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Research Article

IMPACT OF HYPERCHOLESTEROLEMIA ON BONE HEALTH: INSIGHTS FROM A FOUR-MONTH FEEDING STUDY IN MICE

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ABSTRACT

Studies have suggested a link between hypercholesterolemia and osteoporosis, but direct evidence on the impact of cholesterol homeostasis on bone health is limited. In a four-month feeding study with mice subjected to isocaloric high-fat and high-cholesterol diets, micro-computed tomography analysis revealed a significant decrease in bone mineral density, ranging from 60 to 90 mg/mL across different strains. Mechanical testing of femurs demonstrated bone loss, resulting in a reduction in failure load (up to 10%) and energy to failure. The study also highlighted hypercholesterolemia's role in promoting osteoclastogenesis. These findings underscore the connection between hypercholesterolemia and osteoporosis, providing insights for potential intervention strategies.

Keywords :- Hypercholesterolemia, Osteoporosis, Bone Health, Cholesterol Homeostasis, Micro-Computed Tomography, Osteoclastogenesis.

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INTRODUCTION

As a consequence, osteoporosis, fragility fractures occur when bone mass decreases and bone microarchitecture changes. Bone fractures can result in severe, permanent disability, institutionalization, and death as a result of spontaneous and traumatic fractures [1]. For one year after a hip or spine fracture occurs, osteoporotic fractures have a mortality rate of up to 20% [2].

Osteoporosis may also be increased by these conditions in common, as evidence suggests that Cardiovascular disease and metabolic syndrome [3–5] (central obesity associated with insulin resistance, dyslipidemia, and high blood pressure) are interconnected. Cardiovascular disease and hypercholesterolemia are both linked to hypercholesterolemia and metabolic syndrome.

A cholesterol-containing lipoprotein is absorbed from the circulation by osteoblasts (bone-making cells) and osteoclasts (bone-resorbing cells). Consequently, cholesterol distribution is regulated by the control of cholesterol distribution by the organism and cellular metabolic processes. Several processes are involved in maintaining the cholesterol content of cell membranes, including those in the endoplasmic reticulum, this process involves cholesterol-regulated receptor-mediated internalization, intracellular transport mechanisms, and cholesterol efflux via lipoprotein complexes. Even though osteoblasts and osteoclasts maintain cholesterol balance, cells Changes in serum cholesterol concentrations or dysregulation of endogenous synthetic pathways can disrupt this process.

These changes may also complicate bone cell maturation, differentiation, and stabilization. According to epidemiologic data, elevated serum cholesterol levels

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are associated with osteoporosis development. Statin drugs, inhibit cholesterol synthesis by inhibiting the rate-limiting step, have been demonstrated in other studies to increasing bone mineral density, reducing fracture risk, and changing bone marrow, biochemistry of osteoblasts in vivo and in tissue culture. Statins suppress cholesterol synthesis, resulting in a reduction of serum cholesterol concentrations when they are provided in typical dosages. It appears that statins are too small and for too short a duration to exert direct effects on extrahepatic tissues.

Therefore, statins are likely to alter risk of osteoporosis their Osteoblasts and osteoclasts are directly affected are due to their effects on circulating cholesterol levels. Osteoporosis, cardiovascular disease, and metabolic syndrome have been linked in studies suggesting the effects of high cholesterol concentrations. The effects of statins on bone mineral density and fracture risk risk [6-9] we hypothesized that elevated serum cholesterol concentrations osteoblasts and osteoclasts may have different cholesterol levels.

Atherogenic diets in comparison to LFNC diets fed to control animals, the HFHC with sodium cholate diet significantly reduced femur mineral density (15% lower) and mineral content (43% lower). A recent study found rats on HFHC diets displayed a reduced femoral BMD compared to rats on normal chow diets. In neither of these studies was isocaloric diet used, so energy effects were not excluded [10]. This led to an inconclusive interpretation of how cholesterol-sterol reduces BMD.

A study was conducted to determine whether hypercholesterolemia affects bone integrity. It has been shown that hypercholesterolemia alters bone mineral density, number and spacing of trabeculae, bone volume fraction (BVF) between trabeculae, as well as bone mechanical properties and osteoclast activity.

MATERIALS AND METHODS

Animal selection

Over a period of four months, mice were randomly divided into groups receiving either HFHC diets or LFNC diets. However, despite their differences in composition, these diets are fed isocalorically, i.e. the same amount of calories to each type of animal. Blood samples from the terminal phase of the experiment were collected by Serologic cardiac puncture analysis and osteocalcin assay. Manufacturers' instructions were followed for the assays.

Analyses of histomorphology

A fixed femur was decalcified at 4°C with 10% EDTA (pH 7.4) for 2 weeks. A graded ethanol solution (50%, 70%, 90%, and 95%) was used to dehydrate the bones, as well as 100% ethanol twice, followed by xylene and finally paraffin embedding. In accordance with the manufacturer's instructions, Sections of five micrometers were cut, deparaffinized, stained with acid phosphatase, and Hematoxylin was used as a counterstain. Three-nucleated cells are osteoclasts.

X-ray micro computed tomography, mechanical testing, and bones

After the animals were euthanized, reassembled calvaria, right femur, and L4 vertebrae were washed in lactated Ringer's solution. Micro-computed tomography using cone beams with 18 voxels was used to reconstruct all specifications. The calibration density histograms were used to determine a single threshold for the three-dimensional dataset. Diaphyseal segment of 3 mm of the femur was analysed for cortical mineralization and geometrical analysis, and using spline algorithms, Bone is segmented based on a standardized volume it begins and extends to 20% of the length of the distal growth plate. Three dimensional regions of interest were generated by interpolating the spline elements. A three-dimensional mid-diaphysis average was calculated using data from each femur. Inertia bending moment, cross-sectional area, and cortical thickness were measured. Two standardized volumes are measured Vertebral bodies at their proximal and distal ends of the trabecular bone was segmented. An interpolation of the volume of interest was determined each section is digitized and a series of splines is defined. BVF, trabecular thickness, trabecular spacing, anisotropy, and number of trabeculae were evaluated in femoral metaphyses and vertebrae. GE MicroCT systems were used for analysis using software suites for advanced analysis and Micro View.

Biomechanical Properties

We carried out biomechanical testing on preserved frozen specimens at -20°C during the testing process. During the experiment, Ringer's solution was kept at room temperature in order to keep the specimens moist. In order to measure the mechanical properties of the femurs, the mechanical properties of the femurs were measured using a servohydraulic testing machine. As a result of the loading, there was tension on the posterior side of the femur and compression on the anterior side. The top two points of the alignment were independently adjusted so that they were aligned with the bone and in contact with it. A four-point bending load is the result of microCT measurements that translate into four-point bending loads. The load was measured with a load cell and a linear variable differential transducer was used to monitor displacement. MTS Systems' A Test Star IIs system was used to sample load and displacement data at a frequency of 2000 Hz. MATLAB software was used to analyze the load-displacement curves for whole-bone yield load, ultimate load, stiffness, failure energy, and displacement ratio (ultimate displacement: yield

displacement). Micro CT analysis was used to calculate geometric properties and surface mechanics equations for predicted tissue modules. Based on bending, these equations predict extracellular matrix properties.

Analyses Statistical

Student's t-test was used to determine statistical significance. *A significance level of P > .05 was considered.*

RESULTS

The results of four months of isocaloric HFHC and LFNC diets were compared to understanding how hypercholesterolemia affects bone quality. Based on previous studies, HFHC diets are required to raise cholesterol levels in mice, while LFNC diets are well-defined and consist essentially of normal mouse chow. After 4 months of feeding these diets to mice, it was noted that there was no significant difference between the diets were fed by weight, with significant differences in serum cholesterol. Hypercholesterolemia was observed in mice fed the HFHC diet, whereas normal cholesterol concentrations were observed in mice fed the other diet. A liver function test showed no liver toxicity,in the C57BL/6 HFHC diet group, insulin levels were trending higher, however, the diet cohorts were not significantly different. The levels of androgen were not significantly different.

Compared to the HFHC mice, Cortical bone mineral density of the hypercholesterolemic mice was significantly reduced. Despite this, this difference was not statistically significant in mice with SCID. A significant difference in diaphyseal cortical bone thickness and cortical surface area was observed between the two strains. The BMD and moment of inertia (Iyy) of C57BL/6 mice on LFNC and HFHC diet cohorts were also significantly different between the two diet groups. Though the values in SCID mice generally tended to trend in the same direction across different diets, they were not statistically significant. Hypercholesterolemic mice also showed statistically significant changes in trabecular bone in comparison, BVF was smaller in mice with normo-cholesterolemia larger trabecular spacing, fewer trabeculae in both strains and significantly less trabecular thickness and TMD in the LFNC diet cohort versus the HFHC diet cohort of the C57BL/6 strain. In SCID mice, these values trended similarly.

A significant reduction in BMD and mean thickness was observed in both strains of mice fed HFHC. TMD was also significantly higher in C57BL/6 mice fed the LFNC diet, Mineral content of bones and tissues in comparison the trend for these measures was also similar in mice that were fed HFHC diets.

A significant difference was seen between C57BL/6 and SCID mice when HFHC trabecular bone was examined and larger trabecular spacing than in the LFNC cohorts. Additionally, there was a significant a difference in trabecular thickness between C57BL/6 HFHC diet mice and LFNC diet mice.

In contrast to the similarity in diet effects between C57BL/6 and SCID mice, the differences in the effect of diet on the cortical vertebral bone. It was observed that C57BL/6 mice exhibited significantly lower bone mineral density TMD, despite no significant differences, there was a similar trend in measurements of bone mineral content, bone mineral density, and tissue mineral content.

There are significant differences between the HFHC and LFNC diets, as well as their mineral content and microarchitecture, between them, both differ were the reason we investigated whether these differences were also associated with changes in bone structure. The Supplemental Table S1 can be found online at www.ajp.amjpathol.org. A HFHC femor was less stiff and had less energy to fail mechanically incapable of differing from SCID.

In the HFHC and LFNC cohorts, there were no significant differences in osteocalcin levels, which suggests that altered osteoblast activity may not be the cause of these changes in bone mineral density. (data not shown). In collagen I fragments, pyridinoline cross-links were detected as Osteoclast activity. Hypercholesterolemic mice exhibit osteopenic phenotypes because of increased osteoclast activity.Accordingly, histomorphometric analysis revealed that hypercholesterolemic versus normocholesterolemic groups exhibited significantly more osteoclasts in their bones.

DISCUSSIONS

Using diet-induced hypercholesterolemia in mice, we demonstrated that bone quality measures reduced, mimicking osteoporosis-like bone quality in humans.

Since mice were fed isocaloric diets, according to our findings, hypercholesterolemia likely has a direct impact on impact on bone health: In this model, additional calories have no effect, despite the differences in fats and cholesterol between diets;

HFHC diets did not result in any weight gain in mice, and, thus, changes in bone do not reflect weight differences between the animals which loss of bone homeostasis caused by dietary changes.

A 4-month diet of HFHC or LFNC was given to mice, which is approximately 16% to 25% of their lifespan. As long as either diet affects cortical and trabecular bone, this should be sufficient.

C57BL/6 mice exhibited significant mechanical losses in their femors, HFHC cohort showed significant reductions the energy to failure, the ultimate load, and the

stiffness, suggesting that bone mineral loss and trabeculae thinness contributed to bone weakness. Physicomechanical properties resemble osteoporosis in humans. Thinnened diaphyseal cortical bone is thinned and lost, the trabeculae are thinned, and osteoclasts increase activity, and reduced strength and energy to fail are similar to osteoporosis in humans. The thinner bone should have made the bones less brittle; instead, they were more brittle. HFHC diet results in energy loss due to bone failure.

Neither SCID mice nor C57BL/6 mice fed either diet exhibited a comparison of the physical characteristics of HFHC-fed mice and LFNC-fed mice, though the differences were less pronounced.

BMD is influenced by a combination of environmental and genetic factors, although not all factors contribute. As well as serum cholesterol concentrations, osteoporotic phenotypes are also affected by native density of bone mineral. Based on previous studies [11-13] and our own data, SCID mice, which share a background with Balb/C mice, BMD is lower than C57BL/6 mice. Hypercholesterolemia may affect bone density differently depending on the native BMD. C57BL/6 and 129 strains are affected differently by atherogenic diets, as reported in Ishimori et al57. The F2 population variances are related, BMD9, BMD20, BMD21, and BMD22 are the four quantitative trait loci. These genes account is a 21.6% increase in total BMD and a 17.3% increase in vertebral BMD. Osteopenia in mice may be caused by genetic variations affecting bone mineral density.

Because SCID mice cannot perform VDJ recombination, they lack functional T and B cells. These animals have impaired inflammation because macrophages and natural killer cells do not respond. The inflammatory responses of animals were measured in a other study. C57BL/6 mice were found to be more prone to inflammation by HFHC diets, but SCID mice were not. Inflammation and osteoporosis may be linked because proinflammatory cytokines are involved menopause increases osteoclastogenesis and bone resorption. [14] It is clear that inflammation is a mediator of bone loss in hypercholesterolemia, inflammatory as well as noninflammatory pathways could be involved because SCID mice did lose bone.

Hypercholesterolemia increases osteoclast activity and number in bone. In both strains, osteoclast activity could explain the reduced BMD, however the statistically significant effect of hypercholesterolemia was only observed in SCID mice for serum pyridinoline concentration (an osteoclast activity measure), whereas diet had a greater impact on bone in C57BL/6 mice. This is probably due to the timing of the assay i.e., C57BL/6 mice had already reached their peak bone loss at 4 months on diet.

In accordance with our unpublished observations that increased cholesterol availability to macrophages promotes osteoclastogenesis, hypercholesterolemia promotes osteoclastogenesis increased polykaryon numbers and sizes (data not shown). Osteoblasts and osteoclasts have different membrane compositions may be affected by hypercholesterolemia, which could also affect their response to environmental cues. We do not have evidence that alterations in increasing membrane cholesterol levels affects osteoblast growth factors [15, 16]

In humans, high serum cholesterol levels are associated with osteoporosis and low serum cholesterol levels with osteopenia bone mineral density.[17] Concentrations of low-density lipoprotein cholesterol greater than 160 mg/dL in the plasma were associated with more likely than women with a lower LDL cholesterol concentration to have osteopenia (47.9% versus 21.2%). There has been Cholesterol levels (or low-density lipoproteins) have no association with osteoporosis, high cholesterol concentrations did not correlate with osteoporosis, but BMD (femoral head) did. Besides the lack of vertebral measurements,

Using a hypercholesterolemic animal model, we investigated the effects of hypercholesterolemia on bone health. We measured the effect of hypercholesterolemia in mice Controlling calories, body weight, and other factors. According to a previous study, an atherogenic diet increases cholesterol levels concentrations led to bone loss in mice. Additionally, the diet we used in our study raised serum cholesterol concentrations, but was not atherogenic. Mice do not naturally develop atherosclerotic lesions in the aorta similar to those found in humans. [18] An atherogenic diet in mice requires the addition of sodium cholate, a bile acid.63 Sodium cholate is not required to raise cholesterol concentrations, but does cause liver toxicity. The present study used hypercholesterolemic non-atherogenic diets to investigate Hypercholesterolemia and disease.It is important to note that this study is not without caveats, even though the type of analysis used will be useful in hypothesis testing and discrepancy analyses; because mice do not have the same weight distribution as humans, In humans, measuring osteoporosis, there is no way to predict which bones and bone regions will be most affected by hypercholesterolemia.

The authors conclude that a hypercholesterolemic non-atherogenic diet contributes to the development of osteoporotic bone phenotype in mice, including an increase in osteoclasts, the loss of trabeculae, the thinning of the trabeculae and cortex, and a decrease in failure load and energy to fail. There is a possibility that this osteoporosis model can provide the Critical evaluation opportunity interventions designed to counteract hypercholesterolemic diet-induced changes. In

the present study, we suggest that the correlation between hypercholesterolemia and osteoporosis may be caused by the inability to maintain cholesterol homeostasis and the increased activity of osteoclasts. In light of the rising incidence across the globe, hyperlipidemia, metabolic syndrome, and obesity are prevalent, it is expected that diet-induced osteoporosis will also rise, making our novel model particularly relevant at this time.

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