Title of Manuscript: Increased low-density lipoprotein cholesterol level is associated with non-vertebral fractures in postmenopausal women

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Abstract

Purpose: Although a high serum low-density lipoprotein cholesterol (LDL-C) level is an established risk factor for atherosclerosis, it is unclear whether it is associated with osteoporosis. In this study, the associations between the serum LDL-C level and bone mineral density (BMD), bone metabolic markers, and the presence of prevalent vertebral or non-vertebral fractures were examined.

Methods: A total of 211 healthy postmenopausal women (age range, 46-80 years) who visited a community health center was recruited consecutively. Their radiographic and biochemical characteristics were collected.

Results: Prevalent vertebral and non-vertebral fractures were found in 49 (23.2%) and 36 (17.1%) subjects, respectively. Simple regression analyses showed that the serum LDL-C level was not significantly correlated with lumbar or femoral BMD or serum levels of total amino-terminal propeptide of type I collagen (PINP) or carboxy-terminal telopeptide of type I collagen (CTX). Logistic regression analyses adjusted for age and BMI showed that the increased serum LDL-C level was selected as an index affecting the presence of prevalent non-vertebral fractures, but not vertebral fractures. This result was still significant after additional adjustments for years since menopause, physical activity, previous cardiovascular events, bone markers, BMD, serum Ca, P, Cr, 25(OH)D, grip strength, tandem gait test, and use of drugs for hyperlipidemia [odds ratio 1.76 (1.13-2.73), p=0.012].

Conclusions: These findings suggest that a high serum LDL-C level may be a risk factor for prevalent non-vertebral fragility fractures independent of bone turnover, bone mass, vitamin D insufficiency, or frail status in postmenopausal women, and that it may be detrimental to bone, as well as blood vessels.

Introduction

Clinical studies have shown that both atherosclerosis and osteoporosis are frequently present in one patient, and a variety of indices of atherosclerosis is related to the existence of osteoporosis or bone mass reduction [1-3]. Although the two disorders become prevalent with aging, it has been reported that aortic calcification is negatively associated with femoral neck bone mineral density (FN-BMD) in elderly women, even after adjustment for age [3]. Moreover, several studies, including prospective ones, have shown that subjects with aortic calcification have higher risks for vertebral fractures (VFs) and hip fractures than those without aortic calcification [4,5]. Another studies have also shown that subjects with osteoporosis have a higher risk of cardiovascular events resulting from atherosclerosis than those with osteopenia, after adjustments for age and risk factors for cardiovascular diseases [6] and cardiovascular mortality [7]. A recent study showed that the presence of VFs was a risk factor for coronary events independent of BMD [8].

Molecular medicine also indicates an association between atherosclerosis and osteoporosis. Either Klotho- or osteoprotegerin-deficient mice, which is a model for aging, were reported to show advanced stages of both atherosclerosis and osteoporosis [9,10]. Low-density lipoprotein (LDL) receptor-related protein 5 (LRP5) and Frizzled protein are known to act as co-receptors in the Wnt-signal pathway that exerts an anabolic action on bone [11]. LRP5-deficient mice were shown to have both hypercholesterolemia and bone mass reduction [12]. In humans, family members with mutation of LRP6 were shown to have both early-onset cardiovascular diseases and severe osteoporosis complicated by high serum low-density lipoprotein cholesterol (LDL-C) levels, hypertension, and impaired glucose tolerance [13], suggesting that these components of the metabolic syndrome could cause bone fragility, as well as atherosclerosis.

These clinical and experimental observations suggest that common mechanisms underlie osteoporosis and atherosclerosis, and that bone and blood vessels interact with each other. Dyslipidemia is an established risk factor for atherosclerosis, and it may be a candidate that links both disorders. Experiments using mice have shown that dyslipidemia blunted the anabolic action of parathyroid hormone on bone [14,15], and that LDL oxidation products suppressed bone formation by inhibiting differentiation of osteoblasts and by directing progenitor marrow stromal cells to undergo adipogenic instead of osteogenic differentiation [16,17].

It is unclear about the relationship between bone metabolic markers and lipid profiles, although one clinical study showed a positive correlation between them [18]. The association between bone mineral density (BMD) and dyslipidemia, especially high serum LDL-C levels, is also controversial. We

previously found that the serum LDL-C level was significantly and negatively correlated with radial BMD in 214 Japanese postmenopausal women by a multiple regression analysis adjusted for age, years after menopause, body mass index (BMI), and %fat [19]. Another study involving 2248 Chinese postmenopausal women also showed that serum LDL-C was significantly and negatively correlated with whole-body BMD [20]. In contrast, some previous studies showed that serum LDL-C level was not significantly associated with BMD [21,22]. Moreover, little is known about whether serum LDL-C is associated with fractures by affecting bone fragility.

To clarify these issues, we examined the relationships between serum LDL-C and other lipid levels and osteoporosis-related variables such as BMD, bone metabolic markers, and fractures in Japanese postmenopausal women. We especially evaluated the relationships between lipid levels and different types of fractures such as VFs and non-VFs.

Methods

Participants

A total of 211 Japanese postmenopausal women (age range, 46-80 years, mean 63.5 years) underwent health screening for osteoporosis at a community health center and voluntarily participated in this study. All women had been without spontaneous menses for more than 1 year. None had hepatic or renal dysfunction, thyroid diseases, primary hyperparathyroidism, or systemic diseases that might affect bone metabolism. All subjects were free of drugs (estrogens, bisphosphonates, selective estrogen receptor modulators, glucocorticoids, thiazides, antidepressants, thiazolidinediones etc.) known to influence bone metabolism up to the present study. The study was approved by the ethics review boards of our institutions and was in compliance with the Declaration of Helsinki. All subjects agreed to participate in the study and gave their informed consent.

Biochemical measurements

Blood samples were collected after an overnight fast. Concentrations of total cholesterol (TC), high-density lipoprotein cholesterol (HDL-C), triglyceride (TG), calcium (Ca), phosphate (P), and creatinine (Cr) were measured by standard automated techniques (normal ranges: TC 150-219 mg/dl, HDL-C 40-80 mg/dl, TG 50-149 mg/dl, Ca 8.6-10.3 mg/dl, P 2.2-4.6 mg/dl, Cr 0.56-1.23 mg/dl). LDL-C was calculated using Friedewald's formula (LDL-C = TC – HDL-C – TG/5) [23] (normal range: 70-139 mg/dl). Serum levels of intact PTH, total amino-terminal propeptide of type I collagen (PINP), a marker of bone formation, and carboxy-terminal telopeptide of type I collagen (CTX), a marker of bone

resorption, were measured by electrochemiluminescence immunoassay (ECLIA) [24,25] (normal ranges: PTH 11-54 pg/ml, PINP 17.1-64.7 mg/l, CTX 0.100-0.653 ng/ml). The serum 25(OH)D level was measured by ECLIA, as previously described [26].

BMD measurements

BMD values were measured by dual energy X-ray absorptiometry (DXA) using a QDR-4500 (Hologic Inc., Waltham, MA) at the lumbar spine and femoral neck, as previously described [27]. BMD was automatically calculated from the bone area (cm^2) and bone mineral content (BMC) (g) and expressed absolutely in g/cm^2. The Z-score is the number of standard deviations (SDs) by which a given measurement differs from the mean for a sex-, age-, and race-matched reference population. The T-score is the number of SDs by which a given measurement differs from the mean for a normal young adult reference population. The coefficients of variation (precision) of measurements of the lumbar spine and the femoral neck were 0.9% and 1.7%, respectively. The coefficient of variation was obtained in vitro using a 'phantom' with at least four measurements for the same subject. Normative data were obtained from a population-based database of the Japanese Society of Bone and Mineral Research in 1996.

Ascertainment of fractures

For VF assessment, two lateral scans of the vertebrae from T4 to L4 were performed using the single-energy and the dual-energy high-definition scan modes, with the subjects in the supine position (with the C-arm of the scanner rotated through 90°), as previously described [28,29]. DXA images were evaluated by a trained operator using the standard semiautomatic analysis performed by the software supplied by the manufacturer. Marking of vertebral bodies and semi-quantitative analyses were performed on single-energy scans, using the high definition images to aid placement of vertebral markers. VFs were defined using Genant's semiquantitative method, which is commonly used for diagnosis of VFs [30]. We included all of grades 1 to 3 VFs in this study. For non-VF fracture assessment, only low-trauma fractures i.e. those occurring with falls from standing height or less, were taken into account. We included all fractures that occurred in the study subjects at over 40 years old, except fractures of the hand, toes, metacarpals, face and skull as well as pathological and post-procedural fractures [31,32], on the basis of clinical interviews.

Statistical analysis

All data are expressed as the means ± SD for each index. Regression analysis was performed using

the Statistical Package for the Social Sciences (SPSS) version 17 for Windows (IBM Corp., Chicago, IL). Simple regression analysis was used to assess the linear relationships between study parameters, and Pearson's correlation coefficients were calculated. Multiple regression analysis was performed to determine whether the serum LDL-C level was independently and significantly associated with BMD scores when the other parameters were considered. To evaluate the contribution of the serum LDL-C level to the presence of prevalent fractures, multivariate logistic regression analysis was performed. Comparisons between two groups were made with the Mann-Whitney U-test. P values less than 0.05 were considered significant.

Results

Baseline characteristics of the subjects are shown in Table 1. The patients' mean age was 63.5 years, and their mean BMI was 22.9 kg/m^2. The mean L- and FN-BMD Z-scores were 0.3 and 0.1, respectively, indicating that they were close to age-matched controls. Prevalent VFs and non-VFs were found in 49 (23.2%) and 36 (17.1%) subjects, respectively. Prevalent non-VFs were located in the forearm (n=14), ankle (n=10), leg (n=7), rib (n=3), foot (n=3), and hip (n=1). Of these, 2 subjects had prevalent fractures in both a forearm and leg. Medications for dyslipidemia were given to 30 (14.2%) subjects; 28 were given statins and 2 were given ethyl icosapentate.

Simple regression analyses showed that the serum LDL-C level was not significantly correlated with age, years since menopause, BMI, L- or FN-BMD, or serum levels of Ca, Cr, 25(OH)D, PINP, or CTX (data not shown).

Comparisons of serum lipid levels between subjects with and without VFs showed that there were no significant differences in the levels of total-C, LDL-C, HDL-C, or TG between the two groups (Table 2). In contrast, comparisons of those with and without non-VFs showed that the serum LDL-C level, but not other lipids, was significantly higher in those with than in those without non-VFs (p=0.039) (Table 3).

Next, multivariate logistic regression analysis was performed with the presence of non-VFs as a dependent variable and serum LDL-C level as an independent variable (Table 4). The increased serum LDL-C level was selected as an index affecting the presence of prevalent non-VFs [odds ratio per SD increase (OR) = 1.50, 95% confidence interval (CI) = 1.03–2.18, p = 0.034] after adjustments for age and BMI. This association remained significant when multivariate logistic analysis was performed after the addition of years since menopause, physical activity, previous cardiovascular events, serum levels of PINP, CTX, Ca, P, Cr, and 25(OH)D, L- and FN-BMD, grip strength, the tandem gait test, and use of

drugs for dyslipidemia.

Discussion

In this study, serum LDL-C level was a significant risk factor for non-VFs in postmenopausal women, independent of bone markers, BMD, serum vitamin D level, frail status, or use of drugs for dyslipidemia. Since high serum LDL-C level is also known to be an established risk factor for atherosclerosis and cardiovascular events, this finding suggests that high serum LDL-C level would be as detrimental to both bone and blood vessels as type 2 diabetes mellitus [33,34] and chronic kidney disease [35,36]. We also examined the relationship between lipid profiles and bone markers, but found no significant associations between them.

Multiple studies have been conducted on the correlations between serum LDL-C level and BMD. In a study involving 355 Korean postmenopausal women, FN-BMD was significantly lower in the highest quartile of serum LDL-C level than in its lowest quartile [37]. We and others also reported that serum LDL-C level was negatively correlated with radial and whole body BMD, respectively [20,19]. In contrast, in a study involving 13592 Americans aged ≥17 years, there was no significant correlation between the serum LDL-C level and FN-BMD [22]. It has been reported that serum LDL-C level was not significantly correlated with L- or FN-BMD in 2661 Korean postmenopausal women [38]. Our previous study [19] and the present one also showed that serum LDL-C level was not significantly correlated with L- or FN-BMD. A Turkish study showed that LDL-C level was positively correlated with radial BMD after adjustments with age, menopause duration, and BMI [39]. Thus, the presence of a significant correlation between serum LDL-C level and BMD seems to depend on the skeletal site where BMD is measured, and the appendicular bones such as the radius might be more associated with serum LDL-C level than the trunk bones such as the femoral neck and the lumbar spine.

Several studies have also been conducted on the relationship between hypercholesterolemia and fractures. A 20-year-long prospective study involving 1396 men and women aged 25-64 years old showed that the longer the duration of high serum total-C level was, the more significant it became as a risk factor for any osteoporotic fractures [40]. We also found that increased serum LDL-C level was a risk factor for non-VFs in Japanese postmenopausal women in the present study. In contrast, our previous study [19] and the present one showed that serum LDL-C level was not significantly associated with VFs. Bagger et al. also showed that there was no significant difference in serum LDL-C level between elderly women with and without VFs [41]. This discrepancy between VFs and non-VFs might be because effects of LDL-C on bone differ between appendicular sites and truncal sites, given that serum LDL-C level

was more negatively and significantly correlated with radial BMD than L- and FN-BMD [19]. Indeed, in the present study, non-VFs in 36 subjects were found mostly in appendicular bones such as the forearm, ankle, leg, and foot. Another explanation is that the effect of LDL-C on bone might differ between cortical and trabecular bones, because the vertebrae consist mainly of trabecular bone, while the non-vertebrae consist mainly of cortical bone. Experiments with mice have indicated that hyperlipidemia blunted the anabolic action of parathyroid hormone more prominently in cortical bone than in trabecular bone [14,15].

Falls are known to cause non-VFs more often than VFs. Thus, we should exclude the possibility that high serum LDL-C level would enhance non-VF risk by increasing falls. In the present study, although the history or frequency of falls in the subjects was not directly evaluated, grip strength was measured and tandem gait tests were conducted to assess frail status, which are linked to falls. It was found that serum LDL-C level was still significantly associated with prevalent non-VFs after adjustments for grip strength and tandem gait tests, suggesting that this finding is independent of falls.

Parhami et al. showed that minimally oxidized LDL inhibited osteoblastic differentiation of the MC3T3-E1 preosteoblastic cells, as well as M2-10B4 stromal cells, and promoted adipogenic differentiation of the latter cells, as well as 3T3-L1 preadipocytes [42,16], suggesting that LDL oxidation products could also promote osteoporotic loss of bone by inhibiting differentiation of osteoblasts and by directing progenitor marrow stromal cells to undergo adipogenic instead of osteogenic differentiation. Moreover, Tintut et al. showed that treatment of mouse marrow preosteoclasts with oxidized LDL induced RANKL-dependent osteoclastic differentiation of these cells [43]. These experimental findings suggest that high serum LDL-C level may cause bone fragility by suppressing bone formation, as well as increasing bone resorption. However, in the present study, it was found that serum LDL-C level was still significantly associated with prevalent non-VFs after adjustments for serum PINP or CTX, suggesting that this finding is independent of bone turnover.

A low serum 25(OH)D level caused by vitamin D insufficiency or deficiency is known to a risk factor for hip fracture [44]. We also found that a low serum 25(OH)D level enhanced fracture risk in Japanese postmenopausal women [45]. A low serum 25(OH)D is also reported to be one of the causative factors for diabetes mellitus [46] and cardiovascular events [47,48], and to enhance mortality [49], while it is thought to not participate in high serum LDL-C levels [50]. Recent studies showed that lower 25(OH)D level was linked to higher LDL-C level [51,52] and higher incidence of metabolic syndrome [53]. The mean 25(OH)D level of the present subjects was low (16.3ng/ml), which might mediate the relationship between LDL-C level and non-VF incidence in this study. However, we found that serum LDL-C level

was still significantly associated with prevalent non-VFs after adjustments for serum 25(OH)D, suggesting that this finding is independent of vitamin D status.

This study has some limitations. First, it was a cross-sectional study with a small sample size. Second, this study enrolled only Japanese postmenopausal women. Thus, our findings might be applicable to only Japanese population, but not to other ethnic groups. Third, we evaluated morphometric VFs, but not clinical VFs, by X-ray films, which may be a reason for high VF prevalence in this study. Fourth, we did not measure body compositions of the subjects including fat mass in this study. Sarkis KS et al showed that serum LDL-C level was related to BMD independent of fat mass, although serum LDL-C level positively correlated with fat mass [54]. Their findings suggest that measurements of fat mass or other parameters relating to metabolic syndrome may be necessary when performing lipid studies. Finally, this study included 28 (13%) statin users. A meta-analysis and other studies showed that statin use reduced fracture risk [55,56], and this beneficial effect of statins could have affected the outcome of our study. In the present study, the data were also analyzed by excluding statin users, and the significant correlation between the serum LDL-C level and prevalent non-VFs disappeared because of the reduction in statistical power. However, logistic regression analysis showed that the result was still positive after adjustment for use of drugs for dyslipidemia including statins.

In conclusion, we found that high serum LDL-C level may be a risk factor for prevalent non-VFs independent of bone-related confounders, or use of drugs for dyslipidemia in Japanese postmenopausal women, and it may be detrimental to bone, as well as blood vessels, by being involved in both bone fragility and atherosclerosis. Further studies may be needed in larger populations and other ethnic groups to ascertain that the current findings are universal ones.

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Subjects' baseline characteristics

No. of subjects	211	Total-C (mg/dL)	216 ± 29
No. of subjects with prevalent VFs	49 (23.2%)	LDL-C (mg/dL)	131 ± 26
No. of subjects with prevalent non-VFs	36 (17.1%)	HDL-C (mg/dL)	66 ± 16
Age (year)	63.5 ± 7.5	TG (mg/dL)	86 ± 41
Years since menopause (years)	13.4 ± 8.3	25(OH)D (ng/ml)	16.3 ± 4.4
Height (cm)	151.7 ± 5.4	PINP (ng/mL)	54.2 ± 16.5
Body weight (kg)	52.6 ± 7.4	CTX (ng/mL)	0.404 ± 0.150
BMI (kg/m^2)	22.9 ± 3.1	L-BMD (g/cm^2)	0.841 ± 0.147
Ca (mg/dl)	9.1 ± 0.3	Z Score	0.3 ± 1.1
P (mg/dl)	3.5 ± 0.4	T Score	-1.5 ± 1.3
Cr (mg/dl)	0.58 ± 0.06	FN-BMD (g/cm^2)	0.620 ± 0.091
		Z Score	0.1 ± 1.0
		T Score	-1.5 ± 0.8

Abbreviations: C, cholesterol; TG, triglyceride; L, lumbar spine; FN, femoral neck; BMD, bone mineral density

Table 1

Comparison of subjects with and without vertebral fractures

	Presence of prevalent vertebral fractures		
	No	Yes	P value
No. of subjects	162	49	
Age (year)	62.6 ± 7.4	66.4 ± 7.0	0.002 **
Years since menopause (years)	12.3 ± 7.8	17.1 ± 8.8	< 0.001 **
BMI (kg/m^2)	23.0 ± 3.0	22.4 ± 3.6	0.251
Grip strength (kg)	21.9 ± 4.4	21.3 ± 4.7	0.464
Tandem gait test (steps)	9 ± 2	7 ± 3	< 0.001 **
Ca (mg/dl)	9.1 ± 0.3	9.2 ± 0.4	0.473
P (mg/dl)	3.5 ± 0.4	3.5 ± 0.3	0.721
Cr (mg/dl)	0.57 ± 0.09	0.60 ± 0.13	0.214
25(OH)D (ng/ml)	16.6 ± 4.5	15.0 ± 3.7	0.014 *
L-BMD (g/cm^2)	0.852 ± 0.144	0.804 ± 0.150	0.045 *
Z Score	0.3 ± 1.0	0.2 ± 1.2	0.546
T Score	-1.4 ± 1.3	-1.9 ± 1.4	0.042 *
FN-BMD (g/cm^2)	0.626 ± 0.101	0.590 ± 0.091	0.027 *
Z Score	0.1 ± 1.0	0.01 ± 1.1	0.377
T Score	-1.5 ± 0.8	-1.8 ± 0.8	0.009 **
PINP (ng/ml)	54.2 ± 16.7	54.2 ± 15.9	0.991
CTX (ng/ml)	0.398 ± 0.156	0.421 ± 0.126	0.329
Total-C (mg/dl)	216.3 ± 29.0	213.2 ± 26.8	0.496
LDL-C (mg/dl)	131 ± 27	128 ± 25	0.447
HDL-C (mg/dl)	66.5 ± 16.2	66.5 ± 15.6	0.998
TG (mg/dl)	87.6 ± 42.8	80.8 ± 34.3	0.307

*p<0.05, **p<0.01

Table 2

Comparison of subjects with and without non-vertebral fractures

	Presence of prevalent non-vertebral fractures		
	No	Yes	P value
No. of subjects	175	36	
Age (year)	63.0 ± 7.6	65.8 ± 6.8	0.041 *
Years since menopause (years)	12.9 ± 8.3	16.2 ± 7.9	0.032 *
BMI (kg/m^2)	22.7 ± 3.3	23.6 ± 2.4	0.062
Grip strength (kg)	21.6 ± 4.4	22.1 ± 4.9	0.606
Tandem gait test (steps)	9 ± 3	8 ± 3	0.289
Ca (mg/dl)	9.2 ± 0.35	9.0 ± 0.30	0.086
P (mg/dl)	3.5 ± 0.4	3.4 ± 0.4	0.119
Cr (mg/dl)	0.57 ± 0.10	0.60 ± 0.10	0.089
25(OH)D (ng/ml)	16.5 ± 4.6	14.9 ± 2.9	0.009 *
L-BMD (g/cm^2)	0.852 ± 0.146	0.789 ± 0.141	0.019 *
Z Score	0.3 ± 1.1	0.1 ± 1.0	0.178
T Score	-1.4 ± 1.3	-2.0 ± 1.3	0.020 *
FN-BMD (g/cm^2)	0.619 ± 0.100	0.610 ± 0.100	0.621
Z Score	0.1 ± 1.0	0.2 ± 1.1	0.752
T Score	-1.5 ± 0.8	-1.6 ± 0.9	0.488
PINP (ng/ml)	55.0 ± 17.3	51.4 ± 12.1	0.268
CTX (ng/ml)	0.410 ± 0.152	0.371 ± 0.135	0.157
Total-C (mg/dl)	214.3 ± 28.3	222.1 ± 28.8	0.133
LDL-C (mg/dl)	129 ± 26	139 ± 26	0.039 *
HDL-C (mg/dl)	66.6 ± 16.4	65.6 ± 14.0	0.729
TG (mg/dl)	86.0 ± 40.1	86.2 ± 46.0	0.983

*p<0.05, **p<0.01

Table 3

Association between the presence of prevalent non-vertebral fractures and each plasma LDL-C level in the subjects

	Presence of prevalent non-vertebral fractures	
	odds ratio (95%CI)	p value
LDL-C	1.50 (1.03-2.18)	0.034
LDL-C [a]	1.50 (1.02-2.20)	0.041
LDL-C [b]	1.51 (1.03-2.24)	0.037
LDL-C [c]	1.13 (0.97-2.08)	0.074
LDL-C [d]	1.49 (1.03-2.17)	0.035
LDL-C [e]	1.67 (1.05-2.65)	0.030
LDL-C [f]	1.76 (1.13-2.73)	0.012

Multivariate logistic regression analysis was performed with the presence of non-vertebral fractures as a dependent variable and serum levels of LDL-C as an independent variable adjusted for age and BMI.

[a] Additionally adjusted for serum levels of PINP

[b] Additionally adjusted for serum levels of CTX

[c] Additionally adjusted for L-BMD

[d] Additionally adjusted for FN-BMD

[e] Additionally adjusted for years since menopause, physical activity, previous cardiovascular events, grip strength, tandem gait test, serum levels of Ca, P, Cr, 25(OH)D, CTX, L-BMD, history of using drugs for dyslipidemia

[f] Additionally adjusted for years since menopause, physical activity, previous cardiovascular events, grip strength, tandem gait test, serum levels of Ca, P, Cr, 25(OH)D, CTX, FN-BMD, and history of using drugs for dyslipidemia

Abbreviations: CI, confidence interval

Table 4