

EFFECTS OF DIETARY CALCIUM ON CONCENTRATIONS OF LIPIDS, GLUCOSE AND INSULIN IN MALE SPRAGUE-DAWELY RATS

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Abstract

INTRODUCTION: A number of experimental studies have shown that dietary calcium may help improve hypercholesterolemia induced by high-cholesterol/high-fat diets through saponifying cholesterol/fat in the intestine. There is little evidence showing the effect of calcium in a low-fat, low-cholesterol diet on the lipid profile. We evaluated the effect of different levels of dietary calcium, in a cholesterol-free/low-fat diet on serum cholesterol, triglyceride, glucose and insulin, as well as fecal excretion of lipids.

METHODS: Forty-eight male Sprague-Dawely rats were randomly divided to receive three levels of dietary calcium (0.2, 0.5 and 1.2 % W/W) for 10 weeks. Finally, the rats were decapitated and their truncal blood was sampled for biochemical analysis. Fecal fat excretion, total cholesterol, high-density lipoprotein (HDL) cholesterol, triglycerides, glucose, and serum insulin were measured. Low-density lipoprotein (LDL) cholesterol was calculated using the Friedwald equation.

RESULTS: Serum cholesterol and LDL cholesterol of rats receiving a high-calcium diet were significantly lower than those of the other two groups ($P < 0.05$), but serum triglycerides, HDL cholesterol, glucose and insulin and fecal fat excretion were not statistically different in the three groups ($P > 0.05$).

CONCLUSIONS: Our findings suggest that, even with a low-fat low-cholesterol diet, calcium has hypocholesterolemic effects, i.e. there may be hypocholesterolemic mechanisms, other than intestinal saponification of cholesterol and/or fatty acids, including endogenous mechanisms for dietary calcium.

Keywords: Dietary calcium, serum cholesterol, serum LDL cholesterol, serum triglycerides, serum glucose, serum insulin, fecal fat.

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Introduction

Calcium is an essential nutrient required for critical biological functions such as nerve conduction, muscle contraction, cell adhesiveness, mitosis, blood coagulation and structural support of the skeleton.¹⁻³ The findings of previous studies about the role of calcium in plasma lipid profile are controversial. Some studies have reported that dietary calcium may cause favorable changes in plasma lipids and lipoprotein concentrations⁴⁻⁶ and thus reduce the

incidence of ischemic heart disease,⁷⁻¹⁰ while others have suggested that serum calcium may be positively related with higher serum cholesterol levels¹¹⁻¹⁴ and consequently higher incidence of coronary heart disease.¹⁵⁻¹⁷ The potential effects of calcium intake on plasma lipoprotein/lipid concentrations have been investigated in animal experiments¹⁸⁻³⁴ and clinical trials,^{5,18-21,35-38} however, the results have been controversial.

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Serum glucose and insulin may also be affected by dietary calcium and controversies on the effects of dietary calcium in the previous studies raise the issue of the influence of calcium on the glucose and insulin profile.³⁹⁻⁴¹

In addition, in most of the experimental studies on the effects of calcium on circulating lipids, a hypercholesterolemic diet was administered,^{4,23-24,32-34} however, a few studies used a cholesterol-free or low-fat diet.²⁹ Moreover, the effects of dietary calcium on serum glucose are rarely evaluated. Hence, the primary objective of this study was to determine whether dietary calcium affects serum lipid profiles and serum glucose of rats, and whether or not this effect is dose-dependent.

Materials and methods

Forty-five male Sprague-Dawley rats with initial average body weight of 200 g were obtained from Razi Institute, Iran. The rats were housed in individual stainless steel cages.

A 12/12 hr dark-light cycle was applied and the temperature was adjusted at 22-25°C. After a period of adaptation to the new environment and regular diet (AIN-93M), the rats were allocated to three groups of 15. Group 1 received a low-calcium diet (LC) (0.2% calcium W/W), while group 2 received a regular calcium diet (RC) (0.5% W/W) and group 3 received a high-calcium diet (HC) (1.2% W/W).

Other diet compositions are shown in Table 1. Fat, protein, fiber, ash and calcium content of the diets were measured; the results are presented in Table 2.

The vitamin mix was based on AIN-93M-VX.⁴² The Mineral mix - except for CaCO₃ in LC and HC diet - was also based on AIN-93M-MX,⁴² so that in the LC diet the mineral mix contained 140 g/kilogram calcium carbonate, while an extra 14.5 g/kilogram calcium carbonate was added to the RC diet to make the HC diet (Table 1).

The study duration was 10 weeks. Food intakes and spillages of animals were measured to the nearest 0.5 g every three days.

At the end of the study and after eight hours fasting, the rats were decapitated following anesthesia by ether. The trunk blood was drained in 1.5 ml tubes. After 15 minutes of centrifugation at 2000 g, serum samples were drained in 500 μ l microtubes and stored at -80°C immediately.

Ionized calcium concentrations of serum samples were determined by ionized calcium analyzer (Easylyte Na/K/Ca/pH, Medica Corporation).

Serum parathyroid hormone (PTH) and insulin were measured using radioimmunoassay kits (IBL kits).

Serum triglycerides (TG), total cholesterol (C), high-density lipoprotein cholesterol (HDL-C), and serum total calcium and serum glucose were measured by Hitachi 917 automated analyzer, using appropriate kits (Zistshimi Company, Tehran, Iran).

Low-density lipoprotein cholesterol (LDL-C) was computed with the Friedewald formula from total cholesterol ($C_{LDL} = C_{plasma} - C_{HDL} - TG/2.2$).⁴³⁻⁴⁶

The SPSS version 13.5 was used to analyze data. Data are expressed as mean \pm standard deviation. Statistical tests were conducted at the $P < 0.05$ alpha level. Between the groups, comparisons were carried out by one-way ANOVA.

Levene's test was used to test the homogeneity of variances assumption for ANOVA. Scheffe was used as post hoc test to show different groups if the homoscedacity assumption was met. Tamhane test was used to compare serum parathyroid hormone concentrations according to heteroscedacity.

Results

Our findings are shown in Figures 1 and 2 and Table 2. Food intake, as expressed per gram basal body weight [$F(2, 42) = 0.3, P = 0.7$], and also weight gain was similar in groups [$F(2, 42) = 0.26, P = 0.77$].

Serum total calcium was positively correlated with calcium intake [$F(2, 42) = 4, P = 0.026$], however, ionized serum calcium was not different among groups [$F(2, 40) = 1.7, P = 0.2$].

Serum parathyroid hormone was inversely correlated with calcium intake [$F(2, 42) = 10, P = 0.00035$].

Tamhane test showed that serum parathyroid hormone was significantly higher in the HC group compared with other groups ($P < 0.05$).

However, no significant difference in serum parathyroid hormone was observed between RC and LC groups ($P < 0.05$).

Serum Insulin, glucose, triglyceride and HDL-C were not significantly different among groups [$F(2, 42) = 1.37, P = 0.26$; $F(2, 42) = 0.28, P = 0.7$; $F(2, 42) = 1.7, P = 0.2$, respectively].

However, serum total cholesterol was significantly lower in the HC group compared with the LC group [$F(2, 42) = 3.4, P = 0.042$]. Moreover, LDL-C was significantly lower in the HC group compared with other groups [$F(2, 42), P = 0.02$].

TABLE 1. Compositions of diets used in three groups.

	LC, g/kg	RC, g/kg	HC, g/kg
Cornstarch	465.692	465.692	465.692
Casein ($\geq 85\%$ Protein)	140	140	140
Dextrinized corn starch	155	155	155
Sucrose (Finely powdered)	100	100	100
Soybean oil	40	40	40
Fiber (alpha cellulose)	50	50	50
Mineral mix	35	35	35
Vitamin mix	10	10	10
L-cysteine	1.8	1.8	1.8
Choline bitartrate (41.1% Ca)	2.5	2.5	2.5
TBHQ	0.008	0.008	0.008
Calcium carbonate	-	-	12.5

TABLE 2. Results of the analysis

	LC	RC	HC
Protein (%)	13	13	12.7
Fat (%)	4.0	4.0	3.9
Fiber (%)	5	5	5
Ash (%)	4.0	5.1	5.4
Carbohydrate (%)*	74.0	72.9	73.0
Metabolizable energy (Calorie density)/100g	38.3	37.9	37.8

*Carbohydrate content was calculated as: $100 - (\text{protein}\% + \text{fat}\% + \text{fiber}\% + \text{ash}\%)$.

Discussion

The results of this study support the hypothesis that dietary calcium may alter plasma lipid profile favorably and are consistent with previous studies that suggested that dietary calcium reduces serum cholesterol^{4,29,25, 32,33,41,45,47-50} and LDL-cholesterol.^{4,22-23} However, there are studies that did not show calcium to have any significant effect on serum cholesterol^{19,21,24,26,30,36,51-52} and some studies have showed that in different conditions, dietary calcium has different effects on serum/plasma cholesterol.^{34,38,40}

Vitale et al. reported that in low magnesium (Mg) concentrations (24 mg %), rats receiving more calcium (1200 mg %) showed higher levels of serum cholesterol compared with rats receiving less dietary calcium (600 mg %), but in the groups that had received a high-Mg diet (192 mg %), rats receiving more calcium showed lower levels of serum cholesterol.³⁴

Adding calcium to a saturated fat diet caused a decrease of 8 mg/dl in the serum cholesterol level ($P=0.03$) of rats, whereas no change in the serum

cholesterol level was observed when calcium was added to the polyunsaturated fat diet.³⁸ Diersen-Schade et al. did not show any hypocholesterolemic effect for dietary calcium, in the presence of tallow or soybean oil in goats.²⁶ However, Yacowitz et al. reported the hypocholesterolemic and hypotriglyceridemic effects of calcium with both saturated and unsaturated fats, and the effects were more pronounced with saturated fats.³²

We used the same amount of soybean oil as the source of fat in three experimental diets and in accordance with Yacowitz et al.,³² we showed that even with the low levels of fats, dietary calcium could affect serum cholesterol.

We found that serum triglycerides and HDL-C concentrations are not affected by dietary calcium. These findings are consistent with the previous studies that used animal models^{22,24,26,29,30,47} and human subjects.^{20,36,49,52,53-55} However, in some experiments a decrease was shown in serum/plasma triglyceride concentrations of groups receiving higher-calcium diets.^{28,31-33,41,56}

TABLE 3. Serum parameters concentrations in LC, RC and HC groups.

Parameter	group			
	LC	RC	HC	
Food intake (g)	1119±113	1118±134	1087±151	
Serum PTH (ng/l)	42.2±28.5	23.6±8.7	12.4±8.8*	
Serum cholesterol(mmol/l)	Total	1.86±0.26	1.73±0.23	1.65±0.14 ^a
	HDL-C	0.71±0.05	0.69±0.09	0.75±0.1
	LDL-C	0.79±0.3	0.66±0.2	0.53±0.2 ^a
TG (mmol/l)	0.79±0.36	0.85±0.31	0.82±0.30	
Glucose (mmol/l)	6.77±1.05	7.10±1.33	7.10±1.89	
Insulin (ng/ml)	1.9±1.4	2.2±1.4	2.7±1.3	
Fecal fat (g/100)	0.76±0.2	0.77±0.3	0.85±0.3	

*P<0.01 vs. the RC and the LC diet group; ^aP<0.05 vs. the LC diet group

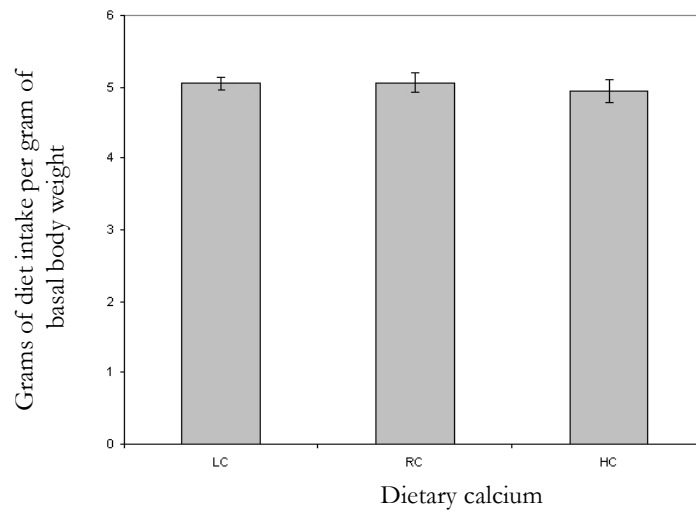


FIGURE 1. Food intake (g/basal body weight) in LC, RC and HC groups. Data are expressed as mean ± SEM (P>0.05), one-way ANOVA.

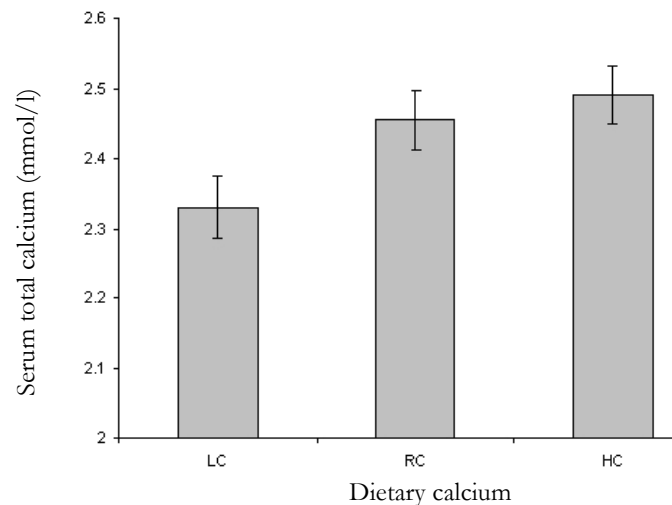


FIGURE 2. Serum total calcium concentrations in LC, RC and HC groups. Data are expressed as mean ± SEM (P>0.05), one-way ANOVA.

Mechanisms underlying the lipid-lowering effects of dietary calcium are not well understood. Previous studies have suggested that calcium interferes with cholesterol absorption and/or metabolism.^{5,32}

One proposed mechanism is the formation of insoluble calcium soaps of fatty acids and/or bile acids and thus reduction of fat absorption.^{32,33,53} It is now known that increasing saturated fatty acid intake increases serum total cholesterol and LDL-C.^{57,58}

Contrary to studies that used cholesterol-supplemented diets,^{4,31-34} we used cholesterol-free diets with unsaturated fat (4% fat) and thus we suggest that the cholesterol-reducing effects of calcium may be mediated by mechanisms other than cholesterol/fatty acid saponification such as bile acid saponification and/or change in endogenous metabolism of cholesterol.

As in the study of Zhang and Tordoff,³⁰ in our study serum concentration of insulin and glucose was not related to calcium concentrations of diets. Two studies^{59,60} showed that high-calcium diets significantly reduced fasting plasma insulin and glucose concentrations.

These two studies involved groups of male aP2-agouti mice fed high-sucrose, high-lard diets that differed in the amount and source of calcium (i.e., 0.4% Ca²⁺ from CaCO₃, 1.2% Ca²⁺ from CaCO₃, 1.2% Ca²⁺ from nonfat dry milk, or 2.4% Ca²⁺ from nonfat dry milk).

In conclusion, we suggest that increasing dietary calcium intake lowers circulating cholesterol and LDL-C in rats. However, the mechanisms whereby Ca may alter serum total cholesterol are not completely elucidated. Further studies are needed to explain the effects of dietary calcium on circulating triglycerides and glucose.

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