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THE INFLUENCE OF GRAPEFRUIT PHENOLIC COMPOUNDS ON MINERAL ABSORPTION IN RATS

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The effect of grapefruit phenols on utilization of some minerals (Ca, P, Mg, Zn, Cu and Fe) in rats was examined. Lyophilised extract from hard parts of grapefruit (stone, peel, white coats) containing mainly flavonones (naringin, hespereridin, hesperetin, nairutin, neohesperedin) was introduced into casein diets in the amount of 0.1% and 0.4% (group C-0.1 and C-0.4, respectively). The addition of tested preparation modified mineral availability to a different extent. No differences in Ca retention between control group (42.72 mg/day) and groups C-0.1 and C-0.4 (40.9 and 43.54 mg/day, respectively) were observed. Compared to control group, retention of P increased from 25.94 mg/day to 42.92 and 44.39; retention of Mg from 2.3 mg/day to 3.55 and 4.15; retention of Zn from 41 μg/day to 96 and 150; retention of Cu from 55.4 μg/day to 64.2 and 71.7 in groups C-0.1 and C-0.4 respectively. Simultaneously, a dose-dependent reduction of Fe retention was observed from 1 μg/day in control group to -39 μg/day in group C-0.1 and -102 μg/day in group C-0.4. However, a 0.1% addition caused only insignificant decrease in retention of this mineral.

The results obtained indicate that small amounts of grapefruit polyphenols in diets can improve availability of some minerals without significant negative influence on Fe status.

INTRODUCTION

Plants contain a wide variety of flavonoids that reveal antimutagenic, antioxidant and anticarcinogenic properties [Bagchi *et al.*, 1998; Hammon *et al.*, 1999; Kuroda & Hara, 1999]. In many experiments, beneficial health-promoting effects of fortification of diets with polyphenol extracts were observed. Increasing antioxidant potential of blood polyphenols decreased concentration of cholesterol and its HDL fraction in serum [Koga *et al.*, 1999; Juśkiewicz *et al.*, 2002; Martin-Carron *et al.*, 2000; Zduńczyk *et al.*, 2002]. This way they reduce the risk of cardiovascular diseases [Hollman *et al.*, 1999; Tijburg *et al.*, 1997]. Besides, polyphenols are substances thought as strong antitumor agents [Bromser *et al.*, 1996; Gupta *et al.*, 1999]. Therefore supplementation of diets with polyphenol extract seems to be a simple and effective way to increase defense of the organism against many diseases [Koga *et al.*, 1999; Bushman, 1998; Waladkhani & Clemens, 1998].

Polyphenols demonstrate a wide range of activity. It has been also reported that polyphenols can modify, in a different way, trace element metabolism [House & Van Campen, 1994; MacDonald *et al.*, 1996; Prystai *et al.*, 1999; Brune *et al.*, 1989; Reddy & Cook, 1991]. From the nutritional and wholesome point of view, the most important seems to be a decrease in iron availability [Brune *et al.*, 1989; Reddy & Cook, 1991]. However, the influence of polyphenols on mineral status in animals and humans is still not obvious and has not been fully explained so far, especially in the case of macro- and microelements other than iron.

The aim of this study was to determine the influence of phenolic compounds extracted from hard parts of grapefruits on the availability of some minerals in rats.

MATERIAL AND METHODS

Lyophilised extract from hard parts of grapefruit (stone, peel, white coats) containing 50% of flavonones (mainly naringin, hespereridin, hesperetin, nairutin, neohesperedin) was used. A commercial preparation was obtained from the company CINTAMANI-POLAND.

45-day-old rats of Wistar strain (from own heard) with the initial weight of 100-110 g were divided into 3 groups consisting of 5 males and 5 females. All animals were fed everyday fresh diet *ad libitum* with permanent access to deionized water. Rats were housed in individual plastic cages in a well-ventilated room with constant temperature of 21-22°C and 12-h dark/light periods. Collection of urine and feces lasted 7 days after 3 days of adaptation. After filtration, urine was conserved in 8% HCl. Diets and faeces, after drying to constant weight, were ashed in an oven at a temperature of 525°C and next dissolved in 6 M HCl. Experimental protocols were approved by the Ethical Council for Animal Experiments.

The composition of diets was based on AIN-1976 recommendations [AIN, 1977]. Standard casein diets (group Control) were supplemented with 0.1% and 0.4% (group C-0.1 and C-0.4, respectively) of tested preparation after reduction of sucrose by about 0.1% and 0.4%. Table 1 presents the composition of control diet.

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TABLE 1. Composition of control casein diet.

Ingredient	Content [g/kg]
Casein	200.0
DL-Methionine	3.0
Corn starch	150.0
Sucrose	500.0
Cellulose	50.0
Corn Oil	50.0
AIN Mineral Mixture[1]	35.0
AIN Vitamin Mixture	10.0
Choline Bitartrate	2.0

[1]-mineral mixture contained (in g/kg): calcium phosphate dibasic ($CaHPO_4$) - 500.0; sodium chloride (NaCl) - 74.0; potassium citrate monohydrate ($K_2C_6H_5O_7$ x H_2O) - 220.0; potassium sulfate (K_2SO_4) - 52.0; magnesium oxide (MgO) - 24.0; manganous carbonate (43-48% Mn) - 3.5; ferric citrate (16-17% Fe) - 6.0; zinc carbonate (70% Zn) - 1.6; cupric carbonate (53-55% Cu) - 0.3; potassium iodate (KIO_3) - 0.01; sodium selenite (Na_2SeO_3 x $5H_2O$) - 0.01; chromium potasium sulphate ($CrK(SO_4)_2$ x 12 H_2O) - 0.55; sucrose - to 1 kg.

A Perkin-Elmer 1100B atomic absorption spectrophotometer was used to determine the contents of Ca, Mg, Cu, Zn, and Fe. Fiske-Subbarow method [Fiske & Subbarow, 1925] was used to measure phosphorus content.

Apparent retention of minerals was calculated as an intake - (fecal + urinary excretion).

Values are expressed as means±SD. The results were worked out statistically using one-way analysis of variance (ANOVA) and significance of differences between groups was determined by the Duncan's multiple range test at the significance level of $p<0.01$ and $p<0.05$.

RESULTS

Table 2 shows a mineral composition of experimental diets. Supplementation of the diets with a small amount of grapefruit preparation had no influence on the content of macro- and microelements. It indicates that the preparation tested was a poor source of minerals.

The intake and absorption of macrolements are presented in Table 3. No differences in the intake, faecal, urinary and retention values of calcium were found. The intake and faecal voidance of phosphorus was also the same in all groups. However, a decrease in urinary excretion of P in rats fed diets with preparation tested resulted in an increase in its retention. In the case of magnesium, a significant ($p<0.01$) dose-dependent increase in its retention was observed in rats fed diets with polyphenols.

Utilization of microelements by rats is shown in Table 3. Supplementation of diets with grapefruit preparation evoked a dose-dependent increase in the retention of Zn and Cu. An opposite situation was reported for iron. The intake of polyphenols enhanced, especially urine, excretion of this microelement that resulted in a negative retention of this microelement. However, a 0.1% addition of extract to diet caused an insignificant reduction of its retention.

TABLE 3. Retention of investigated minerals[1].

Mineral	Control	Group	
		C-0.1	C-0.4
Calcium [mg/d]			
Intake	76.40 ± 9.0	72.14 ± 8.2	72.74 ± 8.1
Faecal	29.19 ± 6.5	26.73 ± 5.6	24.28 ± 5.5
Urinary	4.48 ±1.5	4.43 ± 1.2	4.92 ± 0.9
Retention[2]	42.72 ± 4.4	40.90± 5.8	43.54 ± 5.7
Phosphorus [mg/d]			
Intake	73.53 ± 8.2	69.73 ± 8.6	69.57 ± 8.1
Faecal	20.53 ± 3.9	19.54 ± 4.2	17.23 ± 3.4
Urinary[3]	27.06 ± 4.3^{A}	7.27 ± 1.4^{B}	7.95 ± 0.8^{B}
Retention	25.94 ± 5.1^{B}	42.92 ± 7.6^{A}	44.39 ± 7.6^{A}
Magnesium [mg/d]			
Intake	10.56 ± 1.2	10.70 ± 1.6	10.78 ± 1.4
Faecal	4.35 ± 0.8^{A}	3.21 ± 0.7^{B}	2.78 ± 0.5^{B}
Urinary	3.90 ± 0.8	3.94 ± 0.6	3.86 ± 0.4
Retention	2.30 ± 0.5^{B}	3.55 ± 0.9^{A}	4.15 ± 1.1^{A}
Zinc [μg/d]			
Intake	573 ± 64	538 ± 58	530 ± 60
Faecal	513 ± 60^{A}	423 ± 60^{B}	360 ± 40^{C}
Urinary	19 ± 4	19 ± 4	20 ± 7
Retention[2]	41 ± 20^{C}	96 ± 35^{B}	150 ± 36^{A}
Copper [μg/d]			
Intake	153.9 ±18	149.3 ± 20	151.9 ± 20
Faecal[3]	94.1 ± 13^{A}	78.6 ± 9.0^{B}	72.0 ± 10^{B}
Urinary	4.5 ± 1.0^{C}	6.5 ± 1.3^{B}	8.1 ± 1.2^{A}
Retention	55.4 ± 11^{b}	64.2 ± 13^{ab}	71.7 ± 15^{a}
Iron [μg/d]			
Intake	738 ± 60	712 ± 50	722 ± 52
Faecal	686 ± 60	665 ± 80	719 ± 92
Urinary	51 ± 9^{B}	87 ± 20^{Ab}	105 ± 27^{Aa}
Retention	1 ± 20^{Aa}	-39 ± 50^{ABa}	-102 ± 65^{Bb}

[1] values are means±SD, n=10; [2] apparent retention (micrograms or miligrams a day) = intake - (fecal + urinary); [3] values in the same row with different letters are significantly different (a, b at $p<0.05$; A, B at $p<0.01$).

DISCUSSION

There are some substances of plant origin that influence mineral utilization in humans and animals. Some of them increase mineral absorption whereas others can decrease it. The most known inhibitors are fiber and phytates. Both strongly reduce the utilization of micro- and macroelements [Halrand, 1989; Hallberg *et al.*, 1989; Sandberg *et al.*, 1999; Zduńczyk *et al.*, 1994]. Unfortunately, there are few works only concerning the influence of polyphenols on mineral

TABLE 2. Mineral composition of diets.

Diet	Mineral[1]					
	Ca	P	Mg	Zn	Cu	Fe
Control	4.92 ± 0.2	4.73 ± 0.2	0.68 ± 0.02	36.9 ± 1.5	9.9 ± 0.3	46.5 ± 1.1
C-0.1	4.79 ± 0.2	4.63 ± 0.1	0.71 ± 0.01	35.7 ± 1.4	9.9 ± 0.2	46.4 ± 1.3
C-0.4	4.79 ± 0.2	4.58 ± 0.1	0.71 ± 0.01	34.9 ± 1.3	10.0 ± 0.3	46.1 ± 1.4

[1] Ca, P, Mg expressed in mg/g of dry matter of diet; Zn, Cu, Fe expressed in μg/g of dry matter of diet.

metabolism. It still remains controversial and only interaction with Fe is well explored and seems to be clear.

The results of apparent retention of Mg, Ca, P in the presented experiment are slightly higher than those reported by Lisbona *et al.* [1999] who used diets based on the same recommendations of AIN-76. The utilization of Fe was poor, which is confirmed by other scientists who observed poor availability of non-heme Fe [Lisbona *et al.*, 1999; Siegenberg *et al.*, 1991]. The absorption and retention of Mg, Cu and Zn were higher after supplementation of a control diet with the investigated preparation. In the case of P and Ca, there was no effect on apparent retention. However, because of lower excretion in urine, retention of P was higher than in the control group. Probably, absorbed polyphenols can cause some metabolic changes which reduce P excretion in urine. Such an effect of an increased mineral retention was not observed in earlier works into utilization of polyphenols, neither in humans [Prystai *et al.*, 1999] nor in rats [Coudray *et al.*, 1998; Coudray *et al.*, 2000; Proulx *et al.*, 1993]. Generally, no influence of phenols on Ca, Mg, Cu, Zn metabolism was recorded, even in a long--term experiment [Coudray *et al.*, 2000]. Properties of precipitation of metal ions by polyphenols noted by McDonald *et al.* [1996] *in vitro* suggest that we should expect rather a decrease in the absorption of Zn and Cu in an alimentary tract. However, the mechanism of absorption of these complexes is still unknown. Maybe such a complex of mineral-polyphenol can be more absorbable from the intestine lumen than unbound minerals.

The presented results confirm those obtained previously and indicating that polyphenols can modify non-heme Fe metabolism [Brune *et al.*, 1989; Siegenberg *et al.*, 1991; Tuntawiroon *et al.*, 1991; Zijp *et al.*, 2000]. The investigated preparation reduced the absorption of Fe and its retention. It was a dose-dependent reduction. The addition of a small dose (0.1%) of an extract decreased, though insignificantly, the absorption of Fe. A higher dose of polyphenols applied in a diet (to 0.4%) caused a strong significant reduction of Fe utilization. House & Van Campen [1994] recorded that up to 0.125% addition of polyphenols extracted from beans to rat diets had no influence on Fe utilization. Tuntawiroon *et al.* [1991] and Siegenberg *et al.* [1991] also observed a dose-dependent influence of polyphenols on Fe availability. A possible mechanism of Fe absorption inhibition was explained by Brune *et al.* [1989] who assessed the relation between chemical structure of phenolic compounds and its influence on iron absorption. The inhibition of Fe availability by polyphenols is due to the formation of strong hardly absorbable complexes with iron. It is noted that some substances can enhance iron absorption in humans and rats. The addition of ascorbic acid [Siegenberg *et al.*, 1991; Tuntawiroon *et al.*, 1991] or Na_2EDTA [Davidsson *et al.*, 2001; MacPhail *et al.*, 1994] to a diet containing polyphenols can reduce their negative influence on iron absorption. However, Tuntawiroon *et al.* [1991] observed that even great amounts of ascorbic acid in a diet cannot overcome iron inhibition by phenolic compounds. In addition, according to the suggestions of Reddy and Cook [1991], we should not to extrapolate results of Fe absorption in rats directly to humans. Rats are less sensitive than humans to factors that can modify Fe absorption from the intestine. Despite that, we can still use rats as model animals in experiments where we would like to recognize nutritional determinants of mineral utilization.

CONCLUSIONS

The addition of grapefruit preparation, extracted from hard parts of the fruit, to rat diets significantly increased the availability of P, Mg, Zn and Cu. No influence on Ca status in rats was observed. Like in previous works phenol, extract used caused a dose-dependent reduction of iron availability. However, in the case of its low level, the reduction was insignificant. These results suggest that grapefruit phenols applied in small amounts can act as enhancers of utilization of some minerals without negative effect on Fe availability.

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