ORIGINAL ARTICLE

Effect of Oral Calcium Supplementation on Lipid Profile and Atherogenic Index of Plasma

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Abstract

Objective. To assess the effect of oral calcium supplementation on lipid profile and atherogenic index of plasma (AIP).

Methodology. This study was undertaken in 28 centrally obese male subjects [age 26.4 (6.5) years], BMI 31.6 (4.7) kg/m^2, WC 99.4 (6.4) cm. All participants received six tablets of $CaCO_3$ (250 mg of elemental calcium/ capsule, for a total of 1500 md/day) for 8 weeks. Serum lipid profile including triglyceride, total cholesterol, HDL-C, LDL- C was measured at baseline and after intervention. AIP was calculated by using formula = log (TG/HDL-C).

Results. Oral calcium supplementation achieved a 22% (36 mg/dL, p<0.001) reduction in Triglyceride from baseline [163.4 (37.9) mg/dL] and 19.2% (5.8 mg/dL, p<0.001) increase in HDL-C from baseline [30.4 (7.4) mg/dL]. There were no significant treatment effects on total cholesterol [217.1 (41.21) mg/dL vs 196.3 (46.2) mg/dL] and LDL-C [155.4 (45.1) mg/dL vs 136.3 (45.1) mg/dL]. Significant reduction in serum total cholesterol and LDL-C level was only found in dyslipidemic centrally obese subjects. AIP decreased significantly by 51% with calcium carbonate treatment [median and interquartile range; 0.35 (0.29-0.44) vs 0.17 (0.04-0.44), p<0.01].

Conclusion. Eight-week calcium supplementation at 1500 mg/day led to a significant change in lipid levels and AIP.

Keywords: calcium supplementation, lipid profile, atherogenic index of plasma (AIP)

INTRODUCTION

In developed and developing countries, major risks or causative factors for cardiovascular diseases are elevated blood pressure, lipid profile and obesity.[1] Reduction of these risk factors has been one of the most important fields of medical research. Lifestyle changes, dietary habits and supplements to modify adiposity and hyperlipidemia have become popular as these are simple, less expensive and effective measures to reduce the cardiovascular risks.

Calcium supplementation is widely recommended to postmenopausal women for the prevention of osteoporosis. It has been established over one to two decades that dietary calcium can interact directly with lipid absorption, and can bind to bile acids and increase their excretion and lower serum cholesterol when they are consumed in doses more than the intestine can absorb.[2,3] Lately, a growing body of evidence suggests that calcium supplements also have beneficial effects on insulin sensitivity, blood pressure, body weight and serum lipids.[4-12] In a study by Jacqmain, low daily calcium intake is associated with greater adiposity in women and lipoprotein concentration in both sexes.[4] Some studies have shown that calcium supplementation enhanced weight loss and reduced serum lipid profile in obese subjects.[5,6] On the contrary, significant effect of calcium supplementation on serum lipid or body composition was not seen in healthy older men[7] and normal older postmenopausal women.[8] Some randomized controlled studies demonstrated that calcium supplementation has the ability to lower serum cholesterol level in hypercholesterolemic subjects[9-11] and hyperlipidemic patients.[12,13] However, these studies did not ascribe the beneficial effect of calcium on triglycerides. The results are still unsatisfactory with limited evidence to draw definite conclusions on the effect of calcium supplementation on lipid profile.

Dyslipidemia is commonly associated with an abnormal lipoprotein phenotype which is characterized by increased TG, decreased HDL-C and an accumulation of small dense LDL-C particles even when the levels of LDL-C are often normal. Oxidized LDL-C is taken up by immune system cells which transform to foam cells. These foam cells are trapped in the wall of the blood vessels and contribute to the formation of atherosclerotic plaques that cause arterial narrowing and lead to heart diseases.[14] Serum esterase is associated with HDL-C and destroys the oxidized LDL-C. Therefore HDL-C has ability to protect against heart

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disease. Either the ratio of LDL-c/HDL-c or TC/HDL-c is used as the best predictor of cardiovascular risk.[15] The logarithmically transformed TG/HDL-C ratio is a more accurate predictor of cardiovascular risk than other previously used lipid parameters.[16] Furthermore, in situations where other atherogenic risk parameters appear normal, AIP may be the diagnostic alternative.[17] AIP values increased with increasing cardiovascular risk. AIP value is less than 0.1 in young children and increases up to 0.4 in men and subjects with cardiovascular risk factors such as hypertension, diabetes and dyslipidemia. It has been suggested that AIP values of less than 0.1 are associated with low risk, 0.1 to 0.24 with medium risk and above 0.24 with high CV risk.[18] Khazaál reported that AIP is an index of highest sensitivity for predicting acute coronary events. It is a better screening tool for evaluating the cardiac risk and a monitoring index for any lipid lowering intervention.[19]

While abdominal obesity is more common in women than in men, the risk of cardiovascular diseases is greater in obese men than in obese women.[20] This study has focused on men as cardiovascular disease is a major source of morbidity in males. Therefore, this study intended to find out the effect of oral calcium supplementation on lipid profile and atherogenic index of plasma in centrally obese male subjects.

METHODOLOGY

Apparently healthy centrally obese male subjects (WC > 90 cm), aged between 18-35 years without family history of diabetes mellitus and hypertension (blood pressure >140/90 mmHg), history of regular exercise, chronic smoking and alcohol drinking were recruited to this study. No one was taking medications such as calcium supplements, non-steroidal anti-inflammatory, antihypertensive medications, lipid-lowering drugs, diuretics, or any other drug that affects the lipid metabolism for at least 6 months.

They were instructed to take six $CaCO_3$ capsules daily (each capsule contains 250 mg of elemental calcium, Medicine Supply Co. Ltd., Thailand) for 8 weeks. In the present study, dose of elemental calcium to be given and duration of study period was determined according to previous studies indicating the beneficial effect of oral elemental calcium supplementation on insulin sensitivity in 20 hypertensive patients[21] and in 31 diabetic patients[22] since there was an association between dyslipidemia, obesity and insulin resistance.[23,24]

After a 10-hour fast, morning venous blood sample (10 ml) and morning spot urine sample were taken before and after the 8-week supplementation period. Throughout the study period, they were allowed to take regular diet and also instructed to maintain their dietary patterns, dietary habits and usual sources of diet. Compliance was assessed by biweekly visit and review of the diet diary and physical

activity diaries and tablet counts. Anthropometric data such as BMI and waist circumference, dietary assessment and physical activity assessment were done at the start, visit 2 (4th week of supplementation period) and at the end of supplementation period (8th week of supplementation period).

Total cholesterol (TC) and triglycerides (TG) were assayed by enzymatic colorimetric tests with cholesterol esterase cholesterol oxidase and glycerol phosphate oxidase. High-density lipoprotein cholesterol (HDL-C) was measured after precipitation of the apo-lipoprotein B-containing lipoproteins with phosphotungstic acid. Low-density lipoprotein cholesterol (LDL-C) was calculated from serum TC, TG and HDL-C using the Friedewald formula.[25] Atherogenic index was calculated by using formula = log (TG/HDL-C).[16]

Serum total calcium level was measured by O-Cresolphthaleine-Complexone method. Serum total protein level was measured by Biuret method. Serum albumin level was measured by Bromocresol Green method. Serum ionized calcium level was calculated by using the following formula.[26]

Ionized calcium (mg/dl) = [6Ca − (K/3)] /(K + 6)
Ca = Total calcium (mg/dl)
K = (0.19 x P) + A,
P = Total Protein (g/dl),
A = Albumin (g/dl)]

Serum PTH level was measured by enzyme-linked immunoassay (ELISA). Physical activities assessment was carried out by questionnaire assessment procedure.[27]

Urine creatinine level was measured by Jaffe-Reaction and urine calcium level was measured by O-Cresolphthaleine-Complexone method. Urine calcium excretion was expressed as urine calcium and urine creatinine ratio.

All continuous baseline characteristics, and laboratory parameters were expressed as mean (SD). The changes in parameters before and after calcium supplementation were analyzed by using a paired student's "t" test. Skewed data were expressed as median and interquartile ranges and they were computed by non-parametric tests, Mann-Whitney Signed-Rank test. Differences were considered significant when $p < 0.05$.

RESULTS

A total of 35 subjects participated in this study. Of these, four were dropped out due to incomplete data, three subjects who did not follow the instructions were also excluded from the study. Only 28 subjects remained in this study. Table 1 shows the baseline characteristics of subjects who participated in this study. Body weight, WC and measurement of physical activity was done at the beginning, at the 4th week and at the end of the 8th week of

the study period. Although there was a slight decrease in body weight and WC (0.03% and 2% respectively) at the 8th week of calcium supplementation, it was statistically but not clinically significant. (Table 2). Physical activity was the same during the experimental period.

Table 1. Baseline characteristics of the centrally obese male subjects, mean (SD)

Parameters	Centrally obese subjects (n=28)
Age (yr)	26.4 (6.5)
Weight (kg)	89.6 (15.6)
Height (cm)	168.0 (5.8)
BMI (kg/m^2)	31.6 (4.7)
Waist circumference (cm)	99.4 (6.4)
Hip circumference (cm)	106.0 (10.1)
Waist Hip Ratio	0.98 (0.01)
Resting SBP (mmHg)	118.6 (8.0)
Resting DBP(mmHg)	78.8 (7.9)
Resting MAP (mmHg)	92.1 (7.6)
Heart Rate (beats/min)	74.8 (7.2)
Physical activity (kcal/day)	2817.8 (531.9)

There was no significant change in serum total and ionized calcium before and after calcium supplementation [8.8 (1.3) vs 8.1 (1.1) and 4.2 (1.3) vs 3.9 (1.6) mg/dL]. However, urine calcium excretion (Ca^{2+}/Cr ratio) significantly increased (p<0.01) after calcium supplementation [0.06 (0.03) vs 0.09 (0.04)]. Serum PTH level decreased significantly (p<0.01) from 33.9 (18.6) to 23.4 (9.7) pg/ml after calcium supplementation.

Lipid profile of the subjects is shown in Table 2. HDL-C significantly rose and TG significantly fell with calcium supplementation. There was a reduction in serum TC and LDL-C levels after calcium supplementation, but the changes were not statistically significant. Then, the subjects were subdivided into 2 groups according to the normal serum lipid level. Significant reduction in serum TC and LDL-C levels was found in dyslipidemic centrally obese subjects, but no changes were found in those with normal lipid profile.

Figure 1 showed that AIP decreased significantly by 51% with calcium carbonate treatment [median and interquartile range; 0.35 (0.29-0.44) vs 0.17 (0.04-0.44) (p<0.01)].
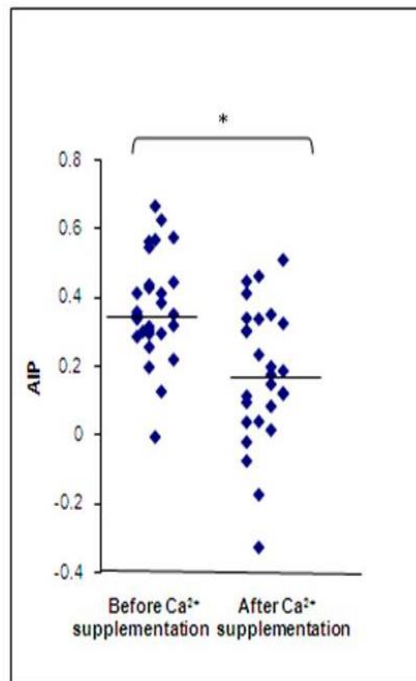

Figure 1. AIP in the centrally obese subjects before and after Ca^{2+} supplementation
Solid line indicates mean values.
*indicates p<0.01
NB: Comparison was done by using non-parametric test (Mann-Whitney Signed-rank test).

Table 2. Body weight, waist circumference and physical activity at different intervals during the study, mean (SD)

Parameters (n=28)	Beginning	4th week	8th week
Weight (kg)	89.6 (15.9)	89.2 (15.8)*	89.1 (15.8)*
WC (cm)	8.28 (1.06)	97.9 (6.0)*	97.4 (6.0) *
Physical activity (kcal/day)	2817.8 (540.4)	2812.5 (522.8)	2807.3 (531.2)

Data are mean (SD).
* indicates p<0.01 (4th week vs beginning and 8th week vs beginning)

Table 3. Serum lipid profiles in centrally obese subjects before and after calcium supplementation

Parameters		Before	After	Changes
TC (mg/dL) (n=28)	mean (SD)	217.1(41.21)	196.3(46.2)	20.80 (60.61)
< 200 mg/dL (n=12)	median and IQ	178(168.9-195.1)	189.7(150-215.7)	3.4(-52-26.6)
> 200 mg/dL (n= 16)		244.6(213,7-277.3)	182(165.7-220.6)	52.4(32.6-79.4)*
TG (mg/dL) (n=28)	mean (SD)	163.4 (37.9)	127.4 (40.2)	35.94 (54.80)***
< 150 mg/dL (n=11)	median and IQ	129(112.4-134.9)	118(86-158.6)	6.3(-14-48.9)
> 150 mg/dL (n= 17)		191.7(166.3-200)	128(107.3-143.2)	53(34.3-84.4)**
HDL-C (mg/dL) (n=28)	mean (SD)	30.4 (7.4)	36.3 (8.9)	-5.84 (12.15)***
> 40 mg/dL (n=2)				
< 40 mg/dL (n=26)	median and IQ	29.1(25.3-31.5)	37.6(29-42.4)	-6.1(-15.4-1.1)**
LDL-C (mg/dL)(n=28)	mean (SD)	155.35 (45.11)	136.34 (44.97)	19 (60.1)
< 130 mg/dL (n=11)	median and IQ	110.8(106.8-121)	137.9(85.76-166.7)	-27.6(-58.8-24.2)
> 130 mg/dL (n= 17)		186(155.9-208.9)	125.8(104.3-158.8)	55.4(18.5-72.1)**

* indicates p<0.05, ** indicates p< 0.01, *** indicates p< 0.001
median and IQ: median and interquartile range

DISCUSSION

The US Institute of Medicine Food and Nutrition Board currently designates a tolerable calcium carbonate upper intake level of 2500 mg/d for adults 19–50 years of age as being free of adverse health effects for nearly all persons in the general population.[28] In this study, the subjects were allowed to take regular diet with supplementation with $CaCO_3$ 1500mg/day, this dose would not pose any health risk.

After calcium supplementation, overall reduction of serum triglyceride level was 22% (n=28). Those with high triglyceride level (>150mg/dL; n=17) exhibited more dramatic change, with a median decrease of 53 mg/dL (27.6%) whereas there was no significant treatment effect in those with normal triglyceride level (n=11). Yacowitz et al., reported that there was a mean decrease of 56.3 mg/dL in serum triglyceride level in 11 hypertriglyceridaemic male subjects with calcium carbonate supplementation (0.89 g) for 21 days.[11] Reid et al., reported no significant effect on triglyceride in 108 healthy older men with 1200 mg elemental calcium supplementation for 2 years.[7] In their study, participants consumed a regular-fat diet (about 28% of total calorie intake) and baseline level of triglyceride was 129.21 mg/dL (69.03). Although the duration of their study was much longer than ours, their result is quite different. The reason may probably be the baseline triglyceride level of the subjects, their study included both normo- and hypertriglyceridaemia.

Contrary to the present study which used 1500 mg/day of calcium carbonate for 8 weeks, Shidfar et al., reported that there was no significant treatment effect on the serum triglycerides in 49 overweight hypertriglyceridaemic male subjects [baseline value 238.7 (81.2) mg/dL] who were treated with 1250 mg/day of calcium for 8 weeks.[13]

Denke et al.,(1993) also reported a non-significant change in TG in 13 healthy men with moderate hypercholesterolemia by high dose (2200 mg per day) of calcium supplementation for 10 day period.[10] It could be assumed that the serum lipid level before the trial and the dose and duration of calcium supplementation might be probable reasons for the beneficial effect of calcium on lipid profile.

In the present study, 16 subjects with the highest TC level (>200 mg/dL) showed a 21.4% decrease in serum cholesterol level with a median decrease of 52.4 mg/dL. Ten of the 16 hypercholesterolaemic subjects had normal cholesterol level (<200 mg/dL) after 8-weeks calcium supplementation. Denke et al.,[10] Yocowitz et al.,[11] and Shidfar et al.,[13] also reported reduction in TC with calcium supplementation. However, amount reduction (52.4 mg/dL) was a bit higher in the present study when compared with these previous studies; i.e., 6%, 23.6 mg/dL and 28.3 mg/dL respectively. Reid et al., reported that there was no significant change in serum total cholesterol

level after intervention because participants had lower cholesterol before the trial.[7,8]

The significant treatment effect of calcium supplementation on HDL-C and LDL-C was also seen in the present study. However, this treatment effect on LDL-C was only seen in subjects with abnormal lipid level but not seen in the subjects with normal lipid concentration. A 29.8% decrease in LDL-C with median decrease of 55.4 mg/dL was seen in 17 subjects with high LDL-C level (>130 mg/dL). Among them, only 7 subjects fell within normal range (60 to 130 mg/dL) after calcium supplementation. HDL-C increased significantly from 30.4 (7.4) mg/dL to 36.3 (8.9) mg/dL (p<0.001) with 19.2% (5.84 mg/dL, p<0.001) increase from baseline. Abnormal low HDL-C level (baseline value <40 mg/dL) was observed in 26 of the 28 subjects and there are just 10 subjects who reached the normal HDL-C level by oral calcium supplementation.

In the present study, median and interquartile range of AIP was 0.35 (0.29-0.44), predicting a higher risk for cardiovascular disease. The study done by Ceska et al., reported that AIP values of less than 0.1 are associated with low risk, 0.1 to 0.24 with medium risk and above 0.24 with high CV risk.[18] The present study also found that AIP decreased significantly by 51% with calcium carbonate treatment. Previous animal studies also reported a protective effect of supplemental calcium against the atherogenic process in young goats.[29] Moreover, supplementation of calcium together with an atherogenic diet has been shown to inhibit atherosclerosis in rabbits.[30]

Atherogenic index of plasma (AIP) is the new marker of atherogenicity, because it is directly related to the atherosclerosis risk. Hypertriglyceridemia increases the activity of hepatic lipase (HL) which results in the increase of HDL-C catabolism (degradation of HDL-C). Each degradation of 1mg HDL-C will correlate with a 2% increase in the risk of coronary heart disease (CHD).[31,16] Some epidemiological studies found a significant inverse correlation between water hardness and mortality from CVD for both males and females.[32] Mortality rate of acute myocardial infarction was 34% lower in the areas supplied with water containing more calcium (>70 mg/L) as compared to those living in an area where the drinking water calcium level was <31 mg/L.

In this study, dietary and physical activity records were taken every fortnight to ensure that all subjects kept their calorie intake as well as physical activity relatively constant for the whole study period. They exhibited only a slight change in body weight (mean difference in BW: 0.5 kg and percent change: 0.03% of the initial weight) and fat loss (mean difference in WC: 2 cm and percent change: 2% of the initial WC). Fat loss (2%) was not clinically significant, even though it was statistically significant.

Both serum total calcium and serum free ionized Ca^{2+} were not modified after calcium supplementation. However, oral calcium supplementation increased urine calcium excretion, expressed as Ca^{2+}/Cr ratio, indicating that calcium intake is high because 10% of ingested calcium is normally excreted in the urine. In this study, it was also noted that serum PTH level significantly decreased after calcium supplementation. This finding was consistent with the studies of Kynast-Gales and Massey (1992),[33] Levey et al.,[34] and Sanchez et al.[21] They showed that an increase in dietary calcium reduces the serum PTH concentration. The observed decrease in serum PTH, increased urine calcium excretion and normal serum calcium level after intervention also support that Ca^{2+} homeostasis seems to be maintained at the expense of serum PTH level in centrally obese subjects before calcium supplementation. Centrally obese individuals who participated in this study might not be meeting the average requirement for calcium in their diet.

Calcium has the ability to modulate energy metabolism through calcitropic hormone concentrations: 1-25 DHCC and PTH.[35-38] Vitamin D and PTH increase calcium uptake by the adipocytes promoting lipogenesis. With oral calcium supplementation, PTH and vitamin D levels are decreased and have opposite effects on the adipocytes.[35] It could explain the reduction in body fat with calcium supplementation due to lipolysis and it can be expected to increase serum lipid profile. In contrast, the present study showed evidence of the serum lipid reduction for individual parameters.

Although fecal calcium excretion was not assessed in this study, observed decrease in serum lipid level after calcium supplementation might probably be attributable to mechanisms related to fat absorption in the gastrointestinal tract. Denke et al.[10] have demonstrated that calcium binds to fatty acids in the intestines and forms insoluble calcium-fatty acid soaps, thereby reducing saturated fatty acid absorption and increasing fecal excretion. Saunders et al. (1988) also found that there was not only an increase in fecal fatty acid excretion but also an increase in fecal bile acid excretion after 2.4 g of calcium supplementation.[39] These reductions in saturated fatty acid absorption and bile acid reabsorption could lower serum cholesterol concentrations.

One limitation of the present study is that a control group on placebo was not included in this study design since a randomized, double-blind, placebo controlled design provides the strength of the study and documents the success of the intervention. Yet, the present study provides preliminary support for an important contributory role of elemental calcium in improving dyslipidemia and thus reducing risk of cardiovascular disease in centrally obese male subjects. Further confirmatory studies using randomized control trial with longer intervention period are recommended.

CONCLUSION

In conclusion, it seems that 1500 mg/day of calcium supplementation led to significant changes in serum lipid level, thereby decreasing AIP. The present study established the contributory role of elemental calcium in decreasing risk factors of cardiovascular diseases in individuals with higher adiposity.

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