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ANALYSIS OF CHANGES IN SELECTED PARAMETERS OF CALCIUM AND MAGNESIUM METABOLISM IN RESPONSE TO DIET COMPOSITION AND B-GROUP VITAMIN SUPPLEMENTATION IN RAT

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Background. The aim of the study was to investigate the effects of a diet modification and supplementation with B-group vitamins, on selected characteristics of calcium and magnesium management in rats.
Material and methods. The experiment was carried out on 60 rats aged 5 months. Animals were fed two different diets. Groups I and II received clean tap water to drink, while group III had water solution of group-B vitamins.
Results. Analysis of blood plasma calcium and magnesium concentrations in the studied animals did not reveal a significant effect of the analysed factors on blood plasma calcium concentration in examined rats. An increase of the plasma level of magnesium was observed with a change in the diet composition. The supplementation reduced magnesium level to those observed in animals fed a basic feed. Diet modification and supplementation exerted the influence on whole blood calcium and magnesium levels. A change in the composition of the diet and its supplementation results also in an increase in bone calcium content in males, and in an increase in bone magnesium content in females.
Conclusions. Lack of changes in blood plasma calcium levels in the studied animals implies the preservation of the homeostatic mechanisms that regulate its concentration, whereas the observed significant changes in the concentration of magnesium, point to a significant effect of this factor on its metabolism. Changes in hematocrit indicator, whole blood concentrations of calcium and magnesium and the absence of changes in concentrations of these elements in blood plasma of supplemented animals may indicate that the elements move to erythrocytes, which may imply a distortion of cellular membrane and an increase in its permeability. Composition of the diet and its supplementation modified also bone calcium and magnesium concentrations in the studied rats.

Key words: B-vitamins supplementation, calcium, magnesium, plasma, whole blood, rat

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INTRODUCTION

Supplementation is commonly viewed as an easy way to compensate for a faulty diet and unhealthy style of life in general, as well as a means of health improvement. Dietary supplementation with water-soluble, easily excreted in urine B-group vitamins is still considered safe.

New reports are being published, however, which state that supplementation is not a proper way of health preservation. The primary objections were formed against administration of fat-soluble vitamins, especially A and E, as human dietary supplements. Many studies have shown that high intake of antioxidant vitamins from food as well as their high concentration in serum is associated with a lower risk of coronary heart disease, and also prevents neoplasm development [Gey et al. 1991, 1993, Verlangieri et al. 1985]. These observations gave way to clinical trials on the application of synthetic vitamin E in the secondary prevention of atherosclerosis. However, administration of vitamin E in the form of pharmaceutical preparations have not proved beneficial in the prevention of CHD, contrary, it have been observed the intensification of the free radical reactions [Virtamo et al. 1998]. As a result of these studies, the views on its application safety have been revised. Unfavorable effects were also observed as a result of higher doses of β-carotene administered to smokers; an increased risk of lung cancer was observed [Omenn et al. 1996].

Dietary supplementation with water-soluble, easily excreted in urine, B-group vitamins is still considered safe.

Our previous studies, carried out on an animal model, demonstrated that diet supplementation containing some B-group vitamins contributed to deposition of visceral and intra-organ adipose tissue, altered the composition of fatty acids of deposited fat, and intensified the processes of their peroxidation [Friedrich and Sadowska 2005]. These observations gave way to a hypotheses that the supplementation may have an influence on the synthesis of steroid hormones and biologically active compounds secreted by adipocytes, which affect bone metabolism and the distribution of calcium and magnesium in tissues.

Hence, in this follow-up study we use an animal model to investigate into the effects of the diet and its B-group vitamin supplementation on selected characteristics of calcium and magnesium management.

MATERIAL AND METHODS

Rats were obtained from the animal husbandry of Chair and Department of Toxicology, Poznań University of Medical Sciences, Poland. The experiment, approved by the Local Ethical Committee in Szczecin (permit no. 35/2007), was carried out in the vivarium of the Department of Human Nutritional Physiology, on 60 SPRD-strain male and female rats aged 5-7 months, of initial body weight: male 362 ±21.0 g, female 238 ±15.2 g.

Following a week long conditioning in the vivarium environment (temperature 21-22°C, 12 h/12 h light/dark cycle), the animals were randomised and sorted into three equinumerous groups of equal body weight, fed *ad libitum* on pelleted feeds composed of the same components, besides those differentiating, produced by the Feeds and Con-

centrates Plant in Kcynia, Poland, after having implemented the procedure 5.14.5. "Cleaning of machines and devices". Group I was fed standard feed (Labofeed H), while groups II and III received modified feed, in which 83.5% of wheat was substituted with wheat flour (type "500"), and 50% of corn grain was substituted with saccharose. The percentage of the remaining components was unchanged (Table 1). All diets were based on the balanced modification of the AIN-93 diet formulation [Reeves et al. 1993].

Table 1. Component composition of feeds used in the experiment

Component	Basic feed, %	Modified feed, %
Wheat	36.4	6
Corn grain	20	10
Wheat bran	20	20
Dry whey	3	3
Fodder salt[a]	0.3	0.3
Soya-bean grain	17	17
Fodder chalk[b]	1.5	1.5
Phosphate 2-CA[c]	0.8	0.8
Premix LRM[d]	1	1
Wheat flour	–	30.4
Saccharose	–	10

[a]Mainly NaCl.
[b]Mainly $CaCO_3$.
[c]$CaHPO_4$.
[d]Vitamin-mineral composition used in animals feeds.

In order to establish the chemical composition of the feeds, basic chemical assays were carried out. We measured the concentration of total nitrogen, converted to quantity of protein, raw fat, dry matter, and ash. The content of carbohydrates was derived from the difference between dry matter and the remaining solid components. The metabolic energy was calculated using commonly applied energy equivalents (Table 2).

Groups I and II received tap water to drink, while group III had water solution of group-B vitamins (B_1, B_2, B_6, and PP) obtained from available pharmaceutical products (Polfa, Kutno, Poland). The supplemented vitamins were administered in 3- to 5-fold higher quantity compared to the difference between their content in the standard and the modified feeds (3.249 mg thiamine, 1.083 mg riboflavin, 2.694 mg pyridoxine, and 9.58 mg nicotinic acid amide per 1 kg of feed), calculated in relation to amount of feed consumed by the animals. The rats received drinking water surplus ad libitum.

The experiment lasted for seven weeks; the amount of feed consumed and fluids drunk by the animals were recorded daily, whereas once a week the animals were weighed.

On the completion of the experiment, the animals were fasted overnight, anesthetised (with Ketanest, Pfizer Ireland Pharmaceuticals), and blood was drawn from the heart to measure the hematocrit, which was done by centrifuging blood in capillary

Table 2. Chemical composition of feeds used in the experiment

Component	Basic feed	Modified feed
Total protein, %	18.1	17.5
Crude fat, %	2.10	2.19
Carbohydrates, %	65.8	66.2
Dry matter, %	92.1	91.6
Total ash, %	6.08	5.69
Brutto energy		
$kcal \cdot g^{-1}$	3.95	3.94
$kJ \cdot g^{-1}$	16.5	16.5
Metabolic energy		
$kcal \cdot g^{-1}$	3.54	3.54
$kJ \cdot g^{-1}$	14.8	14.8

tubes in the hematocrit centrifuge (MPW 52, MPW Med. Instruments, Warsaw, Poland). Blood hemolysates were obtained through addition of 9 ml of deionized water to 1 ml of blood. The remaining blood was centrifuged, at 2000 g for 10 min at 5°C (MPW 350-R, MPW Med. Instruments, Warsaw, Poland).

Calcium and magnesium concentrations were measured in the resulting plasma and whole blood hemolysate by means of atomic absorption spectrometry. Blood plasma was also assayed for the concentrations of ionised calcium (using a ion selective electrode).

The right femoral bones were collected from the animals as well. The bones were dry-mineralised, and calcium and magnesium concentration was determined using atomic absorption spectrometry.

Atomic absorption spectrometry was done using Merck (Darmstadt, Germany) reagents on AAnalyst 400 (Perkin Elmer, Massachusetts, USA).

The resulting data were tested for normality of distribution and processed statistically by means of the Statistica software package, using the Duncan test at the significance level $\alpha = 0.05$ [Statsoft 2009].

RESULTS AND DISCUSSION

Analysis of blood plasma calcium and magnesium concentrations in the studied animals did not reveal a significant effect of the analyzed substances on blood plasma total calcium concentration in either males or females (Table 3). The concentrations remained within the standard range for rats, i.e. 1.33-3.25 mmol/l [Carpenter et al. 2001]. The level of ionized calcium, which is the only biologically active form of this element, did not change either.

Homeostatic regulatory mechanisms in the case of magnesium concentration were not preserved, however. An increase in the plasma level of this element was observed with a change in the diet composition. The supplementation, on the other hand, reduced magnesium levels to those observed in animals fed a basic feed (Table 4).

Table 3. Effect of diet composition and supplementation on chosen indicators of calcium management in rats ($\bar{x}$ ±SD; n = 60)

Examined trait	Sex	Basic feed	Modified feed	Modified feed + supplementation	Influence		
					diet	sex	interaction
Plasma calcium concentration, mmol·l^{-1}	male	2.17 ±0.12 a	2.19 ±0.14 a	2.22 ±0.13 a	–	*	–
	female	2.03 ±0.19 a	2.00 ±0.19 a	2.00 ±0.18 a			
Ionised plasma calcium concentration mmol·l^{-1}	male	0.84 ±0.05 a	0.86 ±0.03 a	0.85±0.05 a	–	–	–
	female	0.89 ±0.04 a	0.86 ±0.03 a	0.86 ±0.1 a			
Whole blood calcium concentration mmol·l^{-1}	male	4.19 ±1.09 a	3.99 ±0.74 a	4.68 ±1.0 b	*	**	*
	female	5.77 ±0.97 a	5.06 ±0.67 a	5.81 ±0.88 a			
Bone calcium content mg·g d.m.$^{-1}$	male	233 ±12.9 a	235 ±14.4 a	258 ±10.5 b	–	–	–
	female	237 ±11.9 a	234 ±12.1 a	229 ±9.2 a			

a, b – means in line denoted with the same letters are not significantly different, $p < 0.05$.
*Statistical difference at $p \leq 0.05$, **$p \leq 0.01$.

Table 4. Effect of diet composition and supplementation on chosen indicators of magnesium management in rats ($\bar{x}$ ±SD; n = 60)

Examined trait	Sex	Basic feed	Modified feed	Modified feed + supplementation	Influence		
					diet	sex	interaction
Plasma magnesium concentration mmol·l^{-1}	male	0.786 ±0.071 a	0.858 ±0.069 b	0.797 ±0.058 a	–	*	–
	female	0.651 ±0.12 a	0.748 ±0.17 b	0.688 ±0.14 a			
Whole blood magnesium concentration mmol·l^{-1}	male	3.95 ±0.41 a	4.05 ±0.54 a	4.23 ±0.53 a	*	**	*
	female	2.31 ±0.37 a	2.92 ±0.51 b	2.81 ±0.40 b			
Bone magnesium content, mg·g d.m.$^{-1}$	male	87 ±4.3 a	86 ±3.1 a	88 ±2.4 a	–	–	–
	female	73 ±2.4 a	78 ±2.0 b	82 ±2.3 c			

a, b, c – means in line denoted with the same letters are not significantly different, $p < 0.05$.
*Statistical difference at $p \leq 0.05$; **$p \leq 0.01$.

Magnesium activates more than 300 enzymes and is involved in a number of metabolic pathways of protein, lipid, and carbohydrate metabolism. Magnesium activates i.a. aspartate aminotransferase. Friedrich et al. [2009], as well as Sadowska and Serwotka [2001], observed an increase in blood aspartate aminotransferase as an effect of the applied diet. Magnesium also takes part in ATP synthesis. Its higher concentrations in blood plasma of the animals fed with modified feed could have been related to enhanced metabolism forced by sucrose present in the diet. It should be stressed, however, that

magnesium level in blood serum does not necessarily remain in a strict association with its concentrations in other tissues, therefore serum level of magnesium is not an ideal indicator of possible disturbances in its metabolism [Malon et al. 2004].

Analysis of the effect of diet and applied supplementation on whole blood concentration of calcium revealed that males fed a diet supplemented with vitamins had its higher blood concentrations, as compared to other groups. In females, the parameter did not change with feeding; however, considering a lack of changes in calcium plasma concentration and a lower hematocrit index observed in females fed a modified unsupplemented and supplemented diet (Table 5), which implied a lower number of erythrocytes or their lower individual volume in blood, one can presume that at animals fed the modified, supplemented feeds, intra-erythrocyte calcium concentration was also higher.

Table 5. Effect of diet composition and supplementation on hematocrite indicator in blood of rats ($\bar{x}$ ±SD; n = 60)

Sex	Basic feed	Modified feed	Modified feed + supplementation
Male	42.3 ±1.31 a	41.1 ±1.28 a	41.8 ±1.52 a
Female	42.3 ±1.07 b	40.5 ±1.85 a	40.7 ±1.01 a

a, b – means in line denoted with the same letters are not significantly different, $p < 0.05$.

The whole blood magnesium concentration in the supplemented males was also higher; however, the differences were statistically non-significant. In females, on the other hand, a change in the diet resulted in a statistically significant increase in the whole blood magnesium concentration, whereas supplementation did not change anything in this respect. This increase in females fed on the modified, unsupplemented diet may have resulted from an increase in blood plasma magnesium; in supplemented females, on the other hand, it was most probably associated with an increase in intracellular magnesium, since no increase was observed in its blood plasma level.

The observed growth in the whole blood calcium concentrations suggests an increase of its intra-erythrocyte concentration. This may imply a distortion of cellular membrane and an increase in its permeability, also as a result of free radicals activity. Friedrich and Dolot [2009] observed enhanced free-radical reactions occurring under B-group vitamin supplementation of the diet. Modification of diet composition and its supplementation with selected B-group vitamins resulted in a disturbance in the antioxidation balance, not only due to a lower content of natural antioxidants in the modified diet, but also due to enhanced lipid metabolism observed in supplemented animals. The latter effect was manifested by, among other symptoms, an increase in the deposition of abdominal fat, which is an independent factor of an enhanced intensity of free-radical processes [Olusi 2002].

The components of biological membranes, especially the side chains of polyunsaturated fatty acids that underly the fluidity of lipid membranes, are particularly vulnerable to free radicals. Free-radical reactions result in lipid peroxidation products which affect the physical properties of cellular membranes, disturbing their integrity and increasing their permeability for polar substances. Moreover, free radicals reduce hydrophobicity of the lipid inner surface of the cellular membranes thus altering the arrangement of the bilayer. This increases the permeability of the membranes in an unspecific way. Lipid

peroxidation also results in inhibition of membrane enzymes and transport proteins activity [Hofmanova et al. 2005, Izzoti et al. 2005]. Oxidative changes in proteins are an inherent effect of free-radical activity. Oxidation of membrane sulfhydryl groups causes the loss of biological activity of proteins. This leads to disruption of the activity of many transporters and enzymes.

Free radicals increase passive permeability of plasma membranes and inactivate the calcium pump, which is responsible for active calcium ion transport out of the cell. In consequence, intracellular concentration of calcium increases [Dargelos et al. 2010].

An elevated intracellular calcium level is not a positive effect, because it has been proven that the element may lead to enhanced lipogenesis and may contribute to insulin-resistance and arterial hypertension [Zemel 1995, Zemel et al. 2000].

Bones represent a calcium reservoir from which it can be relatively easily released. A statistical analysis of the data revealed a significant effect of the applied supplementation on the concentration of calcium in the bones of the studied animals (Table 3). The bones of males that received B-group vitamin supplementation contained more calcium. In the bones of the supplemented females, calcium levels were slightly lower as compared to those measured in the other groups of animals. This seems a striking observation in the context of our previous studies on the effects of B-group vitamin supplemented diet on the quantity of adipose tissue, where we found that supplemented females deposited more fat as compared to other groups [Friedrich and Sadowska 2005]. At present, it has been recognised that the adipose tissue plays a protective role in relation to the osseous tissue. A positive correlation between the body mass index (BMI) and bone mineral content (BMC) was observed by Tremollieres et al. [1993]. A higher BMC and a lower risk of osteoporosis accompanying elevated content of adipose tissue is explained by an increased release of estrogens and a lower concentration of globulin which binds serum sexual hormones, hence a higher concentration of free sexual hormones that protect bone tissue [Monninkhof et al. 2009, Pasquali et al. 1997].

On the other hand, there are reports that demonstrate a lack of this protective effect of adipose tissue in relation to osteoporosis [Czerwińska et al. 2004, Saab et al. 2010], thus the protective properties of fat in relation to bone is not unequivocal. It may be related to the distribution of the adipose tissue. Considering the fact that fat tissue is an endocrine organ and, deposited in various parts of the body, secrets different factors, its effect on bone metabolism probably depends on the location. Subcutaneous adipose tissue allows expression of a range of enzymes participating in the synthesis and metabolism of steroid hormones, including aromatase, which controls the conversion of androstendione to estrone and of testosterone to estradiol. Estrogens act as protectors in relation to bone tissue, both directly, as 17-beta estradiol receptors have been found in bones, and indirectly, through an influence on the secretion of the hormones involved in calcium homeostasis regulation [Arunabh et al. 2003, Kremer et al. 2009, Zallone 2006]. Visceral adipose tissue, on the other hand, secretes tumor necrosis factor – alpha (TNFα) and interleukin-6 (IL-6), which intensify differentiation of stem cells to osteoclasts. Visceral obesity is accompanied by an elevated concentration of glucocorticoids that stimulate osteoclastogenesis and enhance resorption of bone tissue [Śliwa et al. 2006]. In our previous study supplemented animals deposited mainly visceral adipose tissue [Friedrich and Sadowska 2005], which may explain lack of protective effect of adipose tissue accumulation on calcium bone content in examined rats.

A modification in the diet composition was advantageous in relation to magnesium incorporation in the bone tissue of the studied female rats, and the applied supplementa-

tion deepened this effect (Table 4). Bone is an excellent ion exchange system. Ions exchange can take place owing to a large surface area of hydroxylapatite crystals. Calcium ions can be exchanged also with magnesium ions. A question is, whether lower calcium concentrations in bone of supplemented female rats may be balanced by an elevated magnesium level. The process could have been facilitated by vitamin B_6, a component of the supplementation, which improves absorption of magnesium to be next built in bone tissue.

In conclusion it should be stressed that the results obtained exhibit a high level of complexity, multidirectionality, and a far-reaching impact of the studied supplementation on the body. This should not be surprising, however, because the system constitutes a functional integrity, and a fundamental characteristic of all biochemical processes is that they integrate with and subordinate to the regulatory mechanisms functioning in accordance with the needs and capabilities of the system.

CONCLUSIONS

Analysis of the results allowed drawing the following conclusions:

1. Changes in hematocrite indicator, calcium and magnesium concentrations in plasma and whole blood of examined rats feed modified diet with supplementation may indicate that the elements move to erythrocytes.

2. Lack of changes in blood plasma calcium levels in the studied animals implies the preservation of the homeostatic mechanisms that regulate its concentration, whereas the observed significant changes in the concentration of magnesium, occurring as a result of changed composition of the diet, point to a significant effect of this factor on its metabolism.

3. A change in the composition of the diet and its supplementation modified bone calcium and magnesium concentrations in the studied rats, resulting in an increase in calcium in males, and in an increase in magnesium levels in females.

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ANALIZA ZMIAN WYBRANYCH WSKAŹNIKÓW GOSPODARKI WAPNIEM I MAGNEZEM ZACHODZĄCYCH POD WPŁYWEM SKŁADU DIETY ORAZ SUPLEMENTACJI WITAMINAMI Z GRUPY B U SZCZURA

Wstęp. Celem badań była ocena wpływu składu diety oraz suplementacji witaminami z grupy B na wybrane wskaźniki gospodarki wapniem i magnezem u szczura.
Materiał i metody. Badania przeprowadzono na 60 szczurach w wieku 5 miesięcy. Zwierzęta żywiono dwiema paszami o różnym składzie. Do picia grupy I i II otrzymywały czystą wodę, grupie III podawano roztwór witamin z grupy B.
Wyniki. Nie stwierdzono istotnego wpływu badanych czynników na stężenie wapnia w osoczu badanych zwierząt. Zmiana składu diety wpłynęła na wzrost stężenia magnezu w osoczu. Zastosowana suplementacja zmniejszała stężenie do poziomu obserwowanego u zwierząt żywionych paszą podstawową. Modyfikacja diety i suplementacja wywarły wpływ na stężenie wapnia i magnezu w pełnej krwi badanych zwierząt. Zmiana składu diety i zastosowana suplementacja oddziaływały także na wzrost zawartości wapnia w kościach samców oraz magnezu w kościach samic.
Wnioski. Brak zmian w stężeniu wapnia w osoczu badanych zwierząt wskazuje na zachowanie regulujących je mechanizmów homeostatycznych, natomiast różnice w stężeniu magnezu wskazują na silny wpływ badanych czynników na jego metabolizm. Różnice w wartości wskaźnika hematokrytowego, stężenie wapnia i magnezu w pełnej krwi oraz brak zmiany stężenia w osoczu wskazują na przesunięcie badanych pierwiastków do erytrocytów, co może świadczyć o destabilizacji błony komórkowej oraz wzroście jej przepuszczalności.

Słowa kluczowe: suplementacja witaminami z grupy B, wapń, magnez, osocze, krew, szczur

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