[Chem. Pharm. Bull. 31(11)4152—4156(1983)]

Effect of Dietary Calcium on Growth, Calcium, Magnesium and Phosphorus Contents and Fatty Acid Composition of Individual Tissues in Rats

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(Received February 18, 1983)

In order to determine the effects of diet containing three calcium (Ca) levels (none, 0.8 or 2.4%) on rats, the Ca, magnesium and phosphorus contents, fatty acid composition in tissues as well as their growth were examined. In the groups fed with Ca-free and 2.4% Ca diet the growth of rats was suppressed compared with that of a control group (0.8% Ca). In both groups the relative weight of brain with respect to the body weight increased and in the group fed with 2.4% Ca diet, the weight of kidney increased both relatively and absolutely. Ca content in all tissues except the kidney of female rats decreased to various extents in the group fed with Ca-free diet. In the group fed with 2.4% Ca diet, the Ca content increased markedly only in the kidney. In the group fed with 2.4% Ca diet, magnesium content decreased only in bone, and phosphorus content was not affected by any dietary Ca level. Fatty acid compositions of the heart, liver and kidney changed as dietary Ca levels were varied, but no change was observed in the brain. Namely, in the group fed with 2.4% Ca diet, oleic and eicosatrienoic acids increased, but linoleic and arachidonic acids decreased. On the other hand, consistent changes of composition in the Ca-free diet group were not observed in any individual tissue of either sex. From these results, it was concluded that the growth of rats was suppressed in the groups fed not only with Ca-free but also high Ca level diets and that the mineral content and fatty acid composition in various tissues changed as the dietary Ca level was varied.

Keywords——dietary calcium; growth of rat; tissue; calcium; magnesium; phosphorus; fatty acid composition

It is currently believed that one cause of bone fracture of children may be a deficiency of calcium (Ca) intake due to changes of dietary intake, and it is recommended that more Ca should be taken in the diet and/or nutrient supplements in childhood. Diet is also important in biological experiments[1,2] and the benefits of using semisynthetic or semipurified diet in toxicological and/or biochemical experiments have been discussed.[3] It is particularly important to use semipurified diets in studies where an essential nutrient is used.[4]

In the present work, our purpose was to determine the effects of dietary Ca levels on growth, mineral content and fatty acid composition of lipid in individual tissues of rats given semipurified diets.

Materials and Methods

Experimental Diets——Although nutrient forms of Ca include Ca phosphate, carbonate and lactate salts, $CaHPO_4$ was selected as the Ca source for nutrient evaluation. Three different Ca contents in powdered and semipurified diets were used as shown in Table I.

Animals and Feeding——SPF Fischer 344/Nslc male and female rats, 25 d old, were kept in plastic cages with sterilized Beta Chips (Charles River Japan, Inc.). After a preliminary feeding with a sterilized stock diet (Type NMF,

TABLE I. Percentage Composition of Diets[a]

Ingredient	Ca-free	Control[b]	2.4% Ca
Casein, vitamin-free	23.0	23.0	23.0
Sucrose	64.6	64.6	58.0
Vegetable oil[c]	5.0	5.0	5.0
Vitamin mix[d]	1.0	1.0	1.0
Mineral mix[e]	3.0	3.0	3.0
Na_2HPO_4	3.4	—	—
$CaHPO_4$	—	3.4	10.0

a) The diets were prepared by Oriental Yeast Co., Ltd., Japan.
b) Control diet contained 0.8% calcium.
c) The percentage fatty acid composition of vegetable oil was myristic (2.6%), palmitic (32.6%), stearic (11.0%), oleic (37.3%), linoleic (11.8%), linolenic (1.3%) and octadecatetraenoic plus eicosamonoenoic (3.4%) acids.
d) Vitamin mix provided the following in mg/kg diet: α-tocopherol, 50; menadione, 52; thiamine·HCl, 12; riboflavine, 40; pyridoxine, 8; cyanocobalamin, 0.005; ascorbic acid, 300; biotin, 0.2; folic acid, 2; Ca pantothenate, 50; *p*-aminobenzoic acid, 50; niacin, 60; inositol, 60; choline chloride, 2000; retinyl acetate (500 I.U.); ergocarciferol, (1000 I.U.).
e) Mineral mix provided the following in g/kg diet: magnesium chloride, 6.0; potassium citrate, 6.21; potassium chloride, 5.91; sodium chloride, 2.46; potassium sulfate, 0.63; ferric citrate, 0.48; potassium iodide, 0.012; sodium fluoride, 0.012; manganous sulfate, 0.006; potassium aluminate, 0.012; sodium selenate, 0.012; Cupric sulfate·$5H_2O$, 0.012; cobalt chloride·$6H_2O$, 0.012; zinc sulfate·$7H_2O$, 0.012.

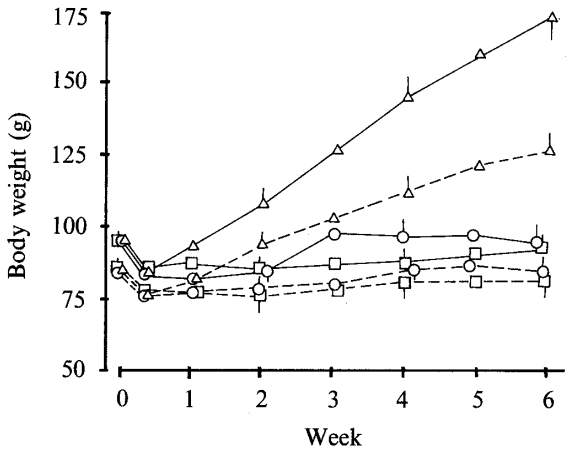

Fig. 1. Growth Curves of Fischer 344 Rats Fed Semipurified Diets Containing Three Levels of Calcium for 6 Weeks

Vertical lines indicate the standard error of the mean. ○=○, calcium-free diet; △=△, control diet; □=□, 2.4% calcium diet. ——, male rats; – – – –, female rats.

Oriental Yeast, Co., Ltd., Japan), the rats were divided into 3 groups of 10 animals and fed with an experimental diet from 4 weeks of age. Both diet and deionized water were allowed *ad libitum* for 6 weeks. The animal room was maintained at 24 °C, 55% relative humidity, 18 air changes per hour, and with a 12 h (0600 to 1800 h) light-dark cycle. After the experimental period of 6 weeks, each rat was exsanguinated by decapitation and the brain, heart, liver, kidney and femur were taken out and weighed immediately. All tissues were frozen and stored at −20 °C until analyzed.

Analytical Methods——After digestion of tissues with nitric acid and hydrogen peroxide, Ca and magnesium were estimated by the use of an atomic absorption spectrophotometer (Hitachi, type 207) in 1.0% $LiCl_3$ solution (final concentration) to exclude the interference of phosphate, and total phosphorus was assayed colorimetrically by the method of Bartlett.[5] Fatty acid compositions of various tissues except the femur were measured by a gas liquid chromatography method as described in our previous paper.[6]

Results and Discussion

Growth and Mineral Contents in Organs

As shown in Fig. 1, male and female rats fed with a control diet (0.8% Ca) grew normally. However, rats of both sexes fed with Ca-free and 2.4% Ca diets grew little, and body weights were significantly lower than in the control group over the experimental period. The transient

loss in body weight observed at the beginning of the experiment may be attributed to reduced food intake due to the unfamiliar taste. In the control group, the food intake of males gradually increased from about 4 g at the 3rd day to 7 g at the 25th day after the beginning of the experiment and was 9 g at the end of the experiment. On the other hand, the food intake of females was somewhat less than that of males over the experimental period. No apparent difference in food intake among the groups was observed during the first 25 d, but the food intake of rats fed with a Ca-free or 2.4% Ca diet scarcely increased during the subsequent part of the experimental period.

The absolute and relative organ weights of male rats fed the control diet were as follows (in grams and ratio to body weight): brain, 1.28 ± 0.04 and 0.73 ± 0.04; heart, 0.59 ± 0.06 and 0.38 ± 0.01; liver, 8.40 ± 0.23 and 4.64 ± 0.14; kidney, 1.77 ± 0.04 and 1.00 ± 0.02; bone, 0.68 ± 0.01 and 0.39 ± 0.02. There was no difference in these values between males and females, except for the kidney. The absolute kidney weights of male and female rats were 1.13 ± 0.05 and 1.02 ± 0.01 g in the Ca-free diet group, 1.77 ± 0.04 and 1.39 ± 0.04 g in the control group and 1.94 ± 0.03 and 1.45 ± 0.05 g in the 2.4% Ca diet group, respectively. The relative kidney weights of male and female rats increased about 100 and 50%, respectively, in the group fed the 2.4% Ca diet. In the groups fed with Ca-free and 2.4% Ca diet, the relative weight of the brain in males and females increased about 70 and 40%, but the absolute weight was not affected. The absolute and relative weights of the other organs did not change in male and female rats fed with any of the diets.

Next, we determined the effects of dietary Ca on the concentrations of minerals in the organs. Ca and magnesium contents in organs of male rats fed the control diet for 6 weeks were 48.9 ± 4.33 and 156 ± 7.81 μg/g in brain, 33.3 ± 5.35 and 193 ± 7.43 μg/g in heart, 39.6 ± 7.85 and 206 ± 4.59 μg/g in liver, 76.8 ± 3.90 and 183 ± 5.35 μg/g in kidney, and 230 ± 6.50 and 4.04 ± 0.06 mg/g in bone, respectively, and there was no significant sex difference. In the group fed the Ca-free diet, the Ca content in all organs, except the kidney of females, decreased to various extents, while the Ca content in the kidney of females increased about 3.5 times. In the group fed the 2.4% Ca diet, the Ca content of males and females increased about 60 and 50 times, respectively, as compared with the control group.

In the group fed the 2.4% Ca diet, the magnesium content decreased only in bone, by about 45 and 20% in males and females, respectively. On the other hand, total phosphorus contents in brain, heart, liver, kidney and bone of male rats of the control group were 3.70 ± 0.13, 3.04 ± 0.02, 3.25 ± 0.14, 3.72 ± 0.35 and 124 ± 0.51 mg/g of tissue, respectively, and these values were independent of sex and dietary Ca level.

The findings in this study suggest that immoderate consumption of Ca results in suppression of growth and alteration of mineral contents in organs such as kidney and bone. This may have important implications for human nutrition.

Fatty Acid Composition of Organs

In experiments with rabbits and rats, it was shown that plasma lipid levels decreased and tissue lipid levels varied as dietary Ca level was increased.[7-9] These findings suggest that lipid mobilization among tissues and lipid metabolism are affected by dietary Ca levels, and consequently the fatty acid composition of tissue lipid is also affected.

In this study, we observed the effects of diets containing three Ca levels on the fatty acid composition of tissue lipid (Table II). The composition of brain lipid was little affected by dietary Ca level. However, the fatty acid compositions of heart, liver and kidney of males and females changed as the dietary Ca level varied. Namely, in the group fed the 2.4% Ca diet, oleic and eicosatrienoic acids increased, but linoleic and arachidonic acids decreased. However, no consistent changes of the compositions in the Ca-free diet group were observed among tissues, and there were no sex differences in the compositions.

TABLE II. Fatty Acid Composition in Individual Tissues from Rats

Fatty acids	Brain		Heart		Liver		Kidney	
	Male	Female	Male	Female	Male	Female	Male	Female
	2.4±0.1	2.6±0.1	2.6±0.2	2.9±0.1	—	—	1.9±0.1	1.7±0.1
C 16:ald	2.2±0.1	2.6±0.1	1.7±0.2	2.2±0.1	—	—	1.7±0.1	1.8±0.2
	2.5±0.1	2.6±0.1	2.1±0.1	1.9±0.1	—	—	1.8±0.1	1.9±0.2
	22.4±0.5	21.2±0.1	14.5±0.6[b]	13.8±0.4	20.4±0.5[c]	21.1±0.2	18.8±0.7[a]	17.7±0.4[b]
C 16:0	23.5±0.6	21.7±0.5	12.5±0.2	14.0±0.4	23.3±0.5	20.1±0.3	21.1±0.7	20.4±0.9
	23.6±0.9	21.7±0.1	13.7±0.7	14.4±0.5	20.6±0.6[b]	21.9±0.7	17.7±0.6[c]	18.1±0.6
	—	—	1.6±0.2	1.6±0.2	3.8±0.2	3.7±0.1	2.7±0.3	3.5±0.3
C 16:1	—	—	2.2±0.3	2.2±0.1	3.7±0.4	4.5±0.1	3.8±0.2	4.4±0.2
	—	—	2.2±0.1	2.3±0.2	4.2±0.2	4.6±0.1	3.8±0.1	4.0±0.4
	4.2±0.2	4.3±0.2	—	—	—	—	—	—
C 18:ald	4.4±0.1	4.7±0.2	—	—	—	—	—	—
	4.2±0.1	4.3±0.1	—	—	—	—	—	—
	23.3±0.1	23.8±0.3	21.5±0.5	20.7±0.3	17.4±0.8	15.9±0.2[d]	18.8±0.4	18.2±0.5
C 18:0	22.8±0.9	22.9±0.6	21.8±0.4	21.0±0.5	15.7±0.8	20.5±0.2	18.3±0.1	17.9±0.1
	22.9±1.0	23.6±0.2	21.1±0.2	20.5±0.6	15.6±0.5	14.4±0.4[d]	18.3±0.5	18.9±0.7
	19.5±0.7	20.3±0.9	17.3±0.6	20.2±0.5	26.8±0.9[c]	33.3±0.2[d]	20.2±0.7	23.6±0.9
C 18:1	19.7±0.8	19.4±0.5	17.2±0.4	19.9±0.4	30.5±0.6	24.8±0.1	20.0±0.3	23.6±0.4
	18.7±0.5	20.6±0.1	20.9±0.1[d]	23.9±0.8[c]	32.5±0.4[a]	36.7±0.8[d]	26.9±0.3[d]	29.5±0.5[d]
	—	—	8.3±0.4[d]	7.9±0.6[c]	4.3±0.3	2.5±0.1	6.7±0.5	5.4±0.3
C 18:2	—	—	15.1±0.1	10.8±0.5	3.4±0.1	2.1±0.4	6.2±0.1	4.9±0.2
	—	—	7.6±0.4[d]	6.6±0.1[d]	2.4±0.2[a]	1.4±0.3	2.4±0.2[d]	2.3±0.4[d]
C 18:4	1.2±0.1	1.2±0.1	—	—	—	—	—	—
plus	1.2±0.2	1.5±0.1	—	—	—	—	—	—
C 20:1	1.1±0.1	1.3±0.2	—	—	—	—	—	—
	—	—	2.4±0.4[d]	3.7±0.6	4.6±0.5	8.9±0.1	3.1±0.2	4.8±0.4
C 20:3	—	—	7.1±0.3	3.7±0.3	5.7±0.4	8.0±0.4	5.2±0.3	3.7±0.4
	—	—	6.7±0.3	8.2±0.4[d]	10.9±0.4[d]	11.2±0.6[c]	7.3±0.1[c]	8.3±0.1[d]
	11.2±0.3	10.3±0.3	22.7±0.6[d]	21.5±0.2	15.8±0.5[c]	9.9±0.1[d]	24.1±0.7[c]	20.2±0.7
C 20:4	10.1±0.3	10.3±0.6	17.8±0.5	20.3±0.3	12.9±0.6	14.2±0.1	20.5±0.5	20.0±0.6
	10.4±0.2	9.9±0.1	18.1±0.1	16.8±0.2[d]	8.6±0.3[d]	6.0±0.3[d]	17.9±0.3[d]	15.0±0.3[d]
	3.2±0.2	2.7±0.2	—	—	—	—	—	—
C 22:4	2.2±0.2	3.4±0.3	—	—	—	—	—	—
	2.8±0.1	2.4±0.1	—	—	—	—	—	—
	—	—	1.5±0.1	1.5±0.2	1.1±0.1	1.0±0.0	1.3±0.3	1.0±0.1
C 22:5	—	—	1.3±0.1	1.8±0.3	1.8±0.1	2.8±0.2	1.4±0.1	1.4±0.2
	—	—	1.1±0.2	1.2±0.2	1.4±0.1	1.1±0.2	1.0±0.1	1.0±0.3
	12.5±0.1	13.5±0.2	7.6±0.4[d]	6.6±0.2[d]	4.9±0.4[b]	3.3±0.4	1.8±0.1	1.6±0.2
C 22:6	11.8±0.5	12.4±0.4	3.0±0.4	3.7±0.1	2.9±0.3	3.4±0.1	1.5±0.1	1.2±0.2
	11.4±1.0	12.3±0.3	5.5±0.2[c]	4.2±0.4	3.4±0.3	2.9±0.2	1.0±0.1	1.0±0.5

Minus signs indicate trace. Values are the mean±S.E. of percentages of fatty acids in 4 experiments. Values in the upper, middle and lower rows are for the calcium-free, control and 2.4% calcium diet groups, respectively. Significant differences from the control groups are as follows: *a*) $p<0.05$; *b*) $p<0.02$; *c*) $p<0.01$; *d*) $p<0.001$.

One of the major functions of fatty acid moieties of tissue lipid as phospholipids is to contribute to the structure and function of cell membranes.[10,11] It is also generally accepted that Ca is important for regulating the permeability and integrity of cell membranes. The fact

that the fatty acid composition of tissue lipid changed as dietary Ca level was varied suggests that dietary Ca may affect metabolic transport and function in organs.

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