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Original Article

The Effects of Calcium Supplement on Serum Lipoprotein in Obese Adults Receiving Energy Restricted Diet

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Abstract

Background: Coronary heart disease is one of the most important problems of public health. One risk factor is dyslipidemia. Evidence from molecular and animal research and epidemiologic investigations indicate that calcium intake may influence lipid metabolism.But intervention studies have produced conflicting results.Objective of this study was to determine the effects of calcium supplementation on serum lipid profiles in the face of caloric restriction in obese adults.

Methods: A double blind randomized placebo-controlled trial on 40 adults with Body Mass Index (BMI)>25kg/m² was conducted in Iran University of Medical Sciences in 2006.Subjects were maintained for 24 weeks on a balanced deficit diet (-500 kcal/d deficit) and randomly assigned to two groups with 1000 mg ca/d as calcium carbonate, or placebo. A General Linear Model was used for the analyses.

Results: There were no significant differences in variables at the 12th and 24th week between the two groups (P> 0.015). But the total cholesterol, LDL–C decreased significantly at the 12th and 24th week in the two groups compared to the initial values (P< 0.05). Ratios of TC/HDL–C,TC/LDL–C and LDL–C/HDL–C decreased significantly at the 12th and 24th week compareddto baseline only in the calcium group (P< 0.05). Similarly, TG and VLDL–C decreased significantly at the 24th week compared to baseline only in the calcium group (P< 0.05).

Conclusions: Twenty four weeks of supplementation with 1000 mg ca/d did not have any effect on serum lipoprotein beyond what can be achieved in an energy restricted diet in obese adults.Therefore further investigations is a necessity.

Keywords: *Calcium supplement, Lipid profile ,Public health, Obesity, Energy restricted diet*

Introduction

According to the Katharina studie's, elevated cholesterol levels and dyslipoproteinemia are metabolic abnormalities that are becoming increasingly significant in industrialized countries, but also world wide (1). On the other hand, the prevalence of obesity has rapidly increased in the past 20 yr and has become a national and global epidemic (2), so WHO latest projections indicates that globally in 2005, at least 400 million adults were obese, and obesity and overweight lead to serious health consenquences. Raised body mass index is a major risk factor for chronic disease such as cardiovascular disease (mainly heart disease and stroke) (3).
There is a proportional increase in the risk of coronary heart disease with rising serum cholesterol levels (4, 5).

Atherosclerosis is the most common cause of coronary heart disease. Endothelial dysfunction initiates atherosclerosis. One of the factors that causes endothelial dysfunction is dyslipidemia (abnormality in any of the lipoprotein fractions), especially elevated LDLs and decreased HDLs (6).
A recently published observational study showed an inverse relationship between calcium intake and the plasma lipoprotein- lipid profile (7). However intervention studies have produced conflicting results (8-12).
Hence there is a demand for further investigation in the context of dietary calcium intake and serum lipid and lipoprotein concentrations. Saponification due to ionic binding of ca+² to fatty acids and bile acids in the gut are the mechanisms that lead to malabsorption of fat (10, 13). It was shown

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in humans that supplementation of the normal diet with large doses of calcium (2-4 g/d) resulted in increased fecal excretion of fat and saturated fatty acids (10, 14). A few studies showed the increasing dietary calcium accelerated weight and fat loss secondary to energy restriction (15, 16).
Thus, the present study was performed to further investigate the relationship between calcium supplement intake and plasma lipoprotein-lipid concentrations, secondary to energy-restriction diets producing an energy deficit of 500 k cal/d.

Materials and Methods

Study design

This study was designed as a placebo-controlled, double-blind trial to determine whether calcium supplementation accompanied with calcium carbonate would accelerate, decreasing the lipoprotein-lipid concentrations induced by caloric restriction in 40 obese adults.

Subjects

Subjects were recruited by placing advertisements in Iran University of Medical Science, Tehran.
One hundered overweight and obese adults were enrolled to participate. Of whom, 53 did not meet the inclusion criteria. The entry criteria was as follows: ages between 20 and 60 yr, Body Mass Index (BMI)>25 kg/m², and an agreement to follow the study diet and tablet intake as prescribed. Subjects were ineligible if they had a history of any of the following problems: endocrine disease except obesity, psychiatric problems, hepatic and renal disease, nephrolitiaze, diabetes, lactose intolerance, malignancy, rheumatic arthritis, respiratory problems, hypertension, cerebral vascular attack, or suffered any form of mal-absorption syndrome, hypo and hypercalcemia, coronary heart disease, hyper and hypo thyroidism, gastrointestinal disorders. The women who were pregnant or lactating were excluded. Finally, subjects were excluded if they were taking calcium supplements in the previous 6 months, used corticosteroid and anticonvulsant or lipid- lowering, oral contraceptive drugs, antiacids and alcohol and abused the drugs. Patients were excluded from participation if they utilized obesity pharmacotherapeutic agents and/or herbal preparations intended for any trial weight change. Of 100 persons enrolled, 47 subjects were eligible for the study and 7 were dropped. So 40 people were observed to complete the study. The study protocol was approved by the Ethics Committee of the Iran University of Medical Science.
Written consents were obtained from the volunteers after they had been informed about the exact nature of the study, including the purpose, the course and the potential risk of the study.

Diets

Subjects were provided with individual assessments of dietary intake by 24 h recalls, food frequency questionnaires, also they were provided with individual instruction and counseling by a registered dietitian (17). A diet was prescribed for every person, for 24 wk. Basal energy expenditure based on the Harris-Benedict equation was used to calculate basal metabolic rate, which was adjusted for activity level. Estimated 1.4-1.5 x BEE for those who engaged in mild daily activities and 1.6-1.7 x BEE for those engaged in strenuous daily activities (18,19). Based on this initial estimate of caloric needs, diet and a food exchange list was prescribed in order to produce a caloric deficit of ~500 kcal/d (Diets were individualized to achieve a 500 kcal/person/day deficit) and comparable levels of macronutrients (fats; 27.5%, carbohydrates; 52.5%, proteins 20% of total kilocalories).
Physical activities, exercise, tobacco and caffeine use were assessed by using questionnaires and maintained at a constant level (baseline level) throughout the study. After completion of baseline testing, participants were maintained on a balanced deficit diet (-500 kcal/d), matching for BMI, sex and age and were randomly assigned into the calcium and placebo groups for 24 wk.
Subjects received 1 g of elemental calcium daily as the carbonate or identical placebo which was produced from lactose, starch, poly vinyl pyrolidone and magnesium estearat.

They were asked to take two 500 mg tablets in the evening with dinner.
Compliance was assessed by interviewing subjects on a weekly basis, monthly FFQ, 24 h recalls, pill counts and a dietitian discussed problems with the subjects to enhance adherence of the diet.

Anthropometric measurement
Body weight and height were measured at baseline, at the 12th and 24th wk, by a qualified technician.
Body weight was measured with a floor scale which was accurate to 0.01kg, with subjects wearing street clothes without shoes, outerwear or accessories. Height was measured with a wall- mounted stadiometer which was accurate to 0.5 cm with subjects without shoes.
BMI was calculated using the standard equation (kilogram per meter squared).

Lipid measurement
Serum lipids were determined from a 6 ml blood sample, collected after the subjects had fasted overnight for at least 12 h at baseline, at the 12th and 24th wk (20).
The blood samples after collection, were immediately centrifuged, and the serum was stored at -8 °C (2). Total cholesterol and triacylglycerol concentrations were determined enzymatically using commerical kits (Parsazmun-Iran), as described elsewhere (20).
To assay the cholesterol, we used cholesterol oxidase. The HDL- C measurement was done by a direct non-precipitation method, using polyethylene glyco- modified enzymes (cholesterol oxidase) using commerical kit (Ziestchem Diagnostics- Iran) and triglycerides were assayed using a glycerol kinase/oxidase method, after initial hydrolysis using lipoprotein lipase. LDL–C and VLDL-C levels were calculated using the Friedwald formula.

Nutrient intake
Daily energy, macronutrient and micronutrient intakes were determined by using one per day 24 h recall and food frequency questionnaires, handed out per assessment period (baseline, the 12th and 24th wk).

Statistical methods
The effect of the supplementation was tested using the GLM (General Linear Model) procedure of repeated measurements. Differences were considered significant at $P< 0.015$.
All data was evaluated for normality of distribution before statistical analysis by Kolmogrov-Smirnov Test. Mann- Whitny non-parametric test used for camparing means of the variables that had not normal distribution, and used Wilcoxon Signed Rank Test for comparing the before and after supplementations within any groups. The Parametric test was used for those variables which were normaly distributed. The Student's- *t*-test was used for comparing means of the varibales between groups, the before and after supplementation and Paired t-test was used for comparing means of the variables within a group before and after supplementation.
Differences were considered significant at $P< 0.05$.
All tests were two- tailed and presented as mean ± SD .
Only subjects who completed the entire study (n= 40) were included in the data analysis.
Information about nutrient intake which were gathered by the 24 h recalls and by FFQ were subsequently coded, and the energy, macronutrient, and micronutrient contents of the diets were calculated by Food Proccessor Nutrition Systerm version II Software.
Data was analyzed using SPSS for Windows version 10 (SPSS. Inc, Chicago , IL).

Results
After baseline testing, 47 participants were randomly assigned, and 40 completed the 6- month protocol (85%).
There were no significant differences in any of the baseline parameters among the two groups. The treated groups were comparable in all indices at baseline. The characteristics of the study participants at baseline are shown in Table 1. As expected from the experimental design, all subjects lost body weight due to the daily energy deficit.

At the 12th wk, body weight (mean±SD) decreased by 2.65 kg ±0.13, at 24th wk 1.4 kg±0.3 in the calcium group and by 1.55kg±0.51, 1.5 kg±0.44 in those taking the placebo, so each of these changes were significant within the two groups (*P*= 0.000) but not between the groups.

The triglyceride and VLDL-C concentrations showed non significant differences between the 2 groups. Comparing of mean of serum triglyceride and VLDL-C concentrations in each group showed a significant reduction at the 12th and 24th wk related to baseline in calcium group (P12th= 0.003, P24th= 0.012) but in the placebo group a significant decrease was observed only in the 12th wk compared to baseline (*P*= 0.043) (Fig. 1).

At the end of the study there were no significant differences in the total cholesterol and LDL–C concentrations between the two groups (TC, *P*= 0.331 LDL–C, *P*= 0.220) but the mean of total and LDL–C concentrations showed significant differences at the 12th and 24th week compared to the initial values in the two groups (TC, *P*= 0.01 to *P*= 0.000) (LDL–C, *P*= 0.042 to *P*= 0.000) (Fig. 2).

The HDL- cholesterol concentration did not have a significant difference between and within (*P*= 0.91 to 0.089) the two groups in any stage of the study (Fig. 3) .

No significant difference were observed for ratios of TC/HDL–C (*P*= 0.886), TC/LDL–C (*P*= 0.886), TG/HDL–C (*P*= 0.167), LDL–C/HDL–C (*P*= 0.555) and HDL–C/LDL–C (*P*= 0.559) between the two groups. The ratios of TC/HDL–C and TC/LDL–C (P12th= 0.004, P24th= 0.011), LDL–C/HDL–C (P12th= 0.009, P24th= 0.032) were significantly decreased only in the calcium group at the 12th and 24th week compared to the beginning (Table 2).

The ratio of TG/HDL–C was significantly decreased at the 12th wk compared to the initial value only in the calcium group (*P*= 0.028) (Table 2).

Diet data for the 40 subjects who completed the study are given in Table 3. There was no significant difference in the calorie and the other macro and micronutrient intakes during the study, among the two groups , at baseline, at the 12th and 24th week (Table 3).

There was no significant difference in the physical activities (Fisher, *P*= 1), exercise (Fisher, *P*= 0.051), tobacco (χ^2, *P*= 1) and caffeine (Fisher, *P*= 0.532) use between the two groups.

Table 1: Baseline characteristics of the subjects

Variable	Calcium group (n=20)	Placebo group (n=20)	*P* Value
Age (year)[1]	36.6 ± 7.8	36.6 ± 8	1
Sex (male,female)[2]	4,16	2 , 18	0.661
BMI (kg/m²)[3]	29.7 ± 3.9	29.8 ± 3.3	0.977
Total cholesterol (mg/dl) [3]	186.2 ± 32.6	193.4 ± 37.6	0.522
LDL cholesterol (mg/dl) [3]	113.7 ± 26.9	121.1 ± 32.4	0.437
HDL cholesterol (mg/dl) [3]	43.4 ± 9.1	46.5 ± 9.4	0.289
Triglycerids (mg/dl) [3]	143.9 ± 38.3	128.3 ± 34.9	0.188
VLDL cholesterol (mg/dl) [3]	28.8 ± 7.7	25.7 ± 7	0.188

All values are mean ± SD.

[1]-There were no significant differences between two groups at baseline (Independent t- test).

[2]- There were no significant differences between two groups at baseline (χ^2).

[3]-There were no significant differences between two groups at baseline (Kolmogrov- Smirnov, Mann- Whitny, Student's- t- Test).

Table 2: Effects of calcium supplementation on ratios of lipid levels

Variable	Calcium group (n=20)			Placebo group (n=20)			*P* value
	Baseline	12th wk	24th wk	Baseline	12th wk	24th wk	
TC / HDL-C	4.39 ± 0.92	3.89 ± 0.75	4.01 ± 0.86	4.29 ± 1.08	4.02 ± 0.99	4.09 ± 0.68	0.886
TC / LDL –C	1.67 ± 0.19	1.73± 0.20	1.74 ± 0.26	1.63 ± 0.17	1.67 ± 0.16	1.62 ± 0.11	0.886
TG / LDL –C	3.45± 1.24	2.91 ± 1.19	3.10± 1.08	2.83 ± 0.83	2.73 ± 1.26	2.65 ± 1.07	0.167
LDL – C / HDL –C	2.69 ± 0.76	2.31 ± 0.60	2.40 ± 0.76	2.72 ± 0.95	2.47 ± 0.78	2.56 ± 0.52	0.555
HDL –C / LDL – C	0.41± 0.15	0.47 ± 0.16	0.47 ± 0.17	0.41 ± 0.14	0.45 ± 0.15	0.41± 0.10	0.559

All values are mean ± SD.

There were no significant differences between groups in 3 stage of study (general linear model).

TC = Total cholesterol, HDL– C= High density lipoprotein, LDL- C= Low density lipoprotein, TG = Triglycerides

Table 3: Diet characteristics

Nutrient	Baseline		12 week		24 week		*P* Value
	Calcium	Placebo	Calcium	Placebo	Calcium	Placebo	
Energy(kcal/d)	1898.10± 1160.56	1617.75 ± 730.90	1329.05 ± 488.90	1146.15 ± 420.29	1151.00 ± 457.40	1130.25±436.10	*P* > 0.05
Protein(g/d)	57.11±32.76	62.24 ± 38.30	50.23 ± 24.40	48.80 ± 25.72	42.68±19.03	52.60±29.22	*P* > 0.05
Charbohydrate (g/d)	235.86±124.28	173.38± 86.40	144.25±58.61	143.40±45.45	143.46± 64.55	147.82±42.60	*P* > 0.05
Total Fat (g/d)	84.56±73.69	76.54 ± 42.73	58.75±33.80	47.91±25.07	46.96±18.37	50.59±31.98	*P* > 0.05
Saturated Fatty Acid(g/d)	20.54±16.72	19.62±10.31	50.19±8.65	50.18 ± 150.49	14.19±6.14	15.67±10.24	*P* > 0.05
MonoUnsaturated Fatty Acid(g/d)	25.74±22.79	22.35±11.68	25.95±14.42	23.52±10.35	16.39±9.13	17.36±7.46	*P* > 0.05
Poly Unsaturated Fatty Acid(g/d)	33.25±40.14	40.29±54.15	11.74±10.55	12.99±10.45	12.54±6.22	15.18±23.38	*P* > 0.05
Cholesterol(g/d)	180.69±187.32	227.38±196.22	241.71±198.37	159.85±128.12	195.21±197.72	125.30± 82.11	*P* > 0.05
Zink(mg/d)	8.56±5.62	7.95±5.17	7.22±4.37	6.91±3.50	6.26±3.27	7.76±3.19	*P* > 0.05
Iron(mg/d)	13.34± 6.83	11.02±4.97	10.61±4.55	8.54±2.50	9.29±4.29	10.96±5.70	*P* > 0.05
Calcium intake(mg/d)	396.24±479.50	394.96±695.10	308.73± 620.70	416.19±588.21	257.24±547.25	581.61±806.15	*P* > 0.05

Values are mean ± SD .

There were no significant differences between groups in 3 stage of study (mann- whitny , independent –*t* – test).

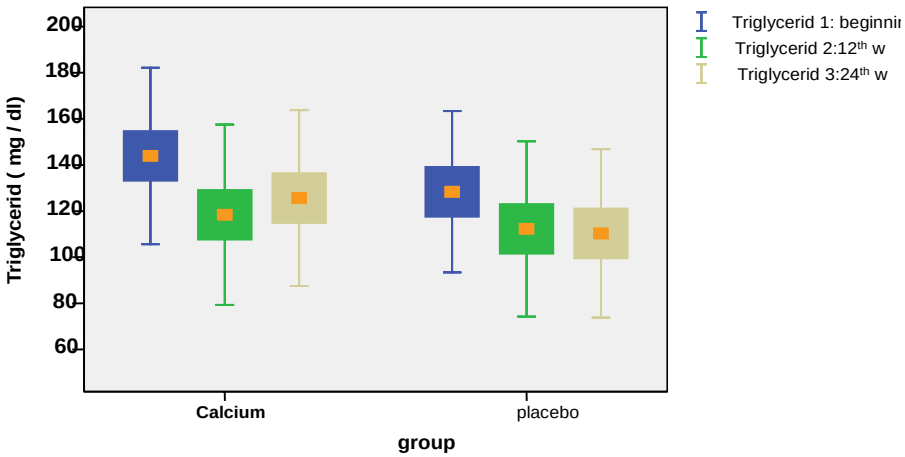

Fig. 1: Effects of calcium carbonate or placebo on plasma triglycerid . Data are the mean±1 SD. There were no significant differences between groups(GLM) (*P*> 0.015)

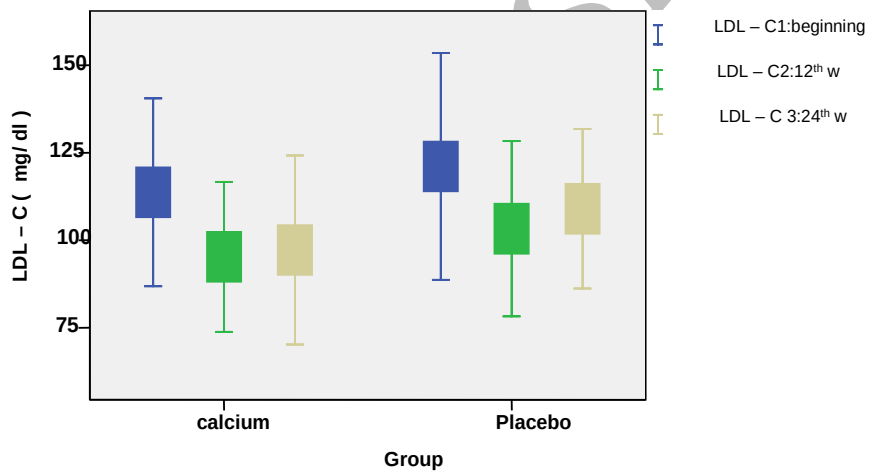

Fig. 2: Effects of calcium carbonate or placebo on plasma LDL-C. Data are the mean±1SD . There were no significant differences between groups(GLM) (*P*> 0.015)

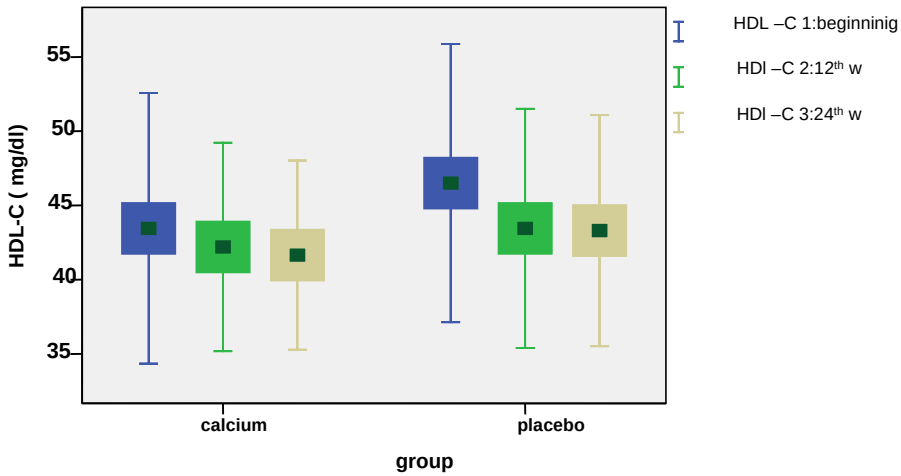

Fig. 3: Effects of calcium carbonate or placebo on plasma HDL-C. Data are the mean ±1 SD . There were no significant differences between groups (GLM) (*P*> 0.015)

Discussion

While so far a few longitudinal clinical studies have been done to examine the association between the calcium supplement (as the carbonate) intake and the plasma lipid- lipoprotein concentrations, in the face of calorie restrictions in obese adults, the other published studies in this area, revealed conflicting findings. At the same time, obesity, high plasma lipid concentrations and cardiovascular disease in the obese are increasing. This research is a contribution to the above mentioned studies. Although the calcium hypolipidemic effects as a predicative factor of lower risk of coronary heart disease, compared with the low calcium intake observed in many studies (7, 9, 10, 12, 13). We found no significant difference in blood lipid profile after 6 month calcium supplementation between the two groups participated.

In one study, researchers assigned 193 adults to calcium carbonate, or placebo for 4 months (12). They found no significant effect on any lipid parameters, which supports our finding. That is probably because the subjects in their study were heterogenous in age, sex and baseline lipid concentrations. But in the present study subjects are homogenous in age, sex and baseline serum lipid concentrations, and intake macronutrients and micronutrients. Also they had a bigger sample size, comparing with our study.

Zemel et al. (15) in a randomized placebo- controlled trial in healthy obese adults, after 24 wk observed the results were consistent with our sutdy. In another study (21) they, placed obese subjects on a balanced deficit (-500 kcal/d) diet and randomized them to control or yogurt treatment. They observed that the participants on the yogurt, exhibited a significant increase in circulating glycerol. Although, duration of our study was longer than Zemel studies (15, 21), the results of the two studies, except for circulating glycerol were identical, possibly because the yogurt caused an increase in lipolysis, more than the calcium supplement.

In a one year calcium supplementation as the calcium citrate in postmenopausal women, researchers observed that the HDL-C level and the HDL-C/LDL-C ratio increased more in the calcium group than in the placebo group(22) .

In CARDIA study, they found no association between dairy intake and incidence of high LDL-C (23). In an observational study, habitual intake of meat, fish, bread and dairy products were assessed. Dairy products intake in men was inversly associated with serum triglycerides, HDL-C after adjustment for age, energy intake and waist to hip ratio (24).

In another study researchers divided participants into three groups on their daily calcium basis. They observed that the lipoprotein-lipid profile receiving from daily dietary calcium intake, regardless of adiposity is inversly correlated with LDL-C, tatal cholesterol, TC/HDL-C concentrations. They also found the ratio of TC/HDL-C was significantly greater in women who consumed lower amounts of calcium (7).

Through a randomized, single- blind study in healthy men with moderate hypercholesterolemia with calcium citrate malate, was observed total and LDL-C ($P< 0.05$) in the high calcium diet reduced significantly, but they found no change in HDL-C (10) .

A double blind crossover study showed whether supplementation of chocolate, reduced plasma LDL-C by 15% ($P< 0.02$), decreased the total cholesterol concentration according to LDL–C and HDL-C, but did not changed triacylglycerols significantly during either of the experimental periods (25).

Another researcher, conducted a palcebo-controlled, double- blind, crossover study with calcium supplementation in young healthy volunteers. They found, serum cholesterol concentrations after 4 wk of supplementation were lower than after 4 wk of placebo ($P= 0.008$), LDL-C concentration and the ratio of LDL-C/HDL-C also tended to be lower after Calcium phosphate suppplementation than after placebo ($P= 0.083$) (26).

In two randomized trial in obese adults, investigators observed that in the weight maintenance phase, subjects on the high dairy diet exhibited a

significant increase in circulating glycerol ($P< 0.01$), whereas there was no significant change observed in the low dairy group. But in the weight loss phase, circulating glycerol was increased significantly in both groups, and there was a significantly greater increase in the high dairy group ($P< 0.01$) (27) .

These outcomes are consistent with their previous observations of the antilipolytic effects of 1, 25-dihydroxyvitamin D in human adipocytes (28-30).

Although the design of our study and Zemel´s are alike, the results of Zemel´s is in contrast to our study observed that the circulating glycerol was increased significantly in the high dairy group, but that consists of other circulating lipid concentrations. On the other hand the Zemel´s findings about other circulating lipid concentrations, supports our findings.

The effects of calcium supplementation on lipid concentrations are likely resulted in calcium binding to fatty acids and bile acids in the gut, thus interfering with lipid absorption. However, the other mechanisms may also be involved. There is evidence that parathyroid hormone and 1, 25-dihydroxyvitamin D regulates adipocyte activity. High concentrations of parathyroid hormone reduce lipolysis in vitro (29, 31). Calcium supplementation suppresses circulating concentrations of parathyroid hormone and 1,25- dihydroxyvitamin D, thereby possibly promoting lipolysis. Studies in transgenic mice have shown that calcium supplementation can increase lipolysis and body temperature, as well as reduce fatty acid synthase activity and body weight (29), implying an effect of calcium intake on thermogenesis. Such effects could contribute to the changes in serum cholesterol levels and its fractions (29, 32). Complexing of lipids in the gut by calcium could also contribute to these effects.

It appears that an increase in dietary calcium intake results in a decrease in $[ca^{2+}]$ i, which in turn increases lipolysis (29).

There are several possible explanations for the above-mentioned discrepancies between these studies and the present data, which could be related to the different duration of different studies or to the kind and the bioavilability of calcium supplement. Examples from two studies (10, 26) show that the elevated cholesterol level might be more susceptible to an intervention with lipid- lowering agante than cholesterol concentrations in normal range. But as in Ditscheid ´s study, the total cholesterol, LDL-C and LDL–C/HDL–C ratio of the healthy subjects were decreased (26).

The above-mentioned discrepancies between different studies, may possibly be related to the different amounts of calcium supplement prescribed in different groups, which participated in the reviewed studies in this paper, or can be related to the period of supplementation, or both, which all can be evaluated as the important factors.

It is not clear how these factors contribute to the different studies, which lead to different results. Following this question is suggested to be considered in further research plans (33).

Our major findings reveal that a calorie restricted diet with intake of 1g/d calcium supplementation as the carbonate, for 24 wk does not significantly lead to alteration in lipoprotein lipid concentrations. We found the lipid index ratio of ischemic heart disease between two groups were not different significantly beyond what can be achieved in a behavioral weight loss intervention.

The strenghts of this study include: a six month follow up, a low drop- out rate (15%), and a randomized double- blind control trial design. Also different from the other studies, these trial subjects were on a calorie restricted approach. And the weakness of our study was the lack of apoliprotein measurement.

But more research is needed to establish whether there is a casual association between calcium intake and plasma lipoprotein- lipid concentrations. There is consistent evidence from randomized controlled trials that calcium supplementation slows postmenopausal bone loss, and there is also some evidence that it prevents fractures in postmenopausal women. Other benefits from the use of calcium supplements have been suggested, including effects on colon cancer, and blood pressure (22). There is also observational evidence that calcium intake is inversely associated with car-

diovascular disease (34). According to the World Health Organization, Cardiovascular disease is the number one cause of death globaly and is projected to remain the leading cause of death. An estimated 17.5 million people died from cardiovascular disease in 2005, representing 30% of all global deaths (35).

In conclusion, if established that calcium intake causes decrease in the blood lipid profile, the use of the calcium supplement or dairy intake leads to prevention of several diseases and public health problems for example cardiovascular diseaes and osteoporosis.

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The authors declare that they have no conflicts of interest.

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