Atherogenic High-Fat Diet Reduces Bone Mineralization in Mice

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ABSTRACT

The epidemiological correlation between osteoporosis and cardiovascular disease is independent of age, but the basis for this correlation is unknown. We previously found that atherogenic oxidized lipids inhibit osteoblastic differentiation in vitro and ex vivo, suggesting that an atherogenic diet may contribute to both diseases. In this study, effects of an atherogenic high-fat diet versus control chow diet on bone were tested in two strains of mice with genetically different susceptibility to atherosclerosis and lipid oxidation. After 4 months and 7 months on the diets, mineral content and density were measured in excised femurs and lumbar vertebrae using peripheral quantitative computed tomographic (pQCT) scanning. In addition, expression of osteocalcin in marrow isolated from the mice after 4 months on the diets was examined. After 7 months, femoral mineral content in C57BL/6 atherosclerosis-susceptible mice on the high-fat diet was 43% lower (0.73 ± 0.09 mg vs. 1.28 ± 0.42 mg; p = 0.008), and mineral density was 15% lower compared with mice on the chow diet. Smaller deficits were observed after 4 months. Vertebral mineral content also was lower in the fat-fed C57BL/6 mice. These changes in the atherosclerosis-resistant, C3H/HeJ mice were smaller and mostly not significant. Osteocalcin expression was reduced in the marrow of high fat-fed C57BL/6 mice. These findings suggest that an atherogenic diet inhibits bone formation by blocking differentiation of osteoblast progenitor cells. (J Bone Miner Res 2001;16:182–188)

Key words: osteoporosis, oxidized lipids, bone, atherosclerosis, high-fat diet

INTRODUCTION

 $E^{\text{PIDEMIOLOGICAL EVIDENCE links osteoporosis with car$ diovascular disease, independently of age.^(1,2) Osteoporosis and the subsequent 1 million fractures in the UnitedStates each year⁽³⁾ results from a combination of increasedbone resorption and decreased bone formation. Lowbone mineral density (BMD) is associated closely withcardiovascular disease mortality,⁽⁴⁻⁶⁾ cardiovascularcalcification,⁽⁷⁻⁹⁾ atherosclerosis,^(10,11) and high lipidlevels.⁽¹⁰⁻¹³⁾ Such correlations raise the possibility of acommon underlying factor or mechanism. We previously found that minimally oxidized low-density lipoprotein (MM-LDL), and other bioactive oxidized lipids that promote atherogenesis and are increased in atherosclerotic lesions,^(14–19) also inhibit osteoblastic differentiation of bone- and marrow-derived preosteoblasts in vitro.^(20,21) Preosteoblasts harvested from the marrow of mice fed a high-fat, atherogenic diet showed significantly less osteoblastic differentiation.⁽²¹⁾ Others have shown a paucity of cells committed to the bone lineage in osteoporotic bone marrow and with aging.^(22,23) These links between lipids, vascular disease, and bone suggest the novel hypothesis that oxidized lipids are the biological link.

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In this study, effects of a high-fat atherogenic diet versus control chow diet on bone mineral content (BMC) and BMD were tested in two strains of mice with genetically different susceptibility to oxidized lipids and atherogenesis. In 1985, Paigen et al. showed differences in the susceptibility of two inbred strains of mice to development of hyperlipidemia and atherosclerotic lesions when fed an atherogenic diet^(24,25); C3H/HeJ were identified as a resistant strain and C57BL/6 as a sensitive strain. Several years later, Liao et al. reported the induction of inflammatory genes by an atherogenic diet in the C57BL/6 but not in the C3H/HeJ strain,⁽²⁶⁾ and Navab et al.⁽²⁷⁾ and Shih et al.⁽²⁸⁾ found differences in the antioxidant defense systems between the susceptible and resistant mouse strains. In the present study, we have compared the susceptibility and resistance of these two strains of mice to the effects of high-fat diet-induced hyperlipidemia on bone. We report that in the atherosclerosis-susceptible C57BL/6 mice, BMC and BMD were significantly lowered by the high-fat diet versus chow diet. These changes were smaller in the atherosclerosis-resistant C3H/HeJ mice. In addition, marrow cells from the high-fat-fed C57BL/6 mice showed reduced osteocalcin expression.

Altogether these results suggest that oxidized lipids adversely affect bone by inhibiting osteoblastic differentiation. If applicable to humans, these studies may result in new therapeutic approaches to osteoporosis.

MATERIALS AND METHODS

Mice and diets

At 1 month of age, male C57BL/6 (atherosclerosissusceptible strain) and C3H/HeJ (atherosclerosis-resistant strain) mice (The Jackson Laboratory, Bar Harbor, ME, USA) were placed on either a control chow diet (National Institutes of Health [NIH]-31 Mouse/Rat Diet 7013 containing 6% fat) or a high-fat (atherogenic) diet (Teklad TD90221; Harlan Teklad, Madison, WI, USA; including 1.25% cholesterol, 15.8% fat, and 0.5% cholate). This atherogenic diet has been found to cause significant hypercholesterolemia in C57BL/6 mice.^(24,25) Femurs and lumbar vertebrae were harvested from 8 animals after 4 months and 14 animals after 7 months. The bones were cleared of soft tissue and fixed in 95% ethanol.

Quantitative computed tomographic scanning

Peripheral quantitative computed tomographic (pQCT) scans were performed on individual bones (left femur, L4 vertebrae) from each mouse. Scanning was done with a STRATEC XCT 960M unit (Norland Medical Instruments, Ft. Atkinson, WI, USA) specifically configured for small bone specimens. Mineral thresholds were set at 1.30 for low-density bone and 2.00 for high-density bone. These thresholds excluded mouse fat, water, muscle, and tendon from true bone. Daily calibration was performed with a manufacturer-supplied phantom (hydroxyapatite in Lucite) of defined density. Calibration with a set of known hydroxyapatite standards (0.05–1000.0 mg/mm³) yielded a correlation of 0.998 with XCT 960M estimation of volumetric

density. Estimates of measurement precision of mineral and volume of femurs and vertebrae were obtained from the middiaphyseal shaft of a B6C3H-F1 femur and from the midbody scans of a B6C3H-F1 L5 vertebra. Six replicate measurements for each bone yielded average values of 1.6, 2.1, and 2.8% for femoral density, mineral, and volume, respectively, and 3.2, 5.9, and 4.7% for L5 vertebral density, mineral, and volume, respectively.

Femurs were scanned full length at 2-mm intervals with a resolution of 0.100 mm/voxel, yielding eight 1-mm-thick cross-sections representing eight axial levels of the femur. Vertebrae were scanned full length at 0.7-mm intervals with the same resolution, yielding three to four 1-mm-thick cross-sections. The center-most scan (based on image morphology) or the mean of two scans sharing the center position was selected for data analyses.

Marrow isolation

After 4 months on the diets, mouse marrow cells were isolated from both femurs from 2 animals in each group as previously described.^(21,29,30) Marrow from both femurs was pooled for each animal and RNA was isolated and analyzed separately by reverse-transcriptase polymerase chain reaction (RT-PCR). RNA was isolated as previously described using the RNA isolation kit from Stratagene (La Jolla, CA, USA).⁽²⁸⁾

RT-PCR

RNA in 3- μ g quantities was reverse-transcribed, and PCR was performed using primers as described previously.⁽³¹⁾ Thermal cycling was carried out for 21 cycles (glyceraldehyde-3-phosphate dehydrogenase [GAPDH]) or 34 cycles (osteocalcin) at 60°C annealing temperature for both GAPDH and osteocalcin. Amplified fragments were isolated on a 6% polyacrylamide gel (29:1 acrylamide to bis-acrylamide), and the autoradiographs were scanned with an AGFA ARCUS II scanner and semiquantitated with NIH Image software, version 1.59, public domain program (National Institutes of Health, Bethesda, MD, USA).

Lipoprotein preparation and oxidation

Human LDL was isolated by density-gradient centrifugation of serum and stored in phosphate-buffered 0.15 M NaCl containing 0.01% EDTA. MM-LDL was prepared by iron oxidation of human LDL as previously described.⁽²⁰⁾ Minimal oxidation of LDL resulted in a 2- to 3-fold increase in conjugated dienes and 2–3 nmol of thiobarbituric acid reactive substances per milligram of cholesterol after dialysis. The concentrations of lipoproteins used in this study are reported in micrograms of protein. The pre- and postoxidation lipopolysaccharide levels in these lipoprotein preparations were <30 pg/ml.

Statistical analysis

Differences in BMC and BMD were assessed using Student's two-tailed *t*-test, allowing for unequal variances and unequal sample sizes where appropriate.

	Mineral content (mg)			Mineral dens	Chow versus high fat	
Slice	Chow	High fat	р	Chow	High fat	р
1	2.29 ± 0.82	0.74 ± 0.19	0.002	0.502 ± 0.05	0.441 ± 0.04	0.01
2	1.05 ± 0.21	0.53 ± 0.20	0.001	0.365 ± 0.05	0.355 ± 0.05	0.70
3	1.02 ± 0.08	0.77 ± 0.10	0.0001	0.447 ± 0.05	0.400 ± 0.06	0.02
4	0.99 ± 0.07	0.72 ± 0.06	0.0003	0.440 ± 0.02	0.391 ± 0.02	0.05
5	1.19 ± 0.14	0.78 ± 0.07	< 0.0001	0.520 ± 0.04	0.410 ± 0.03	0.01
6	1.26 ± 0.12	0.86 ± 0.10	< 0.0001	0.573 ± 0.04	0.465 ± 0.05	< 0.0001
7	1.32 ± 0.16	0.75 ± 0.19	0.0008	0.515 ± 0.05	0.416 ± 0.04	0.1
8	1.13 ± 0.43	0.71 ± 0.31	0.001	0.538 ± 0.03	0.476 ± 0.05	0.01
Mean \pm SD	1.28 ± 0.42	0.73 ± 0.09	0.008	0.488 ± 0.07	0.419 ± 0.04	0.03

TABLE 1. QCT BONE PARAMETERS FOR FEMURS FROM C57BL/6 MICE AFTER 7 MONTHS ON A
CONTROL CHOW OR HIGH-FAT DIET

Scans were performed at 8 longitudinal axis positions (slices) for each femur with 1 being most proximal and 8 most distal. Values of BMC and BMD are expressed as mean \pm SD over all animals in each diet group.

TABLE 2. QCT BONE PARAMETERS FOR FEMURS FROM C3H/HeJ MICE AFTER 7 MONTHS ON A CONTROL CHOW OR HIGH-FAT DIET

	Mineral content (mg)		Chow versus high fat	Mineral dens	Chow versus high fat	
Slice	Chow	High fat	р	Chow	High fat	р
1	2.73 ± 0.79	2.00 ± 0.84	0.12	0.596 ± 0.06	0.542 ± 0.06	0.11
2	1.60 ± 0.24	1.26 ± 0.15	0.01	0.510 ± 0.06	0.426 ± 0.06	0.016
3	1.72 ± 0.16	1.40 ± 0.13	0.002	0.800 ± 0.03	0.720 ± 0.05	0.005
4	1.88 ± 0.24	1.66 ± 0.16	0.07	0.883 ± 0.07	0.909 ± 0.03	0.39
5	2.01 ± 0.18	1.73 ± 0.16	0.009	0.853 ± 0.03	0.846 ± 0.02	0.63
6	2.28 ± 0.27	2.06 ± 0.14	0.09	0.922 ± 0.06	0.911 ± 0.02	0.68
7	2.01 ± 0.41	1.95 ± 0.15	0.75	0.694 ± 0.11	0.807 ± 0.09	0.05
8	1.47 ± 0.40	1.55 ± 0.21	0.67	0.612 ± 0.07	0.562 ± 0.04	0.15
Mean \pm SD	1.96 ± 0.40	1.70 ± 0.29	0.59	0.734 ± 0.15	0.715 ± 0.19	0.26

Scans were performed at 8 longitudinal axis positions (slices) for each femur with 1 being most proximal and 8 most distal. Values of BMC and BMD are expressed as mean \pm SD over all animals in each diet group.

RESULTS

Femoral BMC and BMD

After 4 months, femoral BMD was significantly lower in fat-fed C57BL/6 mice at three of the eight levels scanned (p < 0.04; from 0.488 \pm 0.038 mg/mm³ to 0.423 \pm 0.043 mg/mm³). All three levels were in the middiaphyseal region where variance caused by anatomic complexity is minimized. BMC was not significantly different between the two groups.

After 7 months, femoral BMC was significantly lower in fat-fed C57BL/6 mice compared with control chow-fed mice at all eight levels scanned. Mean mineral content was lowered 43% (from 1.28 ± 0.42 mg to 0.73 ± 0.09 mg; $p \leq 0.002$; Table 1) on the high-fat diet. Changes in mineral content were most significant ($p \leq 0.0003$) at the four middiaphyseal levels (scans 3–6). Femoral mineral density was also significantly lower in fat-fed C57BL/6 mice compared with chow-fed mice at six of eight levels, with a 14.5% mean difference (from 0.488 \pm 0.066 mg/mm³ to 0.419 \pm 0.035 mg/mm³; p = 0.03; Table 1).

In C3H/HeJ mice, which are resistant to the atherogenic effects of a high-fat diet and lipid oxidation products, ^(24,25) the high-fat diet had less effect on bone mineralization. After 4 months on the diet, C3H/HeJ mice showed no significant difference in femoral BMC at any of the eight levels examined (data not shown); BMD was significantly lower at one of eight scanned sites (p = 0.01).

After 7 months on the diet, the fat-fed C3H/HeJ mice had significantly ($p \le 0.01$) lower BMC compared with chow-fed mice at only three of eight levels (Table 2). However, the overall mean difference for all eight levels did not reach statistical significance (p = 0.59). There also was no significant effect of the high-fat diet on femoral mineral density (p = 0.26; Table 2).

Lumbar vertebral mineral content and mineral density

At 4 months, there was no significant difference between chow and high-fat diet groups in either vertebral mineral content or density in either mouse strains. However, at 7

Total bone				Cortical bone			
Mineral content (mg)		Mineral density (mg/mm ³)		Mineral content (mg)		Mineral density (mg/mm ³)	
Chow	High fat	Chow	High fat	Chow	High fat	Chow	High fat
			C5′	7BL/6			
1.20 ± 0.10	0.77 ± 0.10	0.229 ± 0.02	0.212 ± 0.03	0.317 ± 0.08	0.088 ± 0.05	0.455 ± 0.01	0.445 ± 0.01
<i>p</i> <0.001		0.30		< 0.001		0.09	
			C3	H/HeJ			
1.41 ± 0.35	1.31 ± 0.14	0.248 ± 0.03	0.217 ± 0.01	0.624 ± 0.25	0.445 ± 0.13	0.481 ± 0.02	0.474 ± 0.01
р	0.49	0.	03	0.	12	0.	48

TABLE 3. QCT BONE PARAMETERS FOR L4 VERTEBRAE FROM C57BL/6 AND C3H/HeJ MICE AFTER 7 MONTHS ON A CONTROL CHOW OR HIGH-FAT DIET

Values are for the central slice or slices for each of the L4 vertebrae and are expressed as mean \pm SD over all animals in each diet group.

months, vertebral mineral content was significantly lower in the C57BL/6 fat-fed mice (Table 3). Total mineral content of the central section or sections was lower by a mean of 35% (from 1.2 \pm 0.1 mg to 0.77 \pm 0.1 mg; p < 0.001), primarily because of changes in high-density cortical bone (i.e., a 72% decrease). Total mineral density decreased 7% on the high-fat diet, but this change was not statistically significant. In C3H/HeJ mice, a 7% decrease in total mineral content was found, as well as a 29% decrease in cortical mineral content. These changes did not reach statistical significance. Total mineral density of vertebrae from C3H/ HeJ mice decreased 12.5% on the high-fat diet (from 0.248 \pm 0.03 to 0.217 \pm 0.01 mg/mm³; p = 0.03).

Gene expression in marrow cells

After 4 months on the high-fat or chow diets, the marrow isolated from 2 C57BL/6 mice on each diet was analyzed for the expression of three markers of osteoblastic differentiation: alkaline phosphatase, bone sialoprotein, and osteocalcin. All three markers were expressed by the marrow cells. Of the three, only osteocalcin expression was affected by diet, showing a 35% reduction with the high-fat diet when normalized to GAPDH values (Fig. 1).

DISCUSSION

The present study is the first to show that 7-month treatment with an atherogenic high-fat diet lowers BMD and BMC in vivo in atherosclerosis-susceptible C57BL/6 mice, with much smaller effects in the atherosclerosis-resistant C3H/HeJ mice. The atherogenic diet resulted in a significantly lower femoral mineral content and femoral mineral density in the C57BL/6 mice. Smaller changes were seen in the C3H/HeJ mice. The differential effects of the atherogenic diet on bones in the two strains of mice are similar to the effects of that diet on the development of atherosclerosis. Previous reports showed differences in genetically determined factors in response to diet-induced hyperlipidemia and lipid oxidation in these mouse strains to be the



FIG. 1. Effects of a high-fat diet on osteocalcin expression in marrow cells. One-month-old C57BL/6 mice were placed on a high-fat or chow diet for 4 months. The animals were killed and femoral marrow was isolated from each mouse and used to isolate total RNA. RT-PCR analysis showed an expected size band of 360 base pairs (bp). Expression of GAPDH was used for normalization. Each lane represents RNA isolated from an individual mouse.

underlying reason for their degree of susceptibility to atherosclerosis. These differences include: (1) the level of induction of inflammatory genes such as monocyte chemotactic protein-1, colony-stimulating factors, heme oxygenase, and serum amyloid A and activation of nuclear factor κB (NF κB) transcription factor in response to atherogenic diet^(26,27,32) and (2) the ability of high-density lipoprotein (HDL) to protect against the effects of atherogenic diet, because of variability in the level of antioxidant enzyme paraoxonase.⁽²⁸⁾ The latter difference is important in light of the observation that the protective effect of HDL appears to correlate inversely with atherosclerosis,⁽³³⁾ and a direct correlation between HDL levels and BMD in fat-fed mice has been shown (T. Drake, University of California, Los Angeles [UCLA], Department of Pathology, personal communication, 1999). It is intriguing to speculate that similar genetically regulated factors, involved with defense against atherogenic oxidized lipids, also determine susceptibility to osteoporosis.

Because femoral mineral content was more substantially changed by the atherogenic diet than mineral density, the effect may be caused by quantitatively less bone formation and/or shorter bones in the high-fat-fed mice. Although we did not measure femoral size after 7 months in this study, in a separate study, we found no significant change in the femoral or tibial length between chow-fed versus high-fatfed C57BL/6 mice after 4 months on the diet (F. Parhami, unpublished observations, 1999). Because our previous in vitro and in vivo studies showed inhibition of osteoblastic differentiation and bone formation by marrow stromal cells isolated from C57BL/6 mice on the high-fat diet versus chow diet, we speculate that bone formation is inhibited by the atherogenic diet. More direct future studies will further validate this speculation. It is important to note that the mice used in the present study were in their growing stage when peak bone mass is achieved. Inhibition of bone formation during growth stage also would have adverse consequences by reducing peak bone mass. The reducing effects of the dietary fat on BMC and BMD would translate into a reduction in this important determinant of bone strength.

The present results also suggest that increased dietary lipids interfere with osteoblast maturation in vivo, based on dietary inhibition of osteocalcin messenger RNA (mRNA) expression. Although the effect of the high-fat diet on the expression of osteocalcin alone is not sufficient to draw definitive conclusions about differentiation of osteoblasts, this inhibition is consistent with previous ex vivo evidence that exposure to a high-fat diet reduced marrow preosteoblastic maturation in culture,⁽²¹⁾ as well as in vitro evidence that lipid and lipoprotein oxidation products inhibit osteo-blast differentiation and function.^(20,21) Previous studies using the same atherogenic diet in C57BL/6 mice have shown 2- to 3-fold increases in cholesterol levels after 3-4 weeks on this diet, as well as a significant drop in the HDL levels.^(24,25) We therefore speculate that the adverse effects of the high-fat diet on bone in the C57BL/6 mice are caused by dyslipidemia and subsequent increases in lipid oxidation. The diet-induced hyperlipidemia in circulation further translates into increased lipid accumulation in highly vascular tissues and the artery wall because of the diffusion of lipoproteins across the vascular endothelium. Once apart from the protective, antioxidant environment of serum, these lipoprotein particles are oxidized further into biologically active forms responsible for inflammatory processes in atherosclerosis and vascular calcification.^(19,20) Because bone and marrow are both vascularized, circulating lipids can access both sites of active bone remodeling where osteoprogenitor cells are present: (1) the subendothelial space of the osteons and (2) the marrow stroma at the trabecular surface or endosteum. Lipid accumulation⁽³⁴⁾ and monocyte accumulation and plaquing⁽³⁵⁾ have been observed in the vessels of osteons in osteoporotic and aging bone. The presence of circulating lipoproteins in the marrow is expected because marrow is a site for clearance of chylomicrons and chylomicron remnants derived from dietary fat,⁽³⁶⁾ and dietary fat has been found to alter the lipid profile in the marrow.⁽³⁷⁾ Thus, lipid oxidation products may underlie the paradoxical association of cardiovascular disease with osteoporosis.

The findings in the present report are consistent with a preliminary report showing a significant correlation between dietary cholesterol intake and vertebral bone loss in women,⁽³⁸⁾ as well as with population studies showing an association of cholesterol levels with osteoporosis in women⁽¹³⁾ and, preliminarily, in men.⁽³⁹⁾ Recent evidence suggests that 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase inhibitors (statins), lipid-lowering agents commonly used to treat cardiovascular disease, have potent positive effects on bone formation in rodents,⁽⁴⁰⁾ and statin therapy in humans correlates with reduced osteoporosis.^(41–45) Although the mechanism is proposed to be a direct stimulation of osteoblasts, an equally likely mechanism is an indirect effect through lipid-lowering, given that the dominant site of action of these agents, in both humans and rodents,⁽⁴⁶⁾ is in the liver where statins are mostly cleared from circulation.

Evidence suggests that the atherogenic nature of the highfat diet is essential for effects on bone. Wohl et al. previously showed a minimal effect on BMC of a noncholesterol, 8% fat diet in adult roosters.⁽⁴⁷⁾ Because cholesterol feeding is necessary to induce atherosclerosis in roosters,⁽⁴⁸⁾ this finding suggests that a nonatherogenic high-fat diet is not sufficient to induce bone changes.

Collectively, these observations suggest the adverse effects of lipids on bone. The possibility that lipid oxidation products are the biologically active factors linking a high-fat diet with reduced bone formation is supported by the finding of substantially reduced effects in mice that are resistant to the effects of oxidized lipids and by the anabolic effects of the antioxidant vitamin E on bone.⁽⁴⁹⁾ Because cardiovascular disease is the highest risk cause of death for patients with osteoporotic fracture^(4,5) and low BMD is associated with mortality independent of fractures,⁽⁵⁰⁾ elucidation of common lipid- and lipid oxidation-mediated mechanisms has great importance for identifying new preventive measures for both osteoporosis and cardiovascular disease. The possibility that high lipid levels are a common underlying factor in atherosclerosis and bone loss may explain the epidemiological evidence for correlation between cardiovascular disease and osteoporosis.

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REFERENCES

- Boukhris R, Becker KL 1972 Calcification of the aorta and osteoporosis. JAMA 219:1307–1311.
- Frye MA, Melton LJ, Bryant SC, Fitzpatrick LA, Wahner HW, Schwartz RS, Riggs BL 1992 Osteoporosis and calcification of the aorta. Bone Miner 19:185–194.

- Riggs BL, Melton LJ 1992 The prevention and treatment of osteoporosis. N Engl J Med 329:620–627.
- von der Recke P, Hansen MA, Hassager C 1999 The association between low bone mass at the menopause and cardiovascular mortality. Am J Med 106:273–278.
- Browner WS, Seeley DG, Vogt TM, Cummings SR 1991 Nontrauma mortality in elderly women with low bone mineral density. Lancet 338:355–358.
- Naito S, Ito M, Sekine I, Ito M, Hirano T, Iwasaki K, Niwa M 1993 Femoral head necrosis and osteopenia in stroke-prone spontaneously hypertensive rats (SHRSPs). Bone 14:745–753.
- Jie KG, Bots ML, Vermeer C, Witteman JC, Grobbee DE 1996 Vitamin K status and bone mass in women with and without aortic atherosclerosis: A population-based study. Calcif Tissue Int 59:352–356.
- Barengolts EI, Berman M, Kukreja SC, Kouznetsova T, Lin C, Chomka EV 1998 Osteoporosis and coronary atherosclerosis in asymptomatic postmenopausal women. Calcif Tissue Int 62:209–213.
- Ouchi Y, Akishita M, deSouza AC, Nakamura T, Orimo H 1993 Age-related loss of bone mass and aortic/aortic valve calcification—reevaluation of recommended dietary allowance of calcium in the elderly. Ann NY Acad Sci 676:297–307.
- Laroche M, Pouilles JM, Ribot C, Bendayan P, Bernard J, Boccalon H, Mazieres B 1994 Comparison of the bone mineral content of the lower limbs in men with ischaemic atherosclerotic disease. Clin Rheumatol 13:61–64.
- Laroche M, Moulinier L, Bon E, Cantagrel A, Mazieres B 1994 Renal tubular disorders and arteriopathy of the lower limbs: Risk factors for osteoporosis in men? Osteoporos Int 4:309–313.
- 12. Pinals RS, Jabbs JM 1972 Type-IV hyperlipoproteinemia and transient osteoporosis. Lancet **2**:929.
- Broulik PD, Kapitola J 1993 Interrelations between body weight, cigarette smoking and spine mineral density in osteoporotic Czech women. Endocr Reg 27:57–60.
- Yla-Hertualla S 1998 Is oxidized low density lipoprotein present in vivo? Curr Opin Lipidol 9:337–344.
- 15. Watson AD, Leitinger N, Navab M, Faull KF, Horkko S, Witztum JL, Palinski W, Schwenke D, Salomon RG, Sha W, Subbanagounder G, Fogelman AM, Berliner JA 1997 Structural identification by mass spectrometry of oxidized phospholipids in minimally oxidized low density lipoprotein that induce monocyte-endothelial interactions and evidence for their presence in vivo. J Biol Chem 272:13597–13607.
- Haberland ME, Fong D, Cheng L 1988 Malondialdehydealtered protein occurs in atheroma of Watanabe heritable hyperlipidemic rabbits. Science 241:215–218.
- Morrow JD, Minton TA, Mukundan CR, Campbell MD, Zackert WE, Daniel VC, Badr KF, Blair IA, Roberts LJ 1994 Free radical-induced generation of isoprostanes in vivo. Evidence for the formation of D-ring and E-ring isoprostanes. J Biol Chem 269:4317–4326.
- Rosenfeld ME, Khoo JC, Miller E, Parthasarathy S, Palinski W, Witztum JL 1991 Macrophage-derived foam cells freshly isolated from rabbit atherosclerotic lesions degrade modified lipoproteins, promote oxidation of low-density lipoproteins, and contain oxidation-specific lipid-protein adducts. J Clin Invest 87:90–99.
- Berliner JA, Navab M, Fogelman AM, Frank JS, Demer LL, Edwards PA, Watson AD, Lusis AJ 1995 Atherosclerosis: Basic mechanisms. Oxidation, inflammation, and genetics. Circulation 91:2488–2496.
- Parhami F, Morrow AD, Balucan J, Leitinger N, Watson AD, Tintut Y, Berliner JA, Demer LL 1997 Lipid oxidation products have opposite effects on calcifying vascular cell and bone cell differentiation. A possible explanation for the paradox of arterial calcification in osteoporotic patients. Arterioscler Thromb Vasc Biol 17:680–687.

- Parhami F, Jackson SM, Tintut Y, Le V, Balucan JP, Territo MC, 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.
- Mullender MG, van der Meer DD, Huiskes R, Lips PP 1996 Osteocyte density changes in aging and osteoporosis. Bone 18:109–113.
- Bergman RJ, Gazit D, Kahn AJ, Gruber H, McDougall S, Hahn TJ 1996 Age-related changes in osteogenic stem cells in mice. J Bone Miner Res 11:568–577.
- Paigen B, Morrow A, Brandon C, Mitchell D, Holmes P 1985 Variation in susceptibility to atherosclerosis among inbred strains of mice. Atherosclerosis 57:65–73.
- Paigen B, Mitchell D, Reue K, Morrow A, Lusis AJ, LeBoeuf RC 1987 Ath-1, a gene determining atherosclerosis susceptibility and high density lipoprotein levels in mice. Proc Natl Acad Sci USA 84:3763–3767.
- 26. Liao F, Andalibi A, de Beer FC, Fogelman AM, Lusis AJ 1993 Genetic control of inflammatory gene induction and NF-kBlike transcription factor activation in response to an atherogenic diet in mice. J Clin Invest **91**:2572–2579.
- Navab M, Levy-Hama S, Van Lenten BJ, Fonarow GC, Cardinez CJ, Castellani LW, Brennan ML, Lusis AJ, Fogelman AM 1997 Mildly oxidized LDL induces an increased apolipoprotein J/paraoxonase ratio. J Clin Invest 99:2005– 2019.
- Shih DM, Gu L, Hama S, Xia YR, Navab M, Fogelman AM, Lusis AJ 1996 Genetic-dietary regulation of serum paraoxonase expression and its role in atherogenesis in a mouse model. J Clin Invest 97:1630–1639.
- Maniotopoulos C, Sodek J, Melcher A 1988 Bone formation in vitro by stromal cell obtained from bone marrow of young adult rats. Cell Tissue Res 254:317–330.
- Malaval L, Modrowski D, Gupta AK, Aubin JE 1994 Cellular expression of bone related proteins during in vitro osteogenesis in rat bone marrow stromal cultures. J Cell Physiol 158: 555–572.
- Tintut Y, Parhami F, Bostrom K, Jackson SM, Demer LL 1998 Cyclic AMP stimulates osteoblast-like differentiation of calcifying vascular cells: Potential signaling pathway for vascular calcification. J Biol Chem 273:7547–7553.
- 32. Liao F, Lusis AJ, Berliner JA, Fogelman AM, Kindy M, de Beer MC, de Beer FC 1994 Serum amyloid A protein family. Differential induction by oxidized lipids in mouse strains. Arterioscler Thromb Vasc Biol 14:1475–1479.
- 33. Gordon DJ, Probstfield JL, Garrison JR, Neaton JD, Castelli WP, Knoke JD, Jacobs DR Jr, Bangdiwala S, Tyroler HA 1989 High-density lipoprotein cholesterol and cardiovascular disease: Four prospective American studies. Circulation 79:8–15.
- Ramseier E 1962 Untersuchungen uber arteriosklerotische Veranderungen der Knochenarterien. Virchows Arch Path Anat 336:77–86.
- Nyssen-Behets C, Duchesne PY, Dhem A 1997 Structural changes with aging in cortical bone of the human tibia. Gerontology 43:316–325.
- Hussain MM, Mahley RW, Boyles JK, Fainaru M, Brecht WJ, Lindquist PA 1989 Chylomicron-chylomicron remnant clearance by liver and bone marrow in rabbits. J Biol Chem 264: 9571–9582.
- Li Y, Watkins BA 1998 Conjugated linoleic acids alter bone fatty acid composition and reduce ex vivo prostaglandin E2 biosynthesis in rats fed n-6 or n-3 fatty acids. Lipids 33:417– 425.
- Lin YC, Lyle RM, Weaver CM, Teegarden D 1999 Impact of diet variables on changes in spine bone mineral density. FASEB J 13:A244 (abstract).
- Semmler JC 1992 Risk factors for osteoporosis in men. ICCRH, Florence (abstract).

- Mundy G, Garrett R, Harris S, Chan J, Chen D, Rossini G, Boyce B, Zhao M, Gutierrez G 1999 Stimulation of bone formation in vitro and in rodents by statins. Science 286:1946–1949.
- 41. Bauer DC, Mundy GR, Jamal SA, Black DM, Cauley JA, Harris F, Duong T, Cummings SR 1999 Statin use, bone mass and fracture: An analysis of two prospective studies. J Bone Miner Res **14:**S179 (abstract).
- 42. Meier CR, Schlienger RG, Kraenzlin ME, Schlegel B, Jick H 2000 HMG-CoA reductase inhibitors and the risk of fractures. JAMA **283:**3205–3210.
- Wang PS, Solomon DH, Mogun H, Avorn J 2000 HMG-CoA reductase inhibitors and the risk of hip fractures in elderly patients. JAMA 283:3211–3216.
- 44. Chan KA, Andrade SE, Boles M, Buist DSM, Chase GA, Donahue JG, Goodman MJ, Gurwitz JH, LaCroix AZ, Platt R 2000 Inhibitors of hydroxymethylglutaryl-coenzyme A reductase and risk of fracture among older women. Lancet 355: 2185–2188.
- Edwards CJ, Hart DJ, Spector D 2000 Oral statins and increased bone mineral density in postmenopausal women. Lancet 355:2218–2219.
- 46. Hamelin BA, Turgeon J 1998 Hydrophilicity/lipophilicity: Relevance for pharmacology and clinical effects of HMG-CoA reductase inhibitors. Trends Pharmacol Sci **19:**1–38.
- 47. Wohl GR, Loehrke L, Watkins BA, Zernicke RF 1998 Effects of high-fat diet on mature bone mineral content, structure, and mechanical properties. Calcif Tissue Int **63**:74–79.

- Lucas A, Dai E, Liu LY, Nation PN 1998 Atherosclerosis in Marek's disease virus infected hypercholesterolemic roosters is reduced by HMGCoA reductase and ACE inhibitor therapy. Cardiovasc Res 38:237–246.
- Xu H, Watkins BA, Seifert MF 1995 Vitamin E stimulates trabecular bone formation and alters epiphyseal cartilage morphometry. Calcif Tissue Int 57:293–300.
- Center JR, Nguyen TV, Schneider D, Sambrook PN, Eisman JA 1999 Mortality after all major types of osteoporotic fracture in men and women: An observational study. Lancet 353:878– 882.

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