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EFFECT OF APPLE PULP ON CALCIUM, MAGNESIUM AND MANGANESE CONTENT IN ORGANS OF RATS FED ATHEROGENIC DIETS

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Rats of control group were fed a standard diet (LSM). One of the diets was supplemented with 1% cholesterol (LSM+CH), another one with 5% freeze-dried apple pulp (APP), and the last one with both, 1% cholesterol and 5% apple pulp. Dietary addition of apple pulp significantly increased calcium concentration in femur bone from 154.2±5.6 (LSM, LSM+CH) to 180.1±4.8 g/kg wet weight (APP, APP+CH), while bone magnesium content was reduced from 3.34±0.14 to 2.70±0.12 g/kg. Dietary cholesterol (1%) had the similar significant effect on the bone content of these macroelements, and decreased bone manganese content. Moreover, rats fed diets with cholesterol (LSM+CH, APP+CH) revealed the higher Ca concentration in kidney and the lower liver Mg content ($p \leq 0.05$) than in the other groups (LSM, APP). The 5% administration of the freeze-dried apple pulp reduced significantly kidney Mn content. It may be concluded that both dietary factors studied (apple pulp and cholesterol) positively influence calcification of bones but a high level of cholesterol significantly reduces bioavailability and/or storage of the other important macroelement – magnesium.

INTRODUCTION

Diets rich in dietary fibre have been widely recommended because they are thought to decrease the risk of civilization diseases (cancer, atherosclerosis, obesity). These diseases are closely related to a rising intake of sugars, saturated fatty acids and cholesterol. Fruits and vegetables are the main source of dietary fibre in human diets. There are reports that mineral bioavailability can be reduced by fibrous components of the diets [Aoyagi *et al.*, 1993; Kidd *et al.*, 1993]. The mineral binding capacity of fibre fractions can influence mineral absorption [Champagne, 1988]. It was shown that fibre of fruits and vegetables may increase mineral excretion in faeces and absorption of minerals can be impaired in that way [Gralak *et al.*, 1996].

The objective of our study was to determine the effect of the atherogenic diets containing freeze-dried apple pulp on calcium, magnesium and manganese contents in rat liver, kidney and femur bone.

MATERIAL AND METHODS

The experiment was performed on 18 growing male Wistar rats of the initial weight of 130.9±15.3 g divided randomly into four groups. The rats were kept in individual plastic cages in an air-conditioned room at 22°C and 12-h light cycle. All diets were offered in loose form for 21 days and then animals were euthanised in narcosis by bleeding. Organs were removed, weighed and frozen for further analyses.

Rats of the control group were fed the standard diet (LSM) which was composed mainly of natural components. One of the diets was supplemented with 1% cholesterol (LSM+CH), another one with 5% apple pulp (APP), and the last one with both, 1% cholesterol and 5% apple pulp (APP+CH) (Table 1). Whole fruits of apple (v. *Golden delicious*) were washed in distilled water, then immersed in liquid nitrogen, and finally freeze-dried. The freeze-dried apple pulp contained 11.9% of the TDF (total dietary fibre), including an insoluble fraction – 7.7% (IDF) and a soluble one – 4.2% (SDF). The LSM diet contained 88.7% of dry matter and 17.33, 3.29, 7.22, 4.19, and 67.97% (DM basis) of crude protein, crude fibre, ash, ether extract, and N-free extractives, respectively. Dry matter of the diet was determined at 105°C and ash at 550°C in oven. Crude fibre was estimated with Fibertec, ether extract with Soxtec and nitrogen with Kjeltec (all Tecator AB, Hoganas, Sweden). The fibre fractions were estimated according to AOAC [1997].

Samples of the diets (0.5 g), livers (0.5 g), whole kidney and one femur bone were mineralized with a mixture of 5 mL HNO_3 (Merck 1.00441) and 1 mL H_2O_2 (Merck 107298) in hermetic teflon vessels in a microwave oven (Milestone Ethos 900). Mineral elements: Ca, Mg, Fe, Mn, Zn and Cu, were determined by flame atomic absorption spectrometry (Perkin-Elmer 1100B) using hollow cathode lamps. The external standards were prepared on the basis of Titrisol Standards (Merck) for calcium, magnesium, iron, manganese, zinc, and copper, respectively.

For statistical evaluation, one way and two-way (cholesterol*apple pulp) analyses of variance and Scheffe test at $p \leq 0.05$ were applied (Statgraphics Plus 6.0).

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TABLE 1. Mineral composition of the diets (n = 3).

Mineral Item	Diets			
	LSM	LSM+CH	APP	APP+CH
Ca (g/kg)	2.07 ± 0.24	1.75 ± 0.32	2.03 ± 0.10	1.80 ± 0.12
Mg (g/kg)	2.57 ± 0.07	2.42 ± 0.08	2.43 ± 0.11	2.36 ± 0.05
Fe (mg/kg)	427 ± 38	358 ± 76	437 ± 31	384 ± 47
Mn (mg/kg)	148.2 ± 15.7	136.1 ± 19.0	139.0 ± 5.1	133.1 ± 3.9
Zn (mg/kg)	129.1 ± 7.4	118.7 ± 9.8	122.6 ± 5.8	117.5 ± 1.9
Cu (mg/kg)	12.17 ± 1.72	11.98 ± 1.97	11.80 ± 0.23	12.90 ± 1.94

RESULTS AND DISCUSSION

The average daily body gains during 21 days of the trial did not significantly differ and varied from 4.04±0.56 (APP+CH diet), 4.96±0.30 (LSM), 4.99±0.51 (LSM+CH), to 5.43±0.41 g (APP diet). However, the daily gain in the APP group was by over 34% higher than in APP+CH group. Because the feed intake was similar in all groups, it can be suggested that the addition of apple pulp to an atherogenic diet had a negative influence on body gain.

No significant changes of the studied mineral concentration in liver and kidney were observed between groups/diets (Tables 2, 3, 4), although Ca and Mg levels in kidney were apparently lower in the control group. However, significant changes were stated in bone mineral concentration, higher calcium and lower magnesium contents in rats fed diets with apple pulp and/or cholesterol. No significant changes in bone Mn content were observed. In a previous study [Gralak *et al.*, 1996], we found that apple pomace decreased apparent absorption of calcium, magnesium and manganese in rats. Similarly, Hoffman *et al.* [1999] observed that bone mineral content was lower in weanling and yearling foals fed the fat/fibre supplement than in those fed corn and molasses. Those authors stated that the binding of calcium by fat and fibre, may alter the availability of elements necessary for bone development. They used the mixture of corn oil, beet pulp, soybean hulls, oat straw, which contained high amounts of lignin, cellulose and hemicellulose. It was in opposite to present experiment, where diets containing apple pulp improved bone Ca concentration (Figure 1). These differences between experiments can be explained by different composition of dietary fibre. It was found earlier that mineral absorption was negatively correlated with dietary content of the insoluble fibre and not with the soluble fibre [Gralak *et al.*, 1996]. It should be kept on mind that apples contain considerable amounts of pectins and oligosaccharides (4.2% of soluble fibre), which can be very active in the gastrointestinal tract. It seems that apple pulp increased the Ca absorption. Also others found out that fructooligosaccharides could improve calcium and magnesium absorption in the large intestine [Roberfroid & Delzenne, 1998; Wolf *et al.*, 1998]. Ohta *et al.* [1998] suggested that most probably fructooligosaccharides increased trans-cellular absorption of calcium in both, small and large intestines. It is difficult to discuss why the bone Mg content was lowered and not affected in the same way like calcium content.

The cholesterol supplement significantly increased bone calcification, but decreased magnesium content not only in bones, but also in liver (Figure 2). There was a significant interaction

TABLE 2. Calcium content in rat organs (x±SEM).

Diet/Organ	n	Liver (mg/kg)	Kidney (mg/kg)	Femur bone (g/kg)
LSM	3	26.3 ±4.0	37.7 ±8.3	135.7 ±4.9[a]
LSM+CH	5	27.5 ±1.2	66.6 ±4.5	172.6 ±7.2[b]
APP	5	30.7 ±2.7	64.2 ±11.3	178.7 ±7.9[b]
APP+CH	5	29.0 ±3.5	66.6 ±4.0	181.5 ±6.5[b]
Mean	18	28.6 ±1.4	60.9 ±3.5	170.6 ±3.6

[a-b] - means in columns tagged with the different letter differ at p≤0.05

TABLE 3. Magnesium content in rat organs (x±SEM).

Diet/Organ	n	Liver (mg/kg)	Kidney (mg/kg)	Femur bone (g/kg)
LSM	3	264.4 ±6.4	259.8 ±8.3	4.13 ±0.22[a]
LSM+CH	5	234.0 ±7.2	321.6 ±12.0	2.55 ±0.22[b]
APP	5	264.3 ±17.5	317.0 ±24.2	3.00 ±0.14[b]
APP+CH	5	242.0 ±7.1	291.5 ±10.9	2.41 ±0.16[b]
Mean	18	249.7 ±5.8	301.7 ±8.3	2.90 ±0.09

[a-b] - means in columns tagged with the different letter differ at p≤0.05

TABLE 4. Manganese content in rat organs (x±SEM).

Diet/Organ	n	Liver (mg/kg)	Kidney (mg/kg)	Femur bone (mg/kg)
LSM	3	2.75 ±0.22	2.10 ±0.16	2.62 ±0.34
LSM+CH	5	3.02 ±0.14	2.33 ±0.31	1.63 ±0.08
APP	5	3.02 ±0.29	1.52 ±0.13	1.78 ±0.14
APP+CH	5	2.61 ±0.33	1.64 ±0.18	1.48 ±0.14
Mean	18	2.86 ±0.13	1.87 ±0.11	1.74 ±0.08

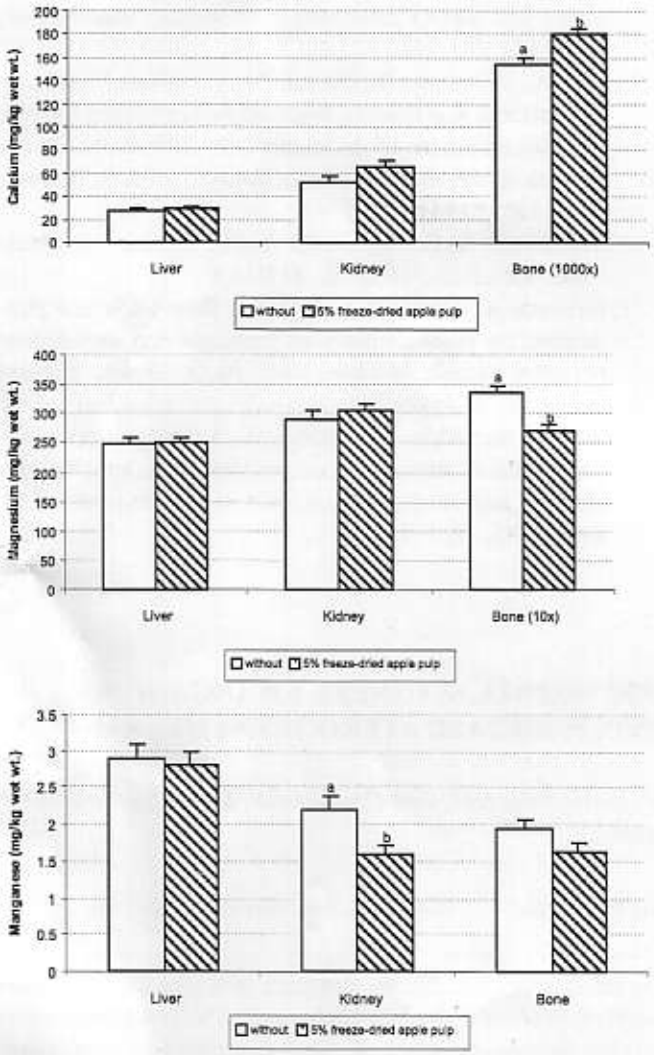

FIGURE 1. The effect of freeze-dried apple pulp (5% in the diets) on mineral concentration in rat organs (x±SEM).

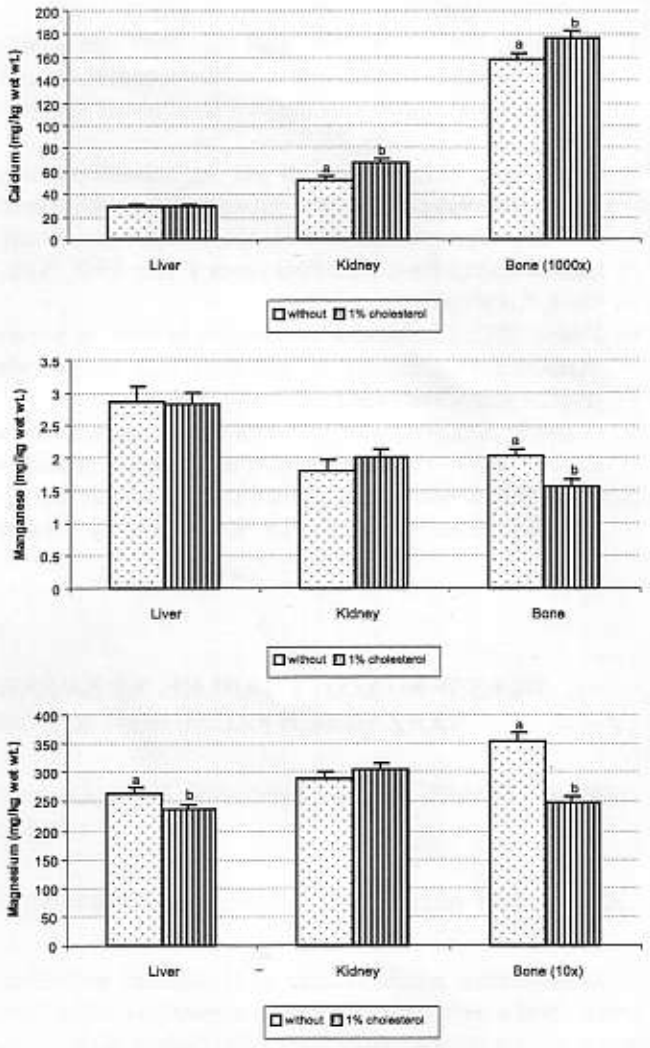

FIGURE 2. The effect of cholesterol (1% in the diets) on mineral concentration in rat organs (x±SEM).

between apple pulp and cholesterol administration in the case of bone mineralisation. The low bone Mg content was correlated (r=-0.44, p=0.07) with high bone Ca content. The positive effect of cholesterol on bone calcification may be related to the metabolism of steroid hormones. Overweighed people often have increased blood cholesterol [Duane, 1997] what predominantly is connected with high cholesterol synthesis [Duane, 1997; Miettinen & Gylling, 2000], but they are said to reveal lower risk of osteoporosis. There is not available literature on the relationship between cholesterol and macroelements, but it was observed earlier that cholesterol might significantly decrease the Mg bioavailability in rats [Stryczek, 2006]. Besides, two-way analysis of variance revealed that cholesterol decreased the bone manganese content. The bone Mn concentration was negatively correlated with the bone Ca content (r=-0.51, p=0.03). We have not found any data allowing an explanation of this phenomenon. Both elements, magnesium and manganese, are involved in antioxidant capacity. Hence their low level in organs of animals fed hypercholesterolemic diets may be an additional risk factor for cardiovascular diseases.

CONCLUSIONS

Generally, the addition of 5% of freeze-dried apple pulp and/or 1% of cholesterol to a diet influences calcium, magnesium and manganese metabolism. It may be concluded

that both dietary factors studied affect positively calcification of bones but a high level of cholesterol significantly reduces bioavailability and/or storage of the other important macroelement – magnesium. Further studies on this topic should be performed for farther explanation of relationships between dietary components and mineral bioavailability.

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WPŁYW MIAZGI Z JABŁEK NA ZAWARTOŚĆ WAPNIA, MAGNEZU I MANGANU W NARZĄDACH SZCZURÓW KARMIONYCH DIETAMI ATEROGENNYMI

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Doświadczenie przeprowadzono na 18 szczurach podzielonych na cztery grupy żywieniowe. Zwierzęta były karmione *ad libitum* paszami przygotowanymi na bazie paszy standardowej LSM. Pasze różniły się od siebie zawartością cholesterolu (1%) i/lub liofilizowanej miazgi z jabłek (11,9% włókna TDF) (5%). Dodatek miazgi jabłczanej i/lub cholesterolu istotnie zwiększył zawartość Ca i jednocześnie istotnie obniżył stężenie Mg w kościach. Równocześnie stwierdzono wyższą zawartość wapnia w nerkach szczurów otrzymujących cholesterol, co może być powiązane z zagrożeniem kamicą nerkową. Wydaje się, że zarówno miazga z jabłek, jak i cholesterol, zwiększają kalcyfikację kości. Wysokie dawki cholesterolu istotnie obniżają biodostępność Mg i mają też wpływ na metabolizm Mn, co z kolei może mieć negatywny wpływ na potencjał przeciwutleniający organizmu.