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Research article

The calciotropic hormone response to omega-3 supplementation during long-term weight-bearing exercise training in post menopausal women

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Abstract
The purpose of this study was to examine the effects of ingestion of omega-3 (n-3) and aerobic exercise intervention on the calcium regulating hormones in healthy postmenopausal women. To this end, 56 healthy sedentary postmenopausal women with mean age 57.7 ± 3.5 yrs participated in this study. Participants were randomly divided into exercise plus supplement (E+S; n = 14), exercise (E; n = 14), supplement (S; n = 14) and control (Con, n = 14) groups. The subjects in E+S and E groups performed aerobic exercise training (walking and jogging) up to 65% of exercise HRmax, three times a week for 16 weeks. Subjects in E+S and S groups were asked to consume 1000 mg/d omega-3 for 16 weeks. The blood ionized Calcium (Ca^{+2}), Parathyroid hormone (PTH), estrogen and Calcitonin (CT) were measured before and after 16 weeks of exercise training. Results indicated that consuming 1000 $mg \cdot day^{-1}$ omega-3 during 16 weeks and or the aerobic exercise, significantly increased CT ($p = 0.001$) in E+S, E and S groups and significantly decreased PTH ($p = 0.001$) levels in E+S and E groups, also significantly increased estrogen ($p = 0.024$) levels in E+S and E groups, but had no significant effects on blood Ca^{+2} ($p = 0.619$) levels. The results of present study demonstrate that omega-3 in combination with regular aerobic exercise training have significant effects on serum CT, estrogen and PTH in non-athletic postmenopausal women, suggesting that participating in moderate intensity weight-bearing exercise and incorporating sources of omega-3 in the diet a possible intervention to help slow the loss of bone that occurs following menopause.

Key words: Postmenopausal women, physical activity, parathyroid hormone, calcitonin, omega-3 fatty acids, bone mineral density.

Introduction

Post-menopausal oestrogen deficiency is associated with the changes in the calcium regulatory hormones, with a pronounced calcitonin (CT) deficiency (Stevenson et al., 1981). The decreased CT level and increased parathyroid hormone (PTH) may result in an increased rate of bone turnover, which, in turn could be an important factor in the pathogenesis of post-menopausal bone loss (Grimston et al., 1993). The skeletal benefits of weight bearing exercise in post-menopausal women have been well documented in a multiple of randomized controlled trails (Feskanich et al., 2002; Kemmler et al., 2002). Regarding that PTH and CT are valuable indirect indicators of bone metabolism and health, the measurement of serum PTH and CT may be helpful in evaluating the effects of exercise on bone metabolism (Brahm et al., 1996; Rong et al., 1997). The effect of exercise on calciotropic hormone level has received limited attention, and studies in this area have not yield consistent results (Iwamoto et al., 2001; Zerath et al., 1997). Nevertheless, long-term moderate intensity exercise has been shown to provide positive effects on bone metabolism. Iwamoto et al. (2001) in a study examining the effects of a 7-11 weeks exercise training on 6-weeks old female Wister rats found a significant decrease in serum PTH, suggested that this decrease can result in an increased bone mass with stimulation of longitudinal bone growth. Alev et al. (2003) evaluated the response of calcium regulatory hormones to aquatic exercise in post-menopausal women and reported an increase in CT and decrease in PTH levels after the exercise program (Alev and Yurtkuran, 2003). A previous prospective study showed that endurance-trained postmenopausal women had lower PTH levels and improved calcium absorption as compared with their sedentary counterparts (Nelsen et al., 1988).

The negative association between PTH and bone mineral density (BMD) is well documented (Grimston et al., 1993). According to a recent report by the women's health initiative (Rossouw et al., 2002) noted, in addition to participating in regular exercise program, improving the diet can minimize bone loss and even increases bone mineral density in postmenopausal women, thereby increasing their ability to prevent bone loss after menopause. One of the potential nutritional candidates is polyunsaturated fatty acids (PUFA). A growing body of evidence indicates that dietary supplementation with PUFAs, in particular omega-3 (n-3), may be beneficial in bone health (Amy et al., 2007; Matsushita et al., 2008), reported that n-3 have an inhibitory activity on osteoclasts and enhance the activity of osteoblasts in animals (Sun et al., 2003). A recent observational study demonstrated that in older adults high intake of omega-3 fatty acids (self-reported) was associated with higher total femoral BMD (Amy et al., 2007). Also, recent epidemiological studies suggest that consumption of n-3 PUFAs may attenuate post-menopausal bone loss (Matsushita et al., 2008; Sun et al., 2003; Vanek and Connor, 2007). Terano et al. (2001) compared the BMD of 132 men and women aged 38–80 years living in a fishing village in Japan with that

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of 332 age-matched urban control subjects and found that the women living in the fishing village, who consumed larger amounts of n-3 fatty acids, had greater radial BMD than did the controls (Terano, 2001). There have been limited studies in humans using PUFAs aimed at preventing bone loss. However, no study has been reported to investigate the effects of PUFAs on calcium homeostasis and calcium regulatory hormones. Calciotropic hormones are valuable indices for assessing bone metabolisms and a positive relationship exists between PUFAs and BMD, and also between long-term weight-bearing exercise and bone health, the present study was designed to assess the combined effects of 16 weeks moderate intensity weight-bearing exercise and n-3 supplementation on calciotropic hormone levels in healthy post-menopausal women. To our knowledge, no study has been conducted concerning the response of calciotropic hormones to n-3 consumption in combination with long-term moderate intensity exercise.

Methods

Subjects

Fifty-six post-menopausal women aged 55-69 years volunteered and gave written consent for the study which was approved by the Human studies committee of Urmia University, IRAN. The women were sedentary, in good health, at least 5 years past-menopause, and taking no medications. Preliminary screening included a medical history, physical examination, and Bruce treadmill test (Bruce and Hornsten, 1969). The serum concentration of Ca^{+2}, CT, and PTH were measured before and after 16 weeks. The participants were randomly assigned to exercise + supplement (E+S, n = 14), exercise (E, n = 14), supplement (S, n = 14), and control (Con, n=14) groups. Leisure, household, and occupational activity was estimated with the Physical Activity Scale for the Elderly Questionnaire (Washburn et al., 1999) (Table 1).

Oral omega-3

The E+S and S groups were supplemented with n-3 capsules [Viva omega-3 fish oil, Canada], containing 180 mg EPA and 120 mg Docosahexaenoic acid (DHA), to supply a total of 1000mg/day (Burr et al., 1989) of n-3 PUFA fatty acids over 16 weeks (Van Papendorp et al., 1995). Both prospective, randomized secondary prevention studies were based on increased intake of fatty fish in addition to other healthy dietary habits, or supplementation of the diet with n-3 PUFA, seem to indicate that ~1 g/d of these fatty acids is beneficial (Burr et al., 1989). The degree of compliance with n-3 supplements, as determined by pill counts, was 98 ± 8%. Also, incorporation of EPA and DHA into the cell membranes of neutrophils was measured. Individual information was collected by trained interviewers in face to face interviews based on a structured and previously validated questionnaire that included the following: socio-demographic data; years since menopause; physical activities, including hours spent sitting, standing, walking, sports, and leisure activities; medications; smoking and drinking alcohol; and other factors that may have possible confounding effects on the relation between dietary omega-3 consumption and metabolism of bone and lipid. (The Ministry of Science and Technology 2004 the Fifth Revision of the Standard Table of Food Composition in Japan). The participants were instructed to record the contents of daily meals, snacks, and beverages in their diet diary. The diary was collected routinely for the first 3 days to confirm the meal intake, and, if necessary, the participants were immediately instructed to adhere to the dietary regimen (Wu et al., 2006). Daily intakes of calcium, vitamin D, carbohydrates, lipids, saturated fatty acid, mono unsaturated fatty acid, polyunsaturated fatty acid, proteins and, total energy were calculated from the daily record by the dietitian on the basis of the fifth revision of the standard tables of food composition in Japan (The Ministry of Science and Technology 2004 the Fifth Revision of the Standard Table of Food Composition in Japan). Data from the diary was used to check compliance with their diet and that dietary intakes did not alter more than would be expected over the 16 weeks of study (Table 4). Information on use of medications and drugs was also obtained through standard questionnaire and self-reported questionnaire in accordance to researcher recommendations (Weiss et al., 2005).

Exercise program

Subjects in E+S and E groups walked or jogged on a treadmill 25–30 $min \cdot day^{-1}$, 3–4 $days \cdot week^{-1}$, 45-55% of their individually determined HR_{Max}. As their exercise tolerance improved, the intensity and duration of walking were increased to 40–45 $min \cdot day^{-1}$, 4–6 $days \cdot week^{-1}$; at an intensity of 55–65% of HR_{Max}. Adherence to the exercise prescription was documented through the use of Polar heart rate monitors. Subjects received feed-back if training intensities were either high or low in comparison with desirable intensities. Attendance was taken at each exercise session to monitor compliance with the program. Subjects were contacted if an exercise session was missed. In those women who completed the interventions, there was > 90% compliance for attendance at the

Table1. Individual characteristics of postmenopausal women. Data are means (±SD).

Variables	E + S (n = 14) 1	E (n = 14) 2	S (n = 14) 3	CON (n = 14) 4	P <
Age (yr)	58.2 (2.3)	57.1 (7.5)	58.5 (2.1)	57.2 (2.2)	.681
Height (m)	1.67 (.06)	1.69 (.09)	1.67 (.11)	1.69 (.11)	.529
Weight (kg)	70.7 (9.8)	71.7 (12.8)	71.3 (15.7)	70.6 (12.6)	.751
BMI ($kg \cdot m^{-2}$)	25.2 (5.5)	27.9 (3.9)	27.4 (2.3)	26.5 (3.1)	.877
Fat (%)	26.5 (3.1)	27.4 (4.7)	28.6 (3.8)	26.4 (5.4)	.617
VO_2max	32.9 (3.1)	33.2 (4.3)	32.8 (2.9)	32.5 (3.4)	.086
PAS	137 (66)	140 (62)	139 (54)	137 (62)	.074

E+S = Exercise + Supplement; E = Exercise; S = Supplement; CON = Control; BMI = Body Mass Index; VO_2max= maksimal oxygen uptake ($ml^{-1} \cdot kg^{-1} \cdot min^{-1}$); PAS = Physical activity score.

Table 2. Baseline and change from baseline (CfB) (After - Before) data of serum calciotropic hormones and Ca^{+2} levels in different groups of postmenopausal women. Data are means (±SD).

Variables		E + S (n = 14) 1	E (n = 14) 2	S (n = 14) 3	CON (n = 14) 4	P <
PTH (pmol·ml^{-1})	Baseline	7.4 (42)	42.2 (5.9)	42.3 (8.6)	42.1 (6.9)	.978
	CfB	-23.6 (8.2) †^{2,3,4}	-14.9 (12.5) †^{1,3,4}	6.9 (12.7) ^{1,2,4}	8.3 (9.9) ^{1,2,3}	**.001**
CT (pg·ml^{-1})	Baseline	1.3 (.4)	1.3 (.6)	1.3 (.5)	1.3 (.7)	.690
	CfB	19.3 (6.7) †^{2,3,4}	13.7 (6.6) †^{1,3,4}	7.8 (3.6) †^{1,2,4}	-.2 (.4) ^{1,2,3}	**.001**
Ca^{2+} (mmol·ml^{-1})	Baseline	1.2 (.4)	1.2 (.4)	1.2 (.2)	1.2 (.5)	.941
	CfB	-.0 (.5)	-.1 (.6)	-.1 (.7)	.1 (.3)	.619
Estrogen (pg·ml^{-1})	Baseline	14.3 (4.6)	15.2 (6.4)	14.5 (6.6)	13.0 (4.0)	.0712
	CfB	11.1 (3.1) †^{2,3,4}	7.3 (1.1) †^{1,3,4}	2.2 (3.2)	-2.8 (1.7)	**.024**

E+S= Exercise + Supplement; E= Exercise; S= Supplement; Con= Control; CfB = Change from Baseline; PTH= Parathyroid Hormone; CT= Calcitonin; Ca^{+2}= Ionized Calcium. Superscripts denote significantly differences among the groups. † $p < 0.05$, significantly different from baseline values (within groups, baseline *vs.* week 16).

exercise sessions. All exercise sessions began between 0600 and 0800 a.m (Seals et al., 1997). S and Con group subjects were instructed to maintain their current physical activity levels during the study (Wu et al., 2006).

Blood sampling

Fasting blood samples were collected 24h before and 16 weeks after the exercise training program. Serum was obtained through centrifugation at 4 ^{0}C and then stored at –70 °C until analyzed for biochemical markers of bone metabolism. Ca^{+2} was assessed on a 634 Ca analyzer (Bayer, USA). PTH was measured with a chemiluminescent luminometer (IMMULITE, Diagnostic products corporation, USA). CT was determined by radioimmunoassay (RIA) with a polyclonal antiserum directed against the carboxyterminus. The antibodies were produced in rabbits against human CT, using a calcitonin kit (CIS Bio International ORIS groups, franc). Serum estrogen levels was detected by a chemiluminescent method (Roche Diagnostics, Indianapolis, IN, USA) using an automatic immunoanalyzer. Neutrophils (>95%) were purified from 10 ml of anticoagulated venous blood by means of dextran sedimentation (Pharmacia, Milton Keynes, Bucks, UK) and centrifugation on a cushion of lymphoprep (Nyegaard, Brimingham, UK) (Boyum et al., 1968) and stored under argon at -70 ^{o}C before extraction of phospholipids using the method developed by Bligh and Dyer (Bligh et al., 1959). Fatty acid composition was analysed using gas chromatography using known standards (Prickett et al., 1981).

Statistical analysis

Mean changes from baseline to 16 weeks were calculated and compared within groups by paired *t*-test. Differences between groups were determined by analysis of variance (ANOVA) for repeated measures, for continuous variables. The statistical software program SPSS for windows, version 13.0 was used for data analysis. All statistical tests were performed and considered significant at a $p \leq 0.05$.

Results

At baseline there were no significant differences between the groups (Table 1). CT levels in E+S, E, and S groups were significantly ($p < 0.05$) increased in post-exercise as compared with baseline values. There was no significant change in CT level for Con group from baseline to post-exercise ($p = 0.591$). E+S and E groups showed significant decrease in PTH level after 16 weeks exercise training ($p < 0.05$), however the slight decline in PTH in S group was not significant. The Con group showed an increase ($p > 0.05$) in PTH level after training period. In contrast, Ca^{+2} did not show any significant difference between baseline and post-exercise values in all groups ($p > 0.05$) (Table 2).

The results of the analysis of variance (ANOVA) for repeated measures revealed that the baseline values of CT, PTH, and Ca^{+2} were not significantly different between the groups ($p > 0.05$). However, significant differences ($p < 0.05$) were observed between the groups in CT and PTH post-exercise. Estrogen levels in E+S and E groups were significantly ($p < 0.05$) increased post-exercise as compared with baseline values. There was no significant change in estrogen levels for S and Con groups from baseline to post-exercise ($p = 0.071$). Ca^{+2} did not show any significant difference between the groups post-exercise ($p > 0.05$) (Table 2). The results also indicated that the observed differences in the PTH levels were more obvious between E+S group with E, S, and Con ($p < 0.05$) groups, and also between E group with S and Con groups ($p = 0.002$). Significant difference was also observed between S and Con groups for the post-exercise PTH levels ($p = 0.047$). Obvious differences in CT levels were observed between E+S group with E, S, and Con ($p = 0.001$) groups, and also between E group with S and Con groups ($p < 0.05$). Significant difference was also observed between S and Con groups in post-exercise CT levels ($p = 0.001$). Therefore, it seems that serum calciotropic hormone levels can mainly be affected by combined actions of exercise training and n-3 supplementation rather than exercise training or n-3 alone. Also, significant differences in the estrogen level observed between E+S and E groups, suggest the importance of exercise to help with this hormone. There were no significant differences between S and Con group in post exercise estrogen levels ($p > 0.05$).

Neutrophil phospholipids PUFA content is expressed as a percentage of total phospholipid. Fatty acid content is presented in Table 3. No significant changes ($p > 0.05$) were observed in neutrophil membrane content when comparing linoleic acid (LA), arachidonic acid (AA), EPA, and DHA values in the E and Con groups before and after the study. However, following n-3

Table 3. Baseline and change from baseline (After - Before) data of fatty acid composition of neutrophil extracts in different groups of postmenopausal women. Data are means (±SD).

Variables		E + S (n = 14) 1	E (n = 14) 2	S (n = 14) 3	CON (n = 14) 4	P <
18:2♀LA	Before	16.8 (1.6)	16.9 (2.8)	16.6 (2.6)	16.7 (2.9)	.591
	After	8.2 (1.1) †[2,4]	17.3 (2.6)[1,3]	8.1 (1.9) †[2,4]	17.3 (2.8)[1,3]	**.002**
20:4♀AA	Before	22.9 (4.6)	23.4 (4.1)	22.8 (4.3)	23.1 (3.9)	.081
	After	13.1 (4.2) †[2,4]	22.9 (3.8) [1,3]	13.2 (3.9) †[2,4]	22.9 (3.6) [1,3]	**.001**
20:5♀EPA	Before	.3 (.9)	.3 (.2)	.3 (.3)	.3 (.2)	.263
	After	4.2 (1.9) †[2,4]	.2 (.2) [1,3]	4.1 (1.8) †[2,4]	.2 (.2) [1,3]	**.006**
22:6♀DHA	Before	2.2 (1.8)	2.2 (1.9)	2.2 (1.7)	2.2 (1.1)	.767
	After	3.6 (2.1) †[2,4]	2.2 (1.7) [1,3]	3.5 (2.1) †[2,4]	2.2 (1.3) [1,3]	**.028**

E+S= Exercise + Supplement; E= Exercise; S= Supplement; Con= Control; LA= Linoleic Acid; AA= Arachidonic Acid; EPA= Eicosapentaenoic Acid; DHA= Docosahexaenoic Acid , ♀ Ratios represent number of carbon-carbon double bounds. Superscripts denote significantly differences among the groups. † $p < 0.05$, significantly different from baseline values (within groups, baseline *vs.* week 16).

supplementation, the neutrophil phospholipids content of DHA and EPA increased significantly ($p < 0.05$) in E+S and S groups, , while the neutrophil phospholipid content of LA and AA was significantly reduced ($p < 0.05$).

Discussion

The measurements of serum calciotropic hormones may be valuable in the examination of the chronic effects of long-term exercise on bone metabolism. Long-term exercise has been demonstrated to increase BMD through the actions of general calciotropic hormones (Iwamoto et al., 2001). In the present study we have examined for the first time the effects of long-term low-moderate intensity exercise in combination with n-3 on calciotropic hormone levels in post-menopausal women. We hypothesized that long-term exercise in combination with n-3 consumption would affect calciotropic hormone levels and that these changes would be related to BMD. Although, the positive effect of either weight-bearing exercise or n-3 on BMD and bone metabolism have been the focus of some studies (Amy et al., 2007; Matsushita et al., 2008), changes in the calciotropic hormones to n-3 consumption that occur during regular exercise are poorly understood.

In the present study, CT concentration was significantly increased after 16 weeks exercise training in E+S, E, and S groups. The increased CT concentration in this study is in accordance with the findings of Alev et al. (2003) who evaluating the hormonal response of sedentary post-menopausal women to aquatic exercise and found a 54% increase in CT level after exercise program (Alev and Yurtkuran, 2003). However, in the study by Thorsen et al. (1996) a single bout of brisk walking did not change the concentration of CT in post-menopausal women (Thorsen et al., 1996). Lin et al. (2005) also, reported a significant increase in the CT level after endurance and strength exercises (Lin et al., 2005). In most of the studies that reported unchanged CT level after exercise, they have used a single exercise bout. However, our participants performed long-term exercise training that

Table 4. Differences in dietary intakes for the women. Data are means (±SD).

Daily intakes		E + S (n = 14) 1	E (n = 14) 2	S (n = 14) 3	CON (n = 14) 4	P <
Total energy (kcal)	Before	2108 (340)	2141 (374)	2161 (344)	2159 (384)	.092
	After	2181 (361) †	2171 (352)	2189 (329)	2198 (363)	.104
Proteins (g)	Before	80 (18)	76 (17)	82 (11)	78 (15)	.521
	After	76 (14)	79 (19)	80 (13)	81 (10)	.065
Carbohydrates (g)	Before	226 (50)	217 (45)	220 (54)	229 (59)	.061
	After	231 (44)	228 (55)	224 (50)	232 (49)	.124
Lipids (g)	Before	70 (17)	72 (20)	73 (12)	74 (27)	.214
	After	68 (27)	70 (22)	66 (31)	71 (25)	.074
SFA (g)	Before	24 (7)	23 (7)	24 (8)	22 (9)	.325
	After	22 (9)	23 (5)	24 (6)	23 (5)	.078
MUFA (g)	Before	29 (8)	28 (9)	28 (6)	27 (7)	.085
	After	28 (5)	26 (8)	28 (8)	26 (7)	.082
PUFA (g)	Before	11 (4)	12 (6)	10 (9)	11 (5)	.088
	After	12 (8)	12 (7)	11 (8)	11 (9)	.091
Total fibers (g)	Before	16 (7)	18 (7)	17 (6)	17 (9)	.064
	After	17 (7)	18 (9)	18 (8)	17 (4)	.093
Calcium (mg)	Before	691.5 (190.9)	703.8 (221.5)	695.8 (253.5)	691.5 (213.3)	.132
	After	683.6 (181.2)	694.8 (221.4)	688.8 (283.7)	697.7 (203.6)	.089
Vitamin D (µg)	Before	9.8 (5.9)	9.5 (7.1)	9.8 (5.7)	9.6 (5)	.068
	After	9.4 (4.7)	9.3 (10.2)	9.7 (6.9)	9.7 (5)	.099
Vitamin K (µg)	Before	429.8 (172.2)	433.6 (207)	426.3 (211)	431.3 (181.9)	.311
	After	433.9 (182.4)	436.1 (218.7)	429.3 (222.2)	439.8 (199.7)	.114

E+S= Exercise + Supplement; E= Exercise; S= Supplement; Con= Control; BMI= Body Mass Index; SFA= Saturated Fatty Acid; MUFA= Mono Unsaturated Fatty Acid; PUFA= polyunsaturated Fatty Acid, † $p < 0.05$, significantly different from baseline values (within groups, baseline *vs.* week 16).

may explain some of the disparities. Exercise intensity can also be another explanation for different results. The underlying mechanisms the exercise training and n-3 induced to raise the CT level in the present study is unknown. What dose appears clear however is that exercise training and n-3 caused an increase in CT level in postmenopausal women and that the increased CT level can possibly decrease osteoclast-mediated bone resorption in physiological concentrations and contributes to a more positive bone balance.

In the present study all groups except the Con group showed a decreased PTH level after study period, however this reduction was not significant for S group. With regard to the response of PTH to exercise, the reported results are conflicting. It has been shown to be unchanged (Thorsen et al., 1996), decreased (Alev and Yurtkuran, 2003; Iwamoto et al., 2001), or increased (Maïmoun et al., 2006; Zerath et al, 1997). Although the PTH response to exercise has been intensively studied, little is known about the effects of long-term exercise on this calciotropic hormone, especially in post-menopausal women. In the present study low-moderate intensity exercise with and without n-3 resulted in a decreased PTH level. This is consistent with those of Alev and Yurtkuran (2003) who demonstrated reduced PTH concentration in response to aquatic exercise training for sedentary postmenopausal women. However, Thorsen et al. (1996) found no change in PTH levels in response to a single bout of brisk walking in post-menopausal women (Thorsen et al., 1996). Maimoun et al. (2006) also, observed a significant increase in PTH following an exercise program (Maïmoun et al., 2006). It is well documented that the secretion of PTH is mainly regulated by the extracellular Ca^{+2} concentration, and increased Ca^{+2} results in suppression of PTH secretion from the chief cells of the parathyroid glands (Rong et al., 1997). In the present study the Ca^{+2} concentrations was not changed at post-exercise in compared with baseline. However, PTH level was decreased in all groups except the control group after 16 weeks. Regarding that Ca^{+2} concentration was not changed in this study and other factors such as acidosis and catecholamines could have influenced the response of PTH but since the intensity of exercise was not too high it probably did not activate these other mechanisms to induce these responses. It is probable that the exercise and n-3 are the main candidate factors modified the secretion of the PTH in our study. The differences between our results and those of studies reported unchanged or increased PTH may be related to different exercise programs used in these studies. The exercises programs have been used in those studies were relatively short, intense, and were performed for short time (i.e., single bout exercise). However, we used long-term moderate intensity and weight-bearing exercise, which may explain the heterogeneity of results.

It is well known that the concentration of Ca^{+2} is altered by physical exercise (Thorsen et al., 1996), We observed no changes in the concentration of Ca^{+2} in this study, representing that neither exercise training nor n-3 did not affect the Ca^{+2} concentrations. Our results are in accordance with the findings of Thorsen et al. (1996) who examining the effect of moderate intensity brisk walking on calcium metabolism in post-menopausal women, reported no change in Ca^{+2} (Thorsen et al., 1996). Other investigators have shown that physical exercise affects calcium homeostasis (Zerath et al., 1997). The absence of significant alterations in Ca^{+2} in this study could possibly be explained by low exercise intensity. The unchanged Ca^{+2} levels during low-moderate intensity exercise training at this study, also may be associated to probable unchanged adrenergic system due to inadequate exercise intensity, since it is widely accepted that the adrenergic system, which is strongly activated by heavy exercise, affects the calcium homeostasis (Zerath et al., 1997).

Our results indicated that the changes in CT and PTH were more obvious in E+S than either E or S groups. This finding apparently indicates that the greater increase in CT level and decreased PTH concentration, which represent enhanced skeletal health, appears to be achieved by the combined actions of exercise and n-3. CT and PTH alterations were E > S > Con, suggesting that both exercise training and n-3 can also significantly alter the CT and PTH response alone. Therefore, it seems that participation in long-term aerobic exercise training rather than n-3 supplementation can be affect serum PTH and CT levels in postmenopausal women. However, that alteration in level of calciotropic hormones was greater when accompanied by exercise training. Therefore, it can be concluded that combined treatments were more effective at skeletal health than either exercise or supplement alone.

These finding clearly supports the view that regular moderate intensity exercise and n-3 improve skeletal health. This improvement is occurred through the actions of general calciotropic hormones. Importantly so far, no study has been reported to evaluate the combined effects of exercise and n-3 or n-3 alone on calcium regulatory hormones. Recent epidemiological studies have shown a positive and significant relationship between dietary n-3 and bone health (Amy et al., 2007; Matsushita et al., 2008). The n-3 may be beneficial as they have been shown to inhibit the activity of osteoclasts and enhance the activity of osteoblasts in animals (Sun et al., 2003). In human studies, Twelve month of EPA supplementation, increased a measure of BMD in post-menopausal women (Terano, 2001). Ishikawa et al. (2000) reported that women 1-5 years past-menopause who consumed fish, which is rich in n-3 PUFAs, had significantly greater metacarpal BMD (Ishikawa et al., 2000). A recent study also showed that a higher ratio of n-6 to n-3 is associated with lower BMD at the hip. While a few studies conducted in pre- and post-menopausal women failed to show a benefit of n-3 fatty acids or fish oil, animal models have also suggested that n-3 may attenuate post-menopausal bone loss (Vanek and Connor, 2007). Ovariectomized mice fed fish oil had significantly less bone loss at the femur and lumbar vertebrae than did ovariectomized mice feed n-6 fatty acids (Sun et al., 2003). Taken together, the epidemiological and supplemental feeding data provide evidence that n-3 PUFAs, can affect bone health in humans.

In the present study, we report that exercise either alone or with S increased estrogen level after the study. It

seems that serum estrogen levels were mainly affected by exercise training but the combination with n-3 supplementation with n-3 tended to be higher. This is in contrast with those of McTiernan et al. (2004) who demonstrated reduced estrogen level in response to a 12-month moderate-intensity exercise intervention in postmenopausal women. Probably, increased level of estrogen in present study affected by negative feed-back system (increased level of CT and decreased level of PTH) that plays an important role in control of circulatory level of hormones. According to the previous investigations, when estrogen is withdrawn, such as after menopause, the rate of bone resorption is increased. Estrogen's major effect on bone tissue, therefore, may be to inhibit bone resorption rather than to promote bone formation. This antiresorptive effect is mediated by the estrogen-induced synthesis and release of paracrine factors from osteoblast cells, which then control osteoclastic activity (Turner et al., 1994). Estrogen increases the release of transforming growth factor-b (TGF-b) from osteoblasts, which subsequently inhibits activity of osteoclasts (Weryha et al., 1995). Interleukin-6 (IL-6), a cytokine that increases osteoclastic activity, is regulated by estrogen, as IL-6 release from osteoblasts has been shown to be inhibited by estrogen. Further evidence of the antiresorptive effect of estrogen is provided by observations of increased synthesis of IL-6 and increased osteoclastic cell number in estrogen-deficient women (Debra et al., 2000). Estrogen not only affects bone metabolism at the cellular level but also alters systemic hormones. Estrogen interacts with many other hormones, including CT and prostaglandins, which result in a response of bone tissue. When the body is in a state of hypercalcemia, the C-cells are stimulated to release calcitonin (Debra et al., 2000), which inhibits bone resorption. Calcitonin seems to be influenced by estrogen, as higher levels of estrogen have been strongly correlated with calcitonin secretory capacity (Duursma et al., 1991). Another calcitropic hormone is the PTH. There is little known about the interaction between estrogen and PTH. Although estrogen does not affect PTH secretion, estrogen does seem to have an indirect effect on PTH action on bone. It has been suggested that estrogen inhibits bone cell responsiveness to PTH, thus reducing bone resorption (Debra et al., 2000). This indirect effect further explains why estrogen withdrawal results in increased bone resorption. We didn't find any research that investigated the effect of PUFAs on estrogen levels, however it seems that n-3 anti-inflammatory properties in one hand (Amy et al., 2007; Matsushita et al., 2008), and regulatory effects of estrogen on cytokines such as IL-6 on the other hand (Debra et al., 2000) can be contributed to these changes.

We found that n-3 supplementation with and without exercise training increased CT level and decreased PTH concentration, indicators of bone formation and resorption, respectively. This effect was greater when was accompanied by exercise training. The mechanisms regarding how n-3 influenced the calcium regulatory hormones remain unclear. However, it has been previously noted that the alterations in prostaglandins production, lipid oxidation, calcium absorption, and osteoblast differentiation may account for the effects of dietary n-3 on bone health (Amy et al., 2007; Matsushita et al., 2008). Increased consumption of n-3 has demonstrated to decrease the ratio of AA to EPA and decrease PGE_2 concentration. This is potentially important, as PGE_2, a major prostaglandin involves in bone metabolism, has reported to stimulate bone resorption. Higher n-3 PUFA intake has also been found to enhance calcium absorption, and hence increases bone calcium (Amy et al., 2007). The increased BMD in their study following n-3 supplementation may be related to inhibited activity of osteoclasts and enhanced activity of osteoblasts in bone tissue. Optimal quantities of n-3 PUFAs, thus, appear to inhibit bone resorption and promote bone formation. The positive effect of n-3 on bone health could be done through the altering calciotropic hormone. In the present study, dietary enrichment with n-3 caused a significant increase in EPA and DHA content and a decrease in AA and LA content of neutrophil phospholipids in the E+S and S groups.

Conclusion

In conclusion, the results of present study revealed that following 16 weeks moderate intensity exercise with n-3 supplementation, the serum CT and estrogen levels were increased and PTH level was decreased in healthy sedentary post-menopausal women. These findings suggest that participating in regular weight-bearing exercise and incorporation sources of n-3 in diet might be a possible intervention to help slow the loss of bone that occurs following menopause. It seems that, the primary factor that altered the CT, estrogen and PTH hormones was exercise and the supplement showed an additive effect. In addition, the ratio of these hormones may be more important than the individual concentration of either hormone. These data are the first to be published regarding the combined effects of aerobic exercise training and n-3 consumption on calcium regulatory hormones level in post-menopausal women, and therefore further elucidations of the physiologic effects of n-3 PUFAs on bone metabolism and calcium regulatory hormones to prevent or treat osteoporosis, are needed.

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Key points

- Long-term weight-bearing exercise was shown to prove positive effects on bone metabolism.
- Serum calciotropic hormone levels and Ca^{+2} can be affected by exercise intensity as well as duration.
- There is a good relationship between dietary omega-3 (n-3) and bone metabolism in post-menopausal women.
- Omega-3 in combination with long-term weight-bearing exercise training has significant effects on serum calciotropic hormone levels in non-athlete post-menopausal women.

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