Concentrations of retinol, α -tocopherol, copper, zinc and iron in plasma of young subjects differing in their engagement in motor activities

Marzena Malara, Elżbieta Hübner-Woźniak, Anna Kurczyńska

Department of Biochemistry, Jozef Pilsudski University of Physical Education, Warsaw, Poland

Summary

Study aim: To assess possible effects of gender and of the level of motor activity on the deficiencies of selected vitamins and minerals in young subjects.

Material and methods: Four groups of physical education (PE) students ($n = 15$ each) were studied: sedentary men (SM) and women (SF), and physically active men (AM) and women (AF) engaged in endurance sports, all aged 18 – 24 years, were studied. Somatic measurements included body height and mass, and body fat content (by Durnin's method, from 4 skinfolds), the biochemical ones included concentrations of retinol, α -tocopherol, copper, zinc and iron in plasma.

Results: Men had significantly higher retinol ($p < 0.05$) and iron ($p < 0.01$) levels than women. The AM and SF groups had significantly ($p < 0.05 - 0.01$) higher plasma levels of copper and zinc than Group SM. Group AF had lower zinc levels than Groups AM and SF, that latter group having lower α -tocopherol compared with Group SM.

Conclusions: Inasmuch the observed differences could be due to the respective intakes, the effect of motor activity and the associated increased elimination of minerals could not be ruled out.

Key words: Antioxidant vitamins – Copper – Zinc – Iron – Motor activity

Introduction

An appropriate supply of vitamins, as well as of macro- and microelements, is known to be indispensable for normal growth and development [28] since ions like copper, zinc, magnesium, iron, etc., are components of many enzymes [2,8]. Those ions, together with vitamins A and E, take part in antioxidant processes directly or indirectly. Specifically, copper (Cu) is a component of e.g. superoxide dismutase (metabolism of free radicals), tyrosinase (melanin synthesis), or cytochrome oxidase (last link in the respiratory chain). Moreover, copper takes part in iron metabolism and haem synthesis, and is indispensable for normal neural functions [26]. Zinc (Zn) is a component of over 300 enzymes, e.g. of DNA and RNA polymerases, superoxide dismutase, alcohol dehydrogenase, carbonic anhydrase, etc. Zinc is also indispensable in the biosynthesis of hormones, e.g. insulin and thyroxine, as well as in the normal functioning of the immune system [22]. Iron is a component of haemoglobin, myoglobin and of many enzymes participating in the electron transport, oxygen storage and transport, in tyrosine iodination, degradation of hydrogen peroxide (enzyme: catalase), prostaglandin biosynthesis and in enhancing the immune system [7].

Retinol, a lipophilic compound of vitamin A-activity, participates in vision processes, cell division and differentiation, normal functioning of the immune system, as well as in growth and reproduction [21]; moreover, retinol is also an antioxidant compound [27]. As to α -tocopherol, the most potent vitamin E form and an antioxidant, it protects blood vessels, red blood cells and cell membranes from damage [18,19]; it is also known to reduce the risk of neoplastic diseases [15,27].

Population studies on the intake of the discussed substances and their concentrations in plasma revealed close associations between incidences of many a disease and a deficient supply of vitamins and minerals, the requirements of which being dependent on age, gender, body mass, dietary habits, life style and physiological status [4,29].

The deficiencies in vitamins A and E and in minerals like copper, zinc and iron, resulting from poorly balanced and diversified diet, remain fairly widespread despite growing knowledge and awareness of the principles of rational nutrition. The high morbidity rate and mortality due to cardiovascular and neoplastic diseases calls for attention regarding an adequate content of antioxidant components of diets [9]. A decreased absorption of vitamins and minerals in the alimentary tract, increased losses with sweat and urine and increased activities of

mitochondrial enzymes that require vitamins and minerals, observed in physically active subjects, calls for a higher supply of those substances compared with sedentary people. A poorly diversified diet used by physically active subjects additionally increases the risk of vitamin and mineral deficiencies in them [5,24]. The aim of this study was thus to assess possible effects of gender and of the level of motor activity on the deficiencies of selected vitamins and minerals in young subjects.

Material and Methods

A total of 60 physical education students aged 18 – 24 years volunteered to participate in the study. They were randomly selected from a greater cohort so as to form 4 groups, $n = 15$ each, representing males and females, and sedentary and physically active ones. Moreover, those who used mineral/vitamin supplementation were eliminated. Sedentary subjects participated in curricular activities only, the active ones were engaged in endurance sports. All subjects supplied their written consents to participate and the study was approved by the local Committee of Ethics. The study was conducted in the spring semester.

Body height was determined with the use of a standard stadiometer with an accuracy of 0.1 cm, body mass was recorded using electronic medical scales with an accuracy of 0.1 kg. Four skinfolds (biceps, triceps, subscapular, hip) were measured using a skinfold caliper (Holtain, U.K.); mean values of 3 replications were used to compute the body fat content using Durnin's method [3].

The volume of physical activity was expressed in hours per week and consisted of curricular motor activities (sedentary groups) and of the declared training volume (active groups).

Blood was sampled in the morning, in the preprandial state, into non-heparinised (0.5 ml) and heparinised polypropylene test tubes and centrifuged for 15 min at 3000 rpm. The collected plasma and serum were stored frozen at -70°C until assayed.

Concentrations of retinol and α -tocopherol were determined by HPLC device (Varian ProStar, USA) equipped with UV-VIS detector (190 – 900 nm). Reference compounds (ChromaDex, USA), plasma and blank samples were applied to a C_{18} DB column (150×4.6 mm, particle size 5 μm ; Restek, USA), eluted with acetonitrile/methanol 70:30 v/v at 1.4 ml/min. Fluorescence density was measured at 280 nm. Retention times for retinol and α -tocopherol amounted to 2.3 and 7.8 min, respectively. Concentrations of zinc and copper (plasma) and of iron (serum) were determined colorimetrically using commercial kits (Randox Labs., U.K.; ZN 2341, CU 2340 and SI 257, respectively).

Using the Statistica 6.0 software, the Shapiro-Wilk's test was used to assess the normality of distributions. The data were subjected to two-way ANOVA with *post-hoc* Student's *t*-test and the chi-square function was applied to frequencies, the level of $p \leq 0.05$ being considered significant.

Results

The results are presented in Tables 1 and 2. No significant differences between active and sedentary subjects were noted in the somatic variables (Table 1). Active groups obviously differed in the declared amount of physical activity from the respective sedentary groups ($p < 0.001$).

As shown in Table 2, no significant between-group differences were noted for retinol but significantly ($p < 0.05$) higher values were found for men than for women. The sedentary female group (SF) had significantly ($p < 0.05$) lowest α -tocopherol concentration and Group SM had significantly ($p < 0.01$) lowest copper concentration. Active men had higher ($p < 0.05$) zinc concentration than active women but in sedentary groups the situation was reversed. Men had significantly ($p < 0.01$) higher iron levels than women. On the other hand, no significant, systematic differences between active and sedentary subjects were noted.

Table 1. Mean values ($\pm$ SD) of somatic variables and of the declared physical activity of students

Variable	Group	AM (n=15)	AF (n=15)	SM (n=15)	SF (n=15)
Age (years)		20.4 $\pm$ 1.3	19.6 $\pm$ 0.7	19.9 $\pm$ 1.0	19.6 $\pm$ 0.7
Body height (cm)		183.2 $\pm$ 5.9	168.6 $\pm$ 6.5**	181.3 $\pm$ 7.2	169.5 $\pm$ 4.7***
Body mass (kg)		78.1 $\pm$ 7.1	60.6 $\pm$ 9.5**	76.0 $\pm$ 7.7	57.9 $\pm$ 5.0***
BMI		23.2 $\pm$ 1.8	21.2 $\pm$ 2.5**	23.0 $\pm$ 1.4	20.1 $\pm$ 1.1***
Body fat content (%)		12.3 $\pm$ 2.8	22.6 $\pm$ 3.8***	12.5 $\pm$ 3.2	23.5 $\pm$ 4.2***
Training experience (years)		10.70 $\pm$ 3.20	7.90 $\pm$ 2.50**	–	–
Physical activity (h/week)		14.0	11.0***	6.0 °	6.0 °

Legend: A – Active subjects; S – Sedentary subjects; M – Male; F – Female; Significantly different from the respective male group: ** $p < 0.01$; *** $p < 0.001$; ° Significantly different from the respective active group

Table 2. Mean values ($\pm$ SD) of retinol, α -tocopherol and metal ion concentrations (μ mol/l) in plasma of students

Variable	Group AM (n=15)	AF (n=15)	SM (n=15)	SF (n=15)	M/F
Retinol	2.66 $\pm$ 0.18	2.49 $\pm$ 0.60	2.91 $\pm$ 0.67	2.49 $\pm$ 0.63	p<0.05
α -Tocopherol	17.46 $\pm$ 3.00	17.79 $\pm$ 4.13	18.65 $\pm$ 3.79	15.69 $\pm$ 2.79*	
Cu	17.20 $\pm$ 3.05	17.27 $\pm$ 7.36	13.64 $\pm$ 2.25 ^{oo}	16.32 $\pm$ 4.06**	
Zn	14.20 $\pm$ 2.10	12.77 $\pm$ 2.69*	11.25 $\pm$ 2.18 ^{oo}	14.74 $\pm$ 2.10 ^o	
Fe	19.79 $\pm$ 5.09	14.38 $\pm$ 4.36*	19.25 $\pm$ 4.90	16.93 $\pm$ 5.94 ^o	p<0.01

Legend: M/F – Gender-related difference; Significantly different from the respective male group: * p<0.05; ** p<0.01; Significantly different from the respective active group: ^o p<0.05; ^{oo} p<0.01; for other explanations see Table 1

Discussion

Retinol concentration in plasma was significantly (p<0.05) higher in men than in women which was in accordance with other authors [14]. However, retinol levels were above the lower limit (0.7 μ mol/l [6,16]) in all subjects in this study.

There are no generally accepted physiological limits of α -tocopherol in plasma. Olmedilla *et al.* [20] recommended 16.2 – 46 μ mol/l; also according to Hallfrisch *et al.* [6] the lower limit amounted to 16.25 μ mol/l. On the other hand, Morrissey *et al.* [18] recommended 11.6 μ mol/l, and Guillard *et al.* [5] 9.3 μ mol/l. When the lower limit of 16.25 μ mol/l was applied, 4 men and 6 women in every respective group had values below that limit but in all subjects the values were above 11.6 μ mol/l. Nevertheless, sedentary women (Group SF) had significantly lowest concentrations of α -tocopherol in plasma; its intake lower than in other groups could have been the likely cause. At any rate, no systematic, significant gender-related difference was detected which tallied with the report of Krasinski *et al.* [14] who found no such difference in subjects aged 19 – 59 years.

The effects of physical training on α -tocopherol in plasma are controversial. Trained kayakers had higher levels of α -tocopherol in plasma than sedentary subjects [25] but lower values were noted in well trained subjects (70.3% of $\dot{V}O_2$ max at the anaerobic threshold) than in the less well trained ones (49.7% $\dot{V}O_2$ max) despite a higher vitamin E intake by the former [10]. Similar observation for rowers was reported in an earlier study [11]. On the other hand, Guillard *et al.* [5] found no significant difference between trained male athletes and sedentary men but insufficient intake of vitamin E was reported in 53% of subjects. The concentrations of retinol and α -tocopherol in plasma reflect the balance of several factors like intake, transport and storage in the body [16] and might explain the lack of relationship between plasma levels of those compounds and their intake [11,14]. On the other hand,

such a relationship was reported, e.g. for men and women aged 20 – 95 years [6,19]; it was also noted that supplementation with vitamin E increased α -tocopherol concentration in plasma [14].

According to König *et al.* [13], copper concentration in plasma is the most popular method of assessing the intake of that element but other factors, like age, health status and physical activity ought to be considered. A more intense loss of macro- and microelements with sweat and urine observed in physically active subjects compared with their sedentary mates leads to respective differences in concentrations of those elements [17]. In this study, a reverse effect was noted. Namely, active subjects tended to have higher concentration of copper in plasma than the sedentary ones (p<0.07). Moreover, as many as 6 sedentary men had copper levels below the recommended limit (11.0 μ mol/l) and the same applied to one woman in every female group.

Zinc concentration in plasma depends on its content in the diet, intestinal absorption and excretion in urine [12]. Alshammari *et al.* [1] found no significant difference in zinc levels between trained female gymnasts and their sedentary mates; those authors found no correlation between zinc and copper intakes and their concentrations in plasma. That lack of correlation for copper was reported also for boys and girls aged 4 – 19 years [13]. No significant effect of gender was found in this study and the between-group differences were rather due to differences in zinc intake and in physical activity; as many as 9 sedentary men and only 2 active women had zinc concentration below the recommended limit (11.1 and 10.7 μ mol/l, respectively) and that difference was significant (p<0.01). Zinc concentrations in all subjects from the two other groups were within normal limits.

Mean iron concentration in serum was significantly lower in women than in men. This is consistent with other reports stating that iron losses in women were additionally enhanced by menstrual bleeding, pregnancy and lactation [23]. Yet, no subject from the entire cohort had

abnormally low iron concentration, i.e. below 10.6 or 6.6 $\mu\text{mol/l}$ for men and women, respectively.

Summing up, no definite conclusions could be drawn from the presented results as to the effects of gender or the degree of physical activity on the concentrations of selected vitamins and minerals in plasma. That prompted us to continue that study under conditions of a controlled intake of those substances. Apart from that, an increased exercise-induced loss of vitamins and minerals with sweat and urine is likely to play some role.

References

1. Alshammari E., S.Shafi, J.Nurmi-Lawton, A.Taylor, S.Lanham-New, G.Ferns (2010) Altered antioxidant and trace-elements status in adolescent female gymnasts. *Int.J.Sport Nutr.Exerc.Metabol.* 20:291-298.
2. Clarkson P.M., E.M.Haymes (1995) Exercise and mineral status of athletes: calcium, magnesium, phosphorus, and iron. *Med.Sci.Sports Exerc.* 27:831-843.
3. Durnin J.V.G.A., J.Womersley (1974) Body fat assessed from total density and its estimation from skinfold thickness: measurements on 481 men and women aged from 16 to 72 years. *Br.J.Nutr.* 32:77-97.
4. Gey K.F. (1995) Cardiovascular disease and vitamins. Concurrent correction of suboptimal plasma antioxidant levels may, as important part of "optimal" nutrition, help to prevent early stages of cardiovascular disease and cancer, respectively. *Bibl.Nutr.Dieta* 52:75-91.
5. Guillaud J.C., T.Penaranda, C.Gallet, V.Boggio, F.Fuchs, J.Klepping (1989) Vitamin status of young athletes including the effects of supplementation. *Med.Sci.Sports Exerc.* 21:441-9.
6. Hallfrisch J., D.C.Muller, V.N.Singh (1994) Vitamin A and E intakes and plasma concentrations of retinol, β -carotene and α -tokoferol in men and women of the Baltimore Longitudinal Study of Aging. *Am.J.Clin.Nutr.* 60: 176-182.
7. Harrison-Findik D.D. (2010) Gender-related variations in iron metabolism and liver diseases. *World J.Hepatol.* 8:302-310.
8. Haymes E.M. (1991) Vitamin and mineral supplementation to athletes. *Int.J.Sport Nutr.* 1:146-169.
9. Herberg S. (1998) The potential role of antioxidant vitamins in preventing cardiovascular disease and cancers. *Nutrition* 14:51351-51358.
10. Hübner-Woźniak E., G.Lutoslawska, B.Panczenko-Kresowska, J.Malczewska (1999) Effect of physical training. graded exercise until exhaustion and diet on lipid peroxidation and plasma α -tokoferol level in men. *Żyw.Człowieka Metabol.* 26:91-98.
11. Hübner-Woźniak E., G.Lutoslawska, B.Panczenko-Kresowska, J.Malczewska, D.Sitkowski (2001) Plasma antioxidant defense status in subjects with different fractional oxygen utilization at the lactate threshold. *Biol.Sport* 18:297-309.
12. King J.C., D.M.Shames., N.M.Lowe, L.R.Woodhouse, B.Sutherland, S.A.Abrams (2001) Effect of acute zinc depletion on zinc homeostasis and plasma zinc kinetics in men. *Am.J.Clin.Nutr.* 74:116-124.
13. König J, I.Elmadfa (2000) Plasma copper concentration as marker of copper intake from food. *Ann Nutr. Metabol.* 44:129-134.
14. Krasinski S.D., R.M.Russell, C.L.Otradivec, J.A.Sadowski, S.C.Hartz, R.A.Jacob, R.B.McGandy (1989) Relationship of vitamin A and vitamin E intake to fasting plasma retinol, retinol-binding protein, retinyl esters, carotene, α -tokoferol and cholesterol among elderly people and young adults: increased plasma retinyl esters among vitamin A-supplemented users. *Am.J.Clin.Nutr.* 49:112-120.
15. Lambert A.J., M.D.Brand (2009) Reactive oxygen species production by mitochondria. *Methods Mol.Biol.* 554:165-181.
16. Machefer G., C.Groussard, H.Zouhal, S.Vincent, H.Youssef, H.Faure, L.Malarde, A.Gratas-Delamarche (2007) Nutritional and plasmatic antioxidant vitamins status of ultra endurance athletes. *J.Am.Coll.Nutr.* 26: 311-316.
17. Maughan R.J. (1999) Role of micronutrients in sport and physical activity. *Br.Med.Bull.* 55:683-690.
18. Morrissey P.A., P.J.A.Sheehy (1999) Optimal nutrition: vitamin E. *Proc.Nutr.Soc.* 58: 495-468.
19. Neuhouwer M.L., C.L.Rock, A.L.Eldridge, A.R.Kristal, R.E.Patterson, D.A.Cooper, D.Neumark-Sztainer, L.J.Cheskin, M.D.Thornquist (2001) Serum concentrations of retinol, α -tokoferol and the carotenoids are influenced by diet, race and obesity in a sample of healthy adolescents. *J.Nutr.* 131:2184-2191.
20. Olmedilla B., F.Granado, S.Southon, A.J.A.Wright, I.Blanco, E.Gil-Martinez, H.van den Berg, B.Corridan, A.M.Roussel, M.Chopra, D.I.Thurnham (2001) Serum concentrations of carotenoids and vitamins A, E and C in control subjects from five European countries. *Br.J.Nutr.* 85:227-238.
21. Pino-Lagos K., Y.Guo, R.J.Noelle (2010) Retinoic acid: a key player in immunity. *Biofactors* 36:430-436.
22. Plum L.M., L.Rink, H.Haase (2010) The essential toxin: impact of zinc on human health. *Int.J.Environ.Res. Public Health* 4:1342-1365.
23. Shams S., H.Asheri, A.Kianmehr, V.Ziaee, L.Koochakzadeh, M.Monajemzadeh, M.Nouri, H.Irani, N.Gholami (2010) The prevalence of iron deficiency anaemia in female medical students in Tehran. *Singapore Med.J.* 51:116-119.
24. Sobal J., P.Sobal, L.F.Marquart (1994) Vitamin/mineral supplement use among athletes: a review of the literature. *Int.J.Sport Nutr.* 4:320-334.
25. Teixeira V., H.Valente, S.Casal, F.Marques, P.Moreira (2009) Antioxidant status, oxidative stress and damage in elite trained kayakers and canoeists and sedentary control. *Int J.Sport Nutr.Exerc.Metabol.* 19:443-456.
26. Turnlund J.R. (1998) Human whole-body copper metabolism. *Am.J.Clin.Nutr.* 67:960-964.
27. Urso M.L., P.M.Clarkson (2003) Oxidative stress, exercise, and antioxidant supplementation. *Toxicology* 189:41-54.

28. Volpe S.L. (2007) Micronutrient requirements for athletes. *Clin.Sports Med.* 26:119-130.

29. Winklhofer-Roob B.M., M.A.Van't Hof, D.H.Shmerling (1997) Reference values for plasma concentrations of vitamin E and A and carotenoids in Swiss population from infancy to adulthood, adjusted for seasonal influences. *Clin.Chem.* 43:146-153.

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