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ORIGINAL ARTICLE

Hiroshi Matsushita · Jill A. Barrios · Jill E. Shea
Scott C. Miller

Dietary fish oil results in a greater bone mass and bone formation indices in aged ovariectomized rats

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Abstract Postmenopausal bone loss and the possible progression to osteoporosis is a major health concern. Until recently, hormone replacement therapy (HRT) was the standard for preventing the development of osteoporosis and possible hip fractures following menopause. However, because of some adverse effects of HRT, new therapies, lifestyle habits, and nutritional interventions are being developed and better characterized in their ability to prevent bone loss after menopause. One such option is to increase the amount of fish oil consumed in the diet. The goal of the current research was to determine the impact of fish oil supplementation on bone mass, density, formation, and resorption in an aged ovariectomized rat model. Twelve-month-old female retired breeder Sprague–Dawley rats were fed a control (Control) or fish oil (Fish) diet. Two weeks following the introduction of the diets, the rats were either sham-operated (Sham) or bilaterally ovariectomized (OVX). Ten weeks after surgery, indices of bone mass and bone histomorphometry were measured. Bone mineral content (BMC) of the whole femur was significantly higher in the Fish/OVX than in the Control/OVX, and the differences were most pronounced in the distal and proximal ends of the femur. However, the Fish/Sham and the Control/Sham did not differ in the measures of BMC. Although the Control/OVX had significantly lower cortical area and greater endosteal perimeter compared with the Control/Sham, the differences were not significant between the Fish/Sham and the Fish/OVX. In addition, the Fish/OVX had a significantly larger percent double-labeled surface and mineral apposition rate at the endocortical surface than the Control/OVX. Our findings suggest that fish oil supplementation has a positive effect on bone metabolism and might be a possible intervention to slow the loss of bone observed following menopause.

Key words bone histomorphometry · bone mineral density · fish oil · menopause · rat

H. Matsushita (✉)
Department of Obstetrics and Gynecology, Niigata City General Hospital, 463-7 Shumoku, Chuo-ku, Niigata 950-1197, Japan
Tel. +81-25-281-5151; Fax +81-25-281-5169
e-mail: matsuh@kf7.so-net.ne.jp

H. Matsushita · J.E. Shea · S.C. Miller
Division of Radiobiology, University of Utah, Salt Lake City, UT, USA

J.A. Barrios · S.C. Miller
Division of Nutrition, University of Utah, Salt Lake City, UT, USA

Introduction

Menopause is a major health concern for women because it is strongly associated with an increased susceptibility to osteoporosis [1]. Osteoporosis, a multifactorial disorder, is characterized by low bone mass and a weakened structural architecture that leads to bone fragility and an increased susceptibility to fractures. Women with a lower peak bone mass in adulthood and a higher rate of bone loss in later life are at higher risk of developing osteoporosis [1,2]. Therefore, therapeutic strategies have focused on maximizing peak bone mass during adolescence and minimizing the bone loss after menopause.

Hormone replacement therapy (HRT) is one treatment option available to prevent postmenopausal bone loss, and some studies have documented that HRT could reduce the risk of fractures [3–6]. However, recent reports by the Women's Health Initiative did not support conventional HRT, with or without progestin, for the prevention of osteoporotic fracture because of some adverse effects [5,7,8]. These undesirable effects have heightened interest in diet and lifestyle changes that minimize bone loss in postmenopausal women, thereby decreasing the necessity for drug therapy in the treatment of osteoporosis.

One potential nutritional candidate is a diet high in fish consumption. Recent epidemiological studies suggest that consumption of fish, a dietary source of calcium and vitamin D, may reduce the risk of hip fractures [9,10]. Fish oil is rich in n-3 polyunsaturated fatty acids (PUFA) such as docosahexaenoic acid (DHA) and eicosapentaenoic acid (EPA), which may favorably affect cardiovascular mortality [11,12],

prevalence of some cancers [13], and activity of inflammatory diseases [14,15]. Ishikawa et al. [16] also reported that women 1–5 years past menopause who consumed fish frequently had significantly greater metacarpal bone mineral density (BMD). A recent study also showed that a higher ratio of n-6 to n-3 is associated with lower BMD at the hip [15]. There have been a limited number of studies in humans using fish oil supplementation aimed at preventing postmenopausal bone loss. Two studies in aged osteoporotic women suggested the beneficial effects of fish oil supplementation [17,18], but another study conducted in pre- and postmenopausal women failed to show a benefit [19]. Animal studies aimed at further investigating the potential positive effects of fish oil on bone metabolism have used chicks, mice, rabbits, and male and female rats [20–25]. Yet, most of these studies were done using young male animals, which might not be comparable to peri- and postmenopausal women. The current Federal Drug Administration approved model for the investigation of menopausal bone changes is the ovariectomized rat model [26]. This model has previously been shown to reproduce the altered bone metabolism that leads to bone loss following menopause. Therefore, the objective of this study was to investigate the influences of fish oil on indices of bone mass, and bone histomorphometry in an aged ovariectomized rat model.

Materials and methods

Animals and diets

The experimental protocol was approved by the Institute of Animal Care and Use Committee (IACUC) at the University of Utah. Female retired breeder Sprague–Dawley rats, 12 months of age, were purchased from Charles River Laboratories (Wilmington, MA, USA). Upon arrival, they were housed in a temperature-controlled room on a daily 14-h light cycle and fed a standard rodent chow (#8640; Harlan Teklad, Madison, WI, USA) and water ad libitum. After a 2-week acclimation period, the rats were randomly divided into five groups based on their body weight: (1) Baseline, (2) control diet + Sham-operated (Control/Sham), (3) control diet + ovariectomized (Control/OVX), (4) fish oil diet + Sham-operated (Fish/Sham), and (5) fish oil diet + ovariectomized (Fish/OVX) (n = 8–10/group).

Isocaloric diets were prepared by Harlan Teklad according to the recommendation of the American Institute of Nutrition [27]. The Baseline, Control/Sham, and Control/OVX groups were fed AIN-93M purified diet (#TD94048), while the Fish/Sham and Fish/OVX groups were fed a 4% fish oil diet, which substituted menhaden oil for soybean oil (Table 1). The manufacturer supplied the fatty acid composition for the different oils (Table 2). To prevent the possible oxidation of the fish oil the amount of *tert*-

Table 1. Diet ingredients of control and fish oil diets

Ingredient	Control diet[a] (g/kg)	Fish oil diet (g/kg)
Casein	140.0	140.0
L-Cystine	1.8	1.8
Cornstarch	465.692	465.684
Maltodextrin	155.0	155.0
Sucrose	100.0	100.0
Soybean oil	40.0	–
Menhaden oil	–	40.0
Cellulose	50.0	50.0
Mineral mix[b]	35.0	35.0
Vitamin mix[c]	10.0	10.0
Choline bitartrate	2.5	2.5
TBHQ[d]	0.008	0.016

[a] AIN-93M Purified Diet (Harlan Teklad, #TD94048)
[b] Mineral mix, AIN-93M-MX (Harlan Teklad, #TD94049)
[c] Vitamin mix, AIN-93-VX (Harlan Teklad, #TD94047)
[d] *tert*-Butylhydroquinone

Table 2. Fatty acid (FA) composition of oils of each diet

Ingredient	Control (soybean oil) (% of total FA)	Fish oil (% of total FA)
C14:0 Tetradecanoic (myristic)	0.1	8.4
C15:0 Pentadecanoic	–	0.9
C16:0 Hexadecanoic (palmitic)	10.3	15.2
C16:1 Hexadecenoic (palmitoleic)	0.2	11.6
C17:0 Heptadecanoic (margaric)	–	0.8
C18:0 Octadecanoic (stearic)	3.8	2.7
C18:1 Octadecenoic (oleic)	22.8	9.5
C18:2 Octadecadienoic (linoleic)	51.0	1.8
C18:3 Octadecatrienoic (linolenic)	6.8	1.8
C18:4 Octadecatetraenoic	–	3.5
C20:0 Eicosanoic (arachidic)	–	0.2
C20:1 Eicosenoic (gadoleic)	0.2	1.3
C20:2 Eicosadienoic	–	0.3
C20:3 Eicosatrienoic	–	0.4
C20:4 Eicosatetraenoic (arachidonic)	–	2.3
C20:5 Eicosapentaenoic	–	16.0
C21:5 Heneicosapentaenoic	–	0.8
C22:1 Docosenoic (Erucic)	–	0.3
C22:4 Docosatetraenoic	–	0.3
C22:5 Docosapentaenoic	–	3.9
C22:6 Docosahexaenoic	–	10.8
C24:1 Tetracosenoic (nervonic)	–	0.3

butylhydroquinone (TBHQ) was doubled and the fish oil diet was vacuum packed in small quantities and stored at –20°C until fed. To prevent possible differences in food intake induced by the surgery and/or taste of the diets, food intakes were matched to the Sham/control group, which was given free access to the control diet, and their food intake was measured daily. The diets were given to the other groups daily with the quantity of food based on the amount the Sham/Control group ate on the previous day. Food was changed daily, and any uneaten portions were removed. The animals remained on their respective diets for the entirety of the experiment.

Two weeks following the introduction of the diets, the Baseline group was killed. The inclusion of the Baseline group provides an initial value for skeletal measures, thereby allowing for the determination of changes in skeletal tissue that result from the surgery and aging. Under anesthesia, the remaining four groups of rats were either bilaterally ovariectomized or sham-operated using the dorsal approach. Ten weeks after surgery, the animals were killed.

Before necropsy the animals were given intraperitoneal injections of fluorochrome markers for evaluation of bone dynamics by histomorphometry. Calcein (Sigma-Aldrich, St. Louis, MO, USA) was administered at a dose of 10 mg/kg body weight 10 days before necropsy, and tetracycline-HCl (Sigma-Aldrich) was administered at a dose of 25 mg/kg body weight 3 days before necropsy.

At necropsy, the uterus and the abdominal fat were collected, and the weights were measured. The uterus was weighed to determine if the ovariectomy was successful. Blood was collected by cardiac puncture; serum was obtained and stored at –80°C until analyzed for serum cross-linked C-terminal telopeptides of type I collagen (CTx). The right femurs were wrapped in saline-soaked gauze and stored at –20°C for subsequent densitometry. The left femurs were cleaned of soft tissue and fixed in 10% neutral buffered formalin for bone histomorphometry.

Bone densitometry

The right femurs were completely thawed at room temperature, and bone mineral content (BMC) and bone mineral density (BMD) were measured by dual-energy X-ray absorptiometry (DEXA) (Norland Medical Systems, Fort Atkinson, WI, USA). BMD and BMC were also evaluated for the proximal, middiaphyseal, and distal parts of the femur by dividing the femur into three equal parts by length. The coefficients of variation for scans and standards were <1.0%.

Tissue preparation for morphometry

The left femurs were dehydrated in graded ethanols and embedded undecalcified in methyl methacrylate. Cross sections of the middiaphysis and frontal sections of the distal femur were cut using a low-speed saw (Buehler, Lake Bluff, IL, USA), mounted on plastic slides, and ground to roughly 30 μm in thickness. The specimens were examined using a fluorescent microscope (Olympus, Tokyo, Japan) equipped with a digital CCD camera (QImaging, Burnaby, BC, Canada) and histomorphometric software (Bioquant Nova Prime version 6.70.10MR for Windows XP; Bioquant Image Analysis, Nashville, TN, USA). The histomorphometric nomenclature is in accordance with the report of the ASBMR Histomorphometry Nomenclature Committee [28].

Histomorphometry of cortical bone

Cortical bone indices were measured on the cross sections of the left femoral middiaphyseal shaft. The primary indices included the total cortical area, periosteal and endosteal surface perimeters, periosteal and endocortical single- and double-labeled surfaces, and interlabel width. From these parameters, the percentages of single- and double-labeled surfaces, and mineralizing surfaces, mineral apposition rate, and surface-referent bone formation rate were calculated for both the periosteal and endosteal surfaces. The bone formation rates were calculated using the double-labeled surfaces.

Histomorphometry of cancellous bone

Structural and dynamic histomorphometric indices were measured in the cancellous bone in the distal metaphysis of the left femurs. The primary indices included the trabecular bone area, trabecular bone perimeter, single- and double-labeled surfaces, and interlabel width. Calculated from these parameters were the percentages of single- and double-labeled surfaces, and mineralizing surfaces, mineral apposition rate, and surface-referent bone formation rate. The mineral apposition rate was corrected for section obliquity, and the bone formation rates were calculated using the double-labeled surfaces.

Serum cross-linked C-terminal telopeptides of type I collagen (CTx)

The cross-linked C-terminal telopeptides of type I collagen were determined in sera samples from six animals in each of the Baseline, Control/OVX, and Fish/OVX groups to evaluate how fish oil affects the bone resorption status. This assay was done using an enzyme-linked immunosorbent assay (RatLaps; Nordic Bioscience Diagnostics A/S, Herlev, Denmark).

Statistical analyses

All data are expressed as the mean ± standard error (SEM), and all data management and statistical analyses were performed with JMP 5.1.1 (SAS Institute, Cary, NC, USA). The baseline, Control/Sham, and Control/OVX were compared by an analysis of variance (ANOVA) followed by Tukey's

HSD; this was done to determine the influence of aging and surgery on measured skeletal parameters. The specific effects of fish oil diet compared with the control diet were tested independently in the Sham (Control/Sham, and Fish/Sham) and OVX (Control/OVX, and Fish/OVX) groups using a one-tailed Student's t test. Differences of $P < 0.05$ were considered significant.

Results

There were early increases in the body weights of the OVX rats supplemented with fish oil, even though the animals were pair fed to the controls (Fig. 1). At the end of the study, both OVX groups tended to have greater body weights than the Sham groups. Overall, the rats consumed about 22–25 g food per day. The uterine weight in the Control/OVX group was significantly less than in the Baseline and Control/Sham groups (data not shown), suggesting that OVX was performed successfully in the experiment. There were no significant differences in uterine weights between the Fish/Sham and Control/Sham nor between the Fish/OVX and Control/OVX (data not shown).

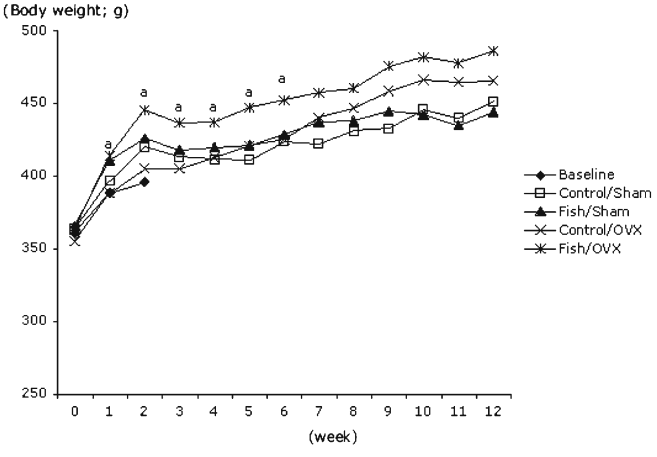

Fig. 1. Body weight changes of the rats. [a]Significantly different from the Control/OVX at $P < 0.05$

The whole femur BMC and BMD in the Control/OVX group were significantly lower than in the Baseline and Control/Sham groups (Table 3). In terms of the skeletal impact of the fish oil diet, the BMC was significantly higher in the Fish/OVX group compared with the Control/OVX group. When the femur was divided into three equal length segments there were differences in the BMC between the Fish/OVX and Control/OVX groups at the proximal and distal ends, and a trend ($P = 0.08$) at the middiaphysis. Additionally, the distal femur BMD was larger in the Fish/OVX compared with the Control/OVX. The Fish/Sham and Control/Sham did not differ in the measures of BMC or BMD.

Cortical bone structural and dynamic indices of the mid-femoral diaphysis are summarized in Table 4. In terms of the ovariectomy effect on cortical structural parameters, the Control/OVX had a lower cortical area ($P < 0.05$) when compared with the Control/Sham, and greater endosteal perimeter ($P < 0.05$) compared with both the Baseline and Control/Sham (Table 4). With regard to the impact of diet on structural parameters, the groups fed the fish oil diet did not differ from the control diet in the Sham and OVX groups for any cortical bone structural parameters. As for the dynamic indices, there were no significant differences among the Baseline, Control/Sham, and Control/OVX, at the periosteal or endocortical surfaces (Table 4). In contrast, the Fish/OVX had a significantly larger percent double-labeled surface and mineral apposition rate at the endocortical surface than the Control/OVX (Table 4). The bone formation rate of the endocortical surface also tended to be higher ($P = 0.05$).

The histomorphometric measures for the distal femoral metaphyseal cancellous bone are presented in Table 5. The double-labeled surface in the cancellous bone was higher in the Control/OVX than in the Baseline and Control/Sham. The Control/OVX also had higher measures for mineralizing surface and bone formation rate than those of the Control/Sham. However, there were no statistical differences between the fish oil groups and their respective control groups.

Serum CTx was significantly increased by ovariectomy, with the Baseline (5.79 ± 0.94 μg/ml) having lower values

Table 3. Femur bone mineral content (BMC) and bone mineral density (BMD)

	Baseline ($n = 10$)	Sham		OVX	
		Control ($n = 9$)	Fish ($n = 8$)	Control ($n = 9$)	Fish ($n = 10$)
Femur BMC (g)					
Whole	0.510 ± 0.017	0.529 ± 0.020	0.508 ± 0.020	0.426 ± 0.013a,b	0.467 ± 0.014c
Proximal	0.180 ± 0.006	0.184 ± 0.007	0.182 ± 0.007	0.154 ± 0.005a,b	0.168 ± 0.005c
Mid	0.136 ± 0.006	0.142 ± 0.006	0.139 ± 0.006	0.121 ± 0.003ab	0.128 ± 0.004
Distal	0.194 ± 0.005	0.197 ± 0.007	0.186 ± 0.007	0.151 ± 0.006a,b	0.170 ± 0.005c
Femur BMD (g/cm^2)					
Whole	0.230 ± 0.007	0.231 ± 0.006	0.225 ± 0.007	0.192 ± 0.005a,b	0.200 ± 0.005
Proximal	0.230 ± 0.007	0.231 ± 0.005	0.228 ± 0.008	0.197 ± 0.005a,b	0.203 ± 0.005
Mid	0.217 ± 0.009	0.217 ± 0.006	0.216 ± 0.008	0.191 ± 0.007a	0.194 ± 0.007
Distal	0.239 ± 0.006	0.236 ± 0.007	0.229 ± 0.007	0.190 ± 0.005a,b	0.202 ± 0.004c

Data are expressed as means ± SEM
Significantly different from Baseline, $P < 0.05$ (a); significantly different from Control/Sham, $P < 0.05$ (b); significantly different from Control/OVX, $P < 0.05$ (c)

Table 4. Cortical bone dynamic indices at the midfemoral diaphysis

	Baseline ($n = 10$)	Sham		OVX	
		Control ($n = 9$)	Fish ($n = 8$)	Control ($n = 9$)	Fish ($n = 10$)
Cortical area (mm^2)	6.58 ± 0.22	7.23 ± 2.6	7.13 ± 1.9	6.26 ± 2.1b	6.51 ± 2.3
Periosteal perimeter (mm)	11.87 ± 0.23	12.78 ± 0.28a	12.93 ± 0.25	12.35 ± 0.21	12.68 ± 0.15
Endosteal perimeter (mm)	7.43 ± 0.31	8.57 ± 0.41	8.77 ± 0.18	8.79 ± 0.31a,b	8.94 ± 0.24
Periosteal surface					
Double-labeled surface (%)	40.3 ± 9.2	34.6 ± 8.9	36.8 ± 9.0	39.5 ± 7.2	39.1 ± 5.2
Mineral apposition rate (μm/day)	0.8 ± 0.1	0.7 ± 0.1	0.7 ± 0.1	0.8 ± 0.1	0.7 ± 0.0
Bone formation rate ($μm^2$/μm/day)	0.40 ± 0.14	0.32 ± 0.10	0.33 ± 0.11	0.33 ± 0.07	0.31 ± 0.05
Endocortical surface					
Double-labeled surface (%)	1.7 ± 1.2	1.8 ± 1.1	4.1 ± 1.4	7.4 ± 3.0	16.7 ± 4.1c
Mineral apposition rate (μm/day)	0.5 ± 0.2	0.5 ± 0.2	0.8 ± 0.2	0.7 ± 0.2	1.1 ± 0.1c
Bone formation rate ($μm^2$/μm/day)	0.02 ± 0.02	0.02 ± 0.01	0.05 ± 0.01	0.08 ± 0.03	0.20 ± 0.06

Data are expressed as means ± SEM
Significantly different from Baseline, $P < 0.05$ (a); significantly different from Control/Sham, $P < 0.05$ (b); significanctly different from Control/OVX, $P < 0.05$ (c)

Table 5. Cancellous bone dynamic indices at the distal femoral diaphysis

	Baseline ($n = 10$)	Sham		OVX	
		Control ($n = 9$)	Fish ($n = 8$)	Control ($n = 9$)	Fish ($n = 10$)
Single-labeled surface (%)	23.8 ± 4.6	16.3 ± 2.2	19.3 ± 2.3	31.8 ± 9.0	22.3 ± 3.7
Double-labeled surface (%)	6.4 ± 2.1	4.8 ± 2.3	5.2 ± 2.7	22.4 ± 7.1a,b	24.0 ± 4.7
Mineralizing surface (%)	18.2 ± 4.1	13.0 ± 3.1	14.9 ± 3.4	38.3 ± 11.1b	35.2 ± 5.3
Mineral apposition rate (μm/day)	0.9 ± 0.1	1.0 ± 0.1	0.9 ± 0.1	0.9 ± 0.0	1.0 ± 0.1
Bone formation rate(s) ($μm^2$/μm/day)	0.06 ± 0.02	0.05 ± 0.02	0.06 ± 0.03	0.21 ± 0.07b	0.25 ± 0.06

Data are expressed as means ± SEM
Significantly different from Baseline, $P < 0.05$ (a); significantly different from Control/Sham, $P < 0.05$ (b)

than the Control/OVX group (11.39 ± 2.2 μg/ml). However, the serum bone resorption marker was not different between the Control/OVX and Fish/OVX (11.67 ± 2.2 μg/ml) groups, suggesting fish oil supplementation did not affect bone resorption at the time point measured in the present investigation.

Discussion

In the present investigation, we demonstrated that aged ovariectomized retired breeder rats on a fish oil diet (Fish/OVX) had significantly higher measures for bone formation, mineralizing surface, and mineral apposition rate at the midfemoral diaphysis and higher femoral BMC than rats fed a control diet (Control/OVX). The larger femoral BMC was most pronounced in the proximal and distal ends. These results suggest that fish oil consumption might mitigate the loss of bone observed following menopause in women, in part through higher bone formation rates. In contrast, there were no differences for any parameters measured between the sham group fed fish oil (Sham/Fish) and the sham group fed a control diet (Sham/Control). This result suggests that the fish oil was able to alter bone metabolism during a dynamic state but not when there were no disturbances to bone metabolism a priori.

The impact of fish oil and n-3 PUFAs on bone metabolism have been investigated in numerous animal models including mice [21], rats [15,20,22,23,29–34], chicks [24,35,36], rabbits [25], and piglets [15,37]. The results of these studies suggested that fish oil and other n-3 PUFAs increase calcium absorption [32,34], increase bone formation [23,24,33], decrease calcium excretion [32], and decrease bone resorption [21,22,37]. However, there have been relatively few studies examining the potential benefits of fish oil in animal models of postmenopausal bone loss, such as the ovariectomy model. One such investigation using ovariectomized mice fed a 5% fish oil diet or a 5% corn oil diet observed that the lower BMD in ovariectomized animals was partly or completely mitigated with fish oil supplementation [21]. The investigators did not report any histomorphometric analysis; therefore, it is not known if the differences in BMD are the result of alterations in bone formation, bone resorption, or both. However, in vitro studies by the authors suggest that mice fed a fish oil diet exhibit a decrease in osteoclastogenesis. Sakaguchi et al. [30] demonstrated that an EPA-enriched diet prevented the decrease in femoral bone weight and strength of ovariectomized rats under calcium restriction. Another investigation using ovariectomized rats examined the effect of various dietary ratio of n-6 : n-3 on calcium homeostasis [29]. There was a positive correlation between femur calcium and both DHA and EPA but no correlation with bone resorption. Another investigation using ovariectomized rats supplemented with EPA and gamma-linolenic acid (GLA) observed that femoral calcium was increased to the level of the sham-operated rats [33]. The findings of the current investigation

corroborate the findings of earlier studies with the Fish/OVX group having higher measures for BMC and bone formation but no difference in bone resorption compared with Control/OVX.

The current study is unique because it is the first to examine the role of fish oil in alterations to bone metabolism, using histomorphometry and DEXA. As stated before, the percent mineralizing surface, mineral apposition rate, and BMC of the ovariectomized fish oil group (Fish/OVX) were higher than the ovariectomized control group (Control/OVX). Additionally, the current investigation utilized ovariectomized retired breeder rats. The reproductive history of an animal is important because dramatic reductions in cancellous bone mass occur after the first lactation that are not fully restored after weaning [38]. The current investigation also used older animals, 1 year old, in contrast with most experiments that utilized younger animals, 2–4 months old. Age has also been shown to alter bone metabolism, with older animals exhibiting a reduced responsiveness to skeletal challenges [39]. Therefore, the reproductive history and age of the animal are important when comparing results with other studies, because most studies of the importance of fish oil in ovariectomized animals have utilized young female rats or mice. Despite these differences in age and reproductive history, the current investigation still observed a positive effect of fish oil (Fish/OVX) compared to animals on the control diet (Control/OVX).

In terms of human intervention studies, one investigation observed an increase in calcium clearance, serum calcium, and serum bone formation markers in older postmenopausal osteoporotic women who supplemented with fish oil for 16 weeks [17]. Previous studies in animals have also shown that fish oil may increase calcium absorption and limit excretion, which would be beneficial to bone metabolism [32,34]. Another human intervention study of older osteoporotic women who were fed a GLA- and EPA-enriched diet for 36 months found a significant increase in lumbar BMD [18]. In contrast, healthy young women and healthy postmenopausal women given evening primrose oil, fish oil, and calcium or calcium supplementation for a year demonstrated no differences in total body BMD or bone turnover markers between the two different supplementations at either age [19]. The lack of results in healthy humans is comparable to that of the present investigation. There were no differences between the sham group fed a fish oil diet (Fish/Sham) compared to those fed a control diet (Control/Sham) in terms of bone mass and bone formation parameters. The small sample size in all the human studies limits the conclusions that can be drawn about the potential benefits of fish oil supplementation on bone metabolism parameters and BMD.

The type of oil used for the present experiment might have been a confounding factor. Soybean oil has a higher n-3 fatty acid content than safflower and corn oils and is the only single fat source that gives an adequate amount and balance of n-3 and n-6 essential fatty acids [27]. The amount of alpha-linolenic acid (n-3) in the diet is important because it is converted to EPA and DHA in the body, which then contribute to eicosanoids production. Therefore, it is possible that soybean oil could raise plasma DHA and EPA levels enough to alter bone metabolism. Thus, this limitation would likely diminish rather than enhance the differences observed, so further investigations with corn or safflower oil would likely increase the importance of the fish oil diet on bone metabolism in an ovariectomized model. In addition, because some n-6 essential fatty acids, such as GLA and dihomo-gamma linolenic acid, are suggested to positively affect bone metabolism [18,29], an appropriate quantity and ratio of n-3 and n-6 essential fatty acids should also be investigated further.

The mechanisms whereby fish oil can conserve skeletal mass and density during estrogen deficiency are not well understood. Estrogen deficiency is known, however, to be associated with increases in proinflammatory cytokines, such as interleukin-1 and -6 (IL-1, IL-6) and tumor necrosis factor-alpha (TNF-α), and changes in these cytokines have been linked with the development of postmenopausal osteoporosis [40]. There are some data suggesting that consumption of fish oils can suppress proinflammatory cytokine production in elderly humans [41]. A recent study conducted in ovariectomized mice reported that fish oil decreased the ability of splenocytes to produce IL-6 and TNF- α and that this was associated with an increase in BMD [42]. These previous findings may help explain the ability of dietary fish oil to improve BMC and BMD and increase bone formation rates during estrogen deficiency as observed in the present study.

In conclusion, a diet containing fish oil resulted in higher BMC and bone formation parameters but did not alter bone resorption at the time point measured in the current investigation using aged ovariectomized rats. In contrast, bone formation and bone resorption were not different between the sham-operated rats fed a diet high in fish oil compared with that fed a control diet. These results agree with other research, in both animals and humans, that fish oil supplementation has a positive effect on bone metabolism and might be a possible intervention to help slow the loss of bone that occurs following menopause.

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