

n-3 Fatty acids are positively associated with peak bone mineral density and bone accrual in healthy men: the NO₂ Study¹⁻³

Magnus Högström, Peter Nordström, and Anna Nordström

ABSTRACT

Background: Knowledge of the influence of nutritional intake on bone health is limited. Polyunsaturated fatty acids have been suggested to influence bone growth and modeling in humans, although data are sparse.

Objective: The objective was to investigate the role of fatty acids in bone accumulation and the attainment of peak bone mass in young men.

Design: The cohort studied consisted of 78 healthy young men with a mean age of 16.7 y at baseline. Bone mineral density (BMD; in g/cm²) of total body, hip, and spine was measured at baseline and at 22 and 24 y of age. Fatty acid concentrations were measured in the phospholipid fraction in serum at 22 y of age.

Results: Concentrations of n-3 fatty acids were positively associated with total BMD ($r = 0.27$, $P = 0.02$) and spine BMD ($r = 0.25$, $P = 0.02$) at 22 y of age. A positive correlation between n-3 fatty acid concentrations and the changes in BMD at the spine ($r = 0.26$, $P = 0.02$) was found between 16 and 22 y of age. Concentrations of docosahexaenoic acid (DHA, 22:6n-3) were positively associated with total BMD ($r = 0.32$, $P = 0.004$) and BMD at the spine ($r = 0.30$, $P = 0.008$) at 22 y of age. A positive correlation was also found between DHA concentrations and the changes in BMD at the spine ($r = 0.26$, $P = 0.02$) between 16 and 22 y of age.

Conclusion: The results showed that n-3 fatty acids, especially DHA, are positively associated with bone mineral accrual and, thus, with peak BMD in young men. *Am J Clin Nutr* 2007;85:803-7.

KEY WORDS Peak bone mineral density, bone accrual, men, docosahexaenoic acid, n-3 fatty acids

INTRODUCTION

Osteoporosis and related fractures are increasing causes of mortality and painful physical impairment in the elderly, especially in the Western world (1, 2). Bone mineral accrual during childhood and adolescence is thought to play a vital role in preventing osteoporosis (3-5). Identifying and optimizing factors influencing peak bone mass is thus important for the prevention of osteoporosis and related fractures.

Genetic factors, estimated to be responsible for ≈70% of the variance in bone mass (6, 7), cannot be influenced; other factors affecting bone, such as nutritional intake, physical activity, and body mass index (BMI), can be influenced, thereby decreasing the risk of osteoporosis and its consequences (8).

Knowledge of the influence of nutritional intake on bone health is limited. Estimated intakes of polyunsaturated fatty acids

(PUFAs) and saturated fatty acids have been suggested to influence bone growth and modeling in humans, although data are sparse (9, 10). A pilot study suggested that dietary supplementation with evening primrose oil rich in γ -linolenic acid (GLA) and fish oil rich in eicosapentaenoic acid (EPA) may decrease bone turnover and increase bone mineral density (BMD) in elderly patients (11).

Animal studies have shown that a dietary intake of long-chain n-3 PUFAs may influence both bone formation and bone resorption (12, 13). Fatty acids have also been reported to have an influence on bone metabolism, including an increase in periosteal bone formation in animal studies (14, 15).

No study to date has investigated the association between concentrations of different fatty acids measured in serum and BMD in men. Therefore, the aim of this 8-y prospective and retrospective study was to investigate a possible role of fatty acids in bone accumulation and the attainment of peak bone mass in young postpubertal men.

SUBJECTS AND METHODS

Subjects

Beginning in 1994, we recruited 95 healthy adolescent white males from high schools and athletic clubs in Umeå in northern Sweden for this longitudinal study—the Northern Osteoporosis and Obesity Study (NO₂ Study). The initial aim was to investigate factors important for the development of BMD (16) and body-composition variables such as fat mass and lean body mass (17, 18). After a mean period of 5 y and 11 mo, 84 of the original participants agreed to participate in a first follow-up examination. At this follow-up, blood samples were obtained. One of the participants received a diagnosis of anorexia nervosa and depression between baseline and the follow-up and was therefore excluded, and 5 subjects were excluded for declining to leave blood samples, which left 78 participants. Seventy-three of these men

¹ From Sports Medicine, Department of Surgical and Perioperative Science (MH, PN, and AN), the Department of Community Medicine and Rehabilitation, (AN), and the Department of Public Health and Clinical Medicine (PN), Umeå University, Umeå, Sweden.

² Supported by grants from the Swedish National Center for Research in Sports.

³ Address reprint requests to A Nordström, Sports Medicine, Department of Surgical and Perioperative Science, Umeå University, SE901 85 Umeå, Sweden. E-mail: anna.nordstrom@drott.umu.se.

Received June 19, 2006.

Accepted for publication October 9, 2006.

participated in the second follow-up conducted after a mean of 7 y and 11 mo.

Questionnaire on lifestyle

Physical activity was measured by questionnaire and defined as the self-reported mean activity associated with sweating or breathlessness each week during the last year. The questionnaire has been used since the data collection began in 1995 (18) and at all follow-ups. In subjects with organized physical training in local sports clubs, the self-reported activity of each subject was also validated by the team coach at the start of this study. The group's physical activity consisted mainly of playing ice hockey, soccer and floor ball, distance running, and some weight training. The questionnaire used at baseline and at the follow-ups also included questions on smoking habits, known illnesses, and medication use. Pubertal stage according to Tanner was determined via self-examination of pubic hair and questions on growth of beard and height development at baseline. None of the participants had any disease or were taking any medication known to affect bone metabolism.

All data were collected at the Sports Medicine Unit at the University Hospital of Northern Sweden. All of the participants gave informed consent, and the Ethics Committee of the Medical Faculty, Umeå University, Umeå, Sweden, approved the study protocol.

Anthropometric measurements

Weight and height were measured in light clothing. Weight was measured to the nearest 0.1 kg by use of a digital scale, and height was measured to the nearest 0.5 cm against a wall-mounted stadiometer.

Bone mineral density measurements

The BMD (g/cm^2) of the total body and right hip was measured by using a Lunar DPX-L (Lunar Co, Waukesha, WI) dual-energy X-ray absorptiometer with version 4.6e software. The CV (CV, ie, SD/mean) was determined by scanning one person 7 times on the same day, with repositioning between each scan. Accordingly, the CVs were 0.7% with the total body scan and $\approx 1\%$ for the total hip scan. The BMD of the spine was derived from the total body scan. One investigator (AN) performed all the analyses. To maximize precision, the scaling option was used and set to 200. To evaluate the region-of-interest program, 2 different persons were scanned. The first person was scanned 7 times on the same day, with repositioning between each scan. The CV was then calculated to be 1.1% for spine BMD. The second person was scanned on different days for a total of 10 times. The CV then increased to 2.5%. The equipment was calibrated each day by using a standardized phantom to detect drifts in BMD measurements. All scans were made by using the same Lunar DPX-L.

Fatty acid profile in serum phospholipids

Serum was obtained under a nonfasting condition from 78 men during the first follow-up, ie, at 22 y of age. Total plasma lipids were extracted according to Folch et al (19), and the phospholipids were isolated on 400 mg aminopropyl solid-phase extraction columns according to Helland et al (20). Phospholipids were eluted from the columns with methanol, evaporated with hot nitrogen, and transmethylated with fresh sodium methoxide. Fatty acid methyl esters were extracted into hexane containing

20 mg butylated hydroxyl toluene (BHT)/L as an antioxidant and separated on a $100 \text{ m} \times 0.25 \text{ mm}$ (internal diameter) capillary gas chromatography column (SP-22566; Supelco, Bellefonte, PA), with hydrogen as the carrier gas and flame ionization detection. The results are expressed as grams fatty acids/g serum phospholipids.

Statistical analyses

All data are presented as means \pm SDs. Bivariate correlations were measured by using Pearson's correlation coefficients (r). The independent contributions of fatty acids to BMD at each site were investigated by using linear regression, including weight, height, and physical activity as independent variables. Differences between 3 groups were tested by using analysis of variance (ANOVA) with Bonferroni's test for post hoc comparisons. The SPSS software for personal computers (version 11.5; SPSS Inc, Chicago, IL) was used for the statistical analyses. P values < 0.05 was considered statistically significant.

TABLE 1

Age, anthropometric data, physical activity, and bone mineral density (BMD) at baseline and at the first and second follow-ups and fatty acid profiles in the phospholipid fraction at the first follow-up in the healthy young men¹

	Baseline ($n = 78$)	First follow-up ($n = 78$)	Second follow-up ($n = 73$)
Age (y)	16.7 ± 0.5^2	22.6 ± 0.7^3	24.6 ± 0.6
Weight (kg)	71.8 ± 9.1^2	81.7 ± 11.1	83.0 ± 10.8
Height (cm)	179 ± 6^4	181 ± 6	181 ± 6
Physical activity (h/wk)	6.9 ± 4.2^2	4.8 ± 3.8	3.6 ± 2.9
BMD (g/cm^2)			
Total body	1.22 ± 0.08^2	1.31 ± 0.07	1.31 ± 0.7
Total hip	1.27 ± 0.13	1.26 ± 0.15	1.23 ± 0.15
Spine	1.16 ± 0.11^2	1.27 ± 0.12	1.30 ± 0.14
Fatty acid profile (g/100 g serum phospholipids)			
Palmitic acid (16:0)	—	29.1 ± 1.2	—
Palmitoleic acid (16:1)	—	0.8 ± 0.4	—
Stearic acid (18:0)	—	15.6 ± 1.4	—
Oleic acid (18:1)	—	11.9 ± 1.9	—
Linoleic acid (18:2n-6)	—	22.9 ± 3.0	—
Eicosatrienoic acid (20:3n-6)	—	3.7 ± 0.8	—
Arachidonic acid (20:4n-6)	—	8.7 ± 1.4	—
Eicosapentaenoic acid (20: 5n-3)	—	1.8 ± 1.0	—
Docosapentaenoic acid (22: 5n-3)	—	1.3 ± 0.3	—
Docosahexaenoic acid (22: 6n-3)	—	3.6 ± 1.0	—
PUFA	—	42.5 ± 2.1	—
MUFA	—	12.7 ± 1.9	—
SFA	—	44.7 ± 1.5	—
n-6	—	35.6 ± 2.3	—
n-3	—	7.0 ± 1.9	—
n-6:n-3	—	5.4 ± 1.3	—

¹ All values are $\bar{x} \pm \text{SD}$. PUFA, polyunsaturated fatty acid; MUFA, monounsaturated fatty acid; SFA, saturated fatty acid.

² Significantly different from first and second follow-ups, $P < 0.01$.

³ Significantly different from second follow-up, $P < 0.01$.

⁴ Significantly different from first follow-up, $P < 0.05$.

TABLE 2

Pearson's correlation coefficients between fatty acids measured in the phospholipid fraction and bone mineral density (BMD, in g/cm^2) at 22 y of age and for the changes in BMD between 16 and 22 y of age ($\Delta 16-22$) and between 22 and 24 y of age ($\Delta 22-24$)¹

Fatty acid	Total			Hip			Spine		
	BMD (n = 78)	$\Delta 16-22$ (n = 78)	$\Delta 22-24$ (n = 73)	BMD (n = 78)	$\Delta 16-22$ (n = 78)	$\Delta 22-24$ (n = 73)	BMD (n = 78)	$\Delta 16-22$ (n = 78)	$\Delta 22-24$ (n = 73)
Palmitic acid	0.16	-0.04	-0.04	0.11	0.01	0.10	0.06	0.01	-0.01
Palmitoleic acid	0.00	0.04	0.10	0.04	0.03	0.09	-0.06	0.02	0.21
Stearic acid	0.07	0.07	-0.01	0.04	0.02	0.09	0.08	0.04	0.01
Oleic acid	-0.26 ²	-0.19	0.04	-0.15	-0.10	-0.18	-0.20	-0.22	0.08
Linoleic acid	-0.20	0.00	0.02	-0.12	-0.06	-0.13	-0.18	-0.15	-0.04
Eicosatrienoic acid	0.06	-0.09	0.08	0.07	-0.04	0.09	0.04	-0.08	0.08
Arachidonic acid	0.17	0.12	0.09	0.12	0.15	0.18	0.18	0.25 ²	-0.04
Eicosapentaenoic acid	0.18	0.04	-0.07	0.08	0.02	0.03	0.15	0.16	-0.19
Docosapentaenoic acid	0.12	0.02	-0.03	0.03	-0.07	0.09	0.12	0.05	-0.11
Docosahexaenoic acid	0.32 ³	0.10	0.08	0.15	0.07	0.13	0.30 ³	0.26 ²	0.10
PUFA	0.09	0.14	-0.04	0.04	0.07	0.04	0.10	0.16	-0.10
MUFA	-0.25 ²	-0.18	0.06	-0.14	-0.09	-0.16	-0.21	-0.21	-0.11
SFA	0.21	0.04	-0.04	0.13	0.03	0.16	0.13	0.05	0.00
n-6	-0.14	0.04	0.05	-0.06	0.00	-0.03	-0.12	-0.07	-0.04
n-3	0.27 ²	0.10	-0.12	0.12	0.07	0.09	0.25 ²	0.26 ²	-0.08
n-6:n-3	-0.12	-0.12	0.09	-0.07	-0.13	-0.09	-0.14	-0.26 ²	0.03

¹ PUFA, polyunsaturated fatty acid; MUFA, monounsaturated fatty acid; SFA, saturated fatty acid.

² $P < 0.05$.

³ $P < 0.01$.

RESULTS

The participants' fatty acid profile, age, weight, height, physical activity, and BMD of the total body, spine, and hip at baseline and follow-up visits are presented in **Table 1**. The concentration of α -linolenic acid could only be detected in $\approx 50\%$ of the subjects; therefore, these data are not presented.

Bivariate correlations between the different fatty acids and BMD measured at 22 y of age and the changes in BMD (Δ BMD) between 16 and 22 y of age and 22 and 24 y of age are presented in **Table 2**. In summary, BMD of the total body measured at 22 y of age showed a significant negative correlation with serum concentrations of oleic acid ($r = -0.26$, $P = 0.02$) and monounsaturated fatty acids ($r = -0.25$, $P = 0.02$) and a significant positive correlation with docosahexaenoic acid (DHA) ($r = 0.32$, $P = 0.004$, **Figure 1**) and n-3 fatty acids ($r = 0.27$, $P = 0.02$). BMD of the spine measured at 22 y of age showed a positive association with DHA ($r = 0.30$, $P = 0.008$) and n-3 fatty acids ($r = 0.25$, $P = 0.02$). Δ BMD of the spine between 16 and 22 y of age showed a positive association with arachidonic acid ($r = 0.25$, $P = 0.02$), DHA ($r = 0.26$, $P = 0.02$), and n-3 fatty acids ($r = 0.26$, $P = 0.02$) and a negative association with the ratio of n-6 to n-3 fatty acids ($r = -0.26$, $P = 0.02$).

The independent relations between the different fatty acids and BMD were also evaluated by using linear regression and including weight, height, and physical activity as explanatory variables. BMD of the total body then showed an independent relation with eicosapentaenoic acid (EPA) ($\beta = 0.31$, $P = 0.02$), DHA ($\beta = 0.31$, $P = 0.02$), and n-3 fatty acids ($\beta = 0.35$, $P = 0.007$) at 22 y of age (data not shown). BMD of the spine measured at 22 y of age showed an independent significant relation with DHA ($\beta = 0.28$, $P = 0.03$) and n-3 fatty acids ($\beta = 0.31$, $P = 0.01$) (data not shown).

The independent association between concentrations of fatty acids and the changes in BMD between 16 and 22 y of age and

between 22 and 24 y were also investigated. The concentrations of different fatty acids were used as explanatory variables together with changes in weight, height, and physical activity during the same period. Δ BMD of the spine between 16 and 22 y of age was then found to be independently related to DHA ($\beta = 0.28$, $P = 0.03$) and n-3 fatty acids ($\beta = 0.28$, $P = 0.04$) measured at 22 y of age. Finally, Δ BMD of the spine between 22 and 24 y of age was independently related to concentrations of palmitoleic acid measured at 22 y of age ($\beta = 0.27$, $P = 0.03$).

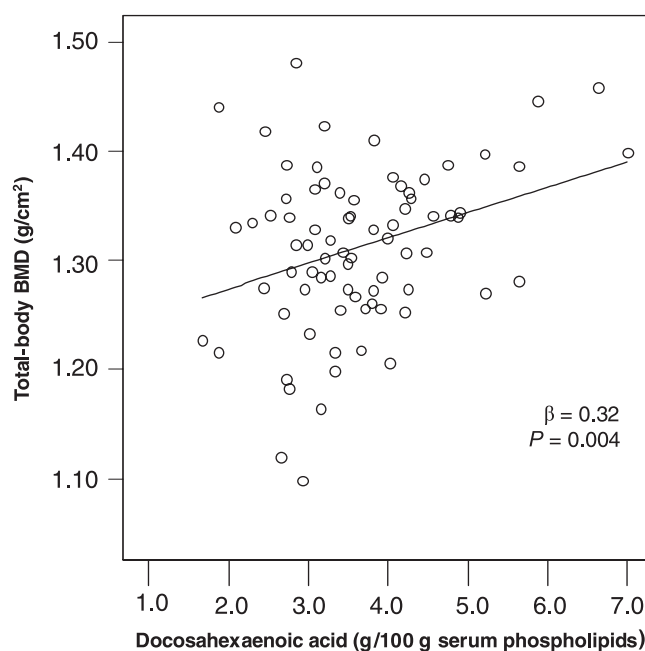


FIGURE 1. Relation between docosahexaenoic acid and bone mineral density (BMD) of the total body in 78 healthy young men.

DISCUSSION

The novelty of our study was in the measurement of fatty acids in the serum phospholipid fraction in healthy men and their association with BMD and bone accrual in our cohort. Our key finding was a positive association between n-3 fatty acids and BMD of the total body and spine and the accumulation of BMD at the spine between 16 and 22 y of age in this cohort of healthy young men.


To our knowledge, studies performed in humans that have investigated the possible role of n-3 fatty acids in skeletal health are sparse, and the few that do exist are supplement studies that did not investigate the concentrations of PUFAs in serum. The data from supplement studies in the elderly suggest that n-3 fatty acid intakes may promote BMD maintenance in this population. In one study, elderly postmenopausal women were given a mixture of fish oil and evening primrose oil with a high content of EPA and γ -linolenic acid (GLA), whereas the control group received coconut oil (11). After 18 mo, both groups had lower concentrations of bone turnover markers such as osteocalcin and deoxyypyridinoline. In the group treated with GLA and EPA, BMD at the lumbar spine was maintained and BMD of the femoral neck even increased. In the control group, BMD decreased at both sites (11). However, it cannot be concluded from this study whether n-3 or n-6 fatty acids or both are beneficial for BMD. In another study, 20 calcium nephrolithiasis patients were given a fish-oil supplement. The results included lower plasma prostaglandin E₂ concentrations, serum calcitriol concentrations, and markers of bone resorption, whereas calcium resorption increased and calcium excretion decreased (21). Thus, it seems that n-3 fatty acids might maintain bone density in the elderly, although the mechanism is not known, and the evidence thus far is sparse at best. This was also confirmed in a large cohort study in which food intakes of PUFAs were investigated in 1532 men and women aged 45–90 y. The ratio of n-6 to n-3 fatty acids showed a negative association with BMD of the hip in both men and women (22). The effects of n-3 fatty acids on bone health may be multifaceted. Postulated mechanisms include effects on calcium absorption in the intestine, reductions in bone resorption because of lower urinary excretion of calcium, and enhanced synthesis of bone collagen (23–25). n-3 Fatty acids have also been put forward as likely candidate inhibitors of the production of cytokines such as interleukin 6, interleukin 1, and tumor necrosis factor, which are implicated in the pathogenesis of osteoporosis (26–31).

No studies of the possible role of n-3 fatty acids in bone mineral accrual in growing humans have been conducted. However, animal studies suggest that n-3 fatty acids may have a positive role in this process. Iwami-Morimoto et al investigated the influence of fish-oil supplementation (rich in n-3 fatty acids) compared with that of corn oil (rich in n-6 fatty acids) on experimental tooth development in rats. n-3 Fatty acid supplementation decreased the number of osteoclasts by 60% and alveolar bone resorption by 80% (13). Reinwald et al (32) found that rats with n-3-deficient bone tissue had a high ratio of n-6 to n-3 fatty acids and diminished bone strength. Repletion with dietary n-3 fatty acids restored the ratio of n-6 to n-3 fatty acids in bone compartments and reversed compromised bone modeling. In contrast, Claassen et al (25) found that growing rats supplemented with increasing ratios of GLA to EPA (ie, n-6 to n-3 fatty acids) had lower concentrations of bone resorption

markers and higher bone calcium contents than did control subjects supplemented with linoleic acid and α -linolenic acid.

The association between n-3 fatty acids and total and spine BMD in our study seemed to mostly be due to the positive correlation of a specific n-3 fatty acid, namely DHA, with both BMD at 22 y of age and changes in BMD of the spine between 16 and 22 y. No studies have investigated the association between serum concentrations of individual PUFAs and bone density in humans. However, experimental animal studies of supplementation support our findings that DHA is positively related to bone density and bone accrual. Kruger and Schollum (33) found that DHA concentrations in red blood cell membranes were associated with BMD, and with calcium absorption in bone, in a cohort of growing rats fed a semisynthetic diet supplemented with tuna oil. Others have found that DHA increases intestinal calcium ATPase activity and may affect calcium absorption through this mechanism (34). Weiler and Fitzpatrick-Wong (35) found that higher concentrations of plasma DHA were associated with lessened bone resorption in piglets fed diets containing different ratios of n-6 to n-3 fatty acids. Thus, other experimental studies support our findings and clearly indicate a possible relation between dietary supplementation with DHA and various bone variables.

The present study had some limitations. The cohort studied was not randomly selected from the general population, but rather consisted of volunteers from high schools and sports clubs. Therefore, inferences with respect to the general population should be made with caution. No information on dietary patterns was available, which may have been useful to further strengthen the relation between fatty acids and BMD. We found relations between changes in BMD and both palmitoleic acid and arachidonic acid. We have no certain theoretical explanation for these findings. In this context it should be noted that, because many correlations were tested, it is more than likely that some of the significant correlations found may have been due to type I errors.

In conclusion, this was the first study to investigate the association between individual PUFAs, BMD, and bone mineral accrual. In a cohort of healthy young men, we found that concentrations of n-3 fatty acids, especially DHA, were positively associated with peak BMD in the total body and spine and with bone accrual in the spine. More studies are needed to confirm our results and investigate the relation between individual PUFAs and BMD further. 

AN and PN collected the material. AN and MH collected background information and drafted the first version of manuscript. All authors worked on the manuscript and approved the final version. None of the authors had any conflicts of interest.

REFERENCES

1. Johnell O, Kanis J. Epidemiology of osteoporotic fractures. *Osteoporos Int* 2005;16(suppl 2):S3–7.
2. Johnell O, Kanis JA, Jonsson B, Oden A, Johansson H, De Laet C. The burden of hospitalised fractures in Sweden. *Osteoporos Int* 2005;16:222–8.
3. MacKelvie KJ, Khan KM, McKay HA. Is there a critical period for bone response to weight-bearing exercise in children and adolescents? a systematic review. *Br J Sports Med* 2002;36:250–7 (discussion 257).
4. Nordstrom A, Karlsson C, Nyquist F, Olsson T, Nordstrom P, Karlsson M. Bone loss and fracture risk after reduced physical activity. *J Bone Miner Res* 2005;20:202–7.
5. Nordstrom A, Olsson T, Nordstrom P. Sustained benefits from previous physical activity on bone mineral density in males. *J Clin Endocrinol Metab* 2006;91:2600–4.



6. Eisman JA. Genetics of osteoporosis. *Endocr Rev* 1999;20:788–804.
7. Seeman E, Hopper JL, Bach LA, et al. Reduced bone mass in daughters of women with osteoporosis. *N Engl J Med* 1989;320:554–8.
8. Cummings SR, Nevitt MC, Browner WS, et al. Risk factors for hip fracture in white women. Study of Osteoporotic Fractures Research Group [see comments]. *N Engl J Med* 1995;332:767–73.
9. Gunnes M, Lehmann EH. Physical activity and dietary constituents as predictors of forearm cortical and trabecular bone gain in healthy children and adolescents: a prospective study. *Acta Paediatr* 1996;85:19–25.
10. Gunnes M, Lehmann EH. Dietary calcium, saturated fat, fiber and vitamin C as predictors of forearm cortical and trabecular bone mineral density in healthy children and adolescents. *Acta Paediatr* 1995;84:388–92.
11. Kruger MC, Coetzer H, de Winter R, Gericke G, van Papendorp DH. Calcium, gamma-linolenic acid and eicosapentaenoic acid supplementation in senile osteoporosis. *Aging (Milano)* 1998;10:385–94.
12. Sakaguchi K, Morita I, Murota S. Eicosapentaenoic acid inhibits bone loss due to ovariectomy in rats. *Prostaglandins Leukot Essent Fatty Acids* 1994;50:81–4.
13. Iwami-Morimoto Y, Yamaguchi K, Tanne K. Influence of dietary n-3 polyunsaturated fatty acid on experimental tooth movement in rats. *Angle Orthod* 1999;69:365–71.
14. Li Y, Seifert MF, Ney DM, et al. Dietary conjugated linoleic acids alter serum IGF-I and IGF binding protein concentrations and reduce bone formation in rats fed (n-6) or (n-3) fatty acids. *J Bone Miner Res* 1999;14:1153–62.
15. Das UN. Interaction(s) between essential fatty acids, eicosanoids, cytokines, growth factors and free radicals: relevance to new therapeutic strategies in rheumatoid arthritis and other collagen vascular diseases. *Prostaglandins Leukot Essent Fatty Acids* 1991;44:201–10.
16. Nordstrom P, Nordstrom G, Thorsen K, Lorentzon R. Local bone mineral density, muscle strength, and exercise in adolescent boys: a comparative study of two groups with different muscle strength and exercise levels. *Calcif Tissue Int* 1996;58:402–8.
17. Nordstrom P, Thorsen K, Bergstrom E, Lorentzon R. High bone mass and altered relationships between bone mass, muscle strength, and body constitution in adolescent boys on a high level of physical activity. *Bone* 1996;19:189–95.
18. Nordstrom P, Thorsen K, Nordstrom G, Bergstrom E, Lorentzon R. Bone mass, muscle strength, and different body constitutional parameters in adolescent boys with a low or moderate exercise level. *Bone* 1995;17:351–6.
19. Folch J, Lees M, Sloane Stanley GH. A simple method for the isolation and purification of total lipids from animal tissues. *J Biol Chem* 1957;226:497–509.
20. Helland IB, Saarem K, Saugstad OD, Drevon CA. Fatty acid composition in maternal milk and plasma during supplementation with cod liver oil. *Eur J Clin Nutr* 1998;52:839–45.
21. Baggio B, Budakovic A, Nassuato MA, et al. Plasma phospholipid arachidonic acid content and calcium metabolism in idiopathic calcium nephrolithiasis. *Kidney Int* 2000;58:1278–84.
22. Weiss LA, Barrett-Connor E, von Muhlen D. Ratio of n-6 to n-3 fatty acids and bone mineral density in older adults: the Rancho Bernardo Study. *Am J Clin Nutr* 2005;81:934–8.
23. Kruger MC, Horrobin DF. Calcium metabolism, osteoporosis and essential fatty acids: a review. *Prog Lipid Res* 1997;36:131–51.
24. Claassen N, Coetzer H, Steinmann CM, Kruger MC. The effect of different n-6/n-3 essential fatty acid ratios on calcium balance and bone in rats. *Prostaglandins Leukot Essent Fatty Acids* 1995;53:13–9.
25. Claassen N, Potgieter HC, Seppa M, et al. Supplemented gamma-linolenic acid and eicosapentaenoic acid influence bone status in young male rats: effects on free urinary collagen crosslinks, total urinary hydroxyproline, and bone calcium content. *Bone* 1995;16(suppl):385S–92S.
26. Endres S, Ghorbani R, Kelley VE, et al. The effect of dietary supplementation with n-3 polyunsaturated fatty acids on the synthesis of interleukin-1 and tumor necrosis factor by mononuclear cells. *N Engl J Med* 1989;320:265–71.
27. Meydani SN, Endres S, Woods MM, et al. Oral (n-3) fatty acid supplementation suppresses cytokine production and lymphocyte proliferation: comparison between young and older women. *J Nutr* 1991;121:547–55.
28. Sun D, Krishnan A, Zaman K, Lawrence R, Bhattacharya A, Fernandes G. Dietary n-3 fatty acids decrease osteoclastogenesis and loss of bone mass in ovariectomized mice. *J Bone Miner Res* 2003;18:1206–16.
29. Manolagas SC, Jilka RL. Bone marrow, cytokines, and bone remodeling. Emerging insights into the pathophysiology of osteoporosis. *N Engl J Med* 1995;332:305–11.
30. Pacifici R. Estrogen, cytokines, and pathogenesis of postmenopausal osteoporosis. *J Bone Miner Res* 1996;11:1043–51.
31. Ralston SH. Analysis of gene expression in human bone biopsies by polymerase chain reaction: evidence for enhanced cytokine expression in postmenopausal osteoporosis. *J Bone Miner Res* 1994;9:883–90.
32. Reinwald S, Li Y, Moriguchi T, Salem N Jr, Watkins BA. Repletion with (n-3) fatty acids reverses bone structural deficits in (n-3)-deficient rats. *J Nutr* 2004;134:388–94.
33. Kruger MC, Schollum LM. Is docosahexaenoic acid more effective than eicosapentaenoic acid for increasing calcium bioavailability? *Prostaglandins Leukot Essent Fatty Acids* 2005;73:327–34.
34. Haag M, Magada ON, Claassen N, Bohmer LH, Kruger MC. Omega-3 fatty acids modulate ATPases involved in duodenal Ca absorption. *Prostaglandins Leukot Essent Fatty Acids* 2003;68:423–9.
35. Weiler HA, Fitzpatrick-Wong SC. Modulation of essential (n-6):(n-3) fatty acid ratios alters fatty acid status but not bone mass in piglets. *J Nutr* 2002;132:2667–72.