

## An Increase in Dietary n-3 Fatty Acids Improves Bone Health in Humans

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## **Abstract**

Animal and in vitro research have shown a beneficial effect of omega-3 (n-3) polyunsaturated fatty acids (PUFA) on bone metabolism. This is the first controlled feeding study in humans to evaluate the effect of dietary n-3 PUFA on bone turnover, assessed by serum concentrations of N-telopeptides (NTx) and bone-specific alkaline phosphatase (BSAP). Subjects (n=23) consumed each diet for 6 weeks in a randomized, 3-period crossover design: 1) Average American Diet (AAD; [34% total fat, 13% saturated fatty acids (SFA), 13% monounsaturated fatty acids (MUFA), 9% PUFA (7.7% LA, 0.8% ALA)]), 2) Linoleic Acid Diet (LA; [37% total fat, 9% SFA, 12% MUFA, 16% PUFA (12.6% LA, 3.6% ALA)]), and 3)  $\alpha$ -Linolenic Acid Diet (ALA; [38% total fat, 8% SFA, 12% MUFA, 17% PUFA (10.5% LA, 6.5% ALA)]). Walnuts and flaxseed oil were the predominant sources of ALA. NTx levels were significantly lower following the ALA diet ( $13.20 \pm 1.21$  nM), relative to the AAD ( $15.59 \pm 1.21$  nM) ( $p < 0.05$ ). Mean NTx level following the LA diet was  $13.80 \pm 1.21$  nM. There was no change in levels of BSAP across the three diets. Concentrations of NTx were positively correlated with the pro-inflammatory cytokine TNF $\alpha$  across all three diets. The results indicate that plant sources of dietary n-3 PUFA may have a protective effect on bone metabolism via a decrease in bone resorption in the presence of consistent levels of bone formation.

## Introduction

Accumulating evidence indicates that dietary fats can influence bone health. The omega-3 (n-3) polyunsaturated fatty acids (PUFA), in particular, may be beneficial, as they have been shown to inhibit the activity of osteoclasts and enhance the activity of osteoblasts in animal studies [1, 2]. Optimal quantities of n-3 PUFA, thus, appear to inhibit bone resorption and promote bone formation. In addition, the ratio of n-6 to n-3 fatty acids in the diet may be important. Lowering the dietary ratio of n-6/n-3 PUFA increased bone marrow cellularity [3], bone strength [4] and bone growth [5] in animals. In contrast, other studies have indicated that dietary saturated fatty acids (SFA) may adversely affect bone health. Animal research [6] and epidemiologic data [7] indicate that diets rich in SFA reduce bone mineral density. Possible mechanisms that may account for the effects of dietary fatty acids on bone include alterations in prostaglandin production, lipid oxidation, calcium absorption, inflammatory processes and osteoblast differentiation [2, 8-12].

Few studies have evaluated the relationship between dietary fats and bone status in humans. Epidemiologic studies have shown inverse relationships between the dietary n-6/n-3 ratio and bone mineral density in older adults [13], as well as between dietary saturated fat and bone mineral density in men [7]. Two studies [14, 15] provided oil supplements containing  $\gamma$ -linolenic acid (n-6), eicosapentaenoic acid (EPA) and docosahexanoic acid (DHA) (n-3) to women. The results were mixed in that n-3 supplementation increased bone density, compared to a placebo containing saturated fatty acids (SFA) in one study [14]; however, in the other study, there was no n-6/n-3 benefit compared to no supplemental fat [15]. Taken together, the epidemiologic and

supplemental feeding data provide suggestive evidence that dietary fatty acids can affect bone health in humans. However, controlled feeding trials are needed in order to convincingly demonstrate a causal relationship between fats and bone health,

The present investigation is the first controlled feeding study in humans to evaluate the effect of increasing dietary  $\alpha$ -linolenic acid (ALA) and, thereby, decreasing the ratio of n-6/n-3 PUFA on bone health. Plant sources of ALA (walnuts and flax) were used. We recently showed that an ALA-rich diet inhibits vascular inflammation and endothelial activation in addition to having substantial lipid lowering effects [16]. A growing body of evidence indicates that many of the pathophysiological events associated with CVD, also are associated with low bone density. Given the literature indicating effects of dietary fatty acids on bone health, as well as reported associations between cardiovascular disease and low bone density/osteoporosis [17], we sought to determine if plant-based n-3 PUFA would also be beneficial to bone health.

## **Subjects and Methods**

### *Subjects*

The present study was part of a larger study [16] that was designed to evaluate the effects of dietary fatty acids on cardiovascular disease risk factors. Twenty-three individuals (20 males and 3 females) participated. Subjects were recruited via advertisements in the local newspaper and fliers distributed across the campus of the Pennsylvania State University. Subjects who met the initial criteria during a phone screen reported to the Metabolic Diet Study Center (MDSC) for anthropometric measurements and baseline blood sampling. The Institutional Review Board at the

Pennsylvania State University approved the experimental protocol and all subjects provided written informed consent.

Baseline subject characteristics are shown in Table 1. Subjects were classified as overweight or obese, i.e., BMI between 25 and 35 kg/m<sup>2</sup>. In addition, subjects were moderately hypercholesterolemic [baseline serum total cholesterol (TC) between 5.17 and 6.21 mmol/L, LDL-cholesterol (LDL-C) between 40<sup>th</sup> and 90<sup>th</sup> percentiles (by NHANES III), with HDL-cholesterol (HDL-C) between 25<sup>th</sup> and 75<sup>th</sup> percentiles (by NHANES III)], and had serum triglyceride (TG) levels less than 3.95 mmol/L. Thus, subjects in the present study were quite representative of the population in the U.S. that is at high risk for cardiovascular disease. The three females were postmenopausal, and had not received hormone replacement therapy (HRT) for at least 6 months prior to the start of the study.

### *Study Design*

A randomized, double-blind, balanced order, three-period crossover design was employed. A Latin-Square protocol was used to randomize the subjects into a sequence of three experimental diets, which differed in their fatty acid composition. Diet periods lasted 6 weeks and were separated by an approximate 3-week compliance break during which subjects consumed their usual diet.

Subjects consumed either breakfast or dinner at the diet center on Monday through Friday; all other meals were prepared and packed for offsite consumption at the subject's home. Diet compliance was monitored by the staff of the diet center and by the review of daily and weekly monitoring forms. Subjects' body weights, usual activities and exercise levels were maintained throughout the course of the study.

### *Experimental Diets*

Three test diets were used: an average American diet (AAD) that served as the control diet, and two high-PUFA diets (low in saturated fat and cholesterol) that had different amounts of linoleic acid [LA, C18:2 n-6, (LA Diet)] and  $\alpha$ -linolenic acid [ALA, C18:3 n-3 (ALA Diet)]. For each of the three experimental diets, eight calorie levels (1800 - 3900 kcal) were developed to meet different energy needs of the subjects. Unit foods (muffins) containing the same macronutrient profile of each of the test diets were used during each diet period to provide incremental adjustments of 100 kcal/day as needed to maintain body weight. The nutrient composition of the three test diets, verified by chemical analyses, is reported in Table 2. All diets were nutritionally adequate and met 2/3 of the established Dietary Reference Intakes [18]. Total fat (~35% energy), carbohydrate (~50% energy) and protein (~15% energy) were kept as constant as possible across the three experimental diets. There were no differences across the 3 diets for calcium and vitamin D.

The ratio of n-6/n-3 fatty acids for the LA and ALA diets were 3.5/1 and 1.6/1, respectively. The n-6/n-3 fatty acid ratio for the AAD was 9/1. Walnuts and walnut oil, which are particularly rich sources of both n-6 and n-3 PUFA, represented half of the total fat in the two high-PUFA diets. The daily consumption of walnuts and walnut oil was 37 g and 15 g, respectively, for the diet providing 2400 kcal/day. Sources of walnuts in the diet included walnut granola, honey walnut butter, walnut pesto, and plain walnuts as a snack. Flaxseed oil, ~20 g/day for the 2400 kcal/day diet, also was used to increase the ALA content of the ALA Diet. Dietary calcium and Vitamin D levels were calculated

using the Nutritionist V database (N-Squared Computing, First DataBank Division, San Bruno, CA).

### *Serum Samples*

Twelve hour fasting blood samples were taken by venipuncture on two consecutive days. Whole blood was centrifuged at 3000 rpm for 15 minutes at -4°C. Serum samples were aliquoted and stored at -80°C until the conclusion of the study when all samples were analyzed together. The serum fatty acid profile was determined using gas chromatography as described by Zhao et al [16]. Percentage of individual fatty acids was calculated according to the peak areas relative to the total area (total fatty acid was set at 100%). Serum N-telopeptides of type I collagen (NTx) were measured using an enzyme-linked immunosorbent assay (ELISA) kit (Ostex International, Seattle); the inter-assay coefficient of variation was 5.5%. Serum NTx is regarded as a reliable indicator of bone resorption since it originates from proteolytic cleavage of bone collagen by osteoclasts, rather than by downstream degradative processes [19]. Serum bone-specific alkaline phosphatase (BSAP) was determined using a chemiimmunoluminescent enzymatic assay (Quest Diagnostics, Chantilly, VA). BSAP provides a general index of bone formation, and a specific index of total osteoblast activity [20]. Serum TNF- $\alpha$ , IL-6, IL-4 and IL-1 $\beta$  levels were measured using ELISAs in the Cytokine Core Laboratory of the Pennsylvania State University General Clinical Research Center using protocols previously described [21].

### *Statistical Analyses*

All statistical analyses were performed using SAS for WINDOWS, release 8.2 (SAS Institute, Cary, NC). Data are expressed as least squares mean  $\pm$  standard error.

Normality testing was conducted for each outcome variable. The Kolmogorov-Smirnov test was used to identify 2 individuals whose NTx levels fell outside of the normal distribution and were classified as outliers. The mixed models procedure (PROC MIXED) was used to test for effects of diet, order of diet presentation, period, and their interactions. Tukey-Kramer adjusted *P* values < 0.05 were used to determine whether the differences in NTx among diets were significant. The magnitude and direction of diet effects were also examined by analyzing change scores (experimental diets – AAD), with AAD values as covariates. Pearson correlation coefficients were used to evaluate the relationship between the levels of NTx and the log-transformed levels of TNF $\alpha$ , IL-6, IL-4, and IL-1 $\beta$  across all diets. In order to account for the repeated measurement of outcome variables across three diet periods, an average correlation for each diet was calculated and the significance of this pooled correlation was then tested using a repeated measures ANOVA with the subject variable as the repeated measure. All analyses were performed on log-transformed values; all means reported represent unadjusted means.

## **Results**

All subjects completed the study (Table 1) and serum fatty acid analysis (Table 3) revealed excellent dietary compliance [16]. Statistical analyses were first completed with all subjects (n=23) and then on all male subjects (n=20). Both sets of analyses yielded similar results, thus men and women were pooled for all analyses presented. Subjects maintained their baseline body weight throughout the study. NTx values for one male and one female subject were classified as significant outliers and excluded from the analyses, based on the Kolmogorov-Smirnov test. The final sample size was 21.

Mean NTx concentrations at the conclusion of the three experimental diets were  $15.59 \pm 1.21$ ,  $13.80 \pm 1.21$ , and  $13.20 \pm 1.21$  nM, for the AAD, LA and ALA diets, respectively (main effect of diet:  $p < 0.05$ ). NTx levels were significantly lower following the ALA diet ( $p < 0.05$ ) and there was a similar trend for the LA diet ( $p = 0.08$ ), compared to the AAD diet (Figure 1). When change scores were analyzed with the control diet (AAD) NTx levels as a covariate, both the ALA and LA diets were associated with significant reductions in NTX concentrations ( $p < 0.05$ ) relative to the AAD. The reduction following the ALA diet was  $2.17 \pm 0.68$  nM (15.3%), while the reduction following the LA diet was  $1.77 \pm 0.68$  nM (11.5%).

Levels of BSAP were unaffected by diet treatment. Mean concentrations of BSAP at the end of each diet period were  $11.78 \pm 0.80$ ,  $12.41 \pm 0.80$ , and  $11.94 \pm 0.81$  mcg/L, for the AAD, LA and ALA diets, respectively ( $p > 0.05$ ). Similarly, the analysis of change scores adjusted for control diet BSAP showed no significant effects of the intervention diets.

The effects of these diets on serum cytokine levels are reported elsewhere [22]. In summary, levels of IL-6, IL-1 $\beta$  and IL-4 did not change after consumption of the three test diets. Levels of serum TNF $\alpha$  however were reduced following the ALA Diet (Median: 10.3 ng/L; Range: (1.2-12,072.7 ng/L), compared to the LA Diet (Median: 13.3 ng/L; Range: (1.2-1,513.0 ng/L) and the AAD (Median: 18.2 ng/L; Range: (1.2-1,232.5 ng/L) (main effect of diet:  $p < 0.08$ ). In subsequent correlational analysis, data from the 16 individuals with detectable levels of TNF $\alpha$  were used. The levels of NTx were positively correlated with the levels of TNF- $\alpha$  ( $r = 0.54$ ;  $p < 0.05$ ), across all three diets. Thus, as

TNF- $\alpha$  decreased with increasing intake of n-3 fatty acids, there were proportional decreases in NTx.

#### *Effects of diet on serum fatty acids*

The serum fatty acid profile for subjects following the consumption of each of the three test diets is presented in Table 3. The changes observed in serum fatty acids validate our assessment of dietary compliance with daily and weekly monitoring forms. As expected, serum total n-6 PUFA were the highest on the LA diet ( $p < 0.001$ ). Serum total n-3 PUFA, ALA, and EPA increased progressively across the LA and ALA diets, with the highest levels on the ALA diet. The ratios of serum SFA:UNSAT, LA:ALA and n-6/n-3 decreased significantly following the consumption of the LA and ALA diets, compared to the AAD ( $p < 0.001$ ), and following consumption of the ALA diet compared to the LA diet ( $p < 0.001$ ). There were no significant correlations between serum concentrations of fatty acids and BSAP or NTx.

## **Discussion**

This is the first controlled feeding study in humans to assess the effects of an ALA-rich diet on bone health, as measured by serum NTx and BSAP, indicators of bone resorption and formation, respectively. We found that a diet rich in ALA provided by walnuts and flaxseed oil significantly reduced serum concentrations of NTx, and maintained levels of BSAP relative to the consumption of a typical American diet, which is lower in n-3 PUFA and higher in SFA. These effects are consistent with a reduction in bone turnover and maintenance of bone formation induced by relatively short-term consumption of the n-3 diet. Although the effects of dietary fats on bone health have

been studied in animals and through supplementation with evening primrose oil in humans, this is the first study to use a whole food source, incorporated into the diet of humans under controlled feeding conditions. Previous studies have shown beneficial effects of walnuts [16, 23] and flaxseed [24] on CVD risk; the present results indicate potential benefits on bone health, as well.

Both ALA (n-3) and LA (n-6) are essential fatty acids because they cannot be synthesized *in vivo* in humans. The dietary reference intakes (DRIs) have established an adequate intake (AI) of 1.6 and 1.1 grams of ALA per day for men and women, respectively [25]. The acceptable macronutrient distribution range (AMDR) for ALA is 0.6 to 1.2% of energy [26]. Ten percent of this recommended AMDR may be achieved by consumption of EPA and DHA. The AI for LA is 17 and 12 g/d for adult men and women, respectively, with an AMDR of 5 to 10% of energy. Each of the three test diets in the present study met or exceeded these recommendations for both ALA and LA (Table 2).

Some scientists believe that the ratio of dietary n-6/n-3 PUFA is important to a variety of health outcomes [27]. The changes in n-6/n-3 ratios in the present study were accomplished by increasing the n-3 PUFA, while maintaining relatively constant levels of n-6 PUFA (Table 2). While these results extend a previous report in animals, showing that reductions in the dietary n-6/n-3 fatty acid ratio can reduce bone resorption [9], the n-6/n-3 ratio reductions in the previous study were achieved by both an increase in n-3 fatty acids and a decrease in n-6 fatty acids. As a result, it is difficult to determine if the reduction in bone resorption was due to the increased consumption of the n-3 fatty acids, or simply due to the decreased consumption of the n-6 fatty acids. In the present study,

NTx levels were significantly lower when subjects consumed the ALA diet than when they consumed the AAD; when subjects consumed the LA diet, NTx levels were marginally significantly lower than when they consumed the AAD ( $p=0.08$ ). This stepwise reduction in NTx across the three diets (ALA < LA < AAD) indicates a possible dose-effect of dietary n-3 fatty acids on bone resorption. The stepwise nature of the results also indicates that the reductions in NTx were not due simply to the lower dietary SFA in the LA and ALA diets. Thus, increasing dietary n-3 PUFA, while maintaining a relatively constant level of dietary n-6 PUFA reduces serum NTx and may reduce bone resorption in humans.

The reductions in serum NTx that occurred when subjects consumed the ALA diet for 6 weeks (15.3%) were somewhat less than have been reported after two weeks and 1 year of hormone replacement therapy (23% and 52%, respectively) or after 6 months of alendronate therapy (30.4%) [28, 29]. Nonetheless, the results presented here may represent a clinically important change. Whether longer-term intake of n-3 PUFA would produce larger decreases in NTx and/or increases in bone density is not known. However, the present results suggest that increasing n-3 PUFA (i.e., ALA) in the diet may be a useful therapeutic adjunct to pharmacological interventions and/or a useful independent intervention for those who elect not to use pharmaceuticals.

The present findings confirm and extend a growing literature indicating beneficial effects of n-3 PUFA on bone health. Work with animals has shown that appropriate amounts of n-3 PUFA can reduce osteoclast activity [1]. One mechanism that might account for this involves local alterations of FA and prostaglandin concentrations within bone tissue [9]. Increased consumption of n-6 PUFA increases the ratio of arachidonic

acid (AA) to EPA, and increases PGE<sub>2</sub> concentration in bone [20]. Conversely, an increased consumption of n-3 PUFA decreases the AA:EPA ratio, decreases PGE<sub>2</sub> concentration and release from bone, and increases the production of PGE<sub>1</sub> [10]. This cascade of effects is potentially important, as PGE<sub>1</sub> inhibits osteoclast activity in vitro [30, 31]. Although measurements of bone prostaglandins were not taken in the present study, the serum fatty acid results showed that serum EPA was significantly greater on the ALA diet than on either the LA diet or the AAD [16], while AA was significantly reduced. It is possible that this shift in fatty acid synthesis increased production of PGE<sub>1</sub> and decreased production of PGE<sub>2</sub> within the bone. This would be expected to reduce osteoclast activity and serum NTx concentrations during the ALA diet.

We also examined the relationship between NTx and cytokines involved in bone remodeling, i.e. tumor necrosis factor-alpha (TNF $\alpha$ ), IL-6, IL-4, and IL-1B [32, 33]. Diet had no significant effect on IL-6, IL-4, and IL-1B. In contrast, TNF-alpha was lower when the ALA diet was consumed relative to the AAD and LA diets, as reported elsewhere [22]. In addition, TNF- $\alpha$  levels were correlated with NTx levels across all of the diets. These findings are consistent with a role for the anti-inflammatory effects of n-3 fatty acids in bone health. TNF- $\alpha$  is a pro-inflammatory cytokine that has been implicated in the dysregulation of bone and cartilage remodeling. The TNF family has pleiotropic functions that include cell proliferation, differentiation, activation, and apoptosis [34]. With regard to bone, TNF- $\alpha$ -induced osteoclast recruitment is hypothesized to be central to the pathogenesis of postmenopausal osteoporosis [35, 36]. TNF- $\alpha$  promotes osteoclastic bone resorption and inhibits bone collagen synthesis in vitro [37], effects that may be mediated by PGE<sub>2</sub> [38]. In addition, TNF- $\alpha$  expression

was enhanced by arachidonic acid in human osteoblast cells, an effect that was attenuated by the n-3 fatty acid EPA [39].

Although TNF- $\alpha$  promotes osteoclastogenesis via an autocrine mechanism [40], systemic TNF- $\alpha$  also has been shown to stimulate bone resorption, increase the number of circulating preosteoclasts [41, 42], and elevate plasma calcium [38]. Furthermore, supplementation with flaxseed oil, which is a rich in ALA, reduced systemic TNF- $\alpha$  by about 30% in humans [43], as well as in wild-type and IL-10 knockout mice [44], and increased bone mineral content in IL-10 knockout mice [44]. Systemic TNF- $\alpha$  therefore, may be an important marker of, and/or contributor to, bone resorptive mechanisms. Taken together, the present results are consistent with literature indicating that dietary ALA can reduce bone resorption possibly through reduced production of TNF- $\alpha$ .

In contrast to the effects of the diets on a marker of bone resorption, there were no effects on a marker of bone formation (BSAP) in the present study. Because others have shown that low ratios of dietary n-6/n-3 fatty acids increase BSAP in rats [9], our results may reflect the age of our subjects and/or that our dietary n-6/n-3 ratios were higher. In the Watkins, et al. study [9], growing rats were used and significant increases in BSAP were induced only when the dietary n-6/n-3 ratio was 1.19. No differences in BSAP were seen among diets with n-6/n-3 ratios ranging from 2.6-23.76 [9]. In the present study, the lowest n-6/n-3 ratio was 1.6, which may not have been low enough for changes in BSAP to be seen. Two recent studies in humans [45] and dogs [46] indicate that the absolute amounts of n-6 and n-3 fatty acids are stronger determinants of the serum bioavailability of these fatty acids, compared to the ratio of n-6/n-3 fatty acids. In addition, effects may fluctuate at different stages of life, i.e. during periods of growth as

opposed to adulthood. No change in plasma alkaline phosphatase activity was seen in adult rats maintained on high n-3 diets [47]. In addition, no change in BSAP occurred when supplements containing a mixture of n-6 and n-3 fatty acids were given to adult women [6, 10] over an extended period of time ( $\geq 12$  mo), even though bone density increased in one of these trials [10]. The effects of n-3 fatty acids on bone health are therefore likely a result of life stage (i.e. growing vs. adult) and the absolute amount of dietary and serum n-3 fatty acids, rather than a function of the ratio of n-6/n-3 fatty acids alone. Because a majority of the above research has been conducted in rats the translation of absolute amounts of n-6 and n-3 fatty acids dosages to achieve these effects in humans is not yet known. The profile of effects reported here (reduced NTx, no change in BSAP) is similar to that reported for a variety of factors including dietary protein and sodium [48, 49], nasal spray calcitonin (after 3 mo) [50], and calcium supplementation [51]. Such a profile suggests a reduction in bone turnover, and a shift in the balance of bone degradation/formation toward formation.

In the present study, most of the subjects were middle-aged men, a population that is generally overlooked in terms of osteoporosis and bone health, but is known to be at high risk for cardiovascular disease. While women represent a majority of the individuals who suffer from osteoporosis, the number of men who are also afflicted continues to grow [52]. In addition, men suffer greater one-year post hip fracture mortality than do women [53]. Although men and women typically consume diets with comparable percentages of fat, men usually consume more total grams of fat [54]. Furthermore, recent epidemiologic data suggest that the effects of dietary fats on bone health may be particularly strong in men (7). Taken together, these reports indicate that

bone health in men bears investigation, and that bone integrity in men, as well as in women, may benefit from manipulations of the dietary fatty acid profile.

Although our subjects were representative of those at risk for cardiovascular disease, the majority of the individuals in this study (n=18) were not at risk for bone loss (NTx >18.1 nM) [28]. However, statistically significant reductions in NTx were still seen. This indicates that a diet high in n-3 PUFA can reduce bone resorption even in individuals who are not currently at an increased risk of osteoporosis. Such a reduction may help delay or prevent the development of bone disease in the future.

The present results do not clearly distinguish if the observed effects are due to the ALA or to the conversion of ALA to EPA and its subsequent effects. The majority of studies investigating the effects of n-3 fatty acids on bone health have used fish oil, which is rich in EPA. However, a recent epidemiologic study showed that reduced LA:ALA ratios were associated with increased hip bone mineral density [13]. In the present study, plant sources of n-3 fatty acids were used; therefore, ALA was the primary n-3 fatty acid provided by the diet. Both serum ALA and EPA were significantly greater in the LA and ALA diet conditions than in the AAD condition. However, serum ALA increased to a greater degree than did EPA, which reflects the well established fact that little ALA is converted to EPA (~ 5-10%), and little is converted to DHA (< 1%) [55, 56]. In the presence of a diet that is relatively high in n-6 PUFA (such as the LA and ALA diets in the present study), this conversion is reduced by 40 to 50% [57]. Therefore, it is possible that in the present study the results are reflective of an increase in ALA or due to potent effects of EPA. We hasten to add that further research is needed to clarify these possibilities.

In summary, the results of the present study indicate that incorporating walnuts and flaxseed into the diet as a means to increase ALA, and consequently decrease the n-6/n-3 ratio, reduces serum NTx and maintains levels of serum BSAP. The reductions in NTx were related to the amount of ALA each diet provided. These results suggest that incorporating plant sources of n-3 PUFA into the diet may provide health benefits not only to the cardiovascular, but also to the skeletal system.

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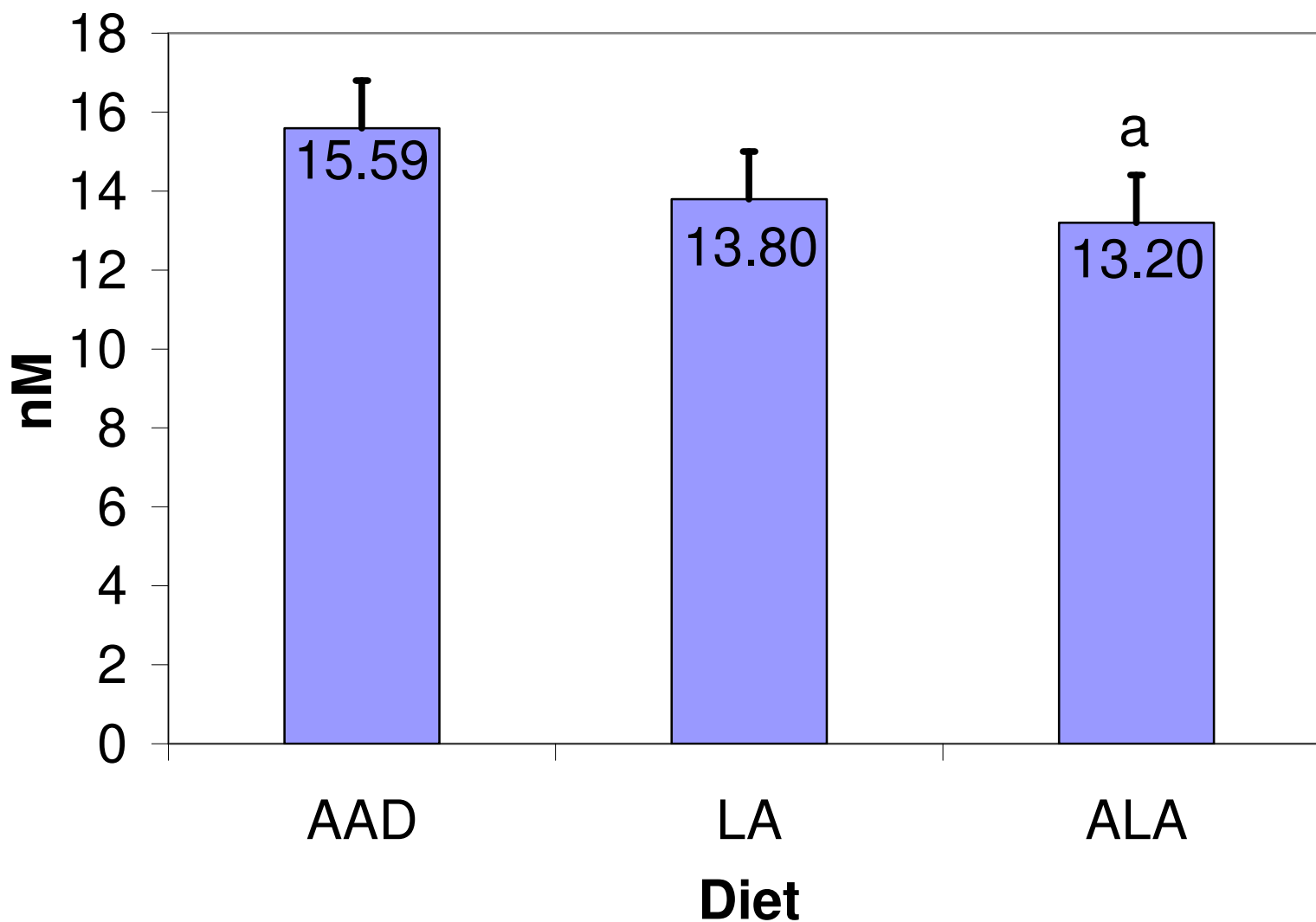


Figure 1: NTx levels when diets differing in fatty acid composition were consumed. <sup>a</sup> $p < 0.05$  when compared to AAD.



**Additional files provided with this submission:**

Additional file 3 : table 3 final.doc : 33Kb

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