHOOL STUDENTS

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Abstract

Health behaviours are actions taken by a person in the field of health. Risky behaviours are those that pose a threat to health. Bioelements are essential for the proper functioning of every organism. Their trace amounts are necessary for life and health. The aim of this study was to determine the relationship between health behaviours and the levels of bioelements in the blood serum of secondary school students. This survey-based study was carried out on a sample of 376 secondary school students aged 13-16 years. It was performed using the HBSC questionnaire (Health Behaviour in School-aged Children: A WHO Collaborative Cross-national Study). Next, cubital vein blood was collected for laboratory tests, and the levels of bioelements (Mg, Ca, Cu, Fe, Zn) in blood serum were determined. The analysis of the research material did not demonstrate substantial differences in the mean serum levels of Ca, Mg, Zn, Cu, Fe between smokers and non-smokers, alcohol consumers and non-consumers, drug users and non-users. There were no statistically significant correlations between the levels of bioelements and the frequency of drinking beer and vodka ($p > 0.05$). A statistically significant correlation was observed between Cu levels and drinking wine ($p \leq 0.05$). Subjects showing aggressive behaviours (getting into scuffles) had lower Mg and Zn levels than other secondary school students. There were weak but statistically significant correlations between Cu levels and the frequency of wine consumption, and between serum Zn levels and smoking marijuana among the subjects.

Keywords: bioelements, health behaviours, secondary school students.

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INTRODUCTION

Health behaviours are actions taken by a person in the field of health. They are usually established in childhood and have a tremendous impact on our physical condition and well-being. Risky behaviours are those that pose a threat to health (Woynarowska-Sołdan, Węziak-Białowolska 2012). As the WHO data from 2002 show, risk factors that contribute most to the overall death rate in European countries are: smoking, alcohol consumption, low consumption of fruit and vegetables, and insufficient physical activity. According to the data of the Global Adult Tobacco Survey (GATS) from 2009-2010, smoking in Poland is on a downward trend, with about 33% of male and 20% of female smokers. Alcohol consumption among adults and adolescents is similar to that observed in other countries of the European Union, and when expressed in pure alcohol it is about 10 litres per person per year (Wojtyniak et al. 2012).

Bioelements are essential for the proper functioning of every organism. Their trace amounts are necessary for life and health.

Calcium (Ca) contributes to metabolic processes in the organism, blood coagulation, and the functioning of the nervous, muscular and endocrine systems (Brzezińska et al. 2004). Its deficiency can be associated with inadequate intake, poor absorption and excessive accumulation of calcium in bones or soft tissues. Consuming beverages with caffeine, drinking too much alcohol and smoking cigarettes may impair Ca assimilation (Hallstőm et al. 2006, Naude et al. 2012).

Magnesium (Mg) is obtained from vegetable and animal products. The processing of food results in the depletion of Mg from a diet. Mg deficiency has negative effects on the immune and nervous systems (Mazur et al. 2007). In children it is manifested by hyperactivity, aggression, lack of concentration, learning problems and anxiety (Ghanizadeh 2013).

Zinc (Zn) is involved in many cellular processes. It also plays an important part in the ethiopathogenesis of depression, but its level does not correspond with the severity of depressive symptoms (Siwek et al. 2005). Zn protects the human body against toxic influence of some elements such as Cd and Pb (Bridges, Zalups 2005).

Copper (Cu) is a component of many enzymes participating in oxidation/reductive processes. Cu deficiency may result from inadequate or no intake, for example due to starving or eating food with low Cu content (Valko et al. 2005).

Iron (Fe) is involved in the process of cellular respiration, and protects cells against toxic oxidation products. Insufficient oxygen supply to tissues may reduce one's ability to make physical effort, impair the psychomotor and mental development (Lieu et al. 2001).

Aim

The aim of this study was to determine the relationship between health behaviours and the levels of bioelements in the blood serum of secondary school students.

MATERIAL AND METHODS

The study was carried out on a sample of 376 secondary school students aged 13-16 years from the West Pomeranian Province (Poland). The students were healthy, did not use dietary supplements, did not suffer from endocrinological disorders or cancerous diseases, and did not receive psychiatric treatment. We analysed four risk behaviours, namely smoking, alcohol consumption, using drugs and getting into scuffles.

The study was conducted with the consent of the Bioethical Commission of the Pomeranian Medical University in Szczecin (BN-001/116/08).

The study consisted of two stages. In the first stage, a survey was performed using an international standardized research tool, the HBSC questionnaire (Health Behaviour in School-aged Children: A WHO Collaborative Cross-national Study). To gather the data, the authors used the method of a diagnostic survey with a questionnaire technique. The authors received the permission for its use from the Mother and Child Institute in Warsaw. Out of the questions included in the questionnaire, the authors selected and used those concerning risky health behaviours analyzed in this study.

The second stage involved laboratory analyses performed to determine the levels of bioelements (Mg, Ca, Cu, Fe, Zn) in blood serum. In order to measure Mg and Zn levels, 5 ml of cubital vein blood were collected into Vacutainer tubes without anticoagulant. The blood was drawn in a treatment room and delivered to a laboratory in accordance with the relevant rules and procedures. The blood was centrifuged at 4,000 rpm for 10 minutes and serum was obtained. Afterwards, the serum samples were diluted with distilled water in the proportion 1 ml of serum : 2 ml of water to determine the concentrations of Zn, Cu, and Fe, and 30 µl of serum : 3 ml of water to determine the concentrations of Ca and Mg. The serum Mg, Zn, Ca, Cu, Fe levels were determined by flame atomic absorption spectrometry (PU 9100X, Philips, Cambridge, UK). Diluted serum samples were inserted directly into the flame. The analytical wavelengths were 285.2 nm for Mg, 213.9 nm for Zn, 422.7 nm for Ca, 354.8 nm for Cu, 248.3 nm for Fe. Concentration values were read from the calibration curve. Internal quality control within the laboratory was performed at two levels, *i.e.* using two types of serum, namely serum with normal Mg, Zn, Ca, Cu, Fe concentrations and serum with Mg, Zn, Ca, Cu, Fe concentrations below normal. A simple 2-2SD Westgard rule was used for verification of the results. This rule states that if one control measure exceeds the mean ± 2SD, it is necessary to repeat the control measurement. If the subsequent result is within the expected values, then we assume that the previous deviation was a random incident, and the run should be accepted as ‘in control’. However, if the control measure exceeds the mean ± 2SD again, a systematic error is likely, and the results cannot be accepted as reliable (the run should be rejected). In such circumstances, trouble-shooting was performed and testing was repeated. The control results were plotted on the Levey-Jennings charts. The laboratory tests were performed in the Department of Biochemistry and Chemistry, the Pomeranian Medical University in Szczecin (Poland) in accordance with the PN-EN ISO 15189 guidelines.

The following concentrations were accepted as laboratory reference norms: Mg: 17-24 mg dm^{-3}, Fe: 0.35-1.5 mg dm^{-3}, Ca: 80-114 mg dm^{-3} for women, and 86-102 mg dm^{-3} for men (Siparsky et al. 2011), Zn: 0.68-1.07 mg dm^{-3}, Cu: 0.76-1.52 mg dm^{-3} (Strzykała 2005).

Statistical methods

Student’s *t*-test for independent samples was used to determine whether the means of dependent variables differed significantly between two samples. The accepted significance level was $\alpha = 0.05$. If $p < 0.05$, the null hypothesis should be rejected in favour of an alternative hypothesis that implicates significant differences between the means of the variables. The Spearman’s R correlation coefficient was applied to test the relationship between the pairs of variables.

All data transformations and calculations were done with MS Excel 2007. Statistical analysis was performed using Statistica 7.1 PL.

RESULTS

The study included a group of 376 secondary school students, composed of 215 boys (57.2%) and 161 girls (42.8%).

Our analysis of the research material did not demonstrate significant differences in the mean serum levels of Ca, Mg, Zn, Cu, and Fe between smokers and non-smokers, alcohol consumers and non-consumers, drug users and non-users (Table 1). It was proven, however, that there were significant differences in Mg and Zn levels between secondary school students who were and those who were not getting into scuffles. Students who were picking fights had significantly lower Mg and Zn levels ($p \leq 0.05$). There were no statistically significant differences between Ca, Cu and Fe levels and getting and not getting into scuffles by secondary school students ($p > 0.05$) – Table 1.

Our analysis of the data did not reveal statistical relationships between the levels of Ca, Mg, Zn, Cu, Fe and smoking cigarettes by secondary school

Table 1

Risky health behaviours vs. levels of bioelements in serum of secondary school students

Risky behaviours	Levels of bioelements				
	Ca (mg dm^{-3}) ($\bar{x}$ ± SD)	Mg (mg dm^{-3}) ($\bar{x}$ ± SD)	Zn (mg dm^{-3}) ($\bar{x}$ ± SD)	Cu (mg dm^{-3}) ($\bar{x}$ ± SD)	Fe (mg dm^{-3}) ($\bar{x}$ ± SD)
Smoking cigarettes					
Yes	90.20 ± 32.10	16.69 ± 6.17	0.77 ± 0.24	0.91 ± 0.29	1.09 ± 0.43
No	86.80 ± 29.60	16.99 ± 5.61	0.80 ± 0.27	0.90 ± 0.29	1.13 ± 0.51
	$z = 0.940$	$z = 0.990$	$z = 0.703$	$z = 0.588$	$z = 0.228$
	$p > 0.05$	$p > 0.05$	$p > 0.05$	$p > 0.05$	$p > 0.05$
Alcohol consumption					
Yes	90.1 ± 29.00	17.11 ± 5.16	0.80 ± 0.23	0.93 ± 0.26	1.17 ± 0.49
No	85.50 ± 30.50	16.81 ± 5.80	0.79 ± 0.28	0.88 ± 0.30	1.09 ± 0.50
	$z = 0.609$	$z = 0.364$	$z = 0.286$	$z = 0.203$	$z = 0.875$
	$p > 0.05$	$p > 0.05$	$p > 0.05$	$p > 0.05$	$p > 0.05$
Using drugs					
Yes	93.40 ± 27.40	17.81 ± 5.21	0.85 ± 0.23	0.95 ± 0.25	1.24 ± 0.59
No	86.70 ± 30.20	16.91 ± 5.74	0.79± 0.27	0.90 ± 0.29	1.11 ± 0.49
	$z = 0.860$	$z = 0.509$	$z = 0.781$	$z = 0.602$	$z = 0.340$
	$p > 0.05$	$p > 0.05$	$p > 0.05$	$p > 0.05$	$p > 0.05$
Getting into scuffles					
Yes	85.31 ± 34.01	16.18 ± 6.37	0.75 ± 0.28	0.86 ± 0.32	1.11 ± 0.56
No	88.33 ± 27.63	17.42 ± 5.27	0.82 ± 0.26	0.92 ± 0.27	1.13 ± 0.47
	$z = 0.079$	$z = 0.129$	$z = 0.633$	$z = 0.235$	$z = 0.697$
	$p > 0.05$	$p \leq 0.05$	$p \leq 0.05$	$p > 0.05$	$p > 0.05$

$\bar{x}$ – arithmetic mean, SD – standard deviation, z – Levene's test, p – level of significance

students ($p > 0.05$). There were also no statistically significant correlations between the levels of bioelements and the frequency of drinking beer and vodka ($p > 0.05$). A statistically significant correlation was observed between Cu levels and drinking wine ($p \leq 0.05$); other bioelements did not significantly correlated with wine consumption ($p > 0.05$). There was a statistically significant correlation between Zn levels and smoking marijuana by secondary school students ($p \leq 0.05$); other bioelements did not statistically significantly correlated with smoking marijuana ($p > 0.05$). An analysis of the relationship between the levels of bioelements in blood serum of secondary school students and getting into scuffles showed that the latter statistically significantly correlated with Mg and Zn levels ($p \leq 0.05$), and did not statistically significantly correlate with Ca, Cu and Fe levels ($p > 0.05$) – Table 2.

Table 2

Correlations between risky health behaviours and levels of bioelements in serum of secondary school students

Risky behaviours	Levels of bioelements									
	Ca	R	Mg		Zn		Cu		Fe	
	R	*p*	*R*	*p*	*R*	*p*	*R*	*p*	*R*	*p*
Smoking cigarettes										
	0.038	>0.05	-0.064	>0.05	-0.047	>0.05	-0.005	>0.05	-0.014	>0.05
Alcohol consumption										
beer	0.024	>0.05	-0.076	>0.05	0.033	>0.05	0.050	>0.05	0.075	>0.05
wine	0.048	>0.05	-0.034	>0.05	-0.010	>0.05	0.128	**≤0.05**	-0.026	>0.05
vodka	-0.066	>0.05	-0.099	>0.05	0.046	>0.05	0.069	>0.05	0.033	>0.05
Using drugs										
marijuana	0.016	>0.05	0.025	>0.05	0.111	**≤0.05**	0.042	>0.05	0.035	>0.05
amphetamine	0.009	>0.05	-0.064	>0.05	-0.036	>0.05	0.058	>0.05	0.010	>0.05
Inhalation drugs	0.026	>0.05	-0.018	>0.05	0.035	>0.05	0.009	>0.05	-0.049	>0.05
Drugs used to stupefy oneself	0.020	>0.05	0.029	>0.05	0.074	>0.05	0.050	>0.05	0.023	>0.05
Getting into scuffles										
	-0.030	>0.05	-0.127	**≤0.05**	-0.110	**≤0.05**	-0.085	>0.05	-0.042	>0.05

R – Spearman's rank correlation coefficient; *p* – level of significance for *R*

DISCUSSION

Disruptive behaviour disorders in children, including aggression, hyperactivity and risky behaviours, are regarded as the main factor predisposing to juvenile delinquency and violence in adult life (Eaton et al. 2006). Therefore, a better understanding of the mechanisms underlying the development of such disorders may help to prevent the occurrence of further problems. There is no dirrect correlation between microelements and human behaviours, but one of the potential contributors to this phenomenon is the lack of well-balanced diet (Liu, Raine 2006), which results in malnourishment and often entails an insufficient supply of micro- and macroelements.

Both active and passive smoking may have effects on mineral metabolism. The study conducted by Serdar et al. on a group of 95 children aged 1-6 proved that the concentration of toxic trace elements, such as: Cd, Pb, Cr, Sb, Fe and Al in the hair of children whose parents smoked cigarettes was significantly higher than in the hair of children whose parents were non-smokers. The number of smokers and the frequency of smoking at children's homes positively correlated with Pb, Cd, Cu, and Ni levels (Serdar et al.

2012). In the study of Unkiewicz-Winiarczyk et al., smokers had lower levels of bioelements (Ca, Mg, Fe, Zn, Cu) in their hair than non-smokers did (Unkiewicz-Winiarczyk et al. 2009). The study performed on a group of 38 girls aged 15-17, coming from rural areas of South Korea, demonstrated similar levels of Cu, Fe, and Mg in smokers and non-smokers. Smokers, however, had higher zinc levels (Kim et al. 2003). In the study presented in this article, the levels of bioelements (Ca, Mg, Zn, Cu, Fe) in blood serum of both groups did not significantly differ (Table 1). The analysis did not reveal statistical correlations between the levels of Ca, Mg, Zn, Cu, and Fe and smoking cigarettes by secondary school students ($p > 0.05$) – Table 2.

Alcohol consumption, and especially alcohol abuse, disturbs the levels of macro- and microelements. The study of 162 adolescents aged between 12 and 16 from South Africa demonstrated that alcohol abuse entails a higher than in the control group risk of vitamin D deficiency and insufficient calcium intake (Naude et al. 2012).

Zn deficiency is a serious nutritional problem. It may have a negative influence on intelectual and sexual development (Salguerio et al. 2002), can lead to behaviour and attention disorders in children, and contribute to reproductive problems, such as hypogonadism, impotence and other diseases of the reproductive system (Mesías et al. 2012). Consequences of the shortage of this element may also include various neurobehavioural disorders (Brown et al. 2001).

In the study of Sobral-Oliveira et al., alcohol abuse had no effects on the serum Zn levels (Sobral-Oliveira et al. 2011). An analysis of the Zn levels in blood of adults in Italy proved that men had higher Zn levels than women. It was also observed that alcohol consumption increased the content of Zn in blood. Age, smoking cigarettes, and domicile did not significantly contribute to the Zn levels of respondents (Bocca et al. 2011).

Alcohol abusers are often malnourished, which may be associated with insufficient distribution of iron and its deficiency. Alcohol consumption causes an increased accumulation of iron in the organism. Even a moderate alcohol intake leads to an increase in the concentartion of iron in an organism, saturation of serum with transferrin and ferritin, as well as a higher content of iron in the liver. Excessive accumulation of this element in the liver may be one of the causes of alcohol-related liver disease (Cylwik et al. 2008). Other studies show that alcohol-abuse does not significantly affect serum iron levels (Sobral-Oliveira et al. 2011).

Magnesium (Mg) is a bioelement whose deficiency is a well-documented phenomenon among alcohol-abusers. It is believed that a diet supplemented with this bioelement alleviates withdrawal symptoms (Hornyak et al. 2004).

According to the study conducted by Bocca et al., in a study carried out in Sardinia alcohol consumption seems to have no effects on the Cu levels in blood (Bocca et al. 2011), which is also confirmed by other researchers (Diaz Romero et al. 2002). Contrary conclusions were drawn by Chinese researchers, who examined 890 residents of the province of Shandong and found that alcohol consumers had lower Cu levels (Zhang et al. 2009).

The authors of this study did not observe significant differences in the levels of bioelements in alcohol consumers and non-consumers. There was just one statistically significant relationship, namely between Cu levels and the frequency of wine consumption by secondary school students ($p \leq 0.05$) – Table 2. This can be associated with the presence of bioactive compounds in wine, such as polyphenols and anthocyanins, and mineral elements: Cu, Zn and Fe.

Young people, especially during the adolescence period, exhibit many behaviours, which are detrimental to health and are often continued in adulthood. An example is experimenting with drugs.

Research on a group of 253 drug-addicted men aged 18-45 demonstrated that they had higher Cu and Zn levels, and lower Fe levels in blood (Hossain et al. 2007). Furthermore, some studies concerning Ca levels in the blood of heroin-addicts showed that their Ca levels were lower than in a control group (Kouros et al. 2010). Studies concerning Mg levels in drug-addicts yielded different results. Sadlik et al., who analyzed a group of opiate addicts during and after detoxification treatment, found that their serum Mg levels were the same as in the control group (Sadlik et al. 2000). Karakiewicz et al. reported contrary data. The mean Mg level in the serum of opiate addicts was considerably lower than in the control group (Karakiewicz et al. 2007).

It was proven in our study that the mean serum levels of bioelements in blood of both drug users and non-users were within normal ranges. Our analysis of the answers given by secondary school students concerning the frequency of using drugs and other intoxicants in the previous 12 months revealed a significant relationship between Zn levels and the frequency of smoking marijuana ($p \leq 0.05$) – Table 2.

Zn deficiency in food is a serious nutritional problem, both in the developed and developing countries (Hamadani et al. 2001). An experiment in which young mice were given food with lower zinc content for two weeks demonstrated that the level of corticosterone in serum significantly increased. It was observed that mice in this group considerably more often showed aggressive behaviours and the latter lasted longer than in a group receiving a diet which met their daily requirements for zinc. Next, the researchers analyzed the concentration of neurotransmitters in the cerebral tissue. They found that homeostatic changes in neurotransmitter release, which were probably caused by an increase in the serum corticosterone level, stimulating aggressive behaviour in mice with Zn deficiency (Takeda et al. 2008).

A high Zn level is observed in the hippocampus. It is believed that this element may play an important part in the modulation of *neurotransmitters* and the functioning of synapses (Cohen-Kfir et al. 2005). Although the role of Zn in an organism has not been fully understood yet, it seems that its deficiency in these areas may contribute to changes in neuronal activity, which in turn has an influence on behaviour. Furthermore, Zn deficiency in an organism leads to an increase in Cu levels in blood, which may cause hyperactivity and schizophrenia (Hagmeyer et al. 2015). Other researchers, however, proved that people with higher serum Zn and Mg levels had less severe depressive symptoms (Stanisławska et al. 2014).

Anaemia caused by iron deficiency is the most common nutritional problem in the world. An analysis of the behaviour pattern and the level of intelligence of anaemic children with iron deficiency and thalassaemia and healthy children revealed that iron deficiency significantly contributes to the development of behavioural disorders and a decrease in IQ score (Mubarak et al. 2010).

The study carried out among ADHD children, in which they were administered oral Mg preparation (MagneB6) for 30 days, demonstrated some improvement in their behaviour, fewer aggressive behaviours and lower anxiety levels, as well as better attention and motor movement comparing to the control group consisting of children with similar disorders, who were given multivitamin supplements (Nogovitsina, Levitina 2006). The analysis of our revealed significant differences in Mg and Zn levels between secondary school students getting and not getting into fights. Subjects who were picking fights had significantly lower Mg and Zn levels ($p \leq 0.05$) – Table 2. Furthermore, the relationship between the levels of bioelements in the serum of secondary school students and the frequency of getting into fights were analyzed, leading to the conclusion that the latter statistically significantly correlated with Mg and Zn levels ($p \leq 0.05$) – Table 2.

Limitations

The authors assessed the levels of selected bioelements (Mg, Ca, Cu, Fe, Zn) in the blood serum of 376 secondary school students. It is necessary to perform further research on a bigger sample to confirm and generalize the results. Moreover, the study would be more valuable if several other key points were analysed. The authors only investigated microlelements in serum, but not in blood cells. Socio-economic factors were not taken into account at all, and they are very important contributors to children's risky behaviours. Furthermore, the authors did not discuss the role of iron deficiency, while recent studies suggest that shortage of this element may play an important role in the dopamine neurotransmission.

CONCLUSIONS

1. Influence of negative health behaviours on the levels of bioelements in blood serum of secondary school students is not easily identifiable.

2. There is a relationship between negative health behaviours and the levels of the selected bioelements (Ca, Zn, Mg, Cu, Fe) in blood serum. Stu-

dents showing aggressive behaviours (getting into fights) have lower Mg and Zn levels than other secondary school students.

3. There are weak but statistically significant correlations between Cu levels and the frequency of wine consumption, and between Zn levels and smoking marijuana among secondary school students.

REFERENCES

Bocca B., Madeddu R., Asara Y., Tolu P., Marchal J.A., Forte G. 2011. *Assessment of reference ranges for blood Cu, Mn, Se and Zn in a selected Italian population*. J. Trace Elem. Med. Biol., 25(1): 19-26. DOI: 10.1016/j.jtemb.2010.12.004

Bridges C.C., Zalups R.K. 2005. *Molecular and ionic mimicry and the transport of toxic metals*. Toxicol. Appl. Pharm., 204(3): 274-308.

Brown K.H., Wuehler S.E., Peerson J.M. 2001. *The importance of zinc in human nutrition and estimation of the global prevalence of zinc deficiency*. Food Nutr. Bull., 22(2): 113-125.

Brzezińska U., Kosicka T., Tykarski A. 2004. *Calcium vs. hypertension*. Nadciśn. Tętn., 8(2): 109-118. (in Polish)

Cohen-Kfir E., Lee W., Eskandari S., Nelson N. 2005. *Zinc inhibition of gamma-aminobutyric acid transporter 4 (GAT4) reveals a link between excitatiory and inhibitory neurotransmission*. P. Natl. Acad. Sci. USA., 102(17): 6154-6159.

Cylwik B., Chrostek L., Szmitkowski M. 2008. *The effect of alcohol on iron metabolism*. Pol. Merk. Lek., 24(144): 561-564. (in Polish)

Diaz Romero C., Henriquez Sánchez P., López Blanco F., Rodriguez E., Serra Majem L. 2002. *Serum copper and zinc concentrations in a representative sample of the Canarian population*. J. Trace Elem. Biol., 16(2): 75-81.

Eaton D.K., Kann L., Kinchen S., Ross J., Hawkins J., Harris W.A., Lowy R., Mcmanus T., Chyen D., Shanklin S., Lim C., Grunbaum J.A., Wechsler H. 2006. *Youth risk behavior surveillance – the United States, 2005*. J. School Health, 76(7): 353-372.

Ghanizadeh A. 2013. *A systematic review of magnesium therapy for treating attention deficit hyperactivity disorder*. Arch. Iran Med., 16(7): 412-417. DOI: 013167/AIM.0010

Hagmeyer S., Haderspeck J.C., Grabrucker A.M. 2015. *Behavioral impairments in animal models for zinc deficiency*. Front. Behav. Neurosci., 8: 443. DOI: 10.3389/fnbeh.2014.00443

Hallstőm H., Wolk A., Glynn A., Micha lsson K. 2006. *Coffee, tea and caffeine consumption in relation to osteoporotic fracture risk in a cohort of Swedish women*. Osteoporosis Int., 17(7): 1055-1064.

Hmadani J.D., Fuchs G.J., Osendarp S.P., Khatun F., Huda S.N., Grantham-Mcgregor S.M. 2001. *Randomized controlled trial of the effect of zink supplemantation on the mental development of Bangladeshi infants*. Am. J. Clin. Nutr., 74(3): 381-386.

Hornyak M., Haas P., Veit J., Gann H., Riemann D. 2004. *Magnesium treatment of primary alcohol-dependent patients during subacute withdrawal: an open pilot study with polysomnography*. Alcohol Clin. Exp. Res., 28(11Ë): 1702-1709.

Hossain K.J., Kamal M.M., Ahsan M., Islam S.K. 2007. *Serum antioxidant micromineral (Cu, Zn, Fe) status of drug dependent subjects: Influence of illicit drugs and lifestyle*. Subst. Abuse Treat. Pr., 2: 12-18.

Karakiewicz B., Kozielec T., Brodowski J., Chlubek D., Noceń I., Starczewski A., Brodowska A., Laszczyńska M. 2007. *Serum magnesium concentration in drug-addicted patients*. Magnes. Res., 20(1): 53-57.

Kim S.H., Kim J.S., Shin H.S., Keen C.L. 2003. *Influence of smoking on markers of oxidative stress and serum mineral concentrations in teenage girls in Korea*. Nutrition, 19(3): 240-243.

Kouros D., Tahereh H., Mohammadreza A., Minoo M.Z. 2010. *Opium and heroin alter biochemical parameters of human's serum*. Am. J. Drug Alcohol Ab., 36(3): 135-139. DOI: 10.3109/00952991003734277

Lieu P.T., Heiskala M., Peterson P.A., Yang Y. 2001. *The role of iron in health and disease*. Mol. Aspects Med., 22(1-2): 1-87.

Liu J., Raine A. 2006. *The effect of childhood malnutrition on externalizing behavior*. Curr. Opin. Pediatr., 18(5): 565-570.

Mazur A., Maier J.A., Rock E., Gueux E., Nowacki W., Rayssiguier Y. 2007. *Magnesium and the inflammatory response: potential physiopathological implications*. Arch. Biochem. Biophys., 458(1): 48-56.

Mesías M., Seiquer I., Navarro M.P. 2012. *Is the Mediterranean diet adequate to satisfy zinc requirements during adolescence?* Public Health Nutr., 15(8): 1429-1436. DOI: 10.1017/S1368980011003429

Mubarak A., Fadel W., Said S., Hammar M.A. 2010. *Profile of behavior and IQ in anemic children*. CNS Spectr., 15(12): 631-638. DOI: 10.1017/S1092852912000089

Naude C.E., Carey P.D., Laubscher R., Fein G., Senekal M. 2012. *Vitamin D and calcium status in South African adolescents with alcohol use disorders*. Nutrients, 4(8): 1076-1094. DOI: 10.3390/nu4081076

Nogovitsina O.R., Levitina E.V. 2006. *Effect of MAGNE-B6 on the clinical and biochemical manifestations of the syndrome of attention deficit and hyperactivity in children*. Eksp. Klin. Farmakol., 69(1): 74-77.

Sadlik J., Pach J., Winniki L., Piekoszewski W. 2000. *Concentration of zinc, copper and magnesium in the serum of drug addicts*. Prz. Lek., 57(10): 563-564. (in Polish)

Salguerio M.J., Zubillaga M., Lysionek A.E., Caro R.A., Weill R., Boccio J.R. 2002. *The role of zinc in the growth and development of children*. Nutrition, 18(6): 510-519.

Serdar M.A., Akin B.S., Razi C., Akon O., Tokgoz S., Kenar L., Aykut O. 2012. *The correlation between smoking status of family members and concentrations of toxic trace elements in the hair of children*. Biol. Trace Elem. Res., 148(1): 11-17. DOI: 10.1007/s12011-012-9337-5

Siparsky G., Accurso F.J. 2011. *Reference ranges in the biochemical and haematological laboratory analyses*. In: *Paediatrics. Diagnosis and treatment*. Hay W.W., Levin M.J., Sondheimer J.M., Deterding R.R. (ed.). Vol. II. Wyd. Czelej, Lublin, 1346-1356. (in Polish)

Siwek M., Wróbel A., Dudek D., Zięba A., Dudek D. 2005. *The role of zinc in the pathogenesis and therapy of affective disorders*. Psychiatr. Pol., 34(5): 899-909. (in Polish)

Sobral-Oliveira M.B., Faintuch J., Guarita D.R., Ooliveira C.P., Carrilho F.J. 2011. *Nutritional profile of asymptomatic alcoholic patients*. Arq. Gastroenterol., 48(2): 112-118.

Stanisławska M., Szkup-Jabłońska M., Jurczak A., Wieder-Huszla S., Samochowiec A., Jasiewicz A., Noceń I., Augustyniuk K., Brodowska A., Karakiewicz B., Chlubek D., Grochans E. 2014. *The severity of depressive symptoms vs. serum Mg and Zn levels in postmenopausal women*. Biol. Trace Elem. Res., 157(1): 30-35. DOI: 10.1007/s12011-013-9866-6

Strzykała K. 2005. *Reference biochemical and haematological values in children*. In: *Clinical norms in paediatrics*. Krawczyński M. (ed.). Wyd. Lek. PZWL, Warszawa, 470-494. (in Polish)

Takeda A., Tamano H., Kan F., Hanajima T., Yamada K., Oku N. 2008. *Enhancement of social isolation-induced aggressive behavior of young mice by zinc deficiency*. Life Sci., 82(17-18): 909-914. DOI: 10.1016/j.lfs.2008.02.005

Unkiewicz-Winiarczyk A., Bagniuk A., Gromysz-Kałkowska K., Szubartowska E. 2009. *Calcium, magnesium, iron, zinc and copper concentration in the hair of tobacco smokers*. Biol. Trace Elem. Res., 128(2): 152-160. DOI: 10.1007/s12011-008-8266-9

Valko M., Morris H., Cronin M.T. 2005. *Metals, toxicity and oxidative stress*. Curr. Med. Chem., 12(10): 1161-1208.

WOJTYNIAK B., GORYŃSKI P., MOSKALEWICZ B. (ed.) 2012. *Report „Health situation of the Polish population and its determinants”*. The National Public Health Institute – The National Institute of Hygiene, Warsaw. http://www.systemochronyzdrowia.pl/index.php/aktualnosci-glowna/raporty/119-raport-sytuacja-zdrowotna-ludnosci-polskiej-i-jej-uwarunkowania. (in Polish)

WOYNAROWSKA-SOŁDAN M., WĘZIAK-BIAŁOWOLSKA D. 2012. *Psychometric analysis of the Positive Health Behaviours Scale for adults*. Probl. Hig. Epidemiol., 93(2): 185-190. (in Polish)

ZHANG H.Q., LI N., ZHANG Z., GAO S., YIN H.Y., GUO D.M., GAO X. 2009. *Serum zinc, copper, and zinc/copper in healthy residents of Jinan*. Biol. Trace Elem. Res., 131(1): 25-32. DOI: 10.1007/s12011-009-8350-9