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The Short Term Impact of a Mediterranean-Style Diet on Bone Markers in Post-Menopausal Women

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The Short Term Impact of a Mediterranean-Style Diet on Bone Markers in Post-Menopausal Women

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Abstract

Osteoporosis is a debilitating skeletal disorder affecting approximately 30% of women over the age of 50 in the United States. The Mediterranean Diet has strong positive effects on cardiovascular health, and so it is of interest to also study its effects on bone health. We conducted a longitudinal, pilot, clinical intervention trial with 16 postmenopausal women. The study had two aims: first to determine if postmenopausal American women were able to adopt a traditional Mediterranean-style diet (MedSD); and second, to determine if adherence to a MedSD resulted in improved bone turnover markers. Participants followed their typical diet for 12 weeks, and then were counseled by a Registered Dietitian to follow the MedSD for 12 weeks. Three-day diet records and the Mediterranean Diet Score questionnaire (MDS) were collected throughout the study period as subjective measures of compliance, while serum fatty acid (FA) profiles were analyzed as an objective measure of compliance to the diet. Serum markers of bone resorption (C-terminal cross-linking telopeptides of type 1 collagen, CTX) and bone formation (procollagen type 1 amino-terminal propeptide, P1NP) were collected to assess changes in bone turnover. Mixed effects longitudinal growth modeling was used to assess changes in primary and secondary outcome variables. The changes observed in 3-day diet records, MDS, and serum FA profiles reflected significant adherence to the MedSD during the intervention phase. Neither serum P1NP nor serum CTX changed significantly throughout the study, however, when individual dietary components were examined, dietary omega-3 had a significant positive effect on serum P1NP, suggesting that this aspect of the MedSD may have a positive impact on bone formation. Future studies should aim to further examine this relationship.

Introduction

Osteoporosis is a skeletal disorder characterized by microarchitectural deterioration of bone, resulting in compromised bone strength and increased risk of fracture (1). In 2008, approximately one in three women over the age of 50 in the United States were diagnosed with osteoporosis (2, 3, 3), and the national prevalence is expected to increase as the population continues to age (1, 2). Health care costs associated with osteoporosis are projected to reach $25.3 billion by the year 2025 (4). Because the disease is essentially irreversible, it is important to develop more effective prevention and treatment strategies. Various modifiable risk factors have already been identified, including low calcium intake, vitamin D insufficiency, high salt intake, high caffeine intake, and excessive alcohol intake (5). Recent research has suggested that omega-3 (n-3) and omega-6 (n-6) polyunsaturated fatty acids (PUFAs) may also play a role in bone health (6, 7, 7-9). While it is beneficial to examine the role of specific nutrients in bone health, it may be more appealing to look at the impact of a whole-diet approach on prevention, because such interventions may be more applicable. Additionally, there is a possibility of synergistic effects between foods and nutrients, and these interactions must be considered when conducting dietary interventions. A whole diet intervention approach mimics the consumption of a variety of foods and therefore encompasses the possible interactions that may exist within a dietary pattern (10).

One dietary pattern that is being studied for its potential impact on bone health is a Mediterranean-Style Diet (MedSD). A traditional MedSD is characterized by high intake of fruits, vegetables, legumes, non-refined grains, moderate to high intake of fish, moderate intake of dairy, low intake of red or processed meat, sweets, and saturated fat, with the main source of dietary fat coming from olive oil (11). Specific components of the MedSD are associated with

higher bone mineral density (BMD), such as increased fruit and vegetable consumption (12, 13), moderate fish intake (14-17), greater intake of olive oil and decreased consumption of red meat (18). Dietary assessment tools have been developed and validated to measure the degree of adherence to a MedSD in an American population. The tools are based on the traditional Mediterranean diet pyramid and take into account the potential consumption of non-Mediterranean foods, thus making them applicable to a US population (19).

Previous studies examining the relationship between the MedSD and bone health are mainly cross-sectional and observational (20, 21). Few intervention trials have assessed the impact of a MedSD on skeletal parameters, and those that have been done, use either a mixed gender sample (22), or a sample of only men (23, 24), thus failing to target the high-risk population of post-menopausal women. Seiquer et al examined calcium retention in young men after a 28-day MedSD intervention and found there to be significant decreases in urinary calcium, and significantly higher calcium retention after the MedSD than during the basal diet period, suggesting that the Mediterranean intervention diet positively impacted calcium utilization and thus could potentially improve peak bone mass in adolescent boys (24). Both Bulló et al and Fernandez-Real et al utilized a subsample of the PREDIMED intervention trial, a study which assigned men and women to either a Mediterranean diet with mixed nuts, Mediterranean diet with virgin olive oil (VOO), or a low-fat control diet in order to assess cardiovascular outcomes. Bulló et al. examined bone mineral density (BMD) as a secondary outcome in 271 men and women via quantitative ultrasound on the calcaneum, and urinary free deoxypyridinoline (a marker of bone resorption) and found that after a one year follow-up period, there were no significant changes in BMD or bone resorption markers (22). Contrastingly, Fernandez-Real et al examined serum total and uncarboxylated osteocalcin levels,

serum CTX and serum P1NP in 127 men at baseline and 2-yr follow-up from fasting blood samples. Serum CTX significantly decreased in all three dietary groups, but serum P1NP significantly increased in only the MedDiet+VOO group, suggesting that a Mediterranean diet supplemented with virgin olive oil for a period of 2 years may have a protective effect on bone in elderly men at cardiovascular risk (23). These studies are inconclusive and fail to address the potential impact of a MedSD in the high-risk population of post-menopausal women.

We therefore undertook a six-month MedSD intervention trial in 16 postmenopausal American women and assessed bone turnover markers as the primary outcome. The overall objectives of this study were 1) to determine if postmenopausal women living in the Unites States could adopt and adhere to a MedSD, and 2) to assess the impact of adherence to a MedSD on bone turnover, measured by serum markers of bone resorption (C-terminal cross-linking telopeptides of type 1 collagen, CTX) and bone formation (procollagen type 1 amino-terminal propeptide, P1NP). The specific hypotheses for each objective are defined in Table 1.

Methods

Participants. Thirty-three women were screened via telephone for participation. Ten women failed telephone screening leaving 23 postmenopausal women who voluntarily enrolled. Seven of these subjects were dropped during the study period secondary to changes in supplement use (meeting exclusion criteria), resulting in a total of 16 participants completing the study. The trial design was a one group, longitudinal pilot clinical intervention. Each subject followed a baseline control diet for 12 weeks and then switched to a Mediterranean-style intervention diet. Subjects visited the research site approximately every 3 weeks for a total of 9 visits.

Exclusion criteria included any disease that may affect bone metabolism, cancers of any kind (except basal or squamous cell of the skin) in the past 5 years, use of medication known to affect bone metabolism, participating in physical activity more than 75 minutes/day for 6 days/week, dietary behaviors or supplementation in excess of DRI upper limits, vitamin D supplementation in excess of 10,000 IU/day, total calcium consumption from food and supplements exceeding 2,000 mg/day, following a medically prescribed diet or dietary pattern similar to the Mediterranean-Style diet (MedSD), history of chronic renal or liver disease, history of hip fracture or known vertebral fracture within the past year, alcoholic beverage intake >3 drinks/day, having an allergy to fish or nuts, achieving a score >81% (45/55) on the Mediterranean-Style Dietary Pattern Score assessment form (MSDPS), consumption of more than 5 servings/day of fruit or vegetables, consumption of 2 or more servings/week of fatty fish, or consumption of 3 or more servings/week of any seafood. The study was approved by the Investigational Review Board at the University of Connecticut Health Center (UCHC). All participants gave their written informed consent.

Diets. Subjects followed their typical diets for the first 12 weeks of the study period. They were instructed by a Registered Dietitian not to make any major changes to their typical diet (i.e. do not introduce new diet habits or eliminate foods or food groups) and not to start any new nutritional supplements. This period served as the control for the intervention phase.

After 12 weeks, participants were educated by a Registered Dietitian to begin the MedSD. This intervention diet included 4 components: 1) incorporation of 3 Tablespoons Extra Virgin Olive Oil (EVOO) daily; 2) incorporation of 3-5 servings/week of high omega-3 fish (salmon or tuna); 3) incorporation of 1.5 ounces of walnuts daily; 4) incorporation of increased amounts of fruits, vegetables and whole grains. Participants were provided with the first three

components of the diet at the research site, and were instructed on how to make the changes involved in the fourth component on their own. Participants could choose any combination of frozen tuna steaks, frozen salmon fillets, or canned tuna in water to meet the requirements of the second dietary component. In order to maintain consistent calorie consumption and prevent weight gain, subjects were counseled about the importance of making the above dietary changes via replacement of already existing foods rather than addition of these foods (i.e. EVOO to replace butter, salmon to replace beef or pork, etc.).

Data Collection. Participants traveled to the Center on Aging at the UCHC in Farmington, Connecticut for each study visit. They recorded dietary intake using 3-day diet records at visits 1, 5, 6 and 9. Diet records were reviewed by a Registered Dietitian and entered into The Food Processor SQL (FoodPro) version 10.1.0 from ESHA research in Salem, Oregon to analyze nutrient composition of recorded foods. Lastly, an 11-question Mediterranean Diet Score (MDS) (19) was administered at visits 1, 5, and 9. MDS scores ranged from 0-55, with higher scores indicating greater adherence to a MedSD.

Serum samples were collected at visits 1, 3, 5, 7 and 9 serum was separated from whole blood and stored at -80°C until measurement. Fatty acids were extracted, methylated, and the resulting methyl esters (FAME) were analyzed by gas chromatography. Individual fatty acids were identified from sample peak comparison to authentic FAME standards and reported as area percentage of total fatty acids. At these visits, P1NP was also collected from the serum to measure bone formation, and CTX was collected to measure bone resorption. These two markers of bone turnover function as the primary outcome measures. Calcium regulation was measured via serum 25-hydroxyvitamin D and serum parathyroid hormone levels.

Statistical Analysis. The primary outcomes in this study were serum P1NP and serum CTX. Secondary outcomes included MDS, serum fatty acids, and dietary components of 3-day food records. A power calculation revealed that the sample size was adequately powered to observe changes in certain serum fatty acids. The study had 99% power to detect a 0.49±0.44% change in serum docosahexanoic acid (DHA), and 70% power to detect a 0.30±0.48% change in serum eicosapentanoic acid (EPA), two omega-3 serum fatty acids of importance. However, as expected, the sample size was not adequately powered to observe an effect of the nutrition intervention on bone turnover markers. In order to have 80% power to detect significant changes in the primary outcome variables P1NP and CTX, a sample size of approximately 150 subjects would be required.

Nutrient data from FoodPro was exported into Microsoft Office Excel 2007 for Windows. Descriptive and mixed effects longitudinal growth analyses were performed using SPSS statistical software (version 21 for Windows). All data were tested for normality using the Shapiro-Wilk test and a p-value of <0.05 indicated that the data differed significantly from a normal distribution. All variables were determined to be normally distributed except the following: total dietary vitamin D (IU) at visits 1, 5, 9; dietary PUFA (g) at visit 5; dietary omega-3 (g) at visits 1, 5, 9; dietary omega-6 (g) at visit 9; dietary ratio of omega-6:omega-3 at visits 1, 5; serum CTX at visits 1, 3, 5, 7; serum fatty acid eicosadienoic acid (20:2n6) at visits 1, 5.

Serum fatty acids that were non-detected by the GC during at least one visit were excluded from analysis. The remaining serum saturated, omega-3, and omega-6 fatty acids were included in the analysis. To address hypotheses 1a), and 1b), mixed-effects longitudinal growth modeling was conducted with time as the independent variable for each of our continuous

dependent variables (MDS score, area percentage of serum omega-3, omega-6, and saturated fatty acids). To address hypotheses 1c), and 1d), time-varying covariates were entered separately into the model (MDS, and dietary factors, respectively). To address hypothesis 2a), one independent variable (IV) was entered into the model (time), and to address hypothesiss 2b), 2c), and 2d), a second IV was entered into each model (MDS, dietary factors, and serum fatty acids, respectively). We chose these statistical methods because modeling the data as a growth pattern allowed us to more accurately capture the slope of the participants' change over time, thus providing more information about the group and individual longitudinal changes observed. Additionally, the addition of a second IV as a time-varying covariate would allow us to explore the effect of that second variable on our DVs. If the additional IV did have a significant effect on the DV, then having this additional IV in the model would help to explain within-person change over time more precisely. Additionally, for those models with one IV that did show a significant change in the dependent variable (DV) over time, a secondary model was tested, in which we recoded baseline data (visits 1 and 5) as zero, and intervention data (visit 9) as 1, in order to confirm that the significant change was attributable to the intervention and not to the placebo effect. Mixed effects longitudinal results are expressed as [β-coefficient (95% CI)] in the text. Tables also include variance components of the models, and goodness-of-fit indices (Akaike Information Criteria, AIC; and Bayesian Information Criteria, BIC).

Results

The enrolled sample at baseline (visit 1) included 22 postmenopausal women, with a mean age of 77±6.8 years, mean weight of 65.4±8.7 kg, and mean BMI of 25.4±2.9 kg/m^2. By the conclusion of the study, three subjects were dropped because they had begun taking fish oil supplements after enrollment, and three were dropped because it was discovered that they had

initially underreported their typical intake of Mediterranean foods, leaving 16 subjects who completed the study. Weight, and thus BMI, remained stable throughout the study period.

Baseline nutrient intake is presented in Table 2. On average, subjects were consuming above the RDA for protein (>0.8g/kg/day), and when supplements were taken into account, subjects were also meeting or exceeding recommendations for calcium (>1200mg/day) and Vitamin D (>600 IU/day). Additionally, their omega-6:omega-3 ratio was approximately equal to that of the typical American n-6:n-3 ratio of about 9.4:1 (6).

The level-1 growth models used to assess hypotheses 1a), 1b), and 2a) were simple linear growth models (DVs listed in Table1). They contained a linear growth slope for time coded as visit 1, visit 5, visit 9, but did not model the treatment effect or the effect of any other time-varying covariates. Level-2 growth models included the addition of a second independent variable in order to create a model with time-varying slopes. The coefficient for these slopes, β_{20}, indicates how much more change we expect participants to make over every unit of the newly added IV. The corresponding equations for both levels of linear growth models are shown in Table 1.

Hypothesis 1a) was that MDS scores would significantly increase during the intervention, indicating subjective adherence to a MedSD. MDS values significantly increased from 32.3±4.3 at baseline (visit 1) to 41.3±3.7 at visit 9 ($p<0.001$), with an average increase of 1.32 (0.67 to 1.97) per visit, indicating a shift toward a more Mediterranean pattern over time. There were no statistically significant variations in the intercept or slopes between subjects for this model, suggesting that participants' initial scores were similar and all changed in a similar pattern. Because of the significant change observed per time point, secondary analysis assessing the intervention effect showed an average increase of 8.86 (7.56 to 10.16; $p<0.001$) after the

intervention. This significant improvement in the MDS after the intervention period indicates that the increase in score was attributable to the intervention and not to the placebo effect.

Hypothesis 1b) was that serum fatty acid profiles would change significantly during the intervention, objectively indicating adherence to a MedSD. Results from these models are presented in Table 3. It was predicted that serum n-6 and saturated FAs would significantly decrease, while serum n-3 FAs would significantly increase during the intervention compared with control period. With the exception of serum linoleic acid (n-6) all of the serum FAs that changed significantly, did so in the way that was expected if a MedSD was adopted. For those fatty acids that changed significantly over each time point, secondary analysis assessing the intervention effect showed that all of these changes were attributable to the intervention. Together, the changes in MDS and serum fatty acid profiles were indicative of successful adherence to the MedSD during the intervention phase.

Hypothesis 1c) was that addition of MDS to the model would predict changes in serum n-3, n-6, and saturated fatty acids. Results are displayed in Table 4.

Hypothesis 1d) was that individual dietary factors would predict changes in serum n-3, n-6 and saturated fatty acids. Results are displayed in Table 5.

The second overall objective was to assess the impact of adherence to a MedSD on serum markers of bone turnover, CTX and P1NP. To address hypothesis 2a), the level-1 growth model with time as the only IV, showed that there were no significant changes in CTX [-.00006 (-0.0117 to 0.0115)] or P1NP [-.282 (-1.31 to 0.743)] throughout the study. To address hypothesis 2b), MDS was added to the model and also showed no significant impacts on CTX [.011 (-.005 to .027)] or P1NP [-.452 (-1.24 to .335)]. To address hypothesis 2c), various dietary factors were added to the model (results shown in table 6). When dietary omega-3 (g) was added to the

model, there was a significant change in P1NP ($p<0.05$), with an average increase of 1.65 µg/L (0.102 to 3.202) per visit. When dietary ratio of n-6:n-3 was added, there was a negative trend in P1NP ($p=.064$), with an average decrease of -0.513 µg/L (-1.06 to 0.033) per visit. When dietary polyunsaturated fat (g) was added to the model, there was a positive trend in P1NP ($p=.082$), with an average increase of 0.296 µg/L (-.042 to .634) per visit. These data indicate a possible beneficial effect of the increased consumption of dietary omega-3, decreased ratio of dietary n-6:n-3 and increased dietary polyunsaturated fat that was seen in the intervention MedSD. Lastly, to address hypothesis 2d), addition of serum FAs to the model showed no significant effects on bone turnover markers, with the exception of docosatetraenoic acid [73.64 (1.79 to 145.48)] on P1NP ($p<0.05$). Results shown in Table 7.

Discussion

This six-month pilot study aimed to determine if postmenopausal, American women could adopt a MedSD and if a MedSD pattern was beneficial to bone health. This sample of older women was in fact able to successfully adopt a MedSD as evidenced by a significant increase in the MDS subjective assessment tool. Objective assessment utilizing serum FA profiles was also supportive of successful adherence to a MedSD during the intervention because of the changes observed in a variety of serum n-3, n-6, and saturated FAs. However, not all serum FAs were reflective of changes in reported dietary fat composition. Of all the major dietary components of a MedSD, dietary n-3 (g) was the most positive predictor of bone formation.

While the MDS assessment tool used has been previously validated (19) for its effective use with non-Mediterranean populations(11), our objective assessment of adherence must be

critiqued. A recent review by Baylin et al addressed the efficacy of various biomarkers as indicators of dietary intake and suggested that the data are conflicting and dependent on a variety of factors (25). Tissue biomarkers may effectively reflect dietary change in fat intake if the macronutrients within the diet are tightly controlled and if the expected change in intake of specific fatty acids is dramatic. Therefore, the variability in our subjects' baseline intake of macronutrients, may have impacted the pattern of change observed throughout the study. Additionally, those fatty acids that cannot be synthesized endogenously, such as linoleic acid, α-linolenic acid, and *trans* FAs may more accurately reflect dietary changes than those that can be synthesized within the body (25), suggesting that the changes we observed in serum LCPUFAs may not be reflecting more than sole dietary intake of those lipids. Despite this, a cross-sectional study in Japan that aimed to determine the association of dietary FA intake and plasma FA concentration of long-chain n-3 FAs found significant correlations for EPA and DHA ($r=0.692$, $r=0.587$, respectively) in a sample of 79 women (26). Previously, plasma FA concentrations were used as a potential marker of dietary compliance in clinical trials, but whole blood was recently suggested as a more reliable indicator (27). It seems that the body of literature would benefit from more research assessing the accuracy of biomarkers of dietary changes in fat intake.

There are various ways to assess bone health, the gold-standard of which is Dual X-ray Absorptiometry (DEXA) to measure bone mineral (BMD) compared to standards using T- and Z-scores (28). However, the use of serum P1NP and CTX was recommended by the International Osteoporosis Foundation (IOF) and the International Federation of Clinical Chemistry and Laboratory Medicine for use in short term, clinical studies for assessment of bone turnover (29). Serum CTX, a marker of bone resorption, is indicative of the proteolytic fragments of the bone collagen matrix (28), while serum P1NP is reflective of the cleavage of type I procollagen to

form type I collagen, the protein that constitutes 90% of bone (30). Together, these markers of resorption and formation, respectively, represent bone turnover. While these bone turnover markers (BTM) have the advantages of being noninvasive and relatively inexpensive, their high variability is an important disadvantage. Serum P1NP and CTX can be affected by both controllable and uncontrollable factors including age, sex, time of day, food intake, physical activity over the past 24 hours, and serum vitamin D (28). The high variability seen with these BTM probably contributed to the lack of significant change seen from our intervention.

While our intervention did not yield significant changes in BTM over time, the inclusion of dietary omega-3 (g) in the model did show a positive impact on serum P1NP. It has been suggested that a potential mechanism for this effect may involve suppression or activation of the gene PPARγ by DHA or arachidonic acid (AA), respectively (31). This mechanism may support our study results because of the significant increase we observed in serum DHA, and trend toward a reduction in AA seen at the end of our study period. Another potential mechanism by which PUFA may affect bone homeostasis is that a shift from n-6 to n-3 FAs may impact complex signaling pathways including those affecting receptor activator nuclear κβ (RANK) (7), a receptor present on osteoclasts; and PGE_2, a prostaglandin shown to regulate bone remodeling (32).

Previous intervention studies examining the impact of a MedSD on bone health were conducted in either a mixed gender sample (22), or a sample of only males (23, 24). Seiquer et al (24) conducted a longitudinal intervention trial with 20 healthy adolescent males aged 11-14 years old, in which participants followed their typical diet for 3 days, and then a MedSD for 28 days. Urine and feces were collected during the 3-day basal diet period and last 4 days of intervention diet period. Apparent calcium absorption, digestibility, and retention were

calculated from calcium intake from food, and calcium excretion from feces and urine. There were significant decreases in urinary calcium excretion and higher calcium retention after the intervention diet than during the basal diet period. While this study used different markers of bone health (calcium utilization) than in our study, it is suggestive that a MedSD may improve peak bone mass in adolescent boys (24). This conclusion is interesting because it provides implications on bone health at a different stage of growth and development than is addressed with our study population. A longitudinal intervention trial by Fernández-Real et al randomly assigned a subsample of 127 community-dwelling elderly men at cardiovascular risk from the Prevención con Dieta Mediterránea (PREDIMED) study to one of three diets: a low-fat control diet, a Mediterranean diet with mixed nuts (MedDiet+mixed nuts), or a Mediterranean diet with virgin olive oil (MedDiet+VOO). Serum total and uncarboxylated osteocalcin levels, serum CTX and serum P1NP were measured at baseline and 2-yr follow-up from fasting blood samples and showed that total osteocalcin significantly increased in the MedDiet+VOO group, but not the MedDiet+mixed nuts or control group. Additionally, serum CTX significantly decreased in all three groups, but serum P1NP significantly increased in only the MedDiet+VOO group, suggesting that a Mediterranean diet supplemented with virgin olive oil for a period of 2 years may have a protective effect on bone in elderly men at cardiovascular risk (23). While this study had a longer follow-up period, their choice of P1NP and CTX as markers of bone status is not ideal because these BTM are recommended for use in shorter term interventions versus longer trials. Bulló et al addressed this limitation, also utilizing a subsample of the PREDIMED study population, but instead measuring quantitative ultrasound on the calcaneum to assess long term changes in BMD. Free deoxypyridinoline was also collected from the urine as a marker of bone resorption. In contrast to the results of the previously cited intervention trials, there were no

significant changes in BMD or bone resorption markers in this study (22). Our study adds to the body of literature on the impact of MedSD interventions on bone health because we were unable to find significant changes on BTM as a result of the MedSD (with the exception of the relationship between dietary omega-3 and serum P1NP). Our results parallel other investigators' findings except we studied a population at high risk for development of osteoporosis that has not previously been evaluated.

The present study has limitations. First, as a pilot study, the small sample size and lack of power to observe changes in our primary outcome variable are most likely our biggest limitation. Additionally, there was a lack of control for certain dietary factors that may impact bone status, such as total calcium and total vitamin D intake. While our exclusion criteria did provide a cut off point for an acceptable range of calcium and vitamin D intake, perhaps changes in bone health are seen more dramatically at lower calcium intakes (33), suggesting potential benefit from a tighter range of these nutrients. Stratifying our participants by calcium intake may have addressed this limitation, but our small sample size made this unfeasible. As with any subjective marker of compliance, the use of the MDS to assess compliance may not have been reliable as it was based on self-report. The measurement of changes in serum FA was used to address this limitation, however, there may also be discrepancy in the efficacy of use of plasma FAs as a measure of dietary compliance as described above. It would be beneficial for future studies to consider including a larger sample size, and tighter control of confounding nutrients such as calcium and vitamin D.

Despite these limitations, our study was still able to capture intriguing results regarding a potential positive impact of dietary omega-3 on the bone formation marker, P1NP. Additionally, this study was novel in that it was able to successfully implement a MedSD in an American

population at high risk for osteoporosis, showing that this dietary pattern can be adopted by non-Mediterranean populations who may benefit from its positive effects.

Conclusion

The results of the present study showed that although our MedSD intervention did not have a direct effect on BTM, the increases in dietary n-3 seen as a result of the intervention did have a positive effect on bone formation. These results are promising because all of our subjects were successful in adopting the MedSD, indicating that the intervention may be a feasible application in clinical practice. Undoubtedly, a MedSD is beneficial for cardiovascular health, and is not harmful to bone health. Our study suggests a benefit to bone health, and so further studies with larger sampling will provide additional insight into this intriguing relationship.

Table 1. Overall objectives, specific hypotheses and corresponding statistical models			
Study Objectives	**Statistical Models**		
	Equation	**Model #**	**Variables**
1. To determine if postmenopausal women following a typical American diet can adopt and adhere to a MedSD	---	---	---
a) MDS will significantly increase during the intervention, indicating adherence to a MedSD	$Y_{ij}=\beta_{00}+\beta_{10}(time)+e_{it}+\epsilon_{0i}+\epsilon_{1i}$	In text	**IV**: time; **DV**: MSDPS
b) Serum fatty acid profiles will change significantly during the intervention, indicating adherence to MedSD	$Y_{ij}=\beta_{00}+\beta_{10}(time)+e_{it}+\epsilon_{0i}+\epsilon_{1i}$ OR $Y_{ij}=\beta_{00}+\beta_{10}(time)+\beta_{20}+e_{it}+\epsilon_{0i}+\epsilon_{1i}+\epsilon_{2i}$	1,1i,2,2i,3,3i,4,5,5i,6,6i,7,7i,8,9,9i,10,10i,11,11i,12	**IV**: time, intervention effect (i); **DV**: serum FAs
c) MDS score will predict changes in serum saturated, omega-6, and omega-3 fatty acids	$Y_{ij}=\beta_{00}+\beta_{10}(time)+\beta_{20}+e_{it}+\epsilon_{0i}+\epsilon_{1i}+\epsilon_{2i}$	13,14,15,16,17,18,19, 20,21,22,23, 24	**IV**: time, MSDPS; **DV**: serum FAs
d) Individual dietary factors from 3-day food records will predict changes in serum saturated, omega-6, and omega-3 fatty acids	$Y_{ij}=\beta_{00}+\beta_{10}(time)+\beta_{20}+e_{it}+\epsilon_{0i}+\epsilon_{1i}+\epsilon_{2i}$	25,26,27,28,29,30,31, 32,33,34,35,36,37,38, 39,40	**IV**: time, dietary factors; **DV**: serum FAs
2. To assess the impact of adherence to a MedSD on bone turnover, measured by serum markers of bone resorption (CTX) and bone formation (P1NP)	---	---	---
a) A significant suppression in bone turnover will be observed during the intervention (decreased P1NP, decreased CTX)	$Y_{ij}=\beta_{00}+\beta_{10}(time)+e_{it}+\epsilon_{0i}+\epsilon_{1i}$	In text	**IV**: time; **DV**: CTX, P1NP
b) MSDPS will predict changes in serum bone turnover markers	$Y_{ij}=\beta_{00}+\beta_{10}(time)+\beta_{20}+e_{it}+\epsilon_{0i}+\epsilon_{1i}+\epsilon_{2i}$	In text	**IV**: time, MSDPS; **DV**: CTX, P1NP
c) Individual dietary factors from 3-day food records will predict changes in serum bone turnover markers	$Y_{ij}=\beta_{00}+\beta_{10}(time)+\beta_{20}+e_{it}+\epsilon_{0i}+\epsilon_{1i}+\epsilon_{2i}$	41,42,43,44,45,46,47, 48,49,50,51,52,53,54, 55,56,57,58,59,60	**IV**: time, dietary factors; **DV**: CTX, P1NP
d) Serum saturated, omega-6, and omega-3 fatty acids will predict changes in bone turnover markers	$Y_{ij}=\beta_{00}+\beta_{10}(time)+\beta_{20}+e_{it}+\epsilon_{0i}+\epsilon_{1i}+\epsilon_{2i}$	61,62,63,64,65,66,67, 68,69,70,71,72,73,74, 75,76,77,78,79,80,81, 82,83,84	**IV**: time, serum FAs; **DV**: CTX, P1NP

Table 2. Baseline (visit 1) Nutrient Data

	Mean	±	SD
Energy			
Energy (kcals)	1701		239
Energy (kcals/kg)	26.6		5.04
Calories from fat (%)	32.22		5.52
Lipids			
Total fat (g)	61.25		14.99
Total fat (g/kg)	0.96		0.27
Calories from saturated fat (%)	10.45		2.63
Saturated fat (g)	19.95		6.32
Saturated fat (g/kg)	0.31		0.11
Monounsaturated fat (g)	16.23		7.4
Monounsaturated fat (%)	8.6		3.86
Monounsaturated fat (g/kg)	0.25		0.13
Polyunsaturated fat (g)	8.33		3.77
Polyunsaturated (%)	4.4		1.94
Polyunsaturated (g/kg)	0.13		0.06
Omega-3 (g)	0.94		0.77
Omega-6 (g)	6.32		3.3
Omega-6:omega-3 ratio	9.31		6.05
Other Macronutrients			
Protein (g)	76.59		14.11
Protein (%)	18.12		2.89
Protein (g/kg)	1.19		0.25
Carbohydrate (g)	214		44.96
Carbohydrate (%)	50.21		7.09
Carbohydrate (g/kg)	3.34		0.83
Micronutrients			
Vitamin D from food (IU)	131.11		94.77
Vitamin D from food & supplements (IU)	1547.46		1754.44
Calcium from food (mg)	794.3		285.94
Calcium from food & supplements (mg)	1339.46		563.95

Table 3. Longitudinal change in serum omega-3, omega-6, and saturated fatty acids

		Fixed Effects			Random Effects				Model Fit Indices		
Model #	***DVs:***	β_{00} (95%CI)	β_{10} (95%CI) (time)	β_{20} (95%CI) (IE)	σ_{ij}	τ_{00}	τ_{01}	τ_{11}	-2 restricted log	AIC	BIC
Serum Saturated FAs											
1	Myristic Acid	.701 (.581 to .819)	-.025 (-.0397 to -.0095)**	N/A	0.010**	0.040*	-0.002	0.001	-23.46	-15.46	-8.41
1i	Myristic Acid[i]	0.698 (0.574 to 0.821)	-0.023 (-0.045 to -0.002)*	-0.011 (-0.142 to 0.121)	0.010**	0.039*	-0.002	0.000	-19.72	-11.72	-4.49
2	Palmitic Acid	20.188 (19.09 to 21.29)	-.168 (-.254 to -.082)***	N/A	0.648**	3.520*	-0.098	0.006	162.26	170.26	177.58
2i	Palmitic Acid[i]	19.892 (18.772 to 21.012)	-1.013 (-1.944 to -0.082)	-1.013 (-1.944 to -0.082)*	0.437**	3.194*	-.782	0.001	157.21	165.21	172.44
3	Margaric Acid	.280 (.258 to .303)	-.003 (-.005 to -.0005)*	N/A	0.000**	0.001*	0.000	0.000	-187.49	-179.49	-172.18
3i	Margaric Acid[i]	0.275 (0.252 to 0.298)	-0.000 (-0.004 to 0.003)	-0.018 (-0.042 to 0.007)	0.000**	0.001*	-0.000	0.000	182.73	-174.73	-167.50
4	Stearic Acid	8.26 (7.53 to 8.99)	.025 (-.052 to .101)	N/A	0.634**	1.160	0.026	0.001	150.57	158.57	165.89
Serum Omega-3 FAs											
5	DHA	1.84 (1.63 to 2.06)	.066 (.034 to .097)***	N/A	0.108**	0.050	0.001	0.000	54.25	62.25	69.56
5i	DHA[i]	2.01 (1.78 to 2.25)	-0.006 (-0.054 to 0.042)	0.577 (0.269 to 0.884)***	0.056**	0.108	-0.007	0.002	44.72	52.72	50.86
6	α-linolenic Acid	.547 (.413 to .681)	.032 (.011 to .053)**	N/A	0.042**	0.016	0.000	0.000	13.02	21.02	28.33
6i	α-linolenic Acid[i]	0.629 (0.484 to 0.775)	-0.003 (-0.038 to 0.031)	0.283 (0.054 to 0.511)*	0.031**	0.029	-0.001	0.001	9.59	17.59	24.81
7	EPA	.726 (.519 to .934)	.032 (-.002 to .066)†	N/A	0.064**	0.080	-0.006	0.002	45.93	53.93	61.20
7i	EPA[i]	0.826 (0.607 to 1.045)	-0.011 (0.058 to 0.036)	0.342 (0.056 to 0.627)*	0.048**	0.099†	-0.009	0.002	42.37	50.37	57.60
8	Clupanodonic acid	.553 (.488 to .619)	-.005 (-.013 to .003)	N/A	0.001**	0.013*	-0.001†	0.000*	-97.37	-88.37	-81.05
Serum Omega-6 FAs											
9	Linoleic acid	30.34 (28.30 to 32.38)	.396 (.185 to .607)***	N/A	5.345***	8.640	0.222	N/A	248.57	256.57	263.88
9i	Linoleic acid[i]	31.25 (29.12 to 33.36)	0.007 (-0.391 to 0.405)	3.11 (0.286 to 5.94)*	4.734**	8.965	0.241	0.007	241.00	249.00	256.23
10	Docosatetranoic acid	.232 (.210 to .253)	-.005 (-.010 to -.001)*	N/A	0.001**	0.001	0.000	N/A	-147.95	-139.95	-132.64
10i	Docosatetranoic acid[i]	.221 (0.189 to 0.235)	0.003 (-0.001 to 0.007)	-0.067 (-0.093 to -0.041)***	0.000**	0.001*	0.000	0.001	-164.84	-156.84	-149.61
11	Arachidonic acid	8.88 (7.64 to 10.11)	-.065 (-.134 to .005)†	N/A	0.547***	4.770*	-0.089	N/A	161.29	169.29	176.61
11i	Arachidonic acid[i]	8.51 (7.28 to 9.74)	0.092 (-0.026 to -0.210)	-1.25 (-2.07 to -0.437)**	0.393**	4.789*	-0.079	0.004	152.41	160.41	167.64
12	Eicosadienoic acid	.282 (.231 to .333)	-.00003 (-.003 to .003)	N/A	0.001**	0.008*	0.000	0.000	-136.70	-128.70	-121.39

β_{00}=average at baseline (visit 1). β_{10} = mean Δ on DV per visit. β_{10}=mean Δ on DV due to intervention effect

τ_{00} = estimate of variance in y-intercepts between subjects. τ01 = estimate of covariance between slopes and intercepts. τ11=estimate of variance in slopes between subjects.

=model including intervention effect as second independent variable

N/A=no random effects were included. IE=intervention effect

*p<.05 **p<.01 ***p<.001 †p<.10

Table 4. Impact of the Mediterranean Diet Score (MDS) on longitudinal change in serum fatty acid profiles

		Fixed Effects			Random Effects				Model Fit Indices		
Model #	***DVs:***	**β_{00} (95%CI)**	**β_{10} (95% CI) (time)**	**β_{20} (95%CI) (MDS)**	σ_{ij}	τ_{00}	τ_{01}	τ_{11}	**-2 restricted log**	**AIC**	**BIC**
Serum Saturated FAs											
13	myristic acid[b]	.784 (.397 to 1.17)	-.021 (-.042 to -.0003)	-.003 (-.015 to .010)	0.010**	0.040**	-0.002	0.001	-15.32	-7.32	-.092
14	palmitic acid[b]	21.75 (18.74 to 24.76)	-.104 (-.246 to 0.038	-.053 (-.150 to .043)	0.585**	3.720*	-0.108	0.008	165.39	173.39	180.62
15	margaric acid[b]	.349 (.283 to .415)	0.0003 (-0.003 to 0.003)	-.002 (-.004 to -.0002)*	0.000**	0.001	-0.000	0.000	-180.25	-172.25	-165.02
16	stearic acid[b]	9.18 (6.36 to 12.00)	0.062 (-0.071 to 0.196	-.031 (-.125 to .062)	0.644**	1.115	0.026	0.001	154.44	162.44	169.66
Serum Omega-3 FAs											
17	a-linolenic acid[b]	-.028 (-.619 to .563)	0.008 (-0.023 to 0.040)	.020 (-.0001 to .039)†	0.036**	0.015	0.000	0.001	16.92	24.92	32.14
18	EPA[b]	-.153 (-.949 to .643)	-0.004 (-0.050 to 0.041)	.030 (.003 to .056)*	0.049**	0.072	-0.005	0.003	48.94	56.94	64.17
19	clupanodonic acid[b]	.683 (.526 to .841)	-0.001 (-0.003 to -0.00004)	-.004 (-.009 to .0005)†	0.001**	0.015**	-0.001*	0.000*	-89.16	-81.16	-73.93
20	DHA[b]	.630 (-.293 to 1.55)	0.016 (-0.031 to 0.063)	.041 (.011 to .072)**	0.074**	0.068	0.001	0.001	55.66	63.66	70.88
Serum Omega-6 FAs											
21	linoleic acid[b]	22.09 (14.26 to 29.92)	0.058 (-0.316 to 0.431)	.281 (.022 to .540)*	4.879**	9.141	0.076	0.019	246.41	254.41	261.63
22	eicosadienoic acid[b]	.328 (.210 to .447)	0.002 (-0.004 to 0.007)	-.002 (-.005 to .002)	0.001**	0.008*	-0.000	0.000	-126.65	-118.65	-111.42
23	arachidonic acid[b]	12.32 (9.49 to 15.14)	0.076 (-0.050 to 0.203)	-.117 (-.206 to -.028)*	0.518***	4.348*	-0.090	N/A	158.90	166.90	174.13
24	docosatetranoic acid[b]	.399 (.310 to .489)	0.001 (-0.003 to 0.006)	-.006 (-.009 to -.003)***	0.001**	0.001	0.000	0.000	-154.67	-146.67	-139.44

β_{00}=average at baseline (visit 1). β_{10} = mean Δ in DV per visit. β_{20}=mean Δ in DV per 1 point of MDS

τ_{00} = estimate of variance in y-intercepts between subjects. τ_{01}=estimate of covariance between slopes and intercepts. τ_{11}=estimate of variance in slopes between subjects.

[b]=serum FA expressed as area percentage of total FA profile

N/A=no random effects were observed.

*p<.05 **p<.01 ***p<.001 †p<.10

Table 5. Impact of dietary factors on longitudinal change in serum fatty acids

Model #	*DVs:*	Fixed Effects: β_{00} (95%CI)	β_{10} (95% CI) (time)	β_{20} (95%CI) (dietary factors)	Random Effects: σ_{ij}	τ_{00}	τ_{01}	τ_{11}	Model Fit Indices: -2 restricted log	AIC	BIC
Dietary saturated fat (g)[a]											
25	myristic acid[b]	0.698 (0.496 to 0.900)	-0.025 (-0.040 to -0.009)**	0.0001 (-0.009 to 0.009)	0.010**	0.040*	-0.002	0.001	-14.40	-6.40	0.831
26	palmitic acid[b]	19.89 (18.29 to 21.50)	-0.169 (-0.254 to -0.083)***	0.016 (-0.049 to .080)	0.698**	3.490*	-0.088	0.003	167.13	175.13	182.36
27	margaric acid[b]	.289 (.253 to .324)	-0.003 (-0.005 to -0.001)*	-.0005 (-.002 to .001)	0.000**	0.001*	-.00004	0.000	-175.28	-167.28	160.05
28	stearic acid[b]	8.26 (6.92 to 9.61)	0.025 (-0.055 to 0.104)	-.0003 (-.061 to .061)	0.653**	1.160	0.026	0.001	155.75	163.75	170.98
Dietary carbohydrate (g)[a]											
29	myristic acid[b]	.795 (.481 to 1.11)	-0.025 (-0.041 to -0.010)**	-.0004 (-.002 to .0009)	0.009**	0.041*	-.003	0.001†	-11.05	-3.05	4.17
30	palmitic acid[b]	20.09 (17.66 to 22.51)	-0.167 (-0.256 to -0.078)***	.0005 (-.010 to .010)	0.663**	3.570*	-.100	0.006	171.05	179.05	186.27
31	margaric acid	.272 (.218 to .326)	-0.002 (-0.005 to -0.000)*	.00004 (-.0002 to .0003)	0.000**	0.001*	-0.000	0.000	-171.24	-163.24	-156.01
32	stearic acid[b]	8.97 (6.92 to 11.02)	0.018 (-0.059 to 0.096)	-.003 (-.012 to .006)	0.621**	1.210†	0.026	0.001	159.07	167.07	174.3
Dietary omega-3 (g)[a]											
33	α-linolenic acid[b]	.534 (.396 to .672)	0.027 (0.002 to 0.052)*	.017 (-.032 to .066)	0.046**	0.011	0.001	0.000	18.2	26.2	33.43
34	EPA[b]	.690 (.490 to .890)	0.018 (-0.019 to 0.055)	.047 (-.020 to .113)	0.073**	0.050	-.002	0.001	49.21	57.21	64.44
35	clupanodonic acid[b]	.553 (.488 to .619)	-0.005 (-0.024 to 0.003)	.0003 (-.011 to .012)	0.002**	0.013**	-.001†	0.000*	-87.77	-79.77	-72.55
36	DHA[b]	1.83 (1.60 to 2.06)	0.059 (0.022 to 0.097)**	.022 (-.053 to .097)	0.111***	0.051	0.001	N/A	58.67	66.67	73.9
Dietary omega-6 (g)[a]											
37	linoleic acid[b]	30.69 (28.54 to 32.83)	0.465 (0.188 to 0.741)**	-.063 (-.206 to .081)	5.590**	8.210	0.250	0.008	251.26	259.26	266.48
38	eicosadienoic acid[b]	.278 (.225 to .330)	-0.001 (-0.005 to 0.003)	.0008 (-.001 to .003)	0.001**	0.008*	-.0003	0.000	-125.31	-117.31	-110.08
39	arachidonic acid[b]	8.89 (7.63 to 10.14)	-0.063 (-0.150 to 0.024)	-.002 (-.048 to .044)	0.573***	4.760*	-.084	N/A	166.97	174.97	182.2
40	docosatetranoic acid[b]	.229 (.204 to .254)	-0.006 (-0.010 to -0.002)**	.0005 (-.002 to .003)	0.001**	0.001	0.000	N/A	-138.62	-130.62	-123.40

β_{00}=average at baseline (visit 1). β_{10} = mean Δ in DV per visit. β_{20}=mean Δ in DV per g of dietary factor

τ_{00} = estimate of variance in y-intercepts between subjects. τ01 = estimate of covariance between slopes and intercepts. τ11=estimate of variance in slopes between subjects.

[a]=IV [b]=serum FA expressed as area percentage of total FA profile.

N/A=no random effects were observed.

*p<.05 **p<.01 ***p<.001 †p<.10

Table 6. Impact of dietary factors on longitudinal change in bone turnover markers

Model #	*DVs:*	Fixed Effects			Random Effects				Model Fit Indices		
		β_{00} (95%CI)	β_{10} (95%CI) (time)	β_{20} (95%CI) (dietary factors)[a]	σ_{ij}	τ_{00}	τ_{01}	τ_{11}	-2 restricted log	AIC	BIC
Omega-3 (g)[a]											
41	Serum CTX[b]	.542 (.364 to .720)	0.002 (-0.013 to 0.018)	-.005 (-.038 to .028)	0.018***	0.089*	-0.001	N/A	2.68	10.68	17.90
42	Serum P1NP[b]	57.88 (42.99 to 72.78)	-0.697 (-1.95 to 0.555)	1.65 (.102 to 3.202)*	24.910**	749.650**	-0.793	4.190*	382.10	390.10	397.40
Omega-6 (g)[a]											
43	Serum CTX[b]	.551 (.370 to .732)	0.003 (-0.011 to 0.018)	-.002 (-.011 to .006)	0.018***	0.090*	-0.001	N/A	5.3	13.30	20.50
44	Serum P1NP[b]	57.40 (42.29 to 72.52)	-0.562 (-1.83 to 0.703)	.320 (-.096 to .736)	27.540**	760.540**	-0.381	4.210*	386.8	394.80	402.00
Omega-6:omega:3 ratio[a]											
45	Serum CTX[b]	.589 (.386 to .793)	-0.002 (-0.015 to 0.011)	-.006 (-.017 to .006)	0.017***	0.092*	-0.0003	N/A	3.90	11.90	19.10
46	Serum P1NP[b]	63.90 (47.72 to 80.09)	-0.445 (-1.65 to 0.761)	-.513 (-1.06 to .033)†	24.770**	829.900**	-8.37	4.185*	385.1	393.10	400.40
Saturated fat (g)[a]											
47	Serum CTX[b]	.506 (.248 to .764)	0.001 (-0.012 to 0.013)	.002 (-.009 to .012)	0.018***	0.089*	-0.0003	N/A	4.90	12.90	20.20
48	Serum P1NP[b]	59.41 (41.26 to 77.57)	-0.208 (-1.399 to 0.984)	-.013 (-.584 to .558)	32.080**	791.300**	-7.84	3.970*	388.6	396.60	403.80
Polyunsaturated fat (g)[a]											
49	Serum CTX[b]	.556 (.374 to .738)	0.004 (-0.011 to 0.019)	-.002 (-.009 to .004)	0.018***	0.090*	-0.001	N/A	5.60	13.60	20.80
50	Serum P1NP[b]	56.92 (41.89 to 71.94)	-0.605 (-1.878 to 0.669)	.296 (-.042 to .634)†	26.398**	747.970**	-2.48	4.331*	386.6	394.60	401.80
Monounsaturated fat (g)[a]											
51	Serum CTX[b]	.584 (.394 to .774)	0.005 (-0.009 to 0.019)	-.003 (-.008 to .002)	0.017***	0.091*	-0.001	N/A	5.2	13.20	20.40
52	Serum P1NP[b]	56.88 (41.28 to 72.49)	-0.433 (-1.71 to 0.848)	.155 (-.119 to .429)	27.811**	785.83**	-8.04	4.470*	388.7	396.70	404.00
Total fat (g)[a]											
53	Serum CTX[b]	.541 (.305 to .778)	0.001 (-0.014 to 0.016)	-.00006 (-.003 to .003)	0.018***	0.089*	-0.0002	N/A	7.60	15.60	22.80
54	Serum P1NP[b]	53.25 (36.17 to 70.34)	-0.501 (-1.803 to 0.800)	.103 (-.051 to .257)	26.780**	783.87**	-8.78	4.586*	389.4	397.40	404.60
Carbohydrate (g)[a]											
55	Serum CTX[b]	.438 (.053 to .824)	0.002 (-0.012 to 0.014)	.0005 (-.001 to .002)	0.018***	0.093*	-0.001	N/A	8.70	16.70	23.90
56	Serum P1NP[b]	59.98 (34.48 to 85.50)	-0.217 (-1.42 to 0.989)	-.004 (-.100 to .093)	31.615**	797.900**	-7.99	4.019*	392.1	400.10	407.30
Total Calcium (mg)[a]											
57	Serum CTX[b]	.329 (.020 to .637)	0.001 (-0.010 to 0.013)	.0002 (-.00004 to .0004)	0.017***	0.078*	0.001	N/A	11.00	19.00	26.30
58	Serum P1NP[b]	46.81 (24.56 to 69.05)	-0.171 (-1.29 to 0.947)	.009 (-.003 to .022)	31.792**	779.320**	1.45	3.353*	394.40	402.40	409.60
Total Vitamin D (IU)[a]											
59	Serum CTX[b]	0.427 (0.214 to .640)	0.0007 (-0.011 to 0.013)	0.000 (-0.000 to 0.000)†	0.017***	0.080*	-0.001	N/A	11.63	19.62	26.85
60	Serum P1NP[b]	40.03 (23.63 to 56.43)	-0.211 (-1.37 to 0.952)	0.013 (0.007 to 0.020)***	31.010**	612.650*	-28.79	3.800*	386.50	394.50	401.73

β_{00}=average at baseline (visit 1). β_{10} = mean Δ in DV per visit. β_{20}=mean Δ in DV per unit of dietary factor

τ_{00}= estimate of variance in y-intercepts between subjects. τ_{01} = estimate of covariance between slopes and intercepts. τ_{11}=estimate of variance in slopes between subjects.

[a]=IV [b]=expressed at nanograms per milliliter

N/A=no random effects were observed.

*p<.05 **p<.01 ***p<.001 †p<.10

Table 7. Impact of serum fatty acids on longitudinal change in bone turnover markers

Model #	*DVs:*	Fixed Effects: β_{00} (95%CI)	β_{10} (95%CI) (time)	β_{20} (95%CI) (serum fatty acids)*	Random Effects: σ_{ij}	τ_{00}	τ_{01}	τ_{11}	Model Fit Indices: -2 restricted log	AIC	BIC
Myristic Acid[a]											
61	Serum CTX[b]	.377 (.092 to .662)	0.006 (-0.008 to 0.021)	.229 (-.099 to .558)	0.017***	0.095*	-.002	N/A	-2.84	5.16	12.39
62	Serum P1NP[b]	51.57 (29.93 to 73.21)	0.058 (-1.25 to 1.36)	10.85 (-11.82 to 33.52)	30.187**	817.800**	-13.21	4.096*	380.28	388.28	395.50
Palmitic acid[a]											
63	Serum CTX[b]	0.622 (-.375 to 1.62)	0.000 (-0.015 to 0.015)	-.004 (-.053 to .045)	0.018***	0.091*	-.0002	N/A	1.92	9.92	17.15
64	Serum P1NP[b]	15.87 (-44.82 to 76.56)	0.152 (-1.12 to 1.42)	2.14 (-.784 to 5.07)	29.760**	770.510**	-12.89	4.060*	383.11	391.11	398.34
Margaric acid[a]											
65	Serum CTX[b]	.742 (.118 to 1.37)	-0.001 (-0.015 to 0.013)	-.730 (-2.88 to 1.42)	0.018**	0.084*	-.0001	0.000	-6.05	1.95	8.18
66	Serum P1NP[b]	57.91 (19.25 to 96.58)	-0.197 (-1.42 to 1.03)	4.49 (-124.25 to 133.24)	31.720**	802.070**	-8.21	3.980*	377.69	385.69	392.92
Stearic acid[a]											
67	Serum CTX[b]	.188 (-.271 to .646)	-0.000 (-0.012 to 0.012)	.042 (-.009 to .094)	0.016***	0.105*	-.002	N/A	-.050	7.95	15.18
68	Serum P1NP[b]	44.35 (14.63 to 74.07)	-0.253 (-1.41 to 0.903)	1.79 (-1.36 to 4.95)	30.530**	833.560**	-10.95	3.722*	383.81	391.81	399.04
a-linolenic acid[a]											
69	Serum CTX[b]	.540 (.332 to .748)	0.001 (-0.013 to 0.015)	-.004 (-.223 to .215)	0.018***	0.089*	-.0002	N/A	-1.05	6.95	14.18
70	Serum P1NP[b]	65.68 (119.15 to 82.21)	0.171 (-1.02 to 1.36)	-11.90 (-23.85 to .048)†	27.950**	816.390**	-14.28	3.680*	378.52	386.52	393.75
EPA[a]											
71	Serum CTX[b]	.543 (.340 to .746)	0.001 (-0.012 to 0.014)	-.007 (-.159 to .144)	0.018***	0.089*	-0.000	N/A	-.322	7.68	14.91
72	Serum P1NP[b]	61.49 (44.76 to 78.22)	-0.108 (-1.35 to 1.14)	-3.19 (-12.57 to 6.19)	29.650**	823.490**	-11.81	4.250*	382.48	390.48	397.71
Clupanodonic acid[a]											
73	Serum CTX[b]	.598 (.178 to 1.02)	0.000 (-0.013 to 0.013)	-.108 (-.807 to .590)	0.018***	0.090*	-0.000	N/A	-3.47	4.53	11.76
74	Serum P1NP[b]	65.27 (33.62 to 96.92)	-0.266 (-1.52 to 0.984)	-11.02 (-61.96 to 39.92)	30.010**	809.020**	-10.32	4.296*	379.35	387.35	394.58
DHA[a]											
75	Serum CTX[b]	.544 (.265 to .882)	0.002 (-0.013 to 0.017)	-.019 (-.161 to .122)	0.018***	0.089*	-0.001	N/A	-.235	7.77	14.99
76	Serum P1NP[b]	70.14 (50.12 to 90.17)	0.183 (-1.13 to 1.50)	-5.95 (-13.47 to 1.57)	26.540**	806.900**	-15.58	4.550*	381.00	389.00	396.22
Linoleic acid[a]											
77	Serum CTX[b]	.065 (-.309 to .639)	-0.005 (-0.020 to 0.009)	.016 (-.003 to .034)†	0.016**	0.081*	0.001	0.000	1.89	9.89	17.11
78	Serum P1NP[b]	76.72 (42.25 to 111.19)	0.021 (-1.21 to 1.25)	-.578 (-1.62 to .462)	30.270**	812.310**	-11.14	3.899*	386.05	394.05	401.28
Eicosadienoic acid[a]											
79	Serum CTX[b]	.653 (.267 to 1.04)	0.001 (-0.011 to 0.013)	-.406 (-1.63 to .821)	0.018***	0.087*	0.000	N/A	-.4.94	3.06	10.28
80	Serum P1NP[b]	73.70 (48.33 to 99.07)	-0.210 (-1.36 to 0.935)	-51.50 (-126.67 to 23.66)	32.120**	734.270**	-2.165	3.602*	376.97	384.97	392.19
Arachidonic acid[a]											
81	Serum CTX[b]	.775 (.306 to 1.24)	-0.001 (-0.014 to 0.012)	-.027 (-.076 to .022)	0.017**	0.091*	-0.001	0.000	0.77	8.77	16.00
82	Serum P1NP[b]	53.63 (23.13 to 84.14)	-0.168 (-1.36 to 1.02)	.624 (-2.40 to 3.65)	31.160**	818.190**	-7.70	3.920*	385.01	393.01	400.24
Docosatetranoic acid[a]											
83	Serum CTX[b]	.622 (.253 to .990)	-0.001 (-0.016 to 0.013)	-.361 (-1.79 to 1.07)	0.018***	0.086*	0.000	N/A	-5.03	2.97	10.20
84	Serum P1NP[b]	42.10 (19.87 to 64.33)	0.189 (-1.04 to 1.42)	73.64 (1.79 to 145.48)*	24.460**	865.970**	-15.57	4.160*	374.86	382.86	390.09

β_{00}=average at baseline (visit 1). β_{10} = mean Δ on DV per visit. β_{20}=mean Δ in DV per 1% increase in serum fatty acid

τ_{00}= estimate of variance in y-intercepts between subjects. τ_{01} = estimate of covariance between slopes and intercepts. τ_{11}=estimate of variance in slopes between subjects.

[a]=IV [b]=expressed as nanograms per milliliter.

N/A=no random effects were observed.

*p<.05 **p<.01 ***p<.001 †p<.10

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