The Effect of an Eight-Week Walking Program on Bone Turnover Markers and sRANKL/Osteoprotegerin Levels in Post-Menopausal Women

Sekiz Haftalık Yürüme Programının Menopoz Sonrası Kadınlarda Kemik Döngüsü Göstergeleri ve sRANKL/Osteoprotegerin Düzeyleri Üzerine Etkisi

Gürbüz BÜYÜKYAZI, MD[a]

[a]Celal Bayar University,
High School of
Physical Education and Sports,
Manisa

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Yazışma Adresi/*Correspondence:*
Gürbüz BÜYÜKYAZI, MD
Celal Bayar University,
High School of
Physical Education and Sports,
Manisa,
TÜRKİYE/TURKEY
gurbuzbuyukyazi@gmail.com

ABSTRACT Objective: To examine the effect of an eight-week moderate intensity walking program on bone turnover markers, serum osteoprotegerin (OPG) and soluble receptor-activator of nuclear factor- kappa β ligand (sRANKL) in post-menopausal women. **Material and Methods:** Twelve post-menopausal women (45-62 years) completed an eight-week walking program at moderate intensity (60-65% maximum heart rate reserve). Non-walking women served as the control group (CG; n= 11). Body weight, percent body fat, body mass index (BMI), estimated maximal oxygen consumption (estimated VO_{2max}), bone turnover markers, sRANKL, serum OPG and sRANKL/OPG ratio were measured before and after the intervention. **Results:** Eight-week walking program produced significant increases in VO_{2max} and reductions in body weight and BMI. Serum intact parathyroid hormone (iPTH), and β- crosslaps (CTx) reduced ($p< 0.01$, for both), sRANKL/OPG ratio ($p< 0.01$) and sRANKL values ($p< 0.05$) increased in the exercise group (EG). We detected significant reductions in the serum OPG levels of EG and CG ($p< 0.01$). No significant differences were observed between the changes in EG and CG in terms of bone turnover markers, OPG and sRANKL values. **Conclusion:** Eight-week moderate intensity walking program is beneficial for improving cardiorespiratory function in post-menopausal women to enable them to lead a less risky and a more independent life; however, it seems not so effective in changing the bone turnover markers and the OPG/sRANKL system.

Key Words: RANK ligand, osteoprotegerin, postmenopausal, osteoporosis, exercise

ÖZET Amaç: Sekiz haftalık yürüme programının menopoz sonrası dönemdeki kadınların, kemik döngüsü göstergeleri, serum osteoprotegerin (OPG) ve solubl nükleer faktör kappa β reseptör aktivatörü (sRANKL) üzerine olan etkisini belirlemek. **Gereç ve Yöntemler:** Menopoz sonrası dönemde 12 kadın (45-62 yaş) orta şiddette (kalp atım sayısı rezervinin %60-65'i) sekiz haftalık bir yürüme programını tamamladı. Yürüyüş programına katılmayan kadınlar kontrol grubunu (KG; n= 11) oluşturdu. Vücut ağırlığı, vücut yağ oranı, beden kütle indeksi (BKİ), tahmini maksimal oksijen tüketimi (tahmini VO_{2max}), kemik döngüsü göstergeleri, sRANKL, serum OPG ve sRANKL/OPG oranları çalışmadan önce ve sonra ölçüldü. **Bulgular:** Sekiz haftalık yürüme programı VO_{2max}'ta anlamlı artışlara vücut ağırlığı ve BKİ'de ise anlamlı azalmalara neden oldu. Egzersiz grubunda (EG) serum intakt paratiroid hormon (iPTH), β-crosslaps (CTx) azaldı (her ikisi için $p< 0.01$). sRANKL değerleri ($p< 0.05$) ve sRANKL/OPG oranı ($p< 0.01$) EG'de anlamlı olarak arttı. Hem EG hem de KG'nin serum OPG düzeylerinde anlamlı azalmalar tespit edildi ($p< 0.01$). EG ve KG'de gözlenen değişimler arasında kemik döngüsü göstergeleri, OPG ve sRANKL değerleri bakımından anlamlı bir farklılık gözlenmedi. **Sonuç:** Orta şiddette yapılan sekiz haftalık yürüme programları menopoz sonrası kadınların daha bağımsız ve daha az riskli bir yaşam sürdürebilmelerini sağlayabilecek kalp-solunum fonksiyonunu geliştirmek için yararlıdır; ancak bu programın kemik döngüsü göstergelerinde ve OPG/sRANKL sisteminde değişiklik yapabilecek kadar etkili olmadığı görülmektedir.

Anahtar Kelimeler: RANK ligand, osteoprotegerin, postmenopoz, osteoporoz, egzersiz

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Osteoporosis is a disease generally believed to occur predominantly in females because estrogen deficiency is a major contributory factor to postmenopausal osteoporosis. The level of circulating estradiol significantly decreases at menopause in all women. Studies have revealed that the decreased levels of serum estradiol are associated with increased levels of bone turnover markers.[1,2]

Although exercise is known to have an important role in the remodeling process of the bone, how the hormonal and mechanical stimuli affect bone and interact throughout life has not been understood thoroughly.[3] It is thought that iPTH as well as mechanical load have a role in this event because PTH is important for the regulation of bone metabolism with catabolic as well as anabolic properties.[4] The majority of the osteocalcin (OC) secreted by the osteobtasts is incorporated into the matrix; the remainder finds its way into the circulation.[5] Alkaline phosphatase (ALP) might be a causative agent for the calcification process.[6] Studies examining β-crosslaps (CTx) levels, a bone resorption marker, determined that elevated CTx levels were related to increased osteoporosis and they were accepted as the determinants of future fracture risks.[1,7] Therefore, researchers have used biochemical markers of bone metabolism, particularly in clinical studies to evaluate bone metabolism in skeletal diseases.[8-12] However, the studies examining the effect of exercise on the skeleton using these markers have revealed conflicting results. In some studies, PTH concentrations were found to be increased, decreased, or unchanged.[3,13,14] Similarly, there are controversial results related to the effect of exercise on serum OC and CTx levels.[7,15,16]

The discovery of osteoprogerin (OPG) has enabled researchers to understand the way in which the processes of bone remodeling are regulated.[17] Researchers have designed some studies to assess the importance of OPG for the skeleton in human populations with conflicting results. In one study, osteoporotic women were determined to have higher circulating levels of OPG than controls did.[18] In another study, the researchers found no difference between serum OPG levels in osteoporotic vs. healthy postmenopausal women.[19] When OPG is administered as a therapeutic agent, a dramatic reduction is observed in bone turnover state, but its long-term effect on bone density is still unclear.[20]

It is still not certain whether circulating OPG and receptor-activator of nuclear factor- κ β ligand (RANKL) reflect changes in bone metabolism as a result of physical activity since there are very few studies with conflicting results indicating the relationship between physical activity and the OPG/solubleRANKL (OPG/sRANKL) system.[7,21] To our knowledge, there are no studies examining the effects of walking exercises on OPG/sRANKL system together with bone turnover markers in postmenopausal women. Therefore, the aim of the present study was to point out to the changes in sRANKL and OPG-serum levels, as well as bone turnover markers in post-menopausal women following an 8-week moderate intensity walking program.

MATERIAL AND METHODS

SUBJECT SELECTION

Female volunteers aged between 45-62 years were recruited through mass mailings of recruitment flyers. Initially, participants were interviewed either face-to-face or over the phone to determine their eligibility for the study.

Recruiting criteria were as follows: (1) not experiencing any menses within the 12 months preceding their participation in the study, (2) to live in Manisa for at least 10 years, (3) not planning to leave the area during the experimental period, (4) being a non-smoker, (5) being sedentary at baseline (people were considered sedentary if they had not performed exercise for 15 minutes or longer more than twice per week for the previous 6 months). Volunteers with a history of cardiovascular disease or diagnosed coronary heart disease, endocrine or metabolic disorders, under medication known to affect bone metabolism, with high resting blood pressure (> 160 mmHg systolic or > 95 mmHg diastolic), musculo-skeletal problems, diabetes mellitus, hyperthyroidism, and a ± 2 kg chan-

ge in body weight during the previous year were excluded from the study. All information about the subjects was determined via questionnaires; the subjects were examined thoroughly at baseline and were informed about the study design. Each subject signed the informed consent form. The participants with a laboratory screening. In the laboratory, the electrocardiography, body compositions of the participants and their blood pressures were measured.

Thirty-two individuals were recruited; six did not meet the initial screening criteria. Twenty-six subjects who underwent laboratory screening were assigned to the walking group and the control group. Participants were not randomized. To maintain compliance, subjects were allowed to choose to participate either in the exercise group (exercise: EG; n= 13) or in the control group (CG; n= 13). Before starting the program, all participants were required to fill out the section related to the eating habits of "The health-profile lifestyle profile", developed by Wakler et al.[22] No statistically significant differences between the groups in terms of their eating habits were found. They were told not to change their dietary habits throughout the study period. One participant from EG and two from CG dropped from the study due to ill health and lack of available time. Therefore, totally 23 participants - 12 in the EG and 11 in the CG-were included in the evaluation (Table 1).

Exercise group members were warned not to take any other form of physical exercise; CG members were also warned not to take part in any physical activity that would make them feel tired. The Ethical Council of the Celal Bayar University, Faculty of Medicine, approved the study. The study was conducted in accordance with the principles of Helsinki Declaration.

STUDY DESIGN

Subjects were accepted to the human performance laboratory and a 400 m-outdoor track in two consecutive weeks between 8:00 and 9:00 a.m. for orientation and measurements. On the first visit, measurements of their height, body weight, body fat, blood pressure, and estimated maximal aerobic capacity (estimated VO_{2max}) were measured. On the second visit, their blood samples were collected at rest. Body composition was measured using bioelectrical impedance analyzer (Model TBF-300, Tanita Corp., Tokyo, Japan). Body fat was expressed as percentage of body weight. The measured values of body weight and height were used to calculate the BMI (BMI, kg/m^2). VO_{2max} was estimated by 2 km walking test.[23,24] For this test, the participants were requested to walk a 2 km-distance as fast as possible. They walked in groups of two. The test was performed on a 400 m outdoor track by completing five tours. The heart rate (HR) recorded at the point of completion of the walk and the duration taken to complete the walk were recorded by exercise specialists. A group of medical staff was available in case of an emergency.

TABLE 1: Initial physical and physiological characteristics of subjects.

	EG (n= 12)		CG (n= 11)		
Variable	**Mean ± SD**	**Median (min-max)**	**Mean ± SD**	**Median (min-max)**	**p**
Age (year)	53.50 ± 4.44	53.00 (45.00-62.00)	54.18 ± 6.70	53.00 (46.00-62.00)	NS
Height (cm)	158.25 ± 5.04	158.50 (150.00-168.00)	153.09 ± 5.87	152.00 (145.00-163.00)	NS
Body weight (kg)	73.78 ± 7.39	73.90 (62.30-84.80)	73.60 ± 11.03	71.50 (47.80-88.70)	NS
BMI (kg/m^2)	29.49 ± 2.89	28.90 (24.70-34.40)	31.45 ± 4.96	31.20 (22.70-39.10)	NS
Percent body fat (%)	36.15 ± 2.70	36.40 (32.50-40.60)	37.85 ± 6.68	36.10 (22.80-44.60)	NS
VO_{2max} ($mL.kg^{-1}.min^{-1}$)	20.85 ± 5.27	20.44 (18.10-23.10)	22.16 ± 1.57	22.43 (17.40-24.52)	NS
Menopause age (y)	3.08 ± 2.15	3.00 (1.10-6.00)	3.45 ± 1.29	3.50 (1.50-6.00)	NS

EG: Exercise group; CG: Control group; Group comparisons were made using Mann- Whitney U test; NS: Not significant.

The following predictive equation developed for women was then used to estimate VO_{2max} from the heart rate, age, BMI and the duration:

116.2-2.98 x duration (min)-0.11 x HR-0.14 x age-0.39 x BMI[23,24]

Subjects were told to avoid any physical activity within 48 hours preceding the assessment day.

EXERCISE PROGRAM

After completion of baseline testing exercise group subjects entered an 8-week walking intervention of moderate intensity. All exercise group members walked on an outdoor track (400 m) for 8 weeks, 5 days per week and they were supervised and monitored by trained exercise specialists. Walking was chosen as the mode of aerobic exercise since it was considered the most common, most feasible, and safest form of sustainable dynamic aerobic exercise for our subjects. The exercise program was planned according to the principles of the American College of Sport Medicine recommendations.[25] The exercise intensity was prescribed based on target heart rates (THRs) calculated from the Karvonen equation:

$[(HR_{maximum}\text{-}HR_{rest}) \times (0.60\text{-}0.65) + HR_{rest}]$

$HR_{maximum}$ was predicted by 220-age formula. In the first four weeks, EG members aimed to walk for 30, 33, 36, and 39 minutes at 60% of maximum heart rate reserve (HRR_{max}); and on the second four weeks, they aimed to walk for 42, 45, 48 and 51 minutes at 65% of HRR_{max}. The participants continuously were warned by the exercise specialists to walk at the THR speed determined for each different training period in all exercise sessions. To ensure compliance with the training intensity (walking speed), at least three heart rate readings were taken by Polar Pacer heart rate monitors (Polar Vantage, Kempele, Finland) and their Rate of Perceived Exertion (RPE) was also taken using a 15-point RPE scale and was noted on training logs. In each exercise session, the supervisor recorded the total walking distances. The subjects warmed-up by walking 5 minutes at a slow pace plus 5-minute stretching activities and cooled-down by 5-minute stretching exercises.

BLOOD ANALYSIS

Following a 12 h overnight fast, venous blood samples were collected from an antecubital vein (20 mL) in the sitting position after a 20-minute rest between 8:00 and 9:00 a.m. Serum was separated by centrifugation, and samples were stored at -80° C until assays were performed (within one month) in all samples.

Total serum ALP levels were analyzed by enzymatic methods on autoanalyzer (D x 800 Beckman Coulter, Galway, Ireland) by commercial reagents. Intact serum PTH levels were assessed by a solid phase, two site chemiluminescent enzyme-labeled immunometric assay on Immulite 2000 hormone analyzer (IMMULITE®, Diagnostics Products Corporation, Los Angeles, USA). Serum OC and CTx were assessed by an electrochemiluminescence immunoassay on Roche E170 immunoassay analyzer (Roche Diagnostics GmbH., Mannheim, Germany). The inter and intra-assay coefficient of variation (CV) for ALP at level 36.5 IU/l were 4.61% and 3.71%, for OC at level 13.7 ng/mL were 3.8% and 3.3%, for CTx at level 0.45 ng/mL were 4.2% and 2.0%, respectively. iPTH at level 54 pg/ml inter-assay precision was 6.3% and intra-assay precision at level 72 pg/mL was 5.7%. Serum concentration of OPG was determined by a commercial sandwich enzyme immunoassay (Biomedica, Vienna). Standard curves were established with provided OPG standards. Sample concentrations were obtained from this standard curve. The method determines the total amount of OPG. The intra-assay CV for OPG measurements at level 4.59 pmol/l was 10% and inter-assay CV at level 5.53 pmol/l was 7%. sRANKL was also determined by an enzyme immunoassay system (Biomedica, Vienna). The system is specific for free soluble RANKL. The intra-assay CV for sRANKL measurements at level 1 pmol/l was 5%; inter-assay CV at level 0.80 pmol/l was 9%. The detection limits for serum OPG and sRANKL assays were 0.14 pmol/l and 0.08 pmol/l, respectively. Serum OPG and sRANKL concentration in pico-moles per liter in the tables were provided as mean ± SD.

STATISTICAL ANALYSIS

Data were analyzed using SPSS package program version 10.0 with non-parametric tests because of low numbers of subjects in groups and lack of homogeneity of variance. Results were presented as mean ± standard deviation (SD). Mann-Whitney U test was used to determine the difference between the two groups. The differences between pre and post values of the intervention period were determined by the Wilcoxon test. All comparisons were considered statistically significant at $p< 0.05$.

RESULTS

EG members aimed to walk at 60-65% of HRR_{max}. The average HR per week during the training for EG was 130.02 ± 7.20 beat.min^{-1} (corresponding to 62% of HRR_{max}). Mean walking speed per week for the whole program was 6.29 ± 0.15 km/h. The RPE reported by EG was 13.95 ± 0.90. EG members totally walked 172123.8 ± 8567.88 m during the intervention period.

At the pre study evaluation, EG and CG were not significantly different with regard to age, body weight, body fat, VO_{2max}, and the menopause age (Table 1).

After 8 weeks, estimated VO_{2max} improved in the exercise group ($p< 0.01$); their body weights ($p< 0.05$) and BMI decreased ($p< 0.01$). However, no significant differences were detected in CG (Table 2).

We observed a significant improvement in aerobic capacity as a result of the walking program. VO_{2max} increased for a mean of 5.46 ± 3.65 mL.kg^{-1}. min^{-1} in EG, which was significantly different from that of CG ($p< 0.01$). The reduction observed in the body weight of EG was significantly different from that of CG ($p< 0.01$; Table 3).

Among the measured bone turnover markers, we detected an insignificant reduction in ALP levels in our study groups. There was a significant reduction in CTx, iPTH and OPG values ($p< 0.01$) and significant increases in sRANKL ($p< 0.05$) levels and sRANKL/OPG ratio ($p< 0.01$) in EG. Apart from a significant reduction in OPG levels, we did not determine any significant changes in any of the bone turnover markers of the CG (Table 4).

When the differences obtained from the measured parameters at the end of the study were compared, no significant difference was determined between EG and CG in terms of their bone turnover markers and OPG/sRANKL values (Table 5).

TABLE 2: Changes in physical and physiological parameters of subjects (mean ± SD).

	EG (n= 12)				CG (n= 11)			
Test (unit)	**Pre**	**Post**	**XΔ ± SD**	**p**	**Pre**	**Post**	**XΔ ± SD**	**p**
Body weight (kg)	73.78 ± 7.39	72.50 ± 7.03	-1.28 ± 1.11	<0.05	73.60 ± 11.03	73.54 ± 10.81	-0.06 ± 1.07	NS
Body fat (%)	36.15 ± 2.70	35.68 ± 3.03	-0.47 ± 1.29	NS	37.85 ± 6.68	37.24 ± 6.18	-0.60 ± 1.01	NS
BMI (kg.m^{-2})	29.46 ± 2.89	28.32 ± 2.82	-1.14 ± 0.96	<0.01	31.45 ± 4.96	30.35 ± 3.80	-1.10 ± 1.74	NS
VO_{2max} (mL.kg^{-1}.min^{-1})	20.85 ± 5.27	26.31 ± 3.41	5.46 ± 3.65	<0.01	22.16 ± 1.57	22.02 ± 1.46	-0.14 ± 0.59	NS

EG = Exercise group; CG = Control group; NS= Not significant Within-group comparisons were made using Wilcoxon Signed Ranks test; NS: No significant.

TABLE 3: The comparison of the differences obtained in the groups.

	Exercise Group (n= 12)	**Control Group (n= 11)**	**p**
Test (unit)	XΔ ± SD	XΔ ± SD	
Body Weight (kg)	-1.28 ± 1.11	-0.06 ± 1.07	<0.01
BMI (kg.m^{-2})	-1.14 ± 0.96	-1.10 ± 1.74	NS
Body fat (%)	-0.47 ± 1.29	-0.60 ± 1.01	NS
VO_{2max} (mL.kg^{-1}.min^{-1})	5.46 ± 3.65	-0.14 ± 0.59	<0.01

Group comparisons were made using Mann- Whitney U test; NS: No significant. BMI= Body Mass Index; VO2max= Maximal Oxygen Consumption; NS= Not significant.

TABLE 4: Changes in sRANKL/OPG system and bone turnover markers of the subjects (mean ± SD).

	EG (n= 12)				CG (n= 11)			
	Pre	Post	XΔ ± SD	p	Pre	Post	XΔ ± SD	p
sRANKL(pmol/l)	0.11 ± 0.06	0.17 ± 0.057	0.06 ± 0.05	<0.05	0.10 ± 0.10	0.11 ± 0.05	0.01 ± 0.14	NS
OPG (pmol/l)	6.90 ± 1.28	4.60 ± 0.93	-2.30 ± 1.13	<0.01	6.71 ± 1.66	4.42 ± 1.35	-2.29 ± 1.10	<0.01
sRANKL/OPG	0.0173 ± 0.009	0.0391 ± 0.011	0.0219 ± 0.009	<0.01	0.01 ± 0.01	0.02 ± 0.01	0.007 ± 0.02	NS
ALP (U/l)	58.08 ± 9.18	52.33 ± 16.84	-5.75 ± 14.30	NS	68.36 ± 14.3	64.36 ± 14.0	-4.00 ± 8.46	NS
iPTH (pg/mL)	49.35 ± 19.98	37.36 ± 13.19	-11.99 ± 11.8	<0.01	64.70 ± 24.4	55.40 ± 18.3	-9.30 ± 20.97	NS
OC (ng/mL)	25.09 ± 6.52	22.88 ± 5.76	-2.20 ± 8.40	NS	22.33 ± 7.65	20.34 ± 8.22	-1.99 ± 6.11	NS
CTx (ng/mL)	0.49 ± 0.16	0.34 ± 0.18b	-0.14 ± 0.09	<0.01	0.36 ± 0.16	0.26 ± 0.19	-0.09 ± 0.17	NS

Within-group comparisons were made using Wilcoxon Signed Ranks test; EG = Exercise group; CG = Control group; sRANKL= soluble receptor-activator of nuclear factor- κ β ligand; OPG= Osteoprotegerin; ALP= Alkaline phosphatase; iPTH= intact parathyroid hormone; OC= Osteocalcin; CTx= β-crosslaps; NS= Not significant

DISCUSSION

In the present study we investigated the effects of 8-week moderate-intensity walking program on bone turnover markers and sRANKL/OPG system in post-menopausal women. The program had beneficial effects on cardio respiratory function (VO_{2max}); and some physical characteristics like reductions in body weight and BMI of the exercising subjects. In addition, we found significant increases in sRANKL levels and sRANKL/OPG ratio in EG. A significant reduction was observed in serum OPG, CTx, and iPTH levels of EG. The reduction in serum OPG was also significant in CG.

Biochemical markers of bone metabolism have been used to evaluate bone metabolism in skeletal diseases.[8-12] However, the results of the studies on the effect of exercise on skeleton using these markers have revealed conflicting results. Rudberg et al determined that PTH levels of young women after 30-40 min jogging were significantly increased, but there was no significant change in PTH levels after cycling.[3] In another study, Thorsen et al found no significant changes in PTH values of postmenopausal women after a single bout of 90-min brisk walking.[14] Brahm et al measured the biochemical markers of bone metabolism of endurance runners exercising for about 12 years and they observed higher body mass density (BMD) values and lower PTH levels in runners than in controls.[13] Similar to Brahm et al we observed significant reductions in iPTH levels in exercising women in this present study. It is well known that chronic hypersecretion of PTH induces bone loss, but injections of PTH can have anabolic effects.[26] The finding that there is a rise in PTH following exercise, but reduced levels in the resting state might indicate that PTH could be involved in the responses of bone to exercise. The significant reduced iPTH levels as a result of walking observed in this present study se-

TABLE 5: The comparison of the differences obtained in the groups.

	Exercise Group (n= 12)	Control Group (n= 11)	p
Test (unit)	XΔ ± SD	XΔ ± SD	
sRANKL (pmol/l)	0.06 ± 0.05	0.01 ± 0.14	NS
OPG (pmol/l)	-2.30 ± 1.13	-2.29 ± 1.10	NS
sRANKL/OPG	0.0219 ± 0.009	0.007 ± 0.02	NS
ALP (U/l)	-5.75 ± 14.30	-4.00 ± 8.46	NS
iPTH (pg/mL)	-11.99 ± 11.84	-9.30 ± 20.97	NS
OC (ng/mL)	-2.20 ± 8.40	-1.99 ± 6.11	NS
CTx (ng/mL)	-0.14 ± 0.09	-0.09 ± 0.17	NS

Group comparisons were made using Mann- Whitney U test; NS: No significant. sRANKL= soluble receptor-activator of nuclear factor- κ β ligand; OPG= Osteoprotegerin; ALP= Alkaline phosphatase; iPTH= intact parathyroid hormone; OC= Osteocalcin; CTx= β-crosslaps; NS= Not significant

em concordant with the positive effects seen on the weight-bearing skeleton in endurance runners. The lack of a significant reduction in CG may also be an indication of the positive effect of the exercise regimen followed by our exercising members. However, in order to interpret the positive effects of the exercise programs, a significant difference on the changes (within group changes) between the groups is necessitated. Therefore, our results need further investigation.

Hatori et al showed that high intensity walking resulted in increases in BMD without a significant change in serum OC; but Milliken et al[15] determined an increasing trend in OC levels in post-menopausal exercising women at the end of 6th months; similarly, the exercisers showed larger positive changes in OC levels over 12 months versus the control group.[15,16] Etherington et al observed reduced OC and ALP levels as a result of 10-week weight-bearing exercise.[27] They concluded that the fall in markers of both bone formation and resorption suggested that there was an overall reduction in bone turnover in response to that level of strenuous exercise. Hinton et al found OC and ALP levels of post-menopausal women increased following 6 weeks of energy restriction and aerobic exercise (walking or jogging at 60% of VO_{2max}).[21] They suggested that weight-bearing, aerobic exercise training may favorably affect the balance between bone resorption and formation during weight loss. Similarly, presumably due to the training might followed, Vincent and Braith found a significant increase in OC levels in both low-intensity and high-intensity resistance training groups.[28] They stated that resistance exercise increased bone turnover, which over time might lead to further changes in BMD. However, Zanker et al found no changes in OC levels of the participants who followed an energy balanced diet regimen and a repeated-periods of prolonged treadmill running.[29] Similar to that, Brooke-Wavell et al found no significant changes in OC levels as a result of 12-month brisk walking in post-menopausal women.[30] Our study shows similarities with the previous research results that found no exercise-bound changes in serum OC levels.[15,29,30] Our study revealed differing results from some of the aforementioned studies. The main reasons of the differences may be the duration of the exercise programs (8 weeks versus 12 months); the exercise intensity (moderate versus strenuous); the different exercise types used (walking versus running plus weight training); energy restriction together with physical activity); and the resistance training that applies a vertical force on the bone.[16,21,27,28]

CTx is a specific marker of bone resorption. The EPIDOS study has determined the relevance of elevated CTx levels in predicting severe clinical events, such as hip fractures.[1] Herrmann and Herrmann stated that the elite endurance athletes exhibited significantly higher levels of CTx than controls, which is neither accompanied by an increase in sRANKL nor a decrease in OPG.[7] According to them, this indicates increased bone resorption in athletes, which is not associated with a shift of OPG and sRANKL toward an osteoclastogenetic constellation. Therefore, they hypothesized that elevated CTx concentrations could be used to identify athletes at risk of osteoporosis and future fractures. We observed significant reductions in CTx levels in EG in the present study, which was not the case in CG members. Therefore, it is possible to say that walking exercises of this intensity may be recommended to post-menopausal women as a protective treatment since higher intensities may result in elevations in their CTx levels, which in turn may increase osteoporosis and future fracture risks.[7] The absence of significant changes in the bone turnover markers apart from the reductions in iPTH and CTx levels in our exercising participants, might suggest that bone turnover markers reflect phases of the bone remodeling cycle which were not affected by our prescribed exercise regime. In addition, the differences between our study and aforementioned studies may be due to the different study populations and designs as well as the assays used. However, the interpretation of the biochemical results in this study is somewhat not clear. Although biochemical markers are believed to serve as precursors to skeleton changes, we are not so sure whether the changes in the biochemi-

cal markers reflect any kind of change in the BMD of post-menopausal women since we did not measure BMD using DXA because we wanted to detect the low-cost effect of exercise on these bone turnover markers. However, the relatively short intervention period of our study (8 weeks) may not have been long enough to allow completion of bone remodeling. Furthermore, the lack of significant difference of the changes between the exercising and control groups apart from maximal oxygen consumption and body weight makes the results we obtained on the positive effects of walking exercise on bone turnover status difficult to interpret.

The receptor activator of nuclear factor-kappa β (RANK)/RANK ligand (RANKL)/OPG pathway has recently been recognized as the final, dominant mediator of osteoclast proliferation and activation.[31] RANKL and its antagonist OPG have led to a detailed molecular and cellular understanding of bone metabolism in health and disease. In most of the disorders including various forms of osteoporosis, scientists detected enhanced osteoclastic bone resorption due to an imbalance of the RANKL-to-OPG ratio, with RANKL exceeding OPG. Although there is no consensus on the serum levels of OPG, elevated serum OPG levels have been found in several forms of osteoporosis and in vascular diseases. Fa'brega et al found that OPG levels significantly increased in patients with alcoholic cirrhosis compared with healthy subjects; Avignon et al also determined that OPG levels were high in subjects with coronary artery disease (CAD) than in those with no CAD.[32,33] We did not find increased OPG levels in neither of our study groups because we included only the participants who met strict criteria that did not allow the participation of people with any vascular or bone related diseases. The significantly reduced levels of OPG obtained in our study groups may be accepted as the indication that our participants are not at risk of some diseases.

The effect of physical activity on OPG and RANKL levels has received considerable attention in recent years. In a study with cross-country skiers and biathlon athletes, Hermann and Hermann detected no significant change in OPG and sRANKL levels of athletes and controls.[7] Hinton et al observed no significant changes in RANKL in post-menopausal women who underwent 6 weeks of energy restriction and aerobic exercise (walking or jogging at 60% VO_{2max}).[21] The exercise-bound changes in our study is controversial with the aforementioned research results since we detected significant increases in sRANKL and decreases in OPG levels in our EG. Elevated levels of OPG have been observed in people with certain disorders and health problems; therefore, determining low levels of OPG in our participants is favorable.[32,33] However, it is not possible to interpret this finding as a positive effect of walking exercises since we also determined a significant reduction in our CG members. In addition, the lack of a significant difference between our exercise and control groups in terms of sRANKL and OPG levels might suggest that these markers were not affected by the prescribed exercise regimen. Therefore, the role of physical training on OPG and sRANKL levels should be reevaluated by different studies with different designs and longer duration with larger numbers of participants from different age and sex groups.

We are aware that it is not possible to draw a solid conclusion from this present investigation since it has some limitations. The first limitation is the relatively small number of the participants in our study groups. We could not include a higher number of subjects due to our very strict participation criteria. In addition, determining the effect of exercise on bone mass is somewhat difficult by measuring only the biochemical markers of bone metabolism because the osteogenic effects of exercise training seems to be site-specific to the anatomic sites at which the mechanical strains occur; thus, it is essential to measure the bone mass at the site of loading.[34] Therefore, together with these biochemical markers of bone metabolism, it would be beneficial to integrate site-specific bone measures into the study designs.

The comparison of our results with the results of other studies is quite difficult the other studies conducted with post-menopausal population beca-

use in most of these studies the participants were allowed or encouraged to take Vitamin D or calcium supplements and in most of them they were given hormone replacement therapy (HRT).[35,36] However, we excluded participants taking vitamin D and calcium supplements and HRT since our main purpose was to highlight the pure exercise-bound effects on the measured parameters in post-menopausal women. Due to the limited number of participants, we were unable to form different groups such as exercisers and non-exercisers with and without HRT treatment and vitamin D and calcium supplements.

The effect of strenuous aerobic exercise and strength training is known to enhance bone mass; however, mild general exercise such as walking is not so effective in preventing post-menopausal bone loss or enhancing bone mass in younger age periods. Even so, training programs that are strenuous enough to cause increases in bone mass or reverse bone loss may not be the most attractive ones for the elderly population since they may pose some risks for them. In order to assure high compliance and attendance, exercise programs should be made attractive for the elderly population. It should be noted that some fractures are the result of falls and they are caused not only by osteoporosis, but also by multiple factors such as low muscle mass and muscle strength, poor balance and coordination, all of which can be modified by exercise. Therefore, in contrast to pharmacologic and nutritional approaches, exercise training may have the potential to prevent osteoporotic fractures by simultaneously influencing multiple risk factors and provide the elderly with an independent life. Thus, exercising for 30-50 min per day, at least five days per week, at moderate intensity level of 60-65% HRR_{max} for most individuals provides at least some health-related benefits, including improved cardiorespiratory fitness, muscle strength and endurance, flexibility and body composition, as well as associated psychological benefits.

CONCLUSION

Despite some limitations, the beneficial results obtained as a result of moderate intensity walking regarding the increases in VO_{2max}, and the reductions in body weight can be accepted as health benefits for post-menopausal women, which may enable them to lead a less risky and a more independent life; however, walking only eight weeks at a moderate intensity seem not so effective in causing favorable changes on bone health to prevent the risk of osteoporosis. It remains to be determined whether walking exercises have favorable effects on bone turnover state in a larger population with longer periods of interventions in which these markers are measured together with BMD.

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