

# Influence of moderate energy restriction and seafood consumption on bone turnover in overweight young adults<sup>1-3</sup>

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## ABSTRACT

**Background:** Overweight and obesity are increasing in young adults. However, moderate energy restriction aimed at lowering body weight may promote bone turnover and bone loss. Inclusion of fish or fish oils in a weight-loss diet may attenuate these adverse skeletal effects.

**Objective:** We examined the effects of incorporating fish or fish oil into an energy-restricted diet on bone turnover markers in young overweight adults.

**Design:** While following a strict hypoenergetic (−30% relative to estimated requirements) diet for 8 wk, 276 overweight men and women [body mass index (in kg/m<sup>2</sup>): 27.5–32.5; age: 20–40 y] were randomly assigned to 1 of 4 dietary groups: sunflower-oil capsules (3 g/d; control), cod (3 × 150 g/wk), salmon (3 × 150 g/wk), and fish-oil capsules (3 g/d). Body weight, bone biomarkers, and 25-hydroxyvitamin D were measured at baseline and endpoint. Data were analyzed with repeated-measures analysis of variance and general linear models.

**Results:** The mean (±SD) weight loss was 5.14 ± 3.0 kg (5.8% ± 3.2% body weight) during the 8 wk in the 4 dietary groups combined. Urinary N-telopeptides of type I collagen and serum C-terminal telopeptide of type I collagen increased ( $P < 0.05$ ), whereas serum osteocalcin (but not bone-specific alkaline phosphatase) decreased ( $P < 0.05$ ) from baseline to endpoint. Increased fish or fish-oil consumption had no effect ( $P > 0.1$ ) on the changes in bone markers induced by weight loss. In contrast, increased salmon consumption increased serum 25-hydroxyvitamin D ( $P < 0.01$ ).

**Conclusions:** A nutritionally adequate but energy-restricted diet, with different contents of n−3 fatty acids, which resulted in modest weight loss, unfavorably altered bone turnover markers in young overweight adults. Such changes were not prevented by increased fish or fish-oil consumption. This trial was registered at the US National Library of Medicine as #NCT00315770. *Am J Clin Nutr* 2008;87:1045–52.

## INTRODUCTION

Obesity is a major public health concern because it is implicated in the development of many chronic diseases (1–3). However, in relation to skeletal health, increased body weight in postmenopausal women is thought to reduce the risk of osteoporosis (4–6). For example, annual bone loss was observed to be significantly lower in postmenopausal women with a higher body mass index [BMI (in kg/m<sup>2</sup>): >25] than in postmenopausal women with a lower BMI (<25) (7). Furthermore, weight loss is associated with increased risk of hip

fracture in older women and men (8, 9). Even moderate weight loss may increase the rate of bone resorption (10–11) and accelerate bone loss (10–13) in overweight and obese postmenopausal women. Some studies (14–16), but not all (17, 18), have shown increased bone loss after weight reduction in premenopausal women, whereas the effects of energy restriction on bone turnover in overweight men has apparently not received much attention (19). Indeed, the effect of energy restriction and accompanying weight loss on skeletal health in young to mid adulthood is still unclear.

Some evidence suggests that the adverse effects of energy restriction on bone turnover may be mitigated by increasing calcium intakes (up to ≈1.7 g/d) in overweight postmenopausal women (10, 12). Such calcium intakes, however, are rarely achieved by dietary means (20, 21). Increased fish consumption is often conducive to a healthy diet, and intake of ≥2 servings/wk (preferably fatty fish) is recommended by the American Heart Association for healthy adults (22). Recently, we showed that including seafood in energy-restricted diets can increase weight loss (23). Fatty fish such as salmon are rich in n−3 fatty acids, and increased fish consumption as part of a weight-loss diet was observed to be more effective in improving glucose-insulin metabolism and dyslipidemia in overweight hypertensive subjects than weight loss alone (24). n−3 Fatty acids were shown to stimulate intestinal calcium absorption and beneficially to alter bone metabolism in rat and cell model systems (25–28). In this context, van Papendorp et al (29) showed, in a randomized trial with 40 osteoporotic patients, that subjects taking a supplement rich in marine-based n−3 fatty acids or these n−3 fatty acids together with evening primrose oil had a greater efficiency of intestinal calcium absorption and higher concentrations of markers of

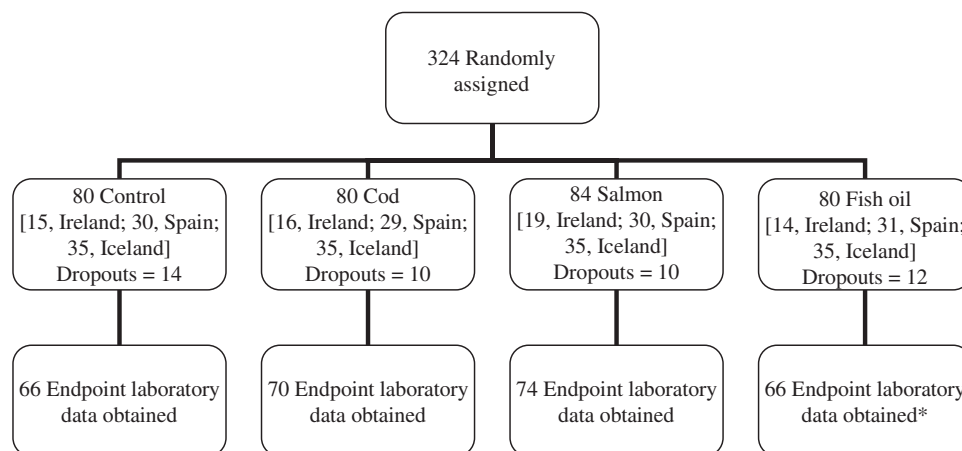
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**FIGURE 1.** Flow of subjects in the study. \*One subject completed the study but failed to provide a blood sample; data from 1 subject was excluded because bone turnover marker concentrations were well outside the normal range.

bone formation than did subjects taking the placebo. Furthermore, a high ratio of n-6 to n-3 fatty acids in the diet is associated with low bone mineral density (BMD) at the hip in men and women > 45 y (30). Although there has been much less investigation of the effect of whole fish on calcium and bone metabolism, Zalloua et al (31) recently reported that increased seafood consumption is significantly associated with increased BMD in women, aged 25–64 y. Thus, increased fish and fish-oil consumption may attenuate the effect of energy restriction-induced weight loss on bone turnover, although this has not been studied. Therefore, the hypothesis of this study was that moderate weight loss, arising from dietary energy restriction, would induce an increase in the rate of bone turnover in young overweight adult men and women and that possibly the inclusion of more fish or fish oil in the diet would ameliorate these negative effects of weight loss on bone turnover.

## SUBJECTS AND METHODS

### Subjects

A total of 324 young adults were recruited into the SEA-FOODplus YOUNG study, which was an 8-wk intervention study of dietary-based weight loss with or without increased consumption of fish or fish oil. The trial was conducted at 3 centers, Iceland ( $n = 140$ ), Spain ( $n = 120$ ), and Ireland ( $n = 64$ ), during the months of October 2004 through May 2005. The progress of these subjects through the trial is shown in **Figure 1**. Details of the subjects were reported previously (23). In brief, inclusion criteria included age 20–40 y, BMI between 27.5 and 32.5, waist circumference minimum of 80 cm (women) and 94 cm (men), and being weight stable within the 3 mo preceding the trial. Volunteers were excluded if they consumed supplements that contained calcium, vitamin D, or fish oil within the previous 3 mo or during the study. Drug treatment of diabetes mellitus; the taking of other prescribed medications, including antiinflammatory and antihypertensive drugs; pregnancy or lactation; drug or alcohol abuse; vegetarianism; dislike of fish; and professional athletes were also reasons for exclusion. The study was approved by the appropriate ethics committee in each collaborating center,

and all participants gave their written consent according to the Helsinki Declaration.

### Study design

Full details of the study design were reported previously (23). Each subject was instructed to follow a diet, energy restricted by 30% from estimated energy requirement ( $\approx 600$  kcal/d; range: 473–718 kcal/d) for 8 consecutive weeks. The energy deficit for each subject was calculated on the basis of their estimated basal metabolic rate (with the use of Harris-Benedict equations), including a correction factor for the overweight status of the subject, and adjusting for physical activity level (set at 1.3), as outlined previously (23). The subjects were randomly assigned to 4 diets with varying dietary protein source and amount of n-3 fatty acids: 1) no seafood [control; included 6 sunflower-oil capsules/d; Loders Croklaan (Lipid Nutrition), Wormerveer, Netherlands; encapsulated by Banner Pharmacaps, Tilburg, Netherlands], 2) lean fish cod (150 g cod  $\times$  3/wk; Samherji, Iceland), 3) salmon (150 g salmon  $\times$  3/wk; Marine Harvest, Nutreco, Norway), or 4) fish-oil capsules [3 g/d; 6 capsules/d; Loders Croklaan (Lipid Nutrition); encapsulated by Banner Pharmacaps]. These 4 groups allowed us to differentiate between a fish and fish-oil effect on bone turnover. The daily provision of n-3 fatty acid was 0.001 g, 0.3 g, 3.0 g, and 1.5 g for the sunflower-oil, cod, salmon, and fish-oil diets, respectively.

To achieve a weight loss of 0.5–1 kg/wk, a 30% energy-deficient diet was formulated by a trained nutritionist for each subject on an individual basis, as described previously (23). In brief, similar diets were formulated for each of the 4 treatment groups and incorporated the particular dietary intervention. All diets were approximately matched across the 4 groups for total fat (30–35% of total energy), carbohydrate (50–55% of total energy), protein (16–20% of total energy), and dietary fiber (20–25 g), and we aimed that each intervention diet would meet the dietary recommendations for macro- and micronutrient intakes. These energy-restricted diets contained similar sources of fruit, vegetables, dairy products, and meat. An exchange list was also formulated to offer a variety of substitute foods of similar caloric content and nutrient composition.



During the intervention phase, each subject made 3 visits to the study center, at baseline (week 0), midpoint (week 4), and endpoint (week 8). In addition, in weeks 2 and 6 subjects were contacted by phone or email by the nutritionist to encourage further compliance with the intervention. The subjects were also encouraged to contact the clinic or metabolic unit on their own initiative if they had any questions about the research during the 8-wk intervention period. A fasting blood sample was drawn and a urine sample was collected at baseline and endpoint. Systolic and diastolic blood pressures were measured at baseline and endpoint with the use of the Medisana blood pressure monitor (Medisana AG, Germany; www.medisana.de).

### Anthropometry and lifestyle data

Anthropometrics and body composition by bioimpedance analysis were measured at baseline, midpoint, and endpoint, as described previously (23). Subjects also answered a health and lifestyle questionnaire at baseline and endpoint, which assessed physical activity, general health, smoking status, and alcohol consumption. Subjects were asked to maintain their normal level of physical activity. Dietary intakes were estimated from 2-d weighed food records completed before baseline and before endpoint visits (between weeks 5 and 8). The dietary records were analyzed with the use of the accepted food-nutrient database in each country. Nutrient intakes in Ireland were calculated with the use of WISP software (version 3; Tinuviel Software, Llanfechell, United Kingdom) that uses data from *McCance and Widdowson's The Composition of Foods*, sixth (32) and fifth (33) editions, plus supplemental volumes to generate nutrient intake data. In Iceland, the nutrient-calculating program is ICEFOOD software (version 2002; Public Health Institute of Iceland, Reykjavik, Iceland), designed for the national dietary survey of The Icelandic Nutrition Council (34), with the use of the National Nutrition Database ISGEM for calculation of nutrient value in the diet; in Spain, the MEDISYSTEM (Sanocare, Madrid, Spain) software was adapted to the Spanish foods. The data were then amalgamated for statistical analysis to describe nutrient intakes.

Compliance with the intervention diets was assessed by the 2-d weighed food records and by a formerly validated food-frequency questionnaire for seafood, first in Iceland for young women and then in a cross-European validation study in Iceland, Ireland, and Spain for young adults of both sexes (35, 36). The food-frequency questionnaire was administered at all 3 visits. Compliance was also assessed by analyzing  $n=3$  and  $n=6$  fatty acids in erythrocyte phospholipids in fasting bloods, as reported elsewhere (23). Results showed good compliance with the intervention diets.

### Collection and preparation of samples

Blood was collected by venepuncture into a evacuated tube with no additive and processed to serum, which was immediately stored at  $-80^{\circ}\text{C}$  until required for analysis. Urine was stored at  $-20^{\circ}\text{C}$  until required for analysis.

### Laboratory methods

Serum osteocalcin (OC) and bone-specific alkaline phosphatase (BAP) were measured with the use of Metra enzyme immunoassay kits (Quidel Corporation, San Diego, CA). Serum C-terminal telopeptide of type I collagen (CTX) was measured

with the use of the Serum CrossLaps enzyme-linked immunosorbent assay (ELISA) kit (Nordic Bioscience Diagnostics A/S, Denmark). Urinary creatinine concentrations were measured with the use of a quantitative, colorimetric assay by Metra (Quidel Corporation). Urinary N-telopeptides of type I collagen (NTx) was measured by the Osteomark ELISA (Unipath Ltd, Bedford, United Kingdom). Urinary NTx concentrations were expressed relative to urinary creatinine concentrations. Serum 25-hydroxyvitamin D [25(OH)D] concentrations were measured with the use of ELISA (OCTEIA 25-Hydroxy Vitamin D; Immuno Diagnostic Systems, Ltd, Boldon, United Kingdom). The quality and accuracy of the serum 25(OH)D analysis in the central biomarker analytic laboratory of the study (University College Cork) are assured on an ongoing basis by participation in the Vitamin D External Quality Assessment Scheme (Charing Cross Hospital, London, United Kingdom). Serum concentrations of intact parathyroid hormone (PTH) were measured by ELISA (OCTEIA intact parathyroid hormone; Immuno Diagnostic Systems, Ltd). Intraassay variability for serum OC, BAP, CTx, 25(OH)D, and PTH and for urinary NTx and creatinine was 8.1%, 6.2%, 8.4%, 7.8%, 8.6%, 8.3%, and 3.4%, respectively. Interassay variability in these variables was avoided by analyzing all samples from a subject in the same run.

### Statistical analysis

The SEAFOODplus YOUNG intervention study was powered to detect  $\approx 1$ -kg difference in weight loss between the 4 diet groups, as the primary outcome (23). The sample size completing the intervention study and included in the present analysis ( $n = 66-74$  per group) provided sufficient power to detect a minimum of a 11.1%, 15.1%, 25.5%, and 30.9% change in the biomarkers of bone turnover: serum OC, BAP, and CTx and urinary NTx, respectively, within a group. Ricci et al (11) previously reported a 12.8%, 36.4%, and 52.5% increase in serum OC, urinary pyridinoline (PYD), and deoxypyridinoline (DPD) (markers of bone resorption), respectively, after moderate weight loss in postmenopausal women. The present study used serum CTx and urinary NTx as markers of bone resorption. Serum markers of bone resorption were shown to be less variable than urinary-based markers, which may be related to variability in creatinine measurement (37). Statistical analysis of the data was conducted with the use of SPSS for WINDOWS version 12.0 (SPSS Inc, Chicago, IL). Descriptive statistics (mean, median, and SD) were determined for all variables. Serum concentrations of the bone biomarkers were not normally distributed and thus were log transformed to achieve near-normal distributions. Baseline characteristics of subjects in the different intervention groups were compared with the use of chi-square test (for male-to-female ratio), one-factor analysis of variance (ANOVA; for normally distributed variables), or Kruskal-Wallis test (for nonnormally distributed variables). Baseline differences in the biochemical markers of bone turnover, serum 25(OH)D and PTH, across the 4 intervention groups were assessed by ANOVA and analysis of covariance, adjusting for sex and country. Differences in changes in nutrient intake from baseline to endpoint across the groups were tested by ANOVA and Tukey's tests (for normally distributed variables) or Kruskal-Wallis test (for nonnormally distributed variables).

Linear models of the response in a repeated-measures analysis for the differences in bone marker concentrations, adjusted for percentage of weight loss, were set up. The main effects included



**TABLE 1**Baseline characteristics of the study participants by treatment group<sup>1</sup>

	Group 1 (n = 66) Sunflower oil	Group 2 (n = 70) Cod	Group 3 (n = 74) Salmon	Group 4 (n = 66) Fish oil	P <sup>2</sup>
Men:women <sup>3</sup>	24:42	31:39	37:37	26:40	0.405
Age (y)	33 ± 5 <sup>4</sup>	32 ± 6	32 ± 5	31 ± 5	0.465
Anthropometric measurements					
Waist (cm)	94.5 ± 7.0 <sup>a,b</sup>	96.8 ± 6.8 <sup>a,b</sup>	97.2 ± 7.9 <sup>a</sup>	94.2 ± 6.5 <sup>b</sup>	0.022
Hip (cm)	110.4 ± 4.9 <sup>a,b</sup>	111.1 ± 5.6 <sup>a</sup>	111.0 ± 4.9 <sup>a</sup>	108.7 ± 5.3 <sup>b</sup>	0.023
Height (m)	1.70 ± 0.1 <sup>a,b</sup>	1.72 ± 0.1 <sup>a,b</sup>	1.73 ± 0.1 <sup>a</sup>	1.70 ± 0.1 <sup>b</sup>	0.029
Weight (kg)	86.7 ± 9.3 <sup>a,b</sup>	89.4 ± 9.7 <sup>a</sup>	91.1 ± 11.8 <sup>a</sup>	84.8 ± 9.9 <sup>b</sup>	0.002
BMI (kg/m <sup>2</sup> )	30.0 ± 1.5	30.2 ± 1.4	30.4 ± 1.4	29.8 ± 1.5	0.062
Fat mass (kg) <sup>5</sup>	29.5 ± 5.9	29.4 ± 5.8	29.1 ± 5.2	28.1 ± 5.5	0.439
Body fat (%) <sup>5</sup>	34.5 ± 8.0	33.3 ± 7.6	32.4 ± 7.0	33.4 ± 7.7	0.530
Fat-free mass (kg) <sup>5</sup>	57.6 ± 11.9	60.0 ± 11.6	62.1 ± 12.4	57.3 ± 11.7	0.059
Blood pressure					
Systolic (mm Hg)	126.1 ± 10.4	125.0 ± 12.1	127.8 ± 13.1	123.2 ± 13.2	0.128
Diastolic (mm Hg)	72.8 ± 7.7	73.2 ± 7.9	73.0 ± 7.7	71.1 ± 7.4	0.257
Dietary intake					
Energy (kcal/d)	2344 ± 648	2390 ± 658	2276 ± 917	2254 ± 716	0.324
Protein (% of energy intake)	17.5 ± 4.4	16.7 ± 3.6	17.3 ± 4.1	17.5 ± 3.8	0.544
Carbohydrate (% of energy intake)	41.2 ± 8.5	41.7 ± 9.5	41.5 ± 9.5	40.9 ± 10.1	0.920
Fat (% of energy intake)	35.9 ± 7.2	36.3 ± 7.1	35.8 ± 8.1	35.4 ± 7.5	0.952
Calcium (mg/d)	1111 ± 392	1064 ± 408	1084 ± 472	1057 ± 395	0.628
Phosphorus (mg/d)	1683 ± 497	1636 ± 432	1598 ± 545	1586 ± 453	0.534
Magnesium (mg/d)	305 ± 95	307 ± 89	301 ± 106	307 ± 90	0.845
Sodium (mg/d)	3177 ± 1236	3233 ± 1153	3154 ± 1530	2975 ± 1044	0.579
Potassium (mg/d)	3692 ± 4632	3138 ± 919	3112 ± 1156	3093 ± 880	0.828
Vitamin D (μg/d)	2.6 ± 2.5	2.9 ± 2.6	3.3 ± 4.4	3.5 ± 4.5	0.915

<sup>1</sup> Different superscript letters denote significant ( $P < 0.05$ ) differences in mean values across the 4 groups (by Tukey's tests).

<sup>2</sup> Analyzed with ANOVA or Kruskal-Wallis test for normally and nonnormally distributed data, respectively.

<sup>3</sup> Analyzed with chi-square test.

<sup>4</sup>  $\bar{x} \pm SD$  (all such values).

<sup>5</sup> Measured by bioelectrical impedance analysis (assessed in 270 subjects).

were dietary treatment, sex, and country. The linear models also included 2-factor interactions between the main effects. Regression analysis was used to examine the association between weight loss and changes in bone marker concentration (unadjusted and adjusted for potential confounded effects of sex, country, and change in dietary intake of bone active nutrients during the course of the 8-wk intervention). A  $P$  value of  $< 0.05$  was taken as being statistically significant.

## RESULTS

Two hundred seventy-eight (120 men and 158 women) of the 324 subjects recruited (86%), completed the study (see Figure 1). The 2 most common reasons for dropout were that the subject was unable to follow the energy-restricted diet or experienced a lack of time to maintain the scheduled clinical visits. Dropouts were equally distributed between intervention groups. For the current analysis, we had complete dietary and anthropometric data and pre- and postintervention bone biomarker values for 277 subjects. However, because concentrations of bone turnover markers in 1 male subject were well outside the normal range, data from this subject were excluded from our analysis.

### Baseline characteristics

The baseline characteristics of subjects across the 4 treatment groups are shown in **Table 1**. Across the 4 treatment groups,

there were no significant differences in male-to-female ratio, mean age, BMI, fat mass, percentage of body fat, and systolic or diastolic blood pressure. Mean waist circumference and height were significantly lower ( $P < 0.05$ ) in the fish-oil group than in the salmon group. Mean hip circumference and body weight were significantly lower ( $P < 0.05$ ) in the fish-oil group than in the cod and salmon groups. Dietary intakes of protein, carbohydrate, fat, or calories did not differ across the 4 dietary treatment groups. Similarly, intakes of other bone-regulating nutrients such as calcium, vitamin D, phosphorus, magnesium, sodium, and potassium did not differ across the 4 dietary treatment groups. In addition, there were no significant differences in mean concentrations of biochemical makers of bone turnover (OC, BAP, CTx, or NTx), 25(OH)D, or PTH among the 4 dietary treatment groups at baseline (**Table 2**).

### Nutrient intakes during the intervention period

As expected, energy intakes, as well as absolute intakes of protein, carbohydrate, and fat, decreased between baseline and endpoint, and the decreases were similar across the study groups for energy, carbohydrate, and fat. The mean ( $\pm SD$ ) decrease in energy in the sunflower-oil, cod, salmon and fish-oil groups was  $-902 \pm 756$ ,  $-943 \pm 624$ ,  $-788 \pm 834$ , and  $-808 \pm 630$  kcal/d, respectively, with no significant difference among groups ( $P = 0.27$ ). The mean decrease in protein intake was smaller ( $P < 0.05$ ) in the salmon group ( $-20.9 \pm 28.0$  g/d) than that in



**TABLE 2**  
Lack of effect of cod, salmon, or fish-oil capsules as part of an energy-restricted diet on biomarkers of bone turnover during 8 wk in young adults<sup>1</sup>

	Sunflower oil (n = 66)		Cod (n = 70)		Salmon (n = 74)		Fish oil (n = 66)		P <sup>4</sup>
	Baseline <sup>2</sup>	Endpoint	Baseline <sup>2</sup>	Endpoint	Baseline <sup>2</sup>	Endpoint	Baseline <sup>2</sup>	Endpoint	
Serum OC (ng/mL)	8.9 ± 1.9 <sup>5</sup>	8.5 ± 1.8	9.1 ± 2.2	9.1 ± 2.3	9.3 ± 2.3	9.4 ± 3.0	8.7 ± 1.9	8.3 ± 1.8	0.049
Serum BAP (U/L)	19.6 ± 5.9	19.5 ± 6.2	21.7 ± 7.0	22.0 ± 7.2	21.0 ± 7.1	21.5 ± 7.5	20.7 ± 5.7	21.2 ± 7.1	0.237
Urinary NTx (nmol/L BCE per mmol/L creatinine)	41.9 ± 28.4	45.6 ± 28.3	46.4 ± 25.2	54.8 ± 35.5	49.1 ± 35.8	50.8 ± 33.9	46.9 ± 28.0	46.2 ± 26.2	<0.0001
Serum CTx (ng/mL)	0.46 ± 0.22	0.51 ± 0.24	0.51 ± 0.19	0.61 ± 0.25	0.54 ± 0.37	0.64 ± 0.36	0.52 ± 0.25	0.60 ± 0.25	0.037
Serum 25(OH)D (nmol/L)	59.2 ± 31.9	56.9 ± 35.6 <sup>a</sup>	59.0 ± 22.1	58.0 ± 23.2 <sup>a</sup>	61.9 ± 33.8	70.3 ± 28.9 <sup>b</sup>	61.7 ± 27.9	64.8 ± 33.2 <sup>a</sup>	0.006
Serum PTH (pmol/L)	3.0 ± 1.5	3.0 ± 1.3	2.9 ± 1.0	3.0 ± 1.5	2.8 ± 1.3	2.8 ± 1.2	3.0 ± 1.1	3.2 ± 1.3	0.532

<sup>1</sup> OC, osteocalcin; BAP, bone-specific alkaline phosphatase; NTx, N-telopeptides of type I collagen; BCE, bone collagen equivalents; CTx, C-terminal telopeptide of type I collagen; 25(OH)D, 25-hydroxyvitamin D; PTH, parathyroid hormone. Different superscript letters denote significant ( $P < 0.01$ ) differences in mean endpoint values across the 4 groups (by Tukey's tests).

<sup>2</sup> No significant differences were evident in any of the biochemical variables across the 4 groups at baseline (by ANOVA or ANCOVA after adjustment for sex and country).

<sup>3</sup> Represents the treatment group × time interaction from repeated-measures ANOVA with log-transformed data and adjusted for percentage of weight loss.

<sup>4</sup> Represents the main time effect by repeated-measures ANOVA with log-transformed data.

<sup>5</sup>  $\bar{x} \pm SD$  (all such values).

the cod ( $-24.9 \pm 26.8$  g/d), fish-oil ( $-28.2 \pm 22.9$  g/d), and sunflower-oil ( $-35.2 \pm 35.1$  g/d) groups. Mean vitamin D intakes decreased from before to after intervention in the sunflower-oil, cod, and fish-oil groups (by  $-1.0 \pm 2.9$ ,  $-1.2 \pm 2.8$ , and  $-1.9 \pm 4.4$   $\mu\text{g/d}$ , respectively) but increased in the salmon group (by  $7.2 \pm 9.6$   $\mu\text{g/d}$ ), a difference between groups that was significant ( $P < 0.001$ ). Intakes of calcium, phosphorus, magnesium, sodium, and potassium decreased during the 8 wk to a similar extent across the 4 treatment groups ( $P > 0.4$ ).

**Effect of weight loss and dietary intervention on biochemical markers of calcium and bone metabolism**

The detailed weight-loss findings, as the primary outcomes of the intervention study, were recently reported in detail elsewhere (23). In brief, during the 8 wk, 95% of subjects ( $n = 263$ ) lost weight ( $>0.5$  kg) with a mean weight loss of  $5.14 \pm 3.0$  kg (percentage of weight loss:  $5.8\% \pm 3.2\%$ ). Mean BMI decreased from  $30.1 \pm 1.5$  to  $28.4 \pm 1.6$ . Mean weight loss during 8 wk across the 4 dietary groups was  $-4.4 \pm 2.8$ ,  $-5.3 \pm 3.2$ ,  $-5.4 \pm 2.7$ , and  $-5.5 \pm 3.3$  kg for the sunflower-oil, fish-oil, cod, and salmon groups, respectively.

Although weight loss as a result of the caloric deficit lead to significant changes in the concentrations of biochemical markers of bone turnover, the inclusion of either lean or fatty fish or fish oil as part of the energy-restricted diet had no effect on these biochemical markers (Table 2), even after controlling for potential confounding effects of sex and country and individual weight loss during 8 wk. Weight loss arising from the energy-restricted diet, and irrespective of fish intervention, lead to a significant reduction in mean concentrations of serum OC ( $-2.2\%$ ) (but not serum BAP) and increases in mean concentrations of serum CTx (15.7%) and urinary NTx (6.9%) (for  $P$  values of main time effects, see Table 2). No significant interactions were observed with sex or country, indicating that effects of weight loss on bone turnover markers were similar across both sexes and across countries. Serum PTH concentration was unaffected by weight loss or fish intervention (Table 2). Serum 25(OH)D concentration was increased by the inclusion of salmon as part of the energy-restricted diet, but there were no significant effects of the other treatments on serum 25(OH)D concentration (Table 2). This finding was not influenced by sex or country.

Regression analysis showed that weight loss during 8 wk was significantly associated with changes in serum OC ( $P = 0.015$ ), serum BAP ( $P = 0.004$ ), serum CTx ( $P = 0.024$ ), and urinary NTx ( $P = 0.0001$ ), even after controlling for possible confounding effects of sex and country. These associations remained after controlling for changes in intake or status of bone-active nutrients (intakes of calcium, phosphorus, sodium, potassium, protein, as well as energy, and vitamin D status) from baseline to endpoint (data not shown).

**DISCUSSION**

The findings of the present trial, which was novel in its design, showed that an energy-restricted diet ( $-30\%$  kcal relative to estimated requirements) for 8 wk lead to significant weight loss in young overweight adult men and women, which was associated with alterations in biochemical markers of bone turnover. Furthermore, the weight-loss-induced increase in the rate of bone resorption in these young overweight adults was not ameliorated



by inclusion of fish or fish oil in the energy-restricted diet, although salmon intake improved both vitamin D intake and status.

The target weight loss was achieved in the majority of participants during the 8-wk period; furthermore, the weight loss achieved by these adult men and women was comparable with other studies carried out predominantly in pre- and postmenopausal women (10, 11, 13, 15, 38); however, the present study had a larger sample size ( $n = 276$ ) than these previous studies. In addition, it showed a larger weight loss with diets that included seafood than did a control diet without seafood (23). Also note that the effect of weight loss on markers of bone resorption in the present study, although in line with findings of many (10–11, 13, 15) but not all (17, 18) studies of dietary-induced weight loss, was evident during a shorter time frame (8 wk) with a more moderate weight loss (5.8%) in our younger adult men and women, aged 20–40 y. For example, Salamone et al (15) reported a modest, but significant, increase in mean annualized percentage change in urinary NTx in overweight premenopausal women who had a weight loss of  $>5.3\%$  during 18 mo, whereas Noakes et al (38), in their 12-wk weight-loss dietary intervention study, reported elevated urinary DPD and PYD in obese women, aged 20–65 y, who lost  $\approx 8.4\%$  body weight, on average.

The lack of effect of weight loss on serum BAP in the present study is in line with that reported in weight-loss studies of overweight postmenopausal women (13) and middle-aged adult men and women (39), whereas the observed weight-loss-induced decrease in serum OC observed in the present study is in contrast to that reported in other studies, which reported either an increase (10–13, 38, 40) or no change in serum OC with dietary-induced weight loss (15, 17, 18, 39). It is possible that the 8-wk intervention in the present study was too short in duration for the increases in bone resorption to be mirrored by increases in the rate of bone formation, which would be expected to occur after a lag phase when the processes of bone resorption and formation are coupled. Generally, the recruitment of osteoblasts occurs  $\approx 6$ –7 wk after resorption (41). Ricci et al (11) measured markers of bone resorption and formation serially during 6 mo in a weight-loss intervention in postmenopausal women ( $n = 27$ ; mean age: 55.9 y) and only observed a significant increase in serum OC from week 5 of weight loss ( $10.2\% \pm 5.5\%$ ) onward (urinary PYD and DPD increased significantly after 1 wk). It is not clear whether the relatively short-term weight-loss-induced changes in bone turnover markers observed in the present study would be translated into changes in BMD. However, several studies of calorie-restricted weight loss have shown an increased rate of bone resorption or bone turnover, which was accompanied by an increased rate of bone loss observed in overweight or obese pre- and postmenopausal women (10–13, 15) and middle-aged men (19). The effects of dietary-induced weight loss on BMD have yet to be investigated in adult men and women aged between 20 and 40 y, a group in which overweightness and obesity are on the increase. Note that obese older women (42) and obese younger men (43) were reported to have higher BMD values at baseline.

The mechanism(s) by which weight loss induces these changes in bone metabolism are unclear. Some investigators have suggested that the bone loss may possibly be due to a reduction in mechanical stress in the weight-bearing skeleton during weight loss, which may influence bone remodelling (15). Others have suggested that reduced calcium intake during dietary-induced weight loss may contribute to increased bone mobilization and loss (44). Ricci et al (11) observed an increase

in serum PTH and in markers of bone resorption after a 6-mo energy-restricted weight-loss intervention, which they suggest may have been associated with the reduced calcium intakes during the period (from 666 to 504 mg/d from baseline to endpoint). In the present study, mean calcium intakes were relatively high at baseline (1079 mg/d) and endpoint (851 mg/d), possibly explaining why serum PTH concentrations were unaffected during the 8-wk intervention. Although not assessed in the present study, it was suggested that energy restriction may decrease calcium absorption [see review by Shapses and Riedt (44)]. A calorie-restricted diet may have lower concentrations of a number of other bone-active nutrients, beyond just calcium, which may have a role in weight-loss-induced elevations in bone turnover. However, in the present study, the change in bone markers associated with weight loss were not ameliorated by controlling for changes in intakes of a number of bone nutrients, including calcium, phosphorus, potassium, and protein. Note again, however, that intakes of these nutrients even in the calorie-restricted diet were still close to that recommended (45). Thus, it seems that the weight-loss-induced bone resorption was independent of changes in nutrient intake. It is also possible that weight reduction induced the changes in bone metabolism by alternative means, such as decreases in serum estrogen, leptin, glucagon-like peptide 2, growth hormone, and insulin-like growth factor I or increased cortisol, as recently discussed by Shapses and Riedt (44).

The inclusion of fish as part of a weight-reducing diet was shown to improve the status of several health biomarkers (23, 46, 47), and there are lines of evidence to suggest that fish and the  $n-3$  fatty acids present in some fish oils may promote calcium absorption and skeletal health (28–30, 48, 49). In the present study, consumption of cod, salmon, or fish oil as part of a weight-reducing diet seemingly had no influence on biomarkers of bone turnover. Fish oils (as opposed to fish liver oils) and cod fish are poor sources of vitamin D (50, 51) and therefore were insufficient to improve 25(OH)D concentrations during the 8 wk in these study groups, unlike the group that received 3 portions of salmon per week, in which an increase ( $\approx 8$  nmol/L) in mean serum 25(OH)D was observed. Salmon is a rich source of vitamin D (typically providing 9.6  $\mu\text{g}/100$  g) (50). In addition, this increased dietary intake of vitamin D was likely to have a significant effect on vitamin D status because of the study being conducted during wintertime when there is no dermal production of the vitamin (52). The improvement in vitamin D status in the salmon group however was not translated into effects on bone marker concentrations or indeed on serum PTH during the 8-wk intervention. Weight loss per se had no effect on serum 25(OH)D concentrations in the adults in the present study, in line with similar findings from other studies (10, 11, 17, 40).

The present study has some limitations, including the absence of data on BMD and physical activity. In addition, the study was powered to observe a change in biomarkers of bone turnover reported from a weight-loss study in postmenopausal women who had calcium intakes of  $\approx 0.5$  g/d (11); therefore, it may have been underpowered for a young adult population undergoing weight loss with a calcium intake of  $\approx 1$  g/d. Nevertheless, the large number of subjects in the current study compared with most previous studies that examined the effects of weight loss on bone turnover is one of its strengths.

In conclusion, this study showed that modest weight loss, achieved by caloric restriction during 8 wk, unfavorably altered



bone metabolism in overweight 20–40-y-old adult men and women. The inclusion of fish or fish oil in the diet was unable to attenuate the effect of weight loss on bone turnover. Against a background of global increases in body weight in young adults, the formulation of nutritionally adequate, energy-restricted diets that do not result in adverse physiologic effects, such as bone loss, is becoming increasingly important. Further longer-term investigations are warranted to investigate the effects of weight loss on bone metabolism and bone mass in overweight and obese subjects at different life stages and to explore possible dietary strategies to minimize the effect of weight loss on bone.

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