Endocrine Journal 2008, **55** (1), 143–151

Increased Bone Turnover in Patients with Hypercholesterolemia

TAKAFUMI MAJIMA*,**,***, AKIRA SHIMATSU*, YASATO KOMATSU**, NORIKO SATOH*, ATSUSHI FUKAO#, KIYOSHI NINOMIYA##, TADASHI MATSUMURA## AND KAZUWA NAKAO**

**Division of Endocrinology and Metabolism, Clinical Research Institute for Endocrine Metabolic Diseases, National Hospital Organization, Kyoto Medical Center, Kyoto, Japan*
***Department of Medicine and Clinical Science, Kyoto University Graduate School of Medicine, Kyoto, Japan*
****Department of Endocrinology and Metabolism, Rakuwakai Otowa Hospital, Kyoto, Japan*
#First Department of Nursing, Aino Gakuin College, Osaka, Japan
##Department of General Medicine, Rakuwakai Otowa Hospital, Kyoto, Japan

Abstract. Osteoporosis has been linked with arteriosclerotic vascular diseases, suggesting that hypercholesterolemia or dyslipidemia may be a common pathogenetic factor underlying these diseases. However, little is known about the relationship between osteoporosis and hypercholesterolemia. The purpose of this study was, therefore, to investigate the effects of hypercholesterolemia upon bone metabolism, by measuring bone turnover markers in hypercholesterolemic patients. This study included 281 Japanese patients with hypercholesterolemia, and 267 control subjects. Serum bone-specific alkaline phosphatase (BAP) of the patients was significantly higher than that of the controls in women. Serum N-terminal telopeptide of type I collagen (NTx) of the patients was significantly higher than that of the controls in both men and women. In addition, both BAP and NTx in men showed a significantly negative correlation with high density lipoprotein cholesterol (HDL-C). On the other hand, in women, both BAP and NTx showed a significantly positive correlation with total cholesterol and low density lipoprotein cholesterol (LDL-C). These results indicate increased bone turnover in hypercholesterolemic or dyslipidemic patients regardless of gender, and suggest the importance of treating hypercholesterolemia or dyslipidemia in order to prevent not only arteriosclerotic complications but also osteoporotic bone loss and subsequent fractures.

Key words: Osteoporosis, Bone turnover, Hypercholesterolemia, Dyslipidemia

(*Endocrine Journal* **55**: 143–151, 2008)

PREVIOUS clinical studies have linked osteoporosis with atherosclerosis [1, 2] and/or cardiovascular diseases [3, 4], suggesting the possibility of a common underlying factor or mechanism among these diseases [5–10]. Hypercholesterolemia has been proposed as one of the most likely candidates [5–10]. However, little is known about the precise nature of the potential relationship between hypercholesterolemia and osteoporosis [5–13]. Several clinical studies [5–13] have examined the relationship between hypercholesterolemia and bone mineral density (BMD), but arrived at quite contradictory results. Some found an association of hypercholesterolemia with lower BMD [5–10], and others with higher BMD [11], while yet others found no association at all [12, 13]. On the other hand, both *in vitro* and *in vivo* animal model studies have demonstrated some detrimental effects of hypercholesterolemia or dyslipidemia on bone metabolism [14–23]. In *in vitro* studies, osteoblastic differentiation has been shown to be inhibited by atherogenic lipids [14, 15]. The mevalonate pathway has recently been proposed as essential for not only the synthesis of cholesterol but also the regulation of bone cell proliferation or apoptosis [16, 17, 24]. In addition, LDL receptor-related protein 5 (LRP5) has recently been identified as a critical regulator of osteoblastic proliferation [18], and a mutation in LRP5 as causing significant reduction in BMD

Received: July 31, 2007
Accepted: November 7, 2007
Correspondence to: Takafumi MAJIMA, M.D., Department of Endocrinology and Metabolism, Clinical Research Institute for Endocrine Metabolic Diseases, National Hospital Organization, 1-1 Mukaihata-cho, Fushimi-ku, Kyoto 612-8555, Japan

in both humans and mice [19, 20]. More recently, a mutation in LRP6, a closely related homolog of LRP5, has been shown to lead to reduced bone mass in mice [21], and to be genetically linked with early coronary disease, metabolic risk factors, and osteoporosis in humans [22]. A mutation in polymorphisms of apolipoprotein E genotype has also been reported to be associated with reduction in BMD in perimenopausal women [23]. Furthermore, animal model studies have found that hypercholesterolemia promoted osteoclastic potential [24] and reduced BMD in mice [25]. All these observations suggest that hypercholesterolemia may promote osteoporotic bone loss, and serve as a warning against the potentially detrimental effects of hypercholesterolemia on bone health.

However, there is a dearth of information in the literature regarding bone metabolism or bone turnover in patients with hypercholesterolemia [5–13]. Clarifying bone metabolism in hypercholesterolemia will be helpful for ascertaining whether the above-mentioned potentially detrimental effects detected in *in vitro* or animal models [14–21, 23–25] also exist in humans. In addition, such clarification should aid the search for an answer for the controversial findings of previous clinical studies [5–13] of the relationship between hypercholesterolemia and osteoporosis, and will promote our understanding of the underlying pathomechanisms, if any, for these two diseases.

The purpose of this study was therefore to investigate bone metabolism in patients with hypercholesterolemia by means of measuring bone turnover markers.

Subjects and Methods

Subjects

The study population comprised 141 men and 140 women with untreated hypercholesterolemia, aged 26–84 (mean ± SD 57.55 ± 12.2) years and 18–89 (mean ± SD 59.61 ± 12.7) years, respectively (Table 1). They were randomly selected from patients who visited the outpatient clinic of Rakuwakai Otowa Hospital between December 2005 and November 2006. The diagnosis of hypercholesterolemia was established on the basis of laboratory findings, including an elevated serum total cholesterol (T-C) level (>220 mg/dl) or an elevated serum low density lipoprotein cholesterol (LDL-C) level (>140 mg/dl).

The control group comprised 167 men and 100 women without hypercholesterolemia, who visited the same clinic during the same period for health checkups. The hypercholesterolemic patients and the control subjects were similar in terms of physical activity and calcium intake.

All subjects completed a questionnaire administered by a doctor or nurse prior to joining the study, and underwent laboratory blood tests. We excluded subjects who had a history of fractures and/or other diseases (type 1 diabetes mellitus, liver disease, renal dysfunction, malignancy, hyperthyroidism, hyperparathyroidism, hypercorticoidism, or hypogonadism) and those taking medications (active vitamin D3, bisphosphonates, selective estrogen receptor modulators, calcitonin, testosterones, steroids, thyroid hormones, diuretics, heparin or anticonvulsants) that could affect bone metabolism. None of the subjects were smokers or drug abusers.

This study was performed in accordance with the recommendations of the Declaration of Helsinki, and the Ethical Committee of Rakuwakai Otowa Hospital approved the protocol. All the subjects gave their informed consent before they were enrolled.

Biochemical measurements

Serum samples were obtained before 9:00 AM after an overnight fast, and were immediately processed and kept frozen at –20°C until the assays were carried out. Serum alkaline phosphatase (ALP), calcium (Ca), phosphate (P), T-C, triglycerides (TG), and high density lipoprotein cholesterol (HDL-C) were measured with standard laboratory methods. LDL-C was calculated with the Friedewald equation (LDL-C = T-C – [HDL-C + TG/5]). Serum bone-specific alkaline phosphatase (BAP) was measured with an enzyme immunoassay kit (Osteolinks-BAP; Sumitomo Pharmaceuticals Inc., Tokyo, Japan) as a specific marker of bone formation. Serum N-terminal telopeptide of type I collagen (NTx) was measured by means of an enzyme-linked immunosorbent assay (Osteomark; Mochida Pharmaceutical Co., Tokyo, Japan) as a specific marker of bone resorption.

Statistical analysis

Data were analyzed with the unpaired t-test for differences between two groups, and with Pearson's cor-

Table 1. Means ± SD of the variables assessed in total subjects classified by gender (A) and in women classified by menopausal status (B).

(A)

	Total subjects (n = 548)					
	Men			Women		
	Patients with hypercholesterolemia (n = 141)	Controls (n = 167)	Total (n = 308)	Patients with hypercholesterolemia (n = 140)	Controls (n = 100)	Total (n = 240)
Age (years)	57.55 ± 12.2^{NS}	59.07 ± 12.7	58.37 ± 12.5	59.61 ± 12.7^{NS}	61.21 ± 16.8	60.28 ± 14.5
Height (cm)	166.51 ± 7.6^{NS}	166.89 ± 6.0	166.72 ± 6.8	153.42 ± 6.7^{NS}	153.32 ± 7.2	153.38 ± 6.9
Weight (kg)	68.45 ± 13.3^{NS}	67.37 ± 11.0	67.86 ± 12.1	56.89 ± 11.9^{NS}	56.06 ± 13.5	56.54 ± 12.6
BMI (kg/m^2)	24.54 ± 3.6^{NS}	24.15 ± 3.5	24.33 ± 3.5	24.12 ± 4.6^{NS}	23.83 ± 5.4	24.00 ± 5.0
ALP (IU/L)	252.27 ± 85.7^{NS}	260.05 ± 122.5	256.49 ± 101.0	264.06 ± 85.4*	239.44 ± 82.0	253.80 ± 84.7
Ca (mg/dL)	9.54 ± 0.4^{NS}	9.40 ± 0.4	9.47 ± 0.4	9.56 ± 0.4^{NS}	9.39 ± 0.4	9.49 ± 0.4
P (mg/dL)	3.23 ± 0.5^{NS}	3.16 ± 0.6	3.19 ± 0.5	3.72 ± 0.5^{NS}	3.55 ± 0.4	3.65 ± 0.5
T-C (mg/dL)	249.57 ± 24.8**	185.31 ± 28.0	214.73 ± 42.3	258.86 ± 29.9**	186.21 ± 25.9	228.59 ± 45.7
TG (mg/dL)	180.70 ± 138.6**	129.43 ± 79.2	154.52 ± 118.8	152.06 ± 79.6**	112.04 ± 56.1	135.38 ± 73.3
HDL-C (mg/dL)	55.09 ± 16.1^{NS}	54.54 ± 14.3	54.79 ± 15.2	62.44 ± 18.4^{NS}	59.64 ± 15.5	61.27 ± 17.3
LDL-C (mg/dL)	158.34 ± 32.5**	104.88 ± 25.8	129.32 ± 40.1	166.01 ± 28.8**	104.16 ± 22.2	140.24 ± 40.3
BAP (U/L)	23.74 ± 7.7^{NS}	24.13 ± 10.0	23.95 ± 9.0	26.08 ± 9.0*	23.38 ± 9.0	24.95 ± 9.1
NTx (nmolBCE/L)	14.64 ± 4.0**	13.34 ± 4.1	13.93 ± 4.1	16.50 ± 4.7**	13.89 ± 4.6	15.41 ± 4.8

(B)

	Women (n = 240)					
	Premenopausal women			Postmenopausal women		
	Patients with hypercholesterolemia (n = 29)	Controls (n = 27)	Total (n = 56)	Patients with hypercholesterolemia (n = 111)	Controls (n = 73)	Total (n = 184)
Age (years)	42.07 ± 8.1^{NS}	39.59 ± 8.0	40.88 ± 8.1	64.20 ± 9.1^{NS}	69.21 ± 11.1	66.19 ± 10.24
Height (cm)	155.21 ± 6.0^{NS}	157.08 ± 5.2	156.11 ± 5.7	152.95 ± 6.8^{NS}	151.92 ± 7.3	152.54 ± 7.0
Weight (kg)	65.24 ± 17.8^{NS}	61.37 ± 15.7	63.38 ± 16.8	54.71 ± 8.6^{NS}	54.09 ± 12.2	54.47 ± 10.2
BMI (kg/m^2)	27.08 ± 7.3^{NS}	24.94 ± 6.5	26.05 ± 6.9	23.35 ± 3.2^{NS}	23.42 ± 4.9	23.38 ± 4.0
ALP (IU/L)	213.69 ± 80.3^{NS}	190.74 ± 71.1	202.63 ± 76.2	277.22 ± 82.1^{NS}	257.45 ± 78.7	269.38 ± 81.1
Ca (mg/dL)	9.55 ± 0.3^{NS}	9.37 ± 0.3	9.46 ± 0.4	9.56 ± 0.4^{NS}	9.41 ± 0.4	9.50 ± 0.4
P (mg/dL)	3.60 ± 0.5^{NS}	3.42 ± 0.4	3.51 ± 0.5	3.75 ± 0.5^{NS}	3.60 ± 0.4	3.69 ± 0.5
T-C (mg/dL)	249.52 ± 19.8**	190.96 ± 25.0	221.29 ± 37.0	261.30 ± 31.6**	184.45 ± 26.2	230.81 ± 47.9
TG (mg/dL)	119.59 ± 78.2^{NS}	121.63 ± 64.1	120.57 ± 71.1	160.54 ± 78.1**	108.49 ± 52.8	139.89 ± 73.6
HDL-C (mg/dL)	68.62 ± 25.9^{NS}	64.74 ± 17.5	66.75 ± 22.2	60.82 ± 15.7^{NS}	57.75 ± 14.4	59.60 ± 15.2
LDL-C (mg/dL)	156.98 ± 20.5**	101.90 ± 19.9	130.42 ± 34.2	168.37 ± 30.2**	105.00 ± 23.1	143.23 ± 41.5
BAP (U/L)	19.40 ± 7.4^{NS}	17.73 ± 6.9	18.59 ± 7.1	27.82 ± 8.6^{NS}	25.47 ± 8.9	26.89 ± 8.8
NTx (nmolBCE/L)	14.13 ± 3.6*	11.99 ± 3.2	13.10 ± 3.55	17.12 ± 4.7**	14.59 ± 4.8	16.12 ± 4.9

Data represent mean ± SD.

BMI, body mass index; T-C, total cholesterol; TG, triglycerides; HDL-C, high density lipoprotein cholesterol; LDL-C, low density lipoprotein cholesterol; Ca, calcium; P, phosphate; ALP, alkaline phosphatase; BAP, bone-specific alkaline phosphatase; NTx, N-terminal telopeptide of type I collagen.

P-values for comparisons between patients with hypercholesterolemia and controls: $^{NS}P>0.05$; $^{*}P<0.05$; $^{**}P<0.01$.

relation test for univariate correlations. Statistics were calculated with StatView version 5.0 (Abacus Concepts, Inc., Berkeley, CA, USA). A p value <0.05 was considered statistically significant.

Results

Table 1 shows a comparison between the hypercholesterolemic patients and the controls, in total subjects

classified by gender (Table 1(A)), and in women classified by menopausal status (Table 1(B)). There were no significant differences between the two groups in age, height, weight, BMI, Ca, or P in men or total, premenopausal, or postmenopausal women. Serum ALP of the patients were significantly higher than those of the controls in total female subjects ($p = 0.026$), but not in men or in women when divided by menopausal status. As expected, T-C, TG and LDL-C of the patients were significantly higher than those of controls in men and total, premenopausal, and postmenopausal women. Meanwhile, HDL-C of the patients was not different from that of controls in men or total, premenopausal, or postmenopausal women. Regarding the specific bone turnover markers, in total female subjects, BAP of the patients was significantly higher than that of controls ($p = 0.0234$), but not in men or in women when divided by menopausal status. On the other hand, serum NTx of the patients was significantly higher than that of controls in men and total, premenopausal, and postmenopausal women ($p = 0.005$, <0.001, 0.016, and <0.001 respectively).

Fig. 1 shows comparisons of bone-specific turnover markers (BAP and NTx) between patients with hypercholesterolemia (open columns) and control subjects (closed columns) in both men (Fig. 1(A) and 1(B)) and women (Fig. 1(C) and 1(B)), divided by age into the four groups (those aged 49 years or less, 50–59 years, 60–69 years, and 70 years or more). In men, the number of the patients and controls in each group divided by age (49 years or less, 50–59 years, 60–69 years, and 70 years or more) was 32 and 38, 37 and 43, 51 and 56, and 21 and 30, respectively. Likewise, in women, that of the patients and controls was 26 and 26, 48 and 20, 28 and 23, 38 and 31, respectively. In men, BAP was not different between the patients and controls in any subgroups divided by age, while NTx was significantly increased in the patients than in controls in all of the subgroups except that aged 50–59 years. In women, BAP was significantly increased in the patients than in

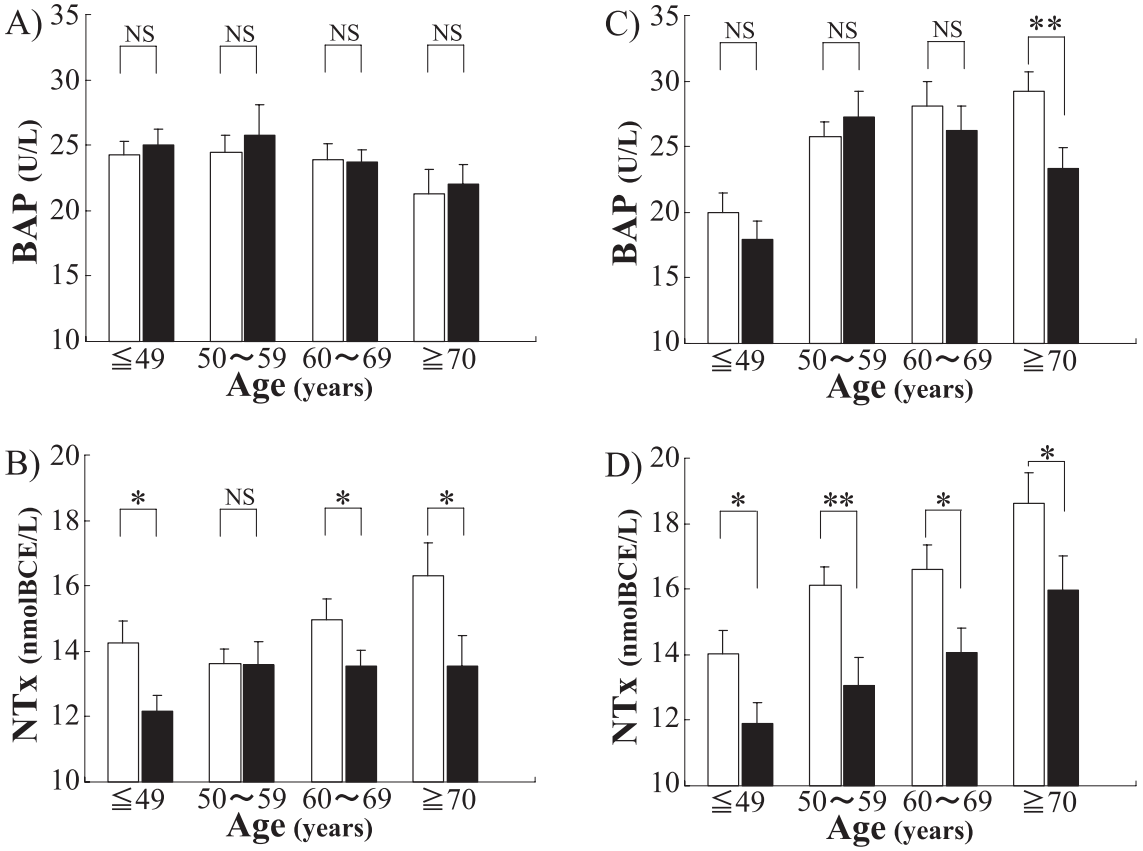

Fig. 1. Comparison of specific bone turnover markers between patients with hypercholesterolemia (open columns) and control subjects (closed columns) in both men (A, B) and women (C, D), stratified by age. Each column represents the mean ± SEM. *P*-values for comparisons between patients with hypercholesterolemia and control subjects: ^{NS}, $P \geq 0.05$; $*P < 0.05$; $**P < 0.01$.

controls in the subgroup aged 70 years or more, but not in the other subgroups. On the other hand, NTx was significantly increased in the patients than in controls in all of the subgroups. None of the intergroup differences in age were significant either in men or women.

Table 2 shows correlations of BAP and NTx with age, height, weight, BMI and the biochemical parameters (except bone turnover markers) in men and total, premenopausal, and postmenopausal women. In men, both BAP and NTx showed a significantly negative correlation with HDL-C ($p = 0.004$, and 0.007, respectively). Otherwise, BAP showed a significantly negative correlation with age ($p = 0.041$), while NTx showed a significantly positive correlation with age ($p = 0.016$), and a significantly negative correlation with weight and BMI ($p = 0.009$, and 0.025, respectively). In total female subjects, on the other hand, both BAP and NTx showed a significantly positive correlation with age ($p<0.001$ for both), T-C ($p<0.001$, and $p = 0.001$, respectively), and LDL-C ($p<0.001$, and $p = 0.001$, respectively). Otherwise, BAP showed a significantly positive correlation with TG ($p = 0.008$), while NTx showed a significantly positive correlation with P ($p = 0.006$), and a significantly negative correlation with weight and BMI ($p = 0.012$, and 0.020, respectively). In premenopausal women, BAP was not correlated with any variables assessed, while NTx showed a significantly positive correlation with T-C ($p = 0.047$) as in total women. In postmenopausal women, as is the case with total women, both BAP and NTx showed a significantly positive correlation with T-C ($p<0.001$, and $p = 0.015$, respectively), and LDL-C ($p<0.001$, and $p = 0.006$, respectively). Otherwise, NTx was significantly correlated with age ($p = 0.031$).

Furthermore, multiple regression analysis was performed between BAP or NTx versus plasma lipids adjusted for age and BMI (Table 3). Even after this procedure, all of those above-described significant correlations between BAP or NTx versus plasma lipids (Table 2) remained statistically significant.

Discussion

In this study we demonstrated for the first time that NTx of patients with hypercholesterolemia was significantly higher than that of controls in both men and women, while BAP of the patients was significantly higher in women but not in men. We also found that both BAP and NTx were significantly correlated positively with both T-C and LDL-C in women. Furthermore, even after multiple regression analysis, these

Table 2. Correlations of BAP and NTx with age, height, weight, BMI and the biochemical parameters (except bone turnover markers) in men and total, premenopausal, and postmenopausal women.

	Age	Height	Weight	BMI	Ca	P	T-C	TG	HDL-C	LDL-C
Men (n = 308)										
BAP	–0.117*	–0.044	–0.067	–0.058	0.054	–0.050	–0.026	0.059	–0.162**	–0.013
NTx	0.137*	–0.106	–0.148**	–0.127*	0.009	0.067	0.007	–0.030	–0.153*	0.073
Women										
Total (n = 240)										
BAP	0.247**	–0.063	–0.001	–0.026	0.085	–0.019	0.253**	0.172*	–0.047	0.245**
NTx	0.268**	–0.066	–0.162*	–0.149*	0.113	0.179*	0.207**	0.067	0.002	0.210**
Pre-M (n = 56)										
BAP	–0.030	0.146	0.190	0.148	–0.088	–0.146	0.087	0.159	–0.033	0.002
NTx	0.171	–0.069	–0.196	–0.185	0.043	0.255	0.266*	–0.013	0.261	0.083
Post-M (n = 184)										
BAP	–0.068	–0.001	0.118	0.129	0.109	–0.071	0.267**	0.136	0.018	0.253**
NTx	0.159*	0.002	–0.060	–0.071	0.118	0.116	0.179*	0.050	–0.031	0.200*

Values are correlation coefficients.

BMI, body mass index; T-C, total cholesterol; TG, triglycerides; HDL-C, high density lipoprotein cholesterol; LDL-C, low density lipoprotein cholesterol; Ca, calcium; P, phosphate; ALP, alkaline phosphatase; BAP, bone-specific alkaline phosphatase; NTx, N-terminal telopeptide of type I collagen; Pre-M, premenopausal women; Post-M, postmenopausal women.

P-values for correlations of BAP and NTx with age, height, weight, BMI and the biochemical parameters in men and total, premenopausal, and postmenopausal women: *$P<0.05$; **$P<0.01$.

Table 3. Correlations between BAP or NTx versus plasma lipids in men and total, premenopausal, and postmenopausal women.

	T-C	TG	HDL-C	LDL-C
Men (n = 308)				
BAP	–0.020	0.071	–0.180**	–0.027
NTx	0.079	0.003	–0.131*	0.085
Women				
Total (n = 240)				
BAP	0.277**	0.138*	0.077	0.245**
NTx	0.228**	0.067	0.023	0.215**
Pre-M (n = 56)				
BAP	0.198	0.133	0.252	–0.030
NTx	0.379**	0.052	0.154	0.133
Post-M (n = 184)				
BAP	0.262**	0.114	0.077	0.247**
NTx	0.229**	0.066	0.010	0.250**

Values are standardized correlation coefficients.
T-C, total cholesterol; TG, triglycerides; HDL-C, high density lipoprotein cholesterol; LDL-C, low density lipoprotein cholesterol; BAP, bone-specific alkaline phosphatase; NTx, N-terminal telopeptide of type I collagen; Pre-M, premenopausal women; Post-M, postmenopausal women.
Multiple regression analysis was performed between BAP or NTx versus plasma lipids adjusted for age and BMI: *P<0.05; **P<0.01.

correlations of both BAP and NTx with both T-C and LDL-C in women remained significant. Meanwhile, both BAP and NTx were found to be significantly correlated negatively with HDL-C in men. Furthermore, even after multiple regression analysis, the correlation between HDL-C and both BAP and NTx in men also remained significant. These findings indicate an increased bone turnover in patients with hypercholesterolemia, especially in female patients, or in male patients with decreased HDL-C, which suggests the importance of improving dyslipidemia to prevent not only arteriosclerotic complications but also osteoporotic bone loss in such patients.

Observational studies have examined the relationship between hypercholesterolemia and bone mass [5–13]. Although the results remain somewhat controversial, [11–13], several studies found a more decreased BMD in patients with hypercholesterolemia [5] or a significant correlation between higher T-C and decreased BMD [6–10]. The possibility has been pointed out that hypercholesterolemia may be the main cause of abnormal bone metabolism in type 2 diabetes mellitus [5, 27]. In addition, findings of an animal model study demonstrated that BMD was reduced in dyslipidemic mice [25]. These findings suggest that hypercholesterolemia may be a risk factor for reducing bone mass, consistent with our findings of accelerated bone resorption in hypercholesterolemia.

However, there have been few clinical studies examining the relationship between hypercholesterolemia and bone metabolism. *In vitro* studies, on the other hand, have found that lipid and lipoprotein oxidation byproducts inhibit osteoblastic differentiation and function [14, 15], although BAP was not reduced in our patients with hypercholesterolemia. Furthermore, Tintut *et al*. have recently reported that hypercholesterolemia promotes osteoclastic differentiation and resorptive activity *in vivo* [24] as well as *in vitro* [15], and have suggested that hypercholesterolemia may cause osteoporotic bone loss via increased bone resorption. Their findings are in agreement with ours, thus indicating the need for evaluation of bone status in patients with hypercholesterolemia for the prevention of osteoporosis and subsequent fractures.

Another finding of significant negative correlation between HDL-C and both BAP and NTx in men in this study are also in line with some of the previous clinical studies showing the correlation of lower HDL-C with lower BMD [6]. In a recent study of ours, we reported that atorvastatin improved accelerated bone turnover in dyslipidemic male patients, especially in those with lower HDL-C [28]. All these observations would suggest that bone turnover markers, preferably along with BMD, should be monitored in dyslipidemic patients, especially male patients with lower HDL-C, to effectively prevent bone loss and subsequent fracture.

On the other hand, it has also been repeatedly suggested that elevated LDL-C is a risk factor for reducing bone mass [6, 7, 9, 10, 13, 29]. Most [6, 7, 9, 10, 13, 29], but not all [11], of the clinical studies have reported a significant correlation of elevated LDL-C with reduced BMD. In support of this epidemiological finding, *in vitro* studies have shown that LDL-C inhibits differentiation of osteoblasts [14], while Tintut *et al.* have reported that LDL-C promotes osteoclastic differentiation *in vitro*, and that elevated LDL-C correlates significantly with increased osteoclast activity in dyslipidemic mice [24]. Consistent with these observations, we also identified a significant correlation between elevated LDL-C and increased bone turnover in women, whose statistical significance sustained after multiple regression analysis. These findings suggest

the potentially adverse effects of LDL-C on bone health to promote osteoporotic bone loss.

Although we identified associations of higher T-C, higher LDL-C and lower HDL-C with higher levels of bone turnover markers in patients with hypercholesterolemia as discussed above, none of these associations was found in our study when limited to controls without hypercholesterolemia (data not shown). Thus, these associations are not applicable to patients without hypercholesterolemia. In fact, Brownbill and Ilich [30] have most recently found no relationship between serum lipids and bone turnover markers in healthy postmenopausal women. However, their results are not applicable to women with hypercholesterolemia, as they have stated in their own report. Additionally, it still remains unclear whether these associations of T-C, LDL-C, and HDL-C with BAP and NTx in our study mean direct effects of T-C, LDL-C or HDL-C on bone metabolism. Because higher T-C, higher LDL-C, and lower HDL-C may be associated with gonadal hypofunction or atherosclerosis, the latter may be the underlying factor in the correlation of T-C, LDL-C or HDL-C with bone turnover markers detected in our study. This is supported by the findings by Pennisi *et al*. [2], of a negative bone remodeling balance in patients with atherosclerosis. Although indirectly, our results thus support the possibility of a relationship between osteoporosis and atherosclerosis.

Since Mundy *et al*. [31] published their report on the stimulatory effects of statins on bone formation, numerous clinical studies have evaluated the beneficial effects of statins on BMD [32], fracture risk [32, 33] and bone metabolism [26, 34–38]. It remains obscure, however, whether the clinical use of statins has beneficial effects on bone health as well [26], even though their numerous pleiotropic effects are well known. Our findings of accelerated bone turnover in patients with hypercholesterolemia may prove useful for a reevaluation of the apparent beneficial effects of statins on bone health reported in the literature. This is because our results suggest that those beneficial effects may be explained by improvements in lipid metabolism *per se*.

There are some limitations in our study. First, the sample size was not large enough to allow us to reach any definite conclusions. Second, due to its cross-sectional nature, our results may be less accurate than those of prospective studies, suggesting that our findings may be confounded by several other factors. Lastly, although the specific bone turnover markers used in our study are known as good predictors for subsequent changes in BMD and the future risk of fractures [39] and, in addition, may be more sensitive detectors of subtle changes in bone metabolism, we did not examine BMD or calculate fracture risk as an odds ratio. Those limitations suggest that the biological/clinical relevance of our findings may be still somewhat obscure. Further studies are thus needed to clarify whether or to what extent those apparently detrimental effects of hypercholesterolemia on bone metabolism found in our study are of clinical relevance.

To summarize, we found a significant increase of BAP in female patients with hypercholesterolemia, and of NTx in both male and female patients compared with the corresponding values in controls. We also found that both BAP and NTx were significantly correlated positively with both T-C and LDL-C in women. These results suggest the detrimental effects of hypercholesterolemia on bone health, especially in women, and therefore probably a higher risk of osteoporosis and future fractures in those patients. In conclusion, we wish to emphasize the importance of treating hypercholesterolemia for the prevention of osteoporotic bone loss as well as cardiovascular complications, and of monitoring bone metabolic markers, preferably in conjunction with BMD, in patients with hypercholesterolemia for the effective prevention of bone loss and subsequent fractures.

References

1. Tanko LB, Bagger YZ, Christiansen C (2003) Low bone mineral density in the hip as a marker of advanced atherosclerosis in elderly women. *Calcif Tissue Int* 73: 15–20.
2. Pennisi P, Signorelli SS, Riccobene S, Celotta G, Di Pino L, La Malfa T, Fiore CE (2004) Low bone density and abnormal bone turnover in patients with atherosclerosis of peripheral vessels. *Osteoporos Int* 15: 389–395.
3. Trivedi DP, Khaw KT (2001) Bone mineral density at the hip predicts mortality in elderly men. *Osteoporos Int* 12: 259–265.
4. Samelson EJ, Kiel DP, Broe KE, Zhang Y, Cupples LA, Hannan MT, Wilson PW, Levy D, Williams SA,

Vaccarino V (2004) Metacarpal cortical area and risk of coronary heart disease: the Framingham Study. *Am J Epidemiol* 159: 589–595.

5. Koshiyama H, Wada Y, Nakamura Y (2001) Hypercholesterolemia as a possible risk factor for osteopenia in type 2 diabetes mellitus. *Arch Intern Med* 161: 1678.
6. Yamaguchi T, Sugimoto T, Yano S, Yamauchi M, Sowa H, Chen Q, Chihara K (2002) Plasma lipids and osteoporosis in postmenopausal women. *Endocr J* 49: 211–217.
7. Wu LY, Yang TC, Kuo SW, Hsiao CF, Hung YJ, Hsieh CH, Tseng HC, Hsieh AT, Chen TW, Chang JB, Pei D (2003) Correlation between bone mineral density and plasma lipids in Taiwan. *Endocr Res* 29: 317–325.
8. Tanko LB, Bagger YZ, Nielsen SB, Christiansen C (2003) Does serum cholesterol contribute to vertebral bone loss in postmenopausal women? *Bone* 32: 8–14.
9. Orozco P (2004) Atherogenic lipid profile and elevated lipoprotein (a) are associated with lower bone mineral density in early postmenopausal overweight women. *Eur J Epidemiol* 19: 1105–1112.
10. Cui LH, Shin MH, Chung EK, Lee YH, Kweon SS, Park KS, Choi JS (2005) Association between bone mineral densities and serum lipid profiles of pre- and postmenopausal rural women in South Korea. *Osteoporos Int* 16: 1975–1981.
11. Adami S, Braga V, Zamboni M, Gatti D, Rossini M, Bakri J, Battaglia E (2004) Relationship between lipids and bone mass in 2 cohorts of healthy women and men. *Calcif Tissue Int* 74: 136–142.
12. Samelson EJ, Cupples LA, Hannan MT, Wilson PW, Williams SA, Vaccarino V, Zhang Y, Kiel DP (2004) Long-term effects of serum cholesterol on bone mineral density in women and men: the Framingham Osteoporosis Study. Bone 34: 557–561.
13. Solomon DH, Avorn J, Canning CF, Wang PS (2005) Lipid levels and bone mineral density. *Am J Med* 118: 1414.
14. Parhami F, Jackson SM, Tintut Y, Le V, Balucan JP, Territo M, Demer LL (1999) Atherogenic diet and minimally oxidized low density lipoprotein inhibit osteogenic and promote adipogenic differentiation of marrow stromal cells. *J Bone Miner Res* 14: 2067–2078.
15. Tintut Y, Parhami F, Tsingotjidou A, Tetradis S, Territo M, Demer LL (2002) 8-Isoprostaglandin E2 enhances receptor-activated NFkappa B ligand (RANKL)-dependent osteoclastic potential of marrow hematopoietic precursors via the cAMP pathway. *J Biol Chem* 277: 14221–14226.
16. Cummings SR, Bauer DC (2000) Do statins prevent both cardiovascular disease and fracture? *JAMA* 283: 3255–3257.
17. Parhami F, Mody N, Gharavi N, Ballard AJ, Tintut Y, Demer LL (2002) Role of the cholesterol biosynthetic pathway in osteoblastic differentiation of marrow stromal cells. *J Bone Miner Res* 17: 1997–2003.
18. Koay MA, Woon PY, Zhang Y, Miles LJ, Duncan EL, Ralston SH, Compston JE, Cooper C, Keen R, Langdahl BL, MacLelland A, O'Riordan J, Pols HA, Reid DM, Uitterlinden AG, Wass JA, Brown MA (2004) Influence of LRP5 polymorphisms on normal variation in BMD. *J Bone Miner Res* 19: 1619–1627.
19. Gong Y, Slee RB, Fukai N, Rawadi G, Roman-Roman S, Reginato AM, Wang H, Cundy T, Glorieux FH, Lev D, Zacharin M, Oexle K, Marcelino J, Suwairi W, Heeger S, Sabatakos G, Apte S, Adkins WN, Allgrove J, Arslan-Kirchner M, Batch JA, Beighton P, Black GC, Boles RG, Boon LM, Borrone C, Brunner HG, Carle GF, Dallapiccola B, De Paepe A, Floege B, Halfhide ML, Hall B, Hennekam RC, Hirose T, Jans A, Juppner H, Kim CA, Keppler-Noreuil K, Kohlschuetter A, LaCombe D, Lambert M, Lemyre E, Letteboer T, Peltonen L, Ramesar RS, Romanengo M, Somer H, Steichen-Gersdorf E, Steinmann B, Sullivan B, Superti-Furga A, Swoboda W, van den Boogaard MJ, Van Hul W, Vikkula M, Votruba M, Zabel B, Garcia T, Baron R, Olsen BR, Warman ML; Osteoporosis-Pseudoglioma Syndrome Collaborative Group (2001) LDL receptor-related protein 5 (LRP5) affects bone accrual and eye development. *Cell* 107: 513–523.
20. Kato M, Patel MS, Levasseur R, Lobov I, Chang BH, Glass DA 2nd, Hartmann C, Li L, Hwang TH, Brayton CF, Lang RA, Karsenty G, Chan L (2002) Cbfa1-independent decrease in osteoblast proliferation, osteopenia, and persistent embryonic eye vascularization in mice deficient in Lrp5, a Wnt coreceptor. *J Cell Biol* 157: 303–314.
21. Kokubu C, Heinzmann U, Kokubu T, Sakai N, Kubota T, Kawai M, Wahl MB, Galceran J, Grosschedl R, Ozono K, Imai K (2004) Skeletal defects in ringelschwanz mutant mice reveal that Lrp6 is required for proper somitogenesis and osteogenesis. *Development* 131: 5469–5480.
22. Mani A, Radhakrishnan J, Wang H, Mani A, Mani MA, Nelson-Williams C, Carew KS, Mane S, Najmabadi H, Wu D, Lifton RP (2007) LRP6 mutation in a family with early coronary disease and metabolic risk factors. *Science* 315: 1278–1282.
23. Gerdes LU, Vestergaard P, Hermann AP, Mosekilde L (2001) Regional and hormone-dependent effects of apolipoprotein E genotype on changes in bone mineral in perimenopausal women. *J Bone Miner Res* 16: 1906–1916.
24. Tintut Y, Morony S, Demer LL (2004) Hyperlipidemia promotes osteoclastic potential of bone marrow cells ex vivo. *Arterioscler Thromb Vasc Biol* 4: e6–10.
25. Parhami F, Tintut Y, Beamer WG, Gharavi N, Goodman W, Demer LL (2001) Atherogenic high-fat diet reduces bone mineralization in mice. *J Bone Miner Res* 16:

182–188.
26. Bauer DC (2003) HMG CoA reductase inhibitors and the skeletal: A comprehensive review. *Osteoporos Int* 14: 273–282.
27. Majima T, Komatsu Y, Yamada T, Koike Y, Shigemoto M, Takagi C, Hatanaka I, Nakao K. (2005) Decreased bone mineral density at the distal radius, but not at the lumbar spine or the femoral neck, in Japanese type 2 diabetic patients. *Osteoporos Int* 16: 907–913.
28. Majima T, Komatsu Y, Fukao A, Ninomiya K, Matsumura T, Nakao K (2007) Short-term Effects of Atorvastatin on Bone Turnover in Male Patients with Hypercholesterolemia. *Endocr J* 54: 145–151.
29. Shilbayeh S (2003) Prevalence of osteoporosis and its reproductive risk factors among Jordanian women: a cross-sectional study. *Osteoporos Int* 14: 929–940.
30. Brownbill RA, Ilich JZ (2006) Lipid profile and bone paradox: higher serum lipids are associated with higher bone mineral density in postmenopausal women. *J Womens Health (Larchmt)* 15: 261–270.
31. Mundy G, Garrett R, Harris S, *et al.* (1999) Stimulation of bone formation *in vitro* and in rodents by statins. *Science* 286: 1946–1949.
32. Watanabe S, Fukumoto S, Takeuchi Y, Fujita H, Nakano T, Fujita T (2001) Effects of 1-year treatment with fluvastatin or pravastatin on bone. *Am J Med* 110: 584–587.
33. Rejnmark L, Olsen ML, Johnsen SP, Vestergaard P, Sorensen HT, Mosekilde L (2004) Hip fracture risk in statin users—a population-based Danish case-control study. *Osteoporos Int* 15: 452–458.
34. Bjarnason NH, Riis BJ, Christiansen C (2001) The effect of fluvastatin on parameters of bone remodeling. *Osteoporos Int* 12: 380–384.
35. Rejnmark L, Buus NH, Vestergaard P, Andreasen F, Larsen ML, Mosekilde L (2002) Statins decrease bone turnover in postmenopausal women: A cross-sectional study. *Eur J Clin Invest* 32: 581–589.
36. Kuzuya M, Suzuki Y, Asai T, Koike T, Kanda S, Nakamura A, Satake S, Umegaki H, Iguchi A (2003) Atorvastatin, 3-hydroxy-3-methylglutaryl coenzyme a reductase inhibitor, reduces bone resorption in the elderly. *J Am Geriatr Soc* 51: 1677–1678.
37. Berthold HK, Unverdorben S, Zittermann A, Degenhardt R, Baumeister B, Unverdorben M, Krone W, Vetter H, Gouni-Berthold I (2004) Age-dependent effects of atorvastatin on biochemical bone turnover markers: a randomized controlled trial in postmenopausal women. *Osteoporos Int* 15: 459–467.
38. Rosenson RS, Tangney CC, Langman CB, Parker TS, Levine DM, Gordon BR (2005) Short-term reductioidin bone markers with high-dose simvastatin. *Osteoporos Int* 16: 1272–1276.
39. Garnero P, Dargent-Molina P, Hans D, Schott AM, Breart G, Meunier PJ, Delmas PD (1998) Do markers of bone resorption add to bone mineral density and ultrasonographic heel measurement for the prediction of hip fracture in elderly women? The EPIDOS prospective study. *Osteoporos Int* 8: 563–569.