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Serum Lipids and Bone Metabolism in Spanish Men: The Camargo Cohort Study

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Abstract. There is growing evidence of a link between lipid and bone metabolism, although data on this association in European men are scarce. This cross-sectional study from a community-based prospective cohort aims to explore the association of serum lipids with different aspects of bone metabolism in Spanish men. Demographic and anthropometric measurements, biochemical parameters including serum lipids, bone remodelling markers and calciotropic hormones, bone mineral density (BMD) assessed by dual X-ray absorptiometry and heel quantitative ultrasound, and prevalent vertebral and non-vertebral fractures, were evaluated in 289 men. Calciotropic hormones or bone markers were not associated with serum lipids. Serum total (TC) and LDL cholesterol, as well as LDL/HDL ratio were positively correlated to BMD at lumbar spine and hip. No significant correlation was noted for triglycerides or HDL. We observed a positive association between triglycerides, LDL/HDL ratio and BUA, and between TC/HDL ratio and both, QUI and BUA. BMD at the femoral neck and total hip was significantly higher in men with hypercholesterolemia after controlling for all the covariates (p=0.007). We did not observe any association between serum lipids and prevalent vertebral fractures. However, we found that TC (p=0.03) and LDL (p=0.04) were lower in subjects with non-vertebral fractures. In conclusion, we have found that a more unfavorable lipid profile (mainly higher LDL-C levels) is associated with higher BMD at lumbar spine and hip in Spanish men. Moreover, we did not observe any association between hypercholesterolemia and prevalent vertebral fractures, but we found lower serum TC and LDL-C levels in men with prevalent non-vertebral fractures.

Key words: Hypercholesterolemia, Bone metabolism, Bone mineral density, Fractures, Bone remodeling markers

THE ASSOCIATION between serum lipids and bone has been suggested as one of the cornerstones of the osteoporosis-atherosclerosis connection [1-7]. Experimental studies have previously suggested serum cholesterol as an important pathogenic factor in the development of bone loss and osteoporosis [8]. In fact, lipid and bone metabolism have recently been linked in several ways. First, adipocytes and osteoblasts share a common progenitor from the stromal cells in the bone marrow, and accumulated evidence of the differentiation switching of these two cell lineages suggests that a large degree of plasticity exists between them, and that this relationship is reciprocal, as shown by the fact that age-related bone loss is accompanied by an increase in marrow adipose tissue [9, 10]. Second, the role of the low-density lipoprotein receptor–related protein 5 (LRP5) gene in the regulation of bone mass and fracture risk has been defined [11, 12]. More recently, a single mutation in LRP6, a closely related homolog of LRP5 which encodes a co-receptor in the Wnt signaling pathway, has been shown to be genetically linked with early coronary disease, features of the metabolic syndrome, osteoporosis and fractures [13]. Finally, there are several drugs such as hormone replacement therapy, statins

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and aminobisphosphonates which exert strong effects on both bone and lipid metabolism [7, 14]. As a matter of fact, the two last agents share the same metabolic mevalonate pathway, recently proposed as essential for not only the synthesis of cholesterol but also the regulation of bone cell proliferation and osteoclast apoptosis [15].

In this context, the relationship between dyslipidemia and bone metabolism has been addressed by several investigators, although results have been inconsistent and even contradictory [8, 16-28]. Moreover, dyslipidemia and bone metabolism, including bone mineral density and osteoporotic fractures, have mainly been analyzed in postmenopausal women. Data about this association in European men are scarce [17, 18, 25].

We have conducted a cross-sectional study that included participants in the Camargo Cohort Study to explore the association of serum lipids levels with different aspects of bone metabolism in men. Furthermore, we have compared in subjects with and without hypercholesterolemia the following: a) Bone mineral density (BMD) measured by dual energy X-ray absorptiometry (DXA) and heel quantitative ultrasound (QUS); b) prevalent vertebral and non-vertebral fractures; and c) calciotropic hormones and bone remodeling markers.

Subjects and Methods

Study design and participants

The study population consisted of 289 white men consecutively included in the Camargo Cohort Study between February 2005 and February 2009. This is a community-based study designed to evaluate the prevalence of metabolic bone diseases and disorders of mineral metabolism, as well as the prevalence of fractures and risk factors for osteoporosis and fragility fractures, in postmenopausal women and men older than 50 attending a primary care center in Northern Spain. The data presented here belong to the cross-sectional part of the study. Camargo is a town of more than 30000 inhabitants, situated near the Cantabrian coast. The age and sex distribution of its population closely resemble the entire population of our region (Cantabria, Spain). The local Ethics Committee approved the study protocol, and all participants gave written informed consent.

At the baseline visit, participants were interviewed by investigators and provided data regarding the risk factors for osteoporosis and fractures using a structured questionnaire including age, race, weight, height, body mass index (BMI), waist perimeter, personal history of fractures after 40 yrs of age, history of osteoporotic fractures among first-degree relatives, tobacco and alcohol use, consumption of dairy products, physical exercise, existence of sensory problems, number of falls in the previous yr, presence of chronic general diseases or disorders affecting bone health, and present or past consumption of medications with influence on bone metabolism.

Height and weight were measured with subjects wearing light indoor clothing but without shoes. BMI was defined as weight (Kg) divided by squared height (m^2). Waist circumference was measured in centimeters at a level midway between the lower rib margin and iliac crest after breathing out, with a flexible tape all around the body, in an erect position with feet together. Subjects with a history of a disease (type 1 diabetes, liver or chronic renal disease, neoplasm, rheumatoid arthritis, connective tissue disorders, hyperthyroidism, hyperparathyroidism, hypogonadism) or medication known to affect bone or lipid metabolism (fibrates, statins, ezetimibe, nicotinic acid, bile acid resins, anticonvulsants, glytazones, thyroid hormones, thiazides, corticosteroids, testosterone, heparin, calcitriol, bisphosphonates, strontium ranelate, calcitonin, teriparatide or parathyroid hormone), were excluded from the analysis.

Laboratory measurements

Blood samples were obtained from an antecubital vein in the morning after a requested 12-hour overnight fast. Serum was divided into 0.5-mL aliquots and stored at -40ºC. Participants were measured for serum total calcium (TCa), phosphate, glucose, creatinine, TC (TC), HDL-cholesterol (HDL-C), LDL-cholesterol (LDL-C) (calculated using the Friedewald formula), triglycerides, albumin, and total alkaline phosphatase by standard automated methods in a Technicon Dax autoanalyser (Technicon Instruments, Co. USA). Hypercholesterolemia was defined as fasting serum TC levels greater than 220 mg/dL or an elevated serum LDL-C level greater than 140 mg/dL [26].

TCa measurements were corrected for albumin con-

centration (cCa) according to a previously published formula [29]. Glomerular filtration rate was calculated using the four-variable MDRD formula and expressed in mL/min/1.73 m^2 [30].

Serum concentrations of aminoterminal propeptide of type I collagen (PINP), C-terminal telopeptide of type I collagen (β-CTX), 25-hydroxyvitamin D3 (25OHD), and intact parathyroid hormone (iPTH) were determined by fully automated Roche electrochemiluminiscence system (Elecsys 2010, Roche Diagnostics, GmbH, Mannheim, Germany). The PINP limit of detection was 5 ng/mL (reference range between 20-76 ng/mL), and its intraassay and interassay coefficients of variation (CV) 3.1% and 3.5% respectively. Intraassay and interassay CV for β-CTX were 4.2% and 4.7%, also respectively (reference range was 0.100-1.000 ng/mL). The detection limit of serum 25OHD was 4 ng/mL. The intraassay CV was 5% and interassay was 8.5%. Regarding iPTH, the detection limit was 6 pg/mL (reference range, 15-65 pg/mL). Intra and interassay CV were 5.4% and 5.9%, respectively.

Bone mineral density assessment

BMD was measured by DXA (Hologic QDR 4500, Bedford, MA, USA) at the lumbar spine (L2-L4), femoral neck (FN), and total hip (TH). *In vivo* precision was 0.4-0.5% at the different measurement sites. Results were expressed as grams per square centimeter and T-score (defined as the number of standard deviations [SDs] below the mean value of young women and men), and Z-score (defined as the number of SDs below the mean for women and men of the same age). Quality control was performed according to the usual standards [31].

Quantitative ultrasound

Heel QUS measurements were performed in all the subjects using the Sahara Clinical Sonometer (Hologic, Bedford, MA, USA). The Sahara device measured both broadband ultrasound attenuation (BUA) (dB/MHz) and speed of sound (SOS) (m/sec) at a fixed region of interest in the mid-calcaneus. The BUA and SOS results are combined to provide the "quantitative ultrasound index" (QUI) using the formula: QUI = 0.41 * (BUA + SOS) – 571. The European reference population we used has been described elsewhere, [32, 33] and yields similar results to the application of normative values from Spanish women for the same QUS device [34]. Quality control checks were performed daily by scanning manufacturer-provided phantoms, prior to scanning the subjects.

Identification of vertebral and non-vertebral fractures

Radiographs of the thoraco-lumbar spine were obtained at the baseline visit. Thoracic radiographs were centered at vertebra T7 and lumbar radiographs at L3. Vertebral fractures were identified according to a semi-quantitative approach [35]. Briefly, anterior, middle, and posterior heights of each vertebra were compared with each other. A reduction between 20 and 25% was considered grade 1; between 25 and 40% grade 2; and greater than 40% grade 3. Radiographs were reviewed by one of the authors, expert in osteoporosis and blinded to any other clinical data of the subjects studied. Non-vertebral clinical fractures not related to major trauma were self-reported, and later confirmed by examination of medical or radiological reports.

Statistical analysis

Results were expressed as mean ± SD or percentages, as appropriate. Levels of laboratory parameters not normally distributed were log-transformed before analysis. Unpaired Student's *t* test or Mann-Whitney *U*-test were used to determine the differences between groups for continuous variables, and x^2-test for categorical variables. Pearson correlation analysis was used for the associations between the lipid profile and BMD. We reassessed the relationship between them by partial correlation analysis after adjustment for the possible confounders. Linear regression adjusted for age, body mass index, and other covariates was used to analyze the association between serum lipids levels and site-specific BMD. Analysis of covariance was performed to compare the site specific BMD of participants with and without hypercholesterolemia adjusting for BMD related covariates, including age, BMI, waist perimeter, prior fracture, current alcohol and tobacco use, calcium intake, physical exercise, type 2 diabetes, hypertension, serum creatinine, serum 25OHD, serum iPTH levels, and bone remodeling markers. We performed multivariate binary logistic regression analysis to determine the influence of serum lipid levels and prevalent vertebral and non-vertebral fractures. Analyses

Table 1. Baseline characteristics and laboratory parameters. Distribution by hypercholesterolemic status.

	Hypercholesterolemia		
	Yes (145) m±SD	No (144) m±SD	*p*
Age (yr)	63.8±8.4	64.9±9.6	0.29
BMI (Kg/m^2)	28.4±3.2	28.2±3.2	0.98
Waist circumference (cm)	100.9±8.9	99.3±9.3	0.73
Dairy calcium (mg/d)	579±354	565±331	0.35
Alcohol (g/d)	24.2±23.2	31.6±55.6	0.28
Tobacco (cigarettes/d)	15.7±16.4	17.4±17.0	0.54
Hypertension*	48 (33.1)	54 (37.5)	0.46
Ischemic heart disease*	5 (3.4)	2 (1.4)	0.44
Cholesterol (mg/dL)	239.6±22.9	185.5±23.6	<0.0001
HDL-C (mg/dL)	51.4±10.8	48.3±12.7	0.03
LDL-C (mg/dL)	163.4±19.5	116.9±18.2	<0.0001
Tryglicerides (mg/dL)	123.0±56.9	98.4±43.8	<0.0001
FPG (mg/dL)	93.8±20.5	97.6±19.6	0.11
Creatinine (mg/dL)	1.11±0.2	1.10±0.2	0.55
GFR (mL/min/1.73 m^2)	72.5±12.2	73.8±14.2	0.41
Alkaline phosphatase (IU/L)	63.3±16.8	66.4±24.1	0.21
Phosphate (mg/dL)	3.1±0.5	3.0±0.5	0.27
Corrected calcium (mg/dL)	9.1±0.4	9.2±0.3	0.76
Albumin (g/dL)	4.6±0.2	4.5±0.3	0.02
iPTH (pg/mL)	51.2±16.8	54.3±18.8	0.15
25OHD (ng/mL)	24.4±7.3	24.5±7.9	0.95
PINP (ng/mL)	37.8±15.6	41.2±18.4	0.08
β-CTX (ng/mL)	0.32±0.2	0.34±0.2	0.33

BMI: Body mass index. HDL: High density lipoproteins. LDL: Low density lipoproteins. FPG: Fasting plasma glucose. iPTH intact parathyroid hormone. 25OHD: 25-hydroxyvitamin D. PINP: Aminoterminal propeptide of type I collagen; β-CTX: C-terminal telopeptide of type I collagen. GFR: Glomerular filtration rate (calculated by means of 4-variables MDRD formula). *Data are expressed as numbers (percentages in parentheses).

were also performed including and excluding subjects with type 2 diabetes mellitus. A value of $p<0.05$ was considered statistically significant. All analyses were conducted using SPSS 15.0 (Chicago, IL).

Results

Baseline characteristics, bone metabolism hormones and bone turnover markers

The study included 289 men, 145 with and 144 without hypercholesterolemia. Mean age was 64±9 (range, 50-92), and the mean BMI was 28.3±3.1 Kg/m^2. BMI highly correlated with waist circumference in men with hypercholesterolemia as well as in those without it (0.790 and 0.795 respectively; $p<0.001$). A total of 40 participants (13.8% of the whole sample) had type 2 diabetes mellitus.

Table 1 shows the clinical characteristics and laboratory parameters, including bone turnover markers, of the subjects studied grouped by cholesterol status. Apart from serum lipid measurements, albumin was slightly lower in subjects with hypercholesterolemia compared with individuals without it ($p=0.02$).

Regarding bone metabolism determinations, in the overall group, when adjusted for age and BMI, serum 25OHD and iPTH levels were not associated with any lipid parameter. Serum β-CTX, but not PINP levels, negatively correlated with BMI ($r=-0.159$; $p=0.007$) and both markers were negatively associated with waist perimeter ($r=-0.203$; $p=0.001$ for β-CTX and $r=-0.134$; $p=0.03$ for PINP) in the whole sample. No correlation was noted between calciotropic hormones or bone markers and serum lipids, whereas 25OHD levels were negatively correlated with waist perimeter ($r=-0.146$; $p=0.02$). Bone turnovers markers were lower in the group of men with hypercholesterolemia

Table 2. Age and BMI-adjusted partial correlations between serum lipids and bone mineral measurements.

	Lumbar BMD	Femoral neck BMD	Total hip BMD	QUI / STIFFNESS	SOS	BUA
TC	0.155*	0.126	0.172**	0.112	0.099	0.117
LDL-C	0.211**	0.151*	0.209**	0.114	0.098	0.118
HDL-C	-0.032	-0.053	-0.007	-0.029	-0.013	-0.035
Triglycerides	-0.001	-0.043	0.020	0.112	0.087	0.131*
TC/HDL ratio	0.103	0.027	0.119	0.131*	0.104	0.141*
LDL/HDL ratio	0.146*	0.058	0.147*	0.126	0.101	0.134*

*$p<0.05$ ** $p<0.01$

TC: Total cholesterol. LDL-C: Low density lipoprotein cholesterol. HDL-C: High density lipoprotein cholesterol.

Table 3. Age and BMI-adjusted linear regression between serum lipids and site-specific BMD measured by DXA.

	β-coefficient	*p*
Lumbar spine BMD		
LDL-C	0.112	0.005
Age	0.080	0.09
BMI	0.167	0.008
Femoral neck BMD		
LDL-C	0.131	0.005
Age	-0.112	0.045
BMI	0.316	<0.0001
Total hip BMD		
LDL-C	0.154	0.001
Age	-0.110	0.049
BMI	0.331	<0.0001

LDL-C: low density lipoprotein cholesterol. HDL-C: high density lipoprotein cholesterol.

Table 4. Densitometric parameters in subjects with and without hypercholesterolemia.

	Hypercholesterolemia		*p*
	Yes (145)	No (144)	
	m±*SD*	*m*±*SD*	
Lumbar BMD	1.029±0.142	0.998±0.163	0.11
T-score	-0.77±1.31	-1.05±1.50	0.10
Z-score	0.01±1.30	-0.25±1.57	0.15
Femoral neck BMD	0.824±0.116	0.782±0.118	0.004
T-score	-0.77±0.85	-1.07±0.88	0.004
Z-score	0.30±0.85	0.03±0.92	0.01
Total hip BMD	0.990±0.126	0.946±0.116	0.003
T-score	-0.26±0.84	-0.56±0.79	0.002
Z-score	0.31±0.85	0.05±0.85	0.01
Osteoporosis*	17 (11.7)	23 (15.9)	0.31

*Defined as a *T-score* <2.5 at lumbar spine, femoral neck or total hip.

but differences were not significant once adjusted by age, BMI and diabetes status ($p=0.06$ for PINP levels). However, when stratified by age subgroup, serum PINP and β-CTX levels were significantly lower only in hypercholesterolemic men aged 70-74 years ($p=0.01$ and $p=0.04$ respectively).

Bone mineral density and osteoporosis

Correlations between serum lipids and bone mineral measurements, adjusted by age and BMI are showed in Table 2. Controlling for all the covariates (waist perimeter, physical activity, dairy calcium intake, current alcohol and tobacco use, type 2 diabetes, prior fragility fracture, hypertension, serum creatinine, serum 25OH levels, PTH levels, and bone turnover markers) did not change these associations. In the case of heel QUS, we observed a positive correlation between serum triglycerides, LDL/HDL ratio and BUA, and between TC/HDL ratio and both, QUI and BUA, in the whole sample, which persisted after adjustment for all other covariates. There were no differences between men with and without hypercholesterolemia in any ultrasonographic parameter.

With regard to BMD measured by DXA, the association between serum cholesterol and BMD was driven by serum LDL-C levels at the three localizations, since when HDL-C or triglycerides entered in the model, the association did not remains statistically significant in age and BMI adjusted linear regression analyses (Table 3). Controlling for all the covariates did not change the association of LDL-C levels and spine or hip BMD.

Moreover, BMD measured by DXA and expressed as g/cm^2 or T and Z-scores was higher in men with hypercholesterolemia than in those without it (Table 4).

Table 5. Crude and adjusted models according to the cholesterol status of the subjects.

		Hypercholesterolemia		
		Yes (145) *m* (g/cm^2)	No (144) *m* (g/cm^2)	*p*
Lumbar spine	Unadjusted	1.029	0.999	0.10
	Age + BMI	1.029	0.996	0.07
	Fully adjusted*	1.027	0.997	0.09
Femoral neck	Unadjusted	0.824	0.783	0.004
	Age + BMI	0.824	0.780	0.003
	Fully adjusted*	0.822	0.781	0.007
Total hip	Unadjusted	0.990	0.946	0.003
	Age + BMI	0.990	0.944	0.002
	Fully adjusted*	0.988	0.945	0.007

m: Mean.

* Include age, BMI, waist perimeter, current alcohol and tobacco use, calcium intake, physical exercise, type 2 diabetes, hypertension, serum creatinine, serum 25OHD, serum iPTH levels, and bone remodeling markers.

Differences were statistically significant at the femoral neck and total hip. Table 5 shows site-specific BMD adjusted by potential confounders according to cholesterol status. In crude and fully adjusted analysis no significant differences were found at lumbar spine. However, BMD at the femoral neck and total hip was significantly higher in men with hypercholesterolemia compared with those without it, and these differences remained significant after controlling for all the covariates. Moreover, no statistically significant differences were seen concerning prevalence of osteoporosis at the lumbar spine, femoral neck and total hip, between normo and hypercholesterolemic men (Table 4). The comparison of serum lipid measurements in men with and without osteoporosis (defined as a T score <-2.5) was not significant in any case. None of the lipid parameters were found to be associated with the existence of osteoporosis when a multivariate binary logistic regression analysis was performed.

Prevalent vertebral and non-vertebral fractures

There were 56 vertebral fractures in 44 men (15.2%). According to Genant's score, 31 were grade 1 vertebral fracture, 11 were grade 2 and 2 were grade 3. Eleven subjects had more than one vertebral fracture. Among men with hypercholesterolemia, 13.8% of them showed prevalent vertebral fractures, which were observed in 16.7% of normocholesterolemic individuals. These differences were not significant, either before or after adjusting for other possible confounders in logistic regression models. We also compared serum lipids levels between men with and without vertebral fractures. Although TC, LDL-C, and triglyceride levels were lower, and HDL-C levels were slightly higher in men with vertebral fractures, differences were non-significant between groups with and without those fractures.

On the other hand, there were 66 non-vertebral fractures reported by 46 (15.9%) participants. Seventeen were traumatic and 49 osteoporotic-related fractures. Again, no differences were noted between both groups. All serum lipid determinations were lower in subjects with non-vertebral fractures, although differences were significant only for TC (202±32 *vs.* 215±36; p=0.03) and LDL-C (132±29 *vs.* 143±29; p=0.04), but not for triglycerides or HDL-C levels. In addition, no association between prevalent non-vertebral osteoporotic fractures and hypercholesterolemia was found in multivariate analyses. In univariate analyses, serum total and LDL-C were associated with an increase in the risk of non-vertebral fractures (OR 1.01; CI95% 1.001-1.023; p=0.04 for each variable). However, in adjusted multivariable models these differences did not remain significant (data not shown).

Repeating all analyses after excluding patients with type 2 diabetes mellitus did not substantially change the results.

Discussion

Although there have been a growing number of clinical studies examining the association between se-

rum lipids and bone metabolism, the literature concerning this relationship is conflicting. The main question probably is whether there is a direct relationship between serum lipids and BMD or whether this association is caused by confounding factors (mainly estrogen status in the case of women). Moreover, most of studies have explored this issue in postmenopausal women, but data in European men are scarce [17, 18, 25]. On the other hand, other studies have included patients under lowering-lipid agents, and are, therefore, difficult to compare. In the present study, we have assessed the relationship between lipid levels and bone metabolism among Spanish men from a prospective community-based cohort, who were not taking any lipid-lowering treatment.

We found a positive correlation between total serum cholesterol levels and BMD (significant at lumbar spine and total hip), and between LDL levels and BMD (at all three sites). Indeed, we have observed a negative, albeit non-significant, association between BMD and HDL-C. These findings are similar to those published by Adami *et al*. [17] in 427 healthy Italian men. They found that subjects with the most favorable lipid profiles consistently had the lowest bone mass values regardless of age, body mass index, smoking habit or dietary calcium intake. We have observed similar results even when adjusting also by other potential confounders such as waist perimeter, physical status, prior fractures, alcohol intake, diabetes, hypertension, serum creatinine, calciotropic hormones and bone turnover markers. In another study, the same Italian investigators analyzed a population-based cohort of 265 men aged 68-75 yrs [18]. As we have found, they observed that hip BMD was significantly related to serum lipids: negatively for HDL-C and positively for triglycerides (we found a positive non-significant association only at total hip), LDL-C, and LDL/HDL ratio. Recently, the Hertforside cohort study performed in UK also showed an increase in total hip BMD as TC and LDL-C levels increased, in the case of the 465 men included, although this relationship did not reach statistical significance [25]. Our data showed that the association between LDL-C and BMD is only partially driven by the relationship of age and BMI with both lipid profile and BMD. Moreover, in our cohort, men with hypercholesterolemia had higher BMD values at lumbar spine, femoral neck and total hip than men without it. These crude differences remains significant only in hip measurements (femoral neck and total hip), and did not change in fully adjusted models.

These are interesting and surprising findings, although contrary to data from North American and Asiatic populations [19, 20, 22, 23]. In fact, a large cross sectional study conducted in the United States using data from NHANES III survey [23], showed no association between lipid levels and hip BMD in either sex, after adjusting by confounders. However, the discrepancy between these findings and those from some European studies suggests that genetic heritage and perhaps dietary and geographical reasons, might play a role in this association. Therefore, our study and those by Adami *et al.* [17, 18] suggest a prevailing higher BMD in hypercholesterolemic men living in Mediterranean countries such as Italy and Spain, and if confirmed in other studies, might be useful to adequately interpreting, for example, the effect of statins on BMD and fractures in these populations.

We have also studied the relationship between heel QUS and serum lipids. The only study published to date regarding this issue was performed by Buizert *et al*. [1] who found a stronger positive association between TC/HDL ratio and QUS parameters (SOS and BUA in both sexes). Moreover, serum levels of HDL-C were significantly inversely associated with QUS. In our cohort, QUS results showed a trend similar to those obtained by DXA, although differences between individuals with and without hypercholesterolemia did not reach significant levels. We found a positive association between serum triglycerides and BUA, although no relationship between serum cholesterol levels (TC, LDL-C or HDL-C) and QUS parameters was detected. However, a significant positive correlation between serum LDL/HDL ratio and BUA and between TC/HDL ratio and BUA and QUI was observed once adjusted for all relevant confounders, indicating that an elevated "bad-to-good" cholesterol ratio might be related to not only BMD but also bone tissue quality. Therefore, our data are in accordance with those by Buizert *et al*. showing that the lipid profile that is favourable in the prevention of cardiovascular disease is at the same time unfavourable for QUS.

We also studied calciotropic hormones and bone turnover markers in our cohort. In a recent study, Majima *et al*. [26] have found an increased bone turnover (measured by serum bone specific alkaline phosphatase and N-terminal telopeptide of type I collagen) in hypercholesterolemic or dyslipidemic patients re-

gardless of gender. However, our data did not support these findings, and in fact, we have observed a lower, albeit non-significant, turnover rate (expressed by lower serum P1NP and β-CTX levels) in hypercholesterolemic men. Such a decrease points to a low bone turnover situation, consistent with the high BMD values found in these subjects. In any case, no clear evidence for a direct influence of serum lipids on bone cells could be drawn from our data of serum bone turnover markers. Only when subjects were stratified by age subgroups, both markers were significantly lower in men aged 70-74 yrs. Unlike Majima *et al*., we have used serum PINP and β-CTX as bone turnover markers, but in any case, both studies are difficult to compare by obvious ethnic and geographical differences, which hind to math the whole results. There were no differences between groups in terms of calciotropic hormones (25OHD and iPTH) in our study, suggesting that probably they do not play a significant role in the association between lipid and bone metabolism in men.

Concerning fractures, we have found an overall prevalence of radiological vertebral fractures not significantly different between subjects with and without hypercholesterolemia (14% *vs.* 17%). Several investigators have found lower levels of serum TC, LDL-C and triglycerides in postmenopausal women with vertebral fractures [19, 27]. Although in our study, these serum lipid levels were also lower in men with vertebral fractures, differences did not reach statistical significance when compared the lipid profiles of the subjects. Sivas *et al*., [27] also found that an increase of 1 mg/dL TC decreases the risk of vertebral fracture by 2.2%. On the other hand, Yamaguchi *et al*. [19] showed that low serum triglyceride levels were associated with the presence of vertebral fractures in postmenopausal women, and for every 1 SD increase in its levels the risk of vertebral fracture was reduced by nearly half. However, our data do not support any association between serum lipid levels and the risk of vertebral fractures in men.

Regarding previous non-vertebral fractures, we have observed that serum TC and LDL-C were significantly lower in men with previous fractures. Similar results have been reported by others [2, 19] in postmenopausal women, but with regard to vertebral fractures. In these women, this has been related to estrogen deprivation, since this hormone is synthesized by cholesterol. On the other hand, as some investigators have pointed out, the mechanism underlying this association of fractures and lower cholesterol levels, may be directly related with the contribution of cholesterol metabolism to the bone structure. Thus, Parhami *et al*. [10] indicate that a baseline level of cholesterol synthesis is necessary for the differentiation of osteoblasts cells. Moreover, some products of cholesterol oxidation, such as oxysterols have been shown to have pro-osteogenic effects on pluripotential mesenchimal stem cells [36]. Whatever the precise mechanism, it seems that lower levels of total and LDL-C would be associated with bone quality. Concerning the risk of non-vertebral fractures, although an association with serum LDL levels was detected in crude analysis, it was not observed in multivariable models. Considering hypercholesterolemia as a whole, no association with prevalent non-vertebral fractures was noted. Therefore, data available on fracture risk in men with hypercholesterolemia are scarce and not entirely consistent, and new studies are needed to clarify this issue.

In the present study a fully approach to the relationship between serum lipids and bone mineral metabolism in men who did not received any lipid-lowering drug has been entertained. We think that this is the main strength of our data. As a matter of fact, we performed a complete BMD analysis, not only by DXA but also by QUS, as well as an assessment of prevalent spine and non-vertebral fractures and a measurement of serum bone markers and bone metabolism hormones in men with and without hypercholesterolemia. We were also able to control for most of the confounders affecting bone and lipid metabolism. However, our study has some weakness. First, it has the usual limitations of the cross-sectional studies, particularly in those aspects referring to the estimation of the temporal relationship between the dyslipidemia and BMD or fractures. These questions could be better addressed in a longitudinal study. Second, recall bias regarding non-vertebral osteoporotic fractures, mainly in older participants, cannot be totally excluded. However, participants were recruited at a primary care center, where medical history is carefully recorded. Third, BMD data do not include body composition. However, correlation between total fat mass and BMI or waist perimeter has showed to be higher in previous studies [19, 25, 28].

In summary, we have found that a more unfavorable lipid profile of higher serum TC and LDL-C levels is associated with higher BMD at lumbar spine and

hip in men. The mechanism underlying this apparent paradox seems to be independent of age, anthropometric measurements, physical activity, dairy calcium intake, alcohol and tobacco use, prior fragility fractures, diabetes, hypertension, serum creatinine, calciotropic hormones or bone turnover markers. However, no relationship was found between HDL-C or triglyceride levels and bone metabolism in our study. Moreover, we did not observe any association between hypercholesterolemia and prevalent vertebral fractures, but we found lower serum TC and LDL-C levels in men with prevalent non-vertebral fractures. Calciotropic hormones and bone markers showed slight differences between men with and without hypercholesterolemia, but an understanding of the precise role of such differences requires further studies. According to our data and those previously published we suggest that plasma lipids might play some physiological role in both bone quantity and quality in men. Future studies need to further investigate the interplay between lipid and bone metabolism, especially at tissue level.

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