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Serum Calcium Increase Correlates With Worsening of Lipid Profile

An Observational Study on a Large Cohort From South Italy

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Abstract: Despite the well-documented role of calcium in cell metabolism, its role in the development of cardiovascular disease is still under heavy debate. Several studies suggest that calcium supplementation might be associated with an increased risk of coronary heart disease, whereas others underline a significant effect on lowering high blood pressure and hyperlipidemia. The purpose of this study was to investigate, in a large nonselected cohort from South Italy, if serum calcium levels correlate with lipid values and can therefore be linked to higher individual cardiovascular risk.

Eight-thousand-six-hundred-ten outpatients addressed to the Laboratory of Clinical Biochemistry, University of Magna Græcia, Catanzaro, Italy from January 2012 to December 2013 for routine blood tests, were enrolled in the study. Total HDL-, LDL- and non-HDL colesterol, triglycerides, and calcium were determined with standard methods.

We observed a significant association between total cholesterol, LDL-cholesterol, HDL-cholesterol, non-HDL cholesterol, triglycerides, and serum calcium in men and postmenopause women. Interestingly, in premenopause women, we only found a direct correlation between serum calcium, total cholesterol, and HDL-cholesterol. Calcium significantly increased while increasing total cholesterol and triglycerides in men and postmenopause women.

Our results confirm that progressive increase of serum calcium level correlates with worsening of lipid profile in our study population. Therefore, we suggest that a greater caution should be used in calcium supplement prescription particularly in men and women undergoing menopause, in which an increase of serum lipids is already known to be associated with a higher cardiovascular risk.

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Abbreviations: HDL = high density lipoprotein, LDL = low density lipoprotein, MANOVA = multiple analysis of variance, MDRD = modification of diet in renal disease, VLDL = very low density lipoprotein.

INTRODUCTION

Calcium is a ubiquitous divalent cation playing a fundamental role in the intra- and extracellular compartments. It is involved in blood coagulation, skeletal mineralization, muscle contraction, and regulation of nerve excitability, and finely regulated by both calcitonin and parathyroid hormone. Despite calcium supplementation being found to be beneficial for bone health in children, young adults, elderly, and menopausal women, there is concern about a possible association with incident cardiovascular disease.[1,2] A meta-analysis published in 2010 reported that calcium supplementation, with and without coadministered vitamin D, associated with a significant increased risk of myocardial infarction in both men and women.[3] On the basis of this meta-analysis the US Preventive Services Task Force recommended against calcium supplementation for the primary prevention of fractures.[4] The relationship between calcium supplementation and risk of cardiovascular disease remains unclear and has unequivocally been explained in the past. First evidences suggested that calcium supplements might have beneficial effects on cardiovascular risk factors as hypertension and hyperlipidemia. The pooled analysis from trials evaluating the role of dietary and nondietary calcium supplements showed a significant though small reduction in systolic and diastolic blood pressure. However, there was a substantial heterogeneity of results across the trials limiting the clinical interpretation of the finding.[5] Likewise, calcium supplementation in a different study was found to increase significantly HDL-cholesterol and slightly, but not significantly, decreased LDL-cholesterol.[6] The potential beneficial effects described in previous studies do not find any comparison by latest and larger clinical trials reporting an increase in cardiovascular mortality among women randomized to calcium supplements.

A number of observational studies associating dietary calcium intake, but not calcium supplementation, and cardiovascular disease have been further published.[7,8]

A more recent meta-analysis has highlighted how the relationship between calcium and/or vitamin D supplementation and cardiovascular disease is influenced by the type of intervention, duration, supplement dosage, and participants. Men assuming vitamin D and calcium, for example, seem to experience more harmful effects compared with women.[9]

An intriguing link between serum calcium and metabolic disorders has been hypothesized.[10–12] High total calcium seems to correlate with metabolic syndrome, insulin-resistance, and a worst lipid profile. Hyperlipidemia represents a

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well-recognized risk factor strictly associated with cardiovascular morbidity and mortality. We have hypothesized that serum calcium might directly correlate with lipid values and therefore might be harmful for individual cardiovascular risk. Therefore, we have designed a cross-sectional study, including a large nonselected cohort of free-living subjects from the urban area of Catanzaro (South Italy) to evaluate the association between total serum calcium and lipids.

METHODS

Study Population

The research conforms to the ethical guidelines of the Declaration of Helsinki as reflected in a priori approval by the Ethical Committee of "Azienda Ospedaliera Mater Domini" (Catanzaro, Italy). All recruited subjects gave written informed consent. Data were collected from records of consecutive 8610 outpatients who attended the Laboratory of Clinical Biochemistry, University of Magna Græcia, Catanzaro, Italy from January 2012 to December 2013 for routine blood tests, and who met the following inclusion criteria: age $\geq$18 and $<$75 years; and glomerular filtration rate, evaluated by Modification of Diet in Renal Disease (MDRD) formula, $>$60 mL/min.[13] The formula is based on serum creatinine value, age, sex, and race. All subjects were from the city of Catanzaro, and provinces around the city.

Biochemical Parameters

Ten milliliters of blood were collected and serum samples were obtained by centrifugation at 3000 rpm for 10 minutes. Total cholesterol, HDL and LDL, triglycerides were performed on Cobas 6000 (Roche Diagnostics, Basel, Switzerland). Total cholesterol, HDL, and LDL were analyzed by using an enzymatic methods (Roche Diagnostics), triglycerides were analyzed by using an enzymatic-colorimetric assay. Non-HDL cholesterol was calculated on the basis of the following formula: (total cholesterol – HDL-cholesterol). Serum calcium level was measured with a colorimetric assay, according to Schwarzenbach, using o-cresolphthalein-complexone. Serum creatinine was measured in the routine laboratory by an automated technique based on a Creatinine Jaffè compensated method for serum and plasma (Roche Diagnostics) implemented in an autoanalyzer. All the assays were carried out according to the manufacturer's instructions.

Statistical Analysis

Men and women were analyzed separately. Women were divided in 2 groups pre- and postmenopause. Menopause was established in arbitrary way at 45 years.[14–16] Men and pre-/postmenopause women were divided in 3 groups based on cholesterol and triglycerides value. In details based on cholesterol: Group 1: total cholesterol $\leq$5.17 mmol/L; Group 2: total cholesterol $>$5.17 and $\leq$6.46 mmol/L; Group 3: total cholesterol $>$6.46 mmol/L. Based on triglyceride: G Group 1: $\leq$1.7 mmol/L; Group 2: $>$1.7 and $\leq$2.3 mmol/L; and Group 3: $>$2.3 mmol/L. Triglycerides and serum calcium were not normally distributed; therefore, they were transformed before applying parametric tests. Triglycerides were log-transformed, whereas a 2-step rank transformation was performed for serum calcium. P value was considered significant if $<$0.05. The comparison between continuous variables was evaluated by t test for unpaired data. The association among serum lipids and serum calcium was investigated by Pearson correlation. The multiple regression analysis was used to evaluate the age-adjusted correlation between serum calcium and lipids.

The multiple analysis of variance (MANOVA) was applied to compare multivariate means among 3 groups (see the above). The independent variables included in the models were age, total cholesterol, triglycerides, HDL-cholesterol, LDL-cholesterol, non-HDL cholesterol, and serum calcium. The P value was considered significant if $<$0.05.

RESULTS

The overall population consisted of 8610 subjects: 4104 women and 4506 men. The age range was 18 to 74 years, median age 55.0 years, mean age and SD 53 $\pm$ 14 years.

Age, serum lipids, and serum calcium of subjects included in the study, and divided according to sex, are displayed in Table 1. Women were significantly younger than men; they showed significantly higher total cholesterol, HDL-cholesterol, LDL-cholesterol, and significantly lower triglycerides. Serum calcium was similar in both sexes.

Among women, 1341 (33%) were in premenopause, and 2763 (67%) in postmenopause. Mean $\pm$ SD age was 34 $\pm$ 7 years in premenopause women, and 59 $\pm$ 8 years in postmenopause women. Age between pre- and postmenopause women, was statistically significant ($P < 0.0001$).

The Pearson correlation showed a significant and direct association between total cholesterol, LDL-cholesterol, HDL-cholesterol, triglycerides, non-HDL cholesterol, and serum calcium in men and postmenopause women. In premenopause women we found a lower, but still significant, direct correlation between total cholesterol, HDL-cholesterol and serum calcium (Table 2).

Furthermore, we have evaluated the association between lipids and serum calcium after adjustment for age. As shown in Table 3, calcium significantly associated with lipids in all groups except with triglycerides in premenopause women.

We further divided men and women (pre- and postmenopause) in 3 groups, based on total cholesterol value and MANOVA results are displayed in Table 4.

Interestingly, serum calcium significantly and progressively increased from Group 1 to 3 in men, and postmenopause women, whereas it was comparable among groups in premenopause women. Age did not differ among postmenopause women divided in 3 groups, whereas significantly differed among men and premenopause women groups.

TABLE 1. Clinical and Biochemical Parameters of Subjects Included in the Study and Divided According to Sex

Variable	Women	Men	P^*
Number (%)	4104 (48)	4506 (52)	—
Age (yr)	51 $\pm$ 14	55 $\pm$ 14	0.0001
Total cholesterol (mmol/L)	5.25 $\pm$ 1.08	4.89 $\pm$ 1.16	0.0001
HDL-cholesterol (mmol/L)	1.55 $\pm$ 0.41	1.24 $\pm$ 0.34	0.0001
LDL-cholesterol (mmol/L)	3.1 $\pm$ 0.96	2.95 $\pm$ 1.01	0.0001
Non-HDL cholesterol (mmol/L)	3.7 $\pm$ 0.67	3.7 $\pm$ 0.82	0.89
Triglycerides (mmol/L)	1.25 $\pm$ 0.74	1.55 $\pm$ 1.05	0.0001
Serum calcium (mmol/L)	2.37 $\pm$ 0.12	2.37 $\pm$ 0.10	0.74

Values are mean $\pm$ standard deviation. NS = not significant.
* Statistical significance on t test.

TABLE 2. Correlation Coefficient Between Lipids and Serum Calcium in Men, and Pre- and Postmenopausal Women

	Serum Calcium (mmol/L)		
Variable	**Men (NR = 4104)**	**Women Premenopause (NR = 1341)**	**Women Postmenopause (Nr = 2763)**
Total cholesterol (mmol/L)	0.16*	0.07^	0.23*
HDL-cholesterol (mmol/L)	0.12*	0.09^	0.13*
LDL-cholesterol (mmol/L)	0.12*	0.04	0.16*
Non-HDL cholesterol (mmol/L)	0.13*	0.07	0.18*
Triglycerides (mmol/L)	0.05*	0.01	0.10*

* $P < 0.0001$.
^ $P < 0.01$.

In Table 5, we have displayed data in subjects according to triglyceride concentration. Age did not differ among groups. Serum calcium progressively and significantly increased, whereas increasing triglycerides in men and postmenopause women. Calcium did not differ among 3 premenopause triglycerides groups.

DISCUSSION

The main finding of the present study is the direct association between serum calcium and total cholesterol and triglycerides in a large cohort of free-living subjects from the urban area of Catanzaro. The proportion of variation shared by total cholesterol and serum calcium was 2.9% in men, 1% in premenopause women, and 4.8% in postmenopause women. The proportion of variation shared by triglycerides and calcium was lower. Indeed, the Pearson coefficient of correlation was 1% in men and postmenopause women. No significant correlation was found between triglycerides and calcium in premenopause women. Similar finding was obtained when men and women were divided in increasing cholesterol and triglyceride groups. Serum calcium significantly increased while increasing lipids in men and postmenopause women, but not in premenopause women.

We further demonstrated that for each increase in serum calcium, lipids significantly increased mainly in men and postmenopause women.

Based on these results we can state that a significant and direct relationship between serum calcium and lipid exists. We hypothesize that, at least in premenopause women, estrogens might neutralize the unfavorable effect of serum calcium on lipid metabolism.

Several mechanisms have been involved in the relationship between calcium, lipids, and estrogens. Some researchers have documented *in-vitro* that calcium supplement might contribute to increase serum cholesterol by decreasing hepatic catabolism in estrogens deficiency condition.[17] In normal conditions, estrogens activate LDL-cholesterol receptor, and increase cholesterol catabolism in the liver.[18] Conversely, calcium decreases cholesterol catabolism, and stimulates lipids synthesis. In detail, calcium supplement decreases the activity of the 7a-hydroxylase (CYP7A), enzyme involved in the cholesterol catabolism, and stimulates Sterol Regulatory Element-Binding Protein (SREBP)-1c expression that is a transcription factor involved in *de-novo* lipid synthesis.[19]

The association between HDL-cholesterol and serum calcium has already been described even if the mechanisms involved are not clear. SREBP pathway might play a role in the regulation of HDL-cholesterol metabolism.[20,21]

Estrogens seem to have a protective role in preventing calcium-induced cholesterol increase mainly in postmenopause women, and in men.[21] The transition from premenopause to postmenopause is critical for many reasons. Indeed, many features of metabolic syndrome, including abdominal obesity, hypertriglyceridemia, elevated LDL-cholesterol, reduced HDL-cholesterol, insulin resistance, and elevated blood pressure frequently occur in postmenopause.[22–24] These abnormalities may be a direct result of ovarian failure or, alternatively, an indirect result of central fat redistribution associated with estrogen deficiency and progressive testosterone predominance.[24] Hormone replacement therapy has beneficial effect on lipid metabolism.[25]

Based on current evidences, we hypothesize that the combination of calcium supplementation and lack of estrogens might affect lipid profile, and as consequence individual cardiovascular risk. Our results are in line with available data. Indeed, first postmenopause women have the worst profile in terms of total-, HDL-, LDL-, non-HDL-cholesterol, and triglycerides, and second higher lipid values associate with higher serum calcium level.

Besides traditional lipid values, we have also included in the analyses non-HDL cholesterol as it represents a better

TABLE 3. Age Adjusted Regression Analysis Between Serum Calcium and Lipids (Total Cholesterol, HDL-Cholesterol, LDL-Cholesterol, Triglycerides, and Non-HDL Cholesterol) Unstandardized Beta Coefficient and [CIs 95%]

	Total Cholesterol (mmol/L)	**HDL-Cholesterol (mmol/L)**	**LDL- Cholesterol (mmol/L)**	**Triglycerides (mmol/L)**	**Non-HDL Cholesterol (mmol/L)**
Men					
Serum calcium	0.40* [0.32–0.47]	0.07* [0.05–0.10]	0.22* [0.17–0.30]	0.14* [0.07–0.22]	0.30* [0.22–0.37]
Premenopause Women					
Serum calcium	0.25* [0.12–0.35]	0.07* [0.02–0.12]	0.15* [0.05–0.22]	NS	0.15* [0.05–0.25]
Postmenopause Women					
Serum calcium	0.52* [0.42–0.60]	0.10* [0.07–0.15]	0.32* [0.25–0.40]	0.14* [0.08–0.20]	0.40* [0.32–0.47]

* $P < 0.01$.

TABLE 4. Age and Serum Calcium of Men and Women (Pre- and Postmenopause) Divided According to Cholesterol Values

Variable	Group 1 ≤5.17 mmol/L	Group 2 >5.17 ≤ 6.46 mmol/L	Group 3 >6.46 mmol/L
Men			
Number	2719	1368	419
Age (yr)	55 ± 15^	54 ± 13	53 ± 11
Serum calcium (mmol/L)	2.36 ± 0.11*	2.39 ± 0.10	2.40 ± 0.09
Premenopause Women			
Number	882	375	84
Age (yr)	32 ± 8*	35 ± 7	36 ± 6
Serum calcium (mmol/L)	2.36 ± 0.11	2.37 ± 0.11	2.38 ± 0.61
Postmenopause Women			
Number	1109	1203	451
Age (yr)	59 ± 9	59 ± 8	59 ± 8
Serum calcium (mmol/L)	2.35 ± 0.12*	2.39 ± 0.12	2.43 ± 0.13

* MANOVA $P < 0.0001$.
^ $P < 0.004$.

marker of cardiovascular risk. Non-HDL cholesterol combines the cholesterol included in VLDL and its subfractions excluded HDL-cholesterol.[26]

CONCLUSIONS

The results of the present study demonstrate a significant correlation between serum calcium and total-, HDL-, LDL-, non-HDL-cholesterol, and triglycerides in postmenopause women and men. This suggests that calcium supplementation in general population should be done with great caution, preferably closely monitoring lipid profile. The research shows some limits as the design of the study and lack of clinical information. First, the study is an observational study; therefore, a cause–effect relationship cannot be established. Second, information about comorbidities or pharmacological treatment were not available. However, the result of the study is in line with some unsatisfactory results of interventional trial aimed to verify the beneficial effect of serum calcium on cardiovascular morbidity and mortality. The huge number of subjects included in our study might overwhelm some limits and offer an explanation to the controversial results available in the literature.

TABLE 5. Age and Serum Calcium of Men and Women (Pre- and Postmenopause) Divided According to Triglycerides Values

Variable	Group 1 ≤1.7 mmol/L	Group 2 >1.7 ≤ 2.3 mmol/L	Group 3 >2.3 mmol/L
Men			
Number	3134	675	697
Age (yr)	54 ± 14	56 ± 12	55 ± 12
Serum calcium (mmol/L)	2.37 ± 0.11*	2.38 ± 0.11	2.38 ± 0.11
Premenopause Women			
Number	1213	85	43
Age (yr)	33 ± 8	35 ± 7	35 ± 6
Serum calcium (mmol/L)	2.37 ± 0.12	2.37 ± 0.13	2.34 ± 0.11
Postmenopause Women			
Number	2086	415	262
Age (yr)	59 ± 8	61 ± 8	60 ± 8
Serum calcium (mmol/L)	2.37 ± 0.12*	2.39 ± 0.12	2.40 ± 0.13

* MANOVA $P < 0.005$.

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