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The effect of n-3 fatty acids on bone biomarkers in Iranian postmenopausal osteoporotic women: a randomized clinical trial

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Abstract Recently, n-3 fatty acids are in the center of attention for their potent anti-inflammatory effects. Osteoporosis as a chronic senile disease is associated with inflammation, and the role of inflammatory mediators has been demonstrated in recent years. The beneficial effects of n-3 fatty acids on bone were proven in many animal studies, while to date, no conclusive data is available in human. The aim of this study was to evaluate the impact of n-3 fatty acids on bone biomarkers in osteoporotic postmenopausal women. Twenty-five osteoporotic postmenopausal women were recruited in the study and randomized in treatment and control groups. The patients received 900 mg n-3 fatty acid capsules or placebo per day for 6 months. Serum levels of osteocalcin, bone alkaline phosphatase (BALP), calcium, vitamin D, and parathormone and urine concentration of pyridinoline (Pyd) were measured at baseline, second month, and sixth month in both groups. In the treatment group, compared with baseline, at the second month, osteocalcin increased slightly; thereafter, it showed decrement trend until the end of the study. In the control group, it decreased all over the study. None of these changes was significant. BALP showed nonsignificant decrease from baseline over the time in both groups. Urine level of Pyd decreased significantly ($P<0.05$) in the treatment group, while no significant change was seen in the control group. Serum calcium and vitamin D increased in both groups; however, changes were not significant. No significant changes were seen in calcium clearance and parathormone. In conclusion, n-3 fatty acids can decrease bone resorption; however, it could not affect bone formation significantly after 6 months treatment. Further investigations are recommended.

Keywords Osteoporosis · n-3 fatty acids · Osteocalcin · Bone alkaline phosphatase · Pyridinoline

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Introduction

Osteoporosis as a common disease in elderly population delineates weak bone density, which follows by bone

fracture (Larijani et al. 2006). The World Health Organization defined osteoporosis as a bone density score at or below 2.5 standard deviations (T-score) below normal peak bone values for young adults (National Institutes of Health (NIH) Consensus Development Panel on Osteoporosis Prevention, Diagnosis, and Therapy) (Anonymous 2001). Osteoporosis encounters elderly population especially postmenopausal women with frequent bone fractures and disability that needs special attention (Chrischilles et al. 1994). Bone fractures due to osteoporosis are caused by both skeletal (bone loss) and nonskeletal problems (impaired balance and muscle loss) NIH 2000). Normal muscle mass causes a direct tension on bone and greater bone formation at remodeling process (Twiss et al. 2009). Bone loss as well as muscle loss occurs by increasing age and almost at the same time. Bone density, which defines bone strength, is a contraction between bone formation and bone resorption. In the bone renewal process, special cells such as osteoclasts and osteoblasts contribute, resulting in production of specific bone resorption and formation markers. From two decades ago, the role of receptor activator of nuclear factor-kappa B ligand (RANKL) and osteoprotegrin (OPG) pathway have been demonstrated in bone remodeling. RANKL is expressed on osteoclasts and stimulates bone resorption, while osteoblasts produce OPG as a receptor for RANKL, then osteoclast formation is inhibited. Therefore, the balance of RANKL/OPG is considered as a controlling module for bone metabolism (Yasuda et al. 1998; Silvestrini et al. 2005). Given this fact, every factor affecting this pathway will influence bone-remodeling cycle. Regarding the pathogenesis of osteoporosis, various investigations have demonstrated the association between inflammation, hormones, growth factors, paracrine and autocrine extractions, homocystein, oxidative stress, and bone fragility (Abdollahi et al. 2005; Yousefzadeh et al. 2006; Salari et al. 2008a, b). In this regard, interleukin-1 (IL-1β) and tumor necrosis factor-α (TNF-α) provoke bone resorption. Over the past decades, strong evidences supported the benefits of n-3 fatty acids in bone health. Several mechanisms have been proposed; however, neither the exact benefit nor the exact mechanism of action of essential fatty acids was determined. It has been postulated that polyunsaturated fatty acids can alter the production of IL-1β, IL-6, and TNF-α by modulating prostaglandin production. Therefore, this modifying effect plays a crucial role in the pathogenesis of osteoporosis. According to our former systematic review, while animal studies support this theory, human studies are not encouraging, and there are no compelling data that can label them as prophylactic agents in osteoporosis (Salari et al. 2008b). This fact guided us into further investigation in this field. The limited numbers of clinical trials for evaluating the relationship between bone health and n-3 fatty acids forwarded us into designing a randomized clinical trial. Biochemical bone markers are secreted during bone remodeling and can be measured in serum or urine, also recently, their use has been increased because of their dose-effect relationships with several drugs, ease of use, and fast change according to the bone status (Cremers and Garnero 2006).

Given their easy application for evaluating bone strength and the above-mentioned possible benefits of polyunsaturated fatty acids on bone, we designed the present study for further assessment of polyunsaturated fatty acids and their role in health, which may provide more evidence for their usage as a supplement.

Materials and methods

The study was authorized by the ethics committee of the endocrinology and metabolism research center of Tehran University of Medical Sciences, and all of the

Table 1 Baseline characteristics of two group patients

	Treatment	Control
Age (year)	60±5.6	63±8.92
T-score	−3.0±0.64	−3.1±0.9
OC (ng/ml)	12.8±9.7	16.4±7.38
BALP (μg/L)	13.2±5.5	13.6±5.49
Ca (mg/dl)	9.97±0.29	9.67±0.3
VitD (nmol/L)	78.5±48.9	39.7±17.6
PTH (pg/ml)	35.4±12.68	50.7±31.26
Pyd (ng/ml)	4.10±52.87	3.93±160.03
CaCl (%)	1.02±0.45	0.64±0.21

Data are mean ± SD

OC osteocalcin, *BALP* bone alkaline phosphatase, *Ca* calcium, *Vit D* vitamin D, *PTH* parathormone, *Pyd* pyridinoline, *CaCl* calcium clearance

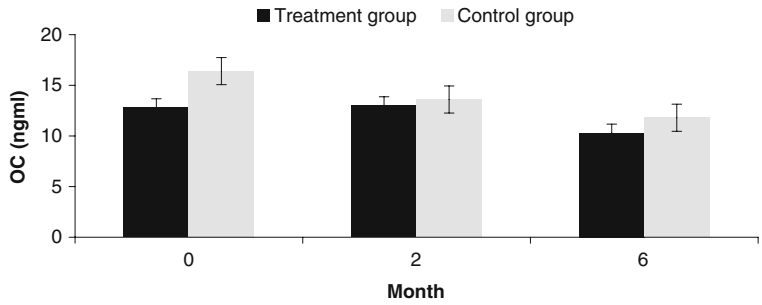

Fig. 1 The changes in serum osteocalcin in two groups in 6 months. Data are in mean ± SD. *OC* serum osteocalcin concentration

patients gave the informed consent. Twenty-five postmenopausal osteoporotic women were entered into the study. All patients' osteoporosis was diagnosed by dual energy X-ray absorptiometry at femur neck and lumbar vertebrae. Their profile was assessed to obtain information about age, menopause state, smoking, alcohol consumption, and past medical history. Patients with history of cancer, diabetes, acute infection, endocrinologic disease, use of special medications such as corticosteroids, hormones, GnRH analogs, anticonvulsive drugs, heparin, aluminum-containing antacids, thyroid hormones, and smoking or alcohol consumption were excluded.

Thirteen patients in the treatment group and 12 patients in the control group were randomized. The patients received three omega 3 capsules (900 mg n-3 fatty acids per day) or three placebo capsules (three times a day after meal) in the treatment and control groups, respectively. All patients were asked to have a normal diet with seafood only zero to one time a week, not to consume much dairy products, and seriously not to change the food regimen until end of the study. Also, they were recommended to inform us upon any change in their medication used or any lifestyle changes. No lifestyle or medication changes were recorded.

Omega 3 capsules and its placebo were provided by Zahravi Pharmaceutical Co. (Iran). Both omega 3 capsules were similar in taste, texture, and appearance. The patients were supplemented for 6 months. Blood and urine samples were collected at baseline, second month, and sixth month post-treatment. At these three time occasions, in the early morning and fasting state, blood and urine samples were collected. Blood samples were centrifuged at 10,000×g for 10 min and kept in a fridge at −70°C until analysis. Serum levels of bone biomarkers such as osteocalcin (OC), bone alkaline phosphatase (BALP), as well as serum creatinine, calcium, vitamin D, and parathormone (PTH) along with urine concentrations of pyridinoline (Pyd), calcium, and creatinine were measured. Serum OC and BALP were measured by N-MiD Ostaocalcin® enzyme-linked-immunosorbent serologic assay (ELISA) Immuno Dignostic Systems (IDS) and OSTASE® BAP EIA (IDS, UK), respectively. Urine Pyd was measured by CUSABIO® (USA). Serum calcium, vitamin D, and PTH concentrations were measured by Ca PARS-AZMON® (Iran), 25 Hydroxy Vitamin D by D-EIA® (IDS, UK), and Intact PTH by ELISA (BIOMERICA®, Germany), respectively.

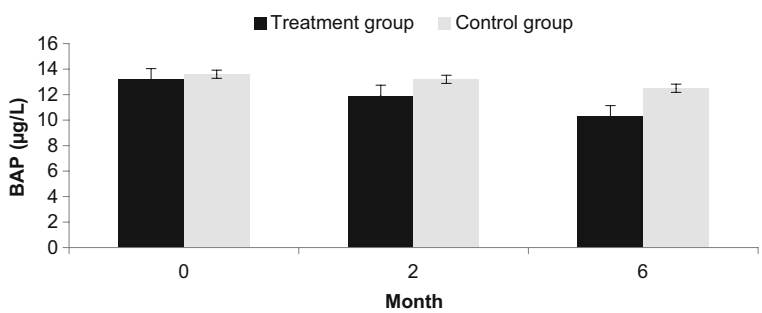

Fig. 2 The changes in serum bone alkaline phosphatase level in two groups in 6 months. Data are in mean ± SD. *BALP* serum bone alkaline phosphatase concentration

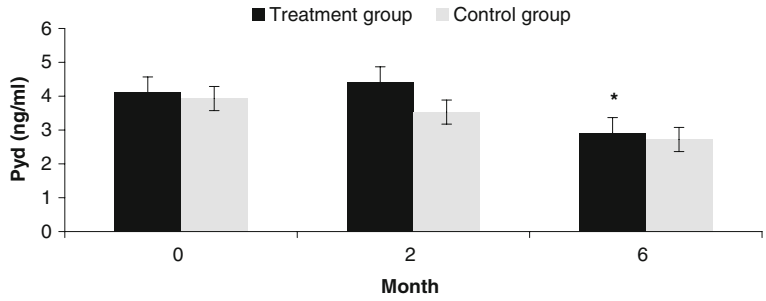

Fig. 3 The changes in urine Pyd concentrations in both groups in 6 months. Data are in mean ± SD. *Asterisk*: significant changes ($P<0.05$), *Pyd* urine pyridinoline concentration

The fractional excretion of Ca (Calcium clearance) was measured via the equation below:

Calcium clearance = Uca. Scr. 100/Sca. Ucr

where Uca=urine Ca concentration, Scr=serum creatinine concentration, Ucr=urine creatinine concentration, and Sca=serum Ca concentration

Statistical analysis

Statistical Package for the Social Sciences software version 16 was used for statistical analysis. Data are expressed as mean ± SD. Results were analyzed by independent samples *t* test and General Linear Model (repeated measures) test. Pearson correlation was used for assessing the correlations. $P<0.05$ was considered significant.

Results

We enrolled a total of 25 postmenopausal osteoporotic women in the study. The mean ± SD of their bone mineral density (BMD) was −3.1±0.74. We randomized the study patients into two groups: treatment (13 patients) and control (12 patients). Only 18 patients (ten in the treatment group and eight in the control group) completed the study. The dropout rate during 6 months was about 28%, which was because of ill health unhooked to the study, family problems, distance problems, frequent decline of appointments, non-compliance, and weight gain which occurred just in one patient. The demographic characteristics of two groups' patients who completed the study are shown in Table 1. All of the data are in mean ± SD. None of these women smoked. Treatment and control groups did not differ significantly at the baseline.

Changes of bone formation markers in 6 months

No significant changes were observed in serum level of bone formation markers. Both OC and BALP decreased after the start of the study. In the treatment group after 2 months, OC level increased slightly, but after 6 months, it decreased in both groups; however, the changes were not significant across the two groups ($P>0.05$, Figs. 1 and 2).

Changes of bone resorption markers in 6 months

Urine Pyd decreased significantly ($P<0.05$) after 6 months in the treatment group, while no significant

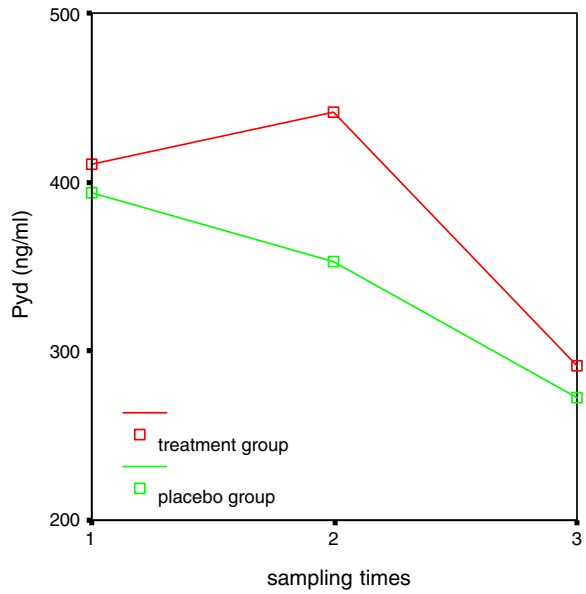

Fig. 4 Changes in urine concentration of Pyd in 6 months in both groups. *Pyd* urine pyridinoline level

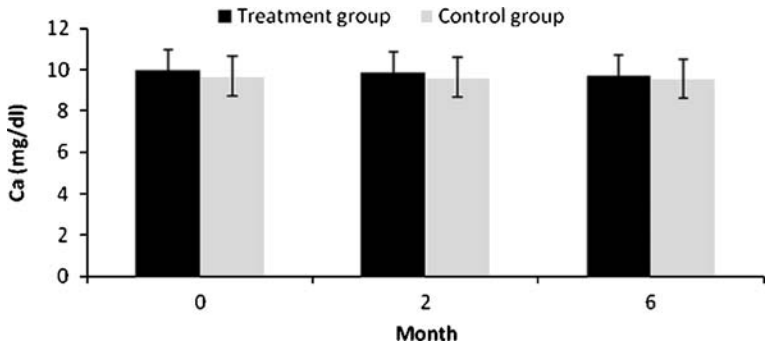

Fig. 5 The changes in serum calcium level in both groups beyond 6 months. Data are in mean ± SD. *Ca* serum calcium level

change was documented in Pyd in the control group (Figs. 3 and 4). The effect-size (r) for this change was 0.30, and the Cohen's d was 0.62.

Changes of calcium, vitamin D, and PTH in 6 months

We found serum calcium changes similar to changes in BALP in 6 months in two groups, which were not significant ($P>0.05$; Fig. 5). Serum vitamin D level increased significantly in both groups after 2 months, and these increments remained statistically significant until 6 months ($P<0.05$, Fig. 6). Serum PTH level decreased after 2 months in both groups and then showed trends toward increasing from baseline, but the changes were not significant ($P>0.05$, Fig. 7).

Changes of calcium clearance in 6 months

No statistically significant change was observed in Ca clearance in both groups in the study period (Fig. 8). The significant relationship between OC and BALP at the beginning was observed ($P<0.05$). Those markers showed significant correlation after 2 months ($P<0.05$). Serum calcium at the baseline was correlated with vitamin D remarkably ($P<0.05$).

Discussion

While the importance of the essential fatty acids in bone health and calcium homeostasis was confirmed in several animal studies (Salari et al. 2008b), to date, no conclusive data is available in human. Although our study demonstrated no significant effect of n-3 fatty acids on bone formation markers (OC and BALP) in osteoporotic women, it showed significant beneficial effects of n-fatty acids on bone resorption marker (Pyd). OC decreased in the study period in both groups, but the changes were not significant ($P>0.05$). In contrast, urine concentration of Pyd decreased in the treatment group significantly at 6 months. It has been shown that the relative amounts of dietary polyunsaturated fatty acids may play a crucial role in maintaining skeletal integrity in the aged patients (Weiss et al. 2005). Another study showed significant increase in serum OC level in the

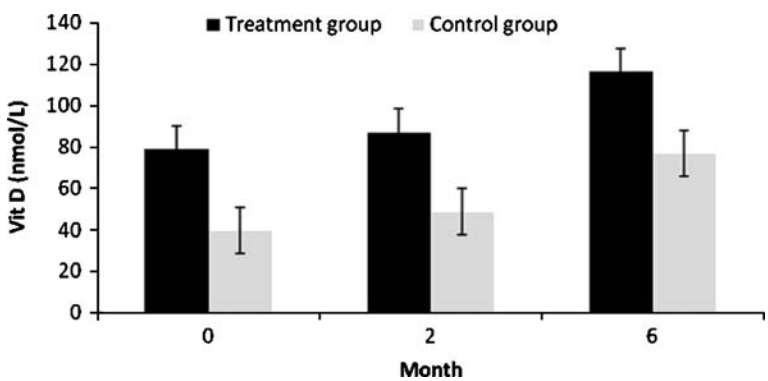

Fig. 6 The changes in serum vitamin D level in both groups beyond 6 months. Data are in mean ± SD. *Vit D* serum vitamin D level

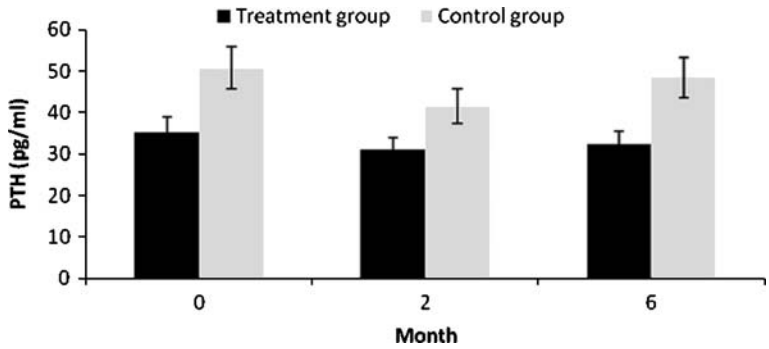

Fig. 7 The changes in serum parathormone in both groups beyond 6 months. Data are in mean ± SD. *PTH* serum parathormone concentration

fish oil patients receiving 4 g/day of fish oil after 16 weeks in comparison to the evening primrose oil group, while BALP decreased significantly in the fish oil group (van Papendrop et al. 1995). Later, it was observed that maintaining lumbar bone mass and increasing femoral bone mass in postmenopausal women is available by consuming polyunsaturated fatty acids for 18 months that accompanied with decreasing OC and deoxypyridinoline levels in both treatment and control groups (Kruger et al. 1998). A significant decrease in the total body BMD in both treatment and control groups after consumption of 440 mg fish oil or calcium for 1 year in the healthy postmenopausal women has been reported while they showed significant decreases in the serum markers of bone formation (OC and BALP) without any significant change in urine N-telopeptide (NTx) concentration in both groups (Bassey et al. 2000). Greater BMD loss in the femoral neck was associated with increased intake of polyunsaturated fatty acids in another study (Macdonald et al. 2004). In renal transplant patients, a link was found between plasma phospholipid n-3 polyunsaturated fatty acids content and BMD (Baggio et al. 2005). Although no change in levels of BALP in n-3 fatty acid consuming group was found, a significant decrease in NTx levels was evident (Griel et al. 2007). Some recent studies indicate a positive relationship between n-3 fatty acids especially DHA and peak BMD in young men (Hőgstrom et al. 2007). Recently, the influence of moderate energy restriction and seafood on bone turnover was evaluated, and a significant decrease in serum OC during 8 weeks in young adults was reported while serum level of BALP did not change significantly. In addition, a significant increases in the bone resorption markers (N-telopeptides of type I collagen in urine and C-terminal telopeptide of type I collagen) was found (Lucey et al. 2008). Some other investigators reported negative impact of polyunsaturated fatty acids on bone as increased risk of fractures in elderly population (Martínez-Ramírez et al. 2007). There are notable animal studies demonstrating controversial effects of n-3 fatty acids on bone as evidenced by negative effects on bone markers and lower urinary calcium (Shen et al. 2006; Watkins et al. 2006).

We calculated calcium clearance as a simple and clinically relevant method for evaluating renal tubular

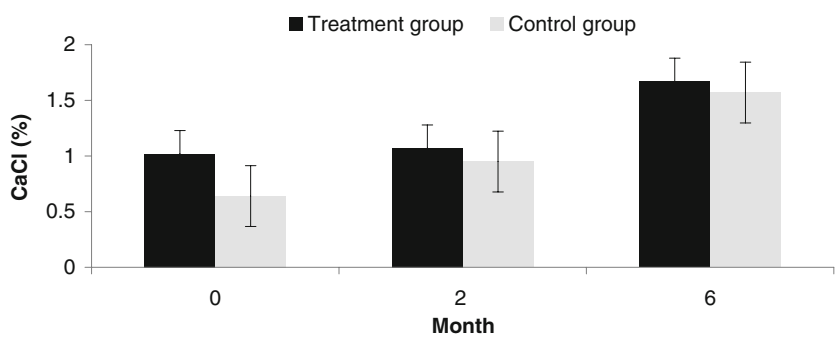

Fig. 8 Changes in calcium clearance in both groups beyond 6 months. Data are in mean ± SD. *CaCl* calcium clearance

handling of calcium. It was observed that one of the mechanisms of actions of fatty acids on bone is orchestrated via increasing intestinal calcium absorption, which can alter bone metabolism (Sun et al. 2004; Gilman and Cashman 2007). We saw trends toward decreasing serum calcium, vitamin D ($P<0.05$), and PTH levels in both treatment and control groups, while there was no significant change in calcium clearance in both groups. In support, no significant changes in serum level of parathyroid hormone and calcium by fatty acids treatment has been already reported (Bassey et al. 2000). A trend toward increasing serum calcium because of higher intestinal calcium absorption in the fish oil group in parallel with increased calcium clearance was found (van Papendrop et al. 1995). It was shown that increased salmon intake is associated with increased serum 25-hydroxyvitamin D ($P<0.05$), while serum PTH was unaffected by fish intervention in overweight young adults (Lucey et al. 2008).

Comparing our results with other studies, our results are similar to some of the studies, while those are not conclusive and mostly confirm the beneficial effects of n-3 fatty acids on bone by decreasing bone resorption. It has been presumed that n-3 fatty acids can affect bone via different mechanisms (by affecting bone formation, bone resorption, serum calcium and vitamin D, and inflammatory mediators), but the exact mechanism of action has not been determined yet. This is because of lack of enough clinical trial studies that mostly have been performed by food questionnaire, and no data is present about the dose of n-3 fatty acids. Our patients took about 900 mg n-3 fatty acids daily which had no significant impact on bone formation markers, while their beneficial effect on bone resorption (Pyd) was observed.

Study limitations

Time shortage was one of our study limitations. Our study was done in 6 months, and it seems more reasonable to design a study with a longer duration of at least 1 to 2 years to evaluate the changes of BMD in parallel with bone markers, which can give us more reliable results. It should be noted that a decrease in bone resorption takes time to be reflected by increasing in bone formation, which is anticipated to happen after a delay. In addition, it was shown that about 6–7 weeks after resorption, osteoblast enrollment occurs (Eriksen et al. 1984). Also, our study lacks data on BMD and physical activity; however, the sample size seems to be insufficient. Insufficient resources forced us into a limited sample size with limited numbers of bone markers. In addition, taking three big soft capsules a day is tough enough to ensure patients compliance.

Therefore, it seems reasonable to perform a multicenter clinical trial in a vast majority of subjects in a longer period of time by providing potent soft capsules of n-3 fatty acids (at least 1 g) along with performing the survey on inflammatory mediators and their relationship with bone markers.

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