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THE EFFECTS OF 8 WEEKS OF WALKING EXERCISES OF DIFFERENT INTENSITY ON sRANKL AND OSTEOPROTEGERIN LEVELS IN PRE-MENOPAUSAL WOMEN

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SUMMARY

This study aimed to clarify the possible positive effects of an eight week walking program of two different intensities on serum osteoprotegerin (OPG) and soluble receptor-activator of nuclear factor κβ ligand (sRANKL), referred as osteoporosis risk factors. Forty-four premenopausal women aged between 30-55 years enrolled in the study and were divided into brisk (BWG; n=15) and moderate tempo walking groups (MTWG; n=16), and controls (CG; n=13). BWG and MTWG walked five days per week starting with 30min per day, steadily increasing up to 51min, at 74% and 54% of their maximum heart rate reserve (HRR_{max}), respectively. Body weight, body fat ratio, body mass index (BMI), estimated maximal oxygen consumption (VO_{2max}), serum OPG, sRANKL, and sRANKL/OPG ratio were measured before and following the program. Walking programs produced significant increases in VO_{2max} favoring BWG; reductions in body weights, BMI, and body fat ratio in both exercise groups. Significant reductions in serum OPG levels of BWG ($p<0.05$) and MTWG ($p<0.01$) were also detected. There were no significant changes in the sRANKL levels and sRANKL/OPG ratios in any group. Significant improvements for protective effects against osteoporosis due to the reduction in serum OPG levels may be attained with either brisk or moderate tempo walking programs. However, brisk walking produces more dramatic effects on cardiorespiratory fitness.

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Key words: Pre-menopause, sRANKL, osteoprotegerin, exercise

ÖZET

FARKLI ŞİDDETTEKİ SEKİZ HAFTALIK YÜRÜME EGZERSİZLERİNİN PREMENOPOZAL KADINLARIN sRANKL ve OSTEOPROTEGERİN DÜZEYLERİ ÜZERİNE ETKİLERİ

Bu çalışmanın amacı farklı iki şiddette yapılan sekiz haftalık yürüyüş programının osteoporoz risk faktörü olarak kabul edilen serum osteoprotegerin (OPG) ve çözülebilir nükleer faktör κβ aktivasyon reseptör ligandı (sRANKL) üzerine olan etkilerini belirlemekti. Çalışmaya katılan 44 premenopozal kadın (30-55 yaşları arasında) hızlı tempo (HTYG; n=15) ve orta tempo yürüyüş grupları (OTYG; n=16) ile kontrol grubuna (KG; n=13) ayrıldı. HTYG ve OTYG haftada beş gün, günde 30 dk'dan başlayarak 51 dk'ya ulaşan sabit süreli artışlarla, maksimum kalp atım hızı rezervinin sırasıyla %74 ve %54'ünde yürüdüler. Vücut ağırlığı, vücut yağ oranı, beden kitle indeksi (BKİ), tahmini maksimal oksijen tüketimi (VO_{2max}), serum OPG, sRANKL, ve sRANKL/OPG oranı program öncesi ve sonrasında ölçüldü. Yürüme programları, HTYG lehine olmak üzere, VO_{2max}'da anlamlı artışlara; vücut ağırlığı, BKİ ve vücut yağ oranında her iki grupta da anlamlı azalmalara neden oldu. HTYG ve OTYG'nin serum OPG seviyelerinde anlamlı düşüşler belirlendi (sırasıyla $p<0.05$ ve $p<0.01$). Hiçbir grubun sRANKL ve sRANKL/OPG oranında anlamlı değişiklik gözlenmedi. Serum OPG düzeylerinde düşüşle ilişkili osteoporoza karşı koruyucu etkide anlamlı gelişmeler hızlı veya orta tempo yürüyüşler ile elde edilebilmekte; ancak kardiyovasküler uygunluk için hızlı tempo yürüyüş çok daha etkili olmaktadır.

Anahtar sözcükler: *Premenopozal, sRANKL, osteoprotegerin, egzersiz*

INTRODUCTION

Physical activity has some effects on bone mass (14,21). However, the overuse of the skeleton may result in bone stress injuries (17). Therefore, in recent years, walking – a milder form of physical activity, has been suggested by physicians. Many people use walking as the basis of their habitual physical activity since it is associated with reduced risk of osteoporotic fracture (4), and carries a low risk of injury (13). It is therefore an ideal intervention that could have a role in the prevention of osteoporosis.

In recent years, osteoprotegerin (OPG) and the receptor-activator of nuclear factor κβ ligand (RANKL) have been used to determine bone status. OPG is a member of the tumor necrosis factor (TNF) receptor super-family. This cytokine binds competitively to RANKL and thereby reduces the impact of RANKL on RANK and preosteoclast. As a result, the transformation of preosteoclast to osteoclasts is reduced, the number of osteoclasts is diminished, and bone formation is promoted. The reduction of RANKL leads to an inactivation of its receptor RANK and stops bone resorption (2). RANKL and OPG play an essential role for osteoclast formation and activation. The RANKL/RANK/OPG pathway has important implications for the pathogenesis, diagnosis and treatment of human bone diseases. Serum OPG levels are increased in patients with CV disease and/or excess bone resorption (5). This may indicate that serum OPG changes are a consequence rather than a cause of disease (8).

Results of cross-sectional studies have revealed either correlations (12,15) or none (9,19) between serum OPG levels and bone density. However, the local expression of OPG mRNA levels in iliac bone biopsies has been correlated with increased fracture susceptibility (1). There are some studies indicating correlations of serum OPG with age (6,9,15,19) and bone turnover (6,9,19). Thus one may hypothesize that circulating OPG can relate to metabolic changes within the bone compartment. However, it is still not certain whether circulating OPG and RANKL reflect changes in bone metabolism as a result of physical activity. There are few studies with conflicting results indicating correlations between physical activity and the OPG/RANKL system (18,20); and no studies examining the effects of walking exercises with different intensities on the OPG/RANKL system exist. Therefore, the aim of the present study is to evaluate the expectedly positive changes in serum sRANKL and OPG levels following eight week walking programs of two different intensities in pre-menopausal women.

MATERIALS AND METHODS

Thirty-four sedentary pre-menopausal women aged 30 to 55 years participated in the study. The ones wishing to participate in the exercise groups were randomly categorized as the brisk (BWG; n=15) or the moderate tempo (MTWG; n=16) walking groups. Thirteen women formed the control group (CG). No statistically significant differences existed for their initial physical and physiological characteristics (Table 1).

Table 1. Subjects' baseline physical and physiological characteristics (mean ± SD)

Parameter/Group	BWG (n=15)	MTWG (n=16)	CG (n=13)
Age (yr)	40.3 ± 3.9	43.5 ± 5.3	42.1 ± 7.5
Height (cm)	161.8 ± 7.4	160.0 ± 6.0	160.0 ± 9.9
Body weight (kg)	73.5 ± 12.1	77.0 ± 10.4	71.6 ± 10.8
BMI ($kg.m^{-2}$)	28.0 ± 3.9	30.8 ± 4.6	28.1 ± 5.0
Body fat (%)	33.8 ± 4.8	36.4 ± 4.8	35.2 ± 5.5
VO_{2max} ($ml.min^{-1}.kg^{-1}$)	28.0 ± 5.0	25.1 ± 4.2	25.4 ± 5.2

BMI: Body mass index

All information about the subjects was gathered via questionnaires. They were examined thoroughly before the beginning of the study, were informed about the study design, and were asked to sign the informed consent form. The ethical council of the Celal Bayar University Faculty of Medicine approved the study.

Subjects with previous cardiovascular disease history or diagnosed for coronary heart disease, endocrine or metabolic disorders; those with a high resting blood pressure (>160 mmHg systolic or >95 mmHg diastolic), DM, hyperthyroidism, musculo-skeletal problems or irregular menses; and those with a ± 5 kg change in body weight during the previous year; and/or being treated for osteoporosis, or being under medication capable of affecting bone metabolism; and smokers were excluded from the study.

Dietary intake was measured by using two dietary questionnaires related to fat consumption and fruit, vegetable and fiber consumption (3). The participants were determined to have a balanced and sufficient dietary intake and were warned not to change their dietary habits throughout the study. Body composition was measured using a bioelectrical impedance analyzer (Model TBF-300, Tanita Corp., Tokyo, Japan). VO_{2max} was estimated via a 2 km walking test (10,16). Subjects were told to avoid any physical activity within the 48 h preceding the assessment day. Walking speeds, causing 50-55% and 70-75% maximal heart rate reserve (HRR_{max}) were calculated with the Karvonen formula. On the first four weeks, BWG aimed to walk for 30, 33, 36, and 39 min at 70% of HRR_{max}. On the next four weeks, they aimed to walk for 42, 45, 48 and 51 min at 75% HRR_{max}. MTWG aimed to walk for the same duration as BWG, but their walking speed was targeted to be at 50% HRR_{max} for the first four weeks and 55% HRR_{max} for the second four weeks. At least three heart rate readings were taken with heart rate monitors (Polar Vantage, Kempele, Finland) and their rate of perceived

exertion (RPE) was also taken using a 15-point scale. The subjects warmed up and cooled down performing 5 min of stretching exercises.

Blood samples (20 ml) were collected after a 20 min rest between 8:00 and 9:00 a.m. Serum was separated by centrifugation, and samples were stored at -80°C until assays were run (within one month) in all samples. Serum OPG levels were determined with a commercial sandwich enzyme immunoassay (Biomedica, Vienna, Austria). Standard curves were drawn using the serum standards provided. The intra-assay CV for serum OPG measurements at the 4.59 pmol/l level was 10%, and the inter-assay CV at the 5.53 pmol/l level was 7%. sRANKL was determined with an enzyme immunoassay system (Biomedica, Vienna, Austria). The system is specific for free soluble RANKL. The intra-assay CV for RANKL measurements at the 1.00 pmol/l level was 5%, and the inter-assay CV at the 0.80 pmol/l level was 9%. The lower detection limits for OPG and RANKL assays were 0.14 pmol/l and 0.08 pmol/l.

Data were analyzed by means of the Wilcoxon Signed Ranks test for within-group comparisons, the Kruskal-Wallis test for group comparisons, and the Mann-Whitney U test to determine the differences between the two groups. Results are presented as mean ± SD. Statistical significance was defined at the $p<0.05$ level.

RESULTS

The average heart rate during the training for BWG was 152.1 ± 7.3 bpm (74% of HRR_{max}), and it was 131.4 ± 7.3 bpm for MTWG (54% of HRR_{max}). Mean walking speed in the program for the BWG was 7.26 ± 0.67 kph, and it was 5.36 ± 0.34 kph for the MTWG. The RPE reported by the BWG was 15.9 ± 0.8 and it was 13.2 ± 0.5 for the MTWG. Total distance walked for the whole program was 189423 ± 6237 m for the BWG, and it was 163474 ± 6154 m for the MTWG.

After eight weeks, the estimated VO_{2max} improved significantly in both exercise groups. Their body weights, BMIs, and body fat ratios decreased significantly (Table 2). The change observed in both exercise groups in their body weights and body fat was significantly different from the change that occurred in CG ($p<0.01$ and $p<0.05$, respectively). The change in BMI in the MTWG was significantly different from that in the CG ($p<0.01$). The change in VO_{2max} found in the BWG was significantly different from those in the MTWG and CG ($p<0.01$). The change in VO_{2max} in the MTWG was different from that in the CG ($p<0.05$) too.

Table 2. Changes in physical and physiological parameters (mean ± SD).

Period/ Variables	Pre-intervention	Post-intervention	Mean differences
Body weight (kg)			
BWG	73.5 ± 12.1	72.1 ± 11.6[b]	-1.39 ± 1.30[d]
MTWG	77.0 ± 10.4	75.4 ± 10.1[b]	-1.62 ± 1.19[d]
CG	71.6 ± 10.8	71.8 ± 10.7	0.26 ± 0.77
Body fat (%)			
BWG	33.8 ± 4.8	32.5 ± 5.2[a]	-1.23 ± 1.34[c]
MTWG	36.4 ± 4.8	35.1 ± 5.1[a]	-1.35 ± 1.80[c]
CG	35.2 ± 5.5	35.7 ± 4.7	0.47 ± 1.96
BMI ($kg.m^{-2}$)			
BWG	28.0 ± 3.9	27.4 ± 3.2[a]	-0.63 ± 0.78
MTWG	30.8 ± 4.6	29.2 ± 4.2[b]	-1.58 ± 2.70[d]
CG	28.1 ± 5.0	27.6 ± 3.6	-0.50 ± 1.82
VO_{2max} ($ml.min^{-1}.kg^{-1}$)			
BWG	28.0 ± 4.0	35.4 ± 3.8[b]	7.04 ± 4.81[d,e]
MTWG	25.1 ± 4.2	27.9 ± 4.2[a]	2.37 ± 3.12[c]
CG	25.4 ± 4.9	24.9 ± 4.4	-0.50 ± 3.35
sRANKL (pmol/l)			
BWG	0.21 ± 0.20	0.20 ± 0.15	-0.01 ± 0.28
MTWG	0.23 ± 0.22	0.18 ± 0.25	-0.05 ± 0.32
CG	0.27 ± 0.21	0.30 ± 0.12	0.03 ± 0.26
OPG (pmol/l)			
BWG	6.12 ± 1.49	4.26 ± 3.08[a]	-1.86 ± 3.60
MTWG	5.70 ± 1.43	3.47 ± 1.82[b]	-2.23 ± 1.99
CG	4.89 ± 1.17	4.19 ± 0.48	-0.69 ± 0.97
sRANKL/OPG			
BWG	0.03 ± 0.03	0.05 ± 0.05	0.02 ± 0.06
MTWG	0.04 ± 0.04	0.05 ± 0.04	0.01 ± 0.04
CG	0.06 ± 0.05	0.07 ± 0.05	0.02 ± 0.03

[a]$p<0.05$ change from baseline; [b]$p<0.01$ change from baseline; [c]$p<0.05$ vs. CG; [d]$p<0.01$ vs CG; [e]$p<0.01$ vs MTWG

Pre- and post-test differences in serum OPG levels of the BWG ($p<0.05$) and the MTWG ($p<0.01$) were observed. The reduction observed in serum OPG levels for the CG was not statistically significant. There were no significant changes in RANKL values and RANKL/OPG ratios in any of the groups (Table 2).

DISCUSSION

The most outstanding finding of this study is the significant reduction in serum OPG levels of both exercise groups without any changes in their sRANKL levels. The significant decrease in the serum OPG levels of the exercising subjects in this study may be interpreted as the beneficial effects of exercise on the protective mechanism against osteoporosis and vascular diseases.

The effect of physical activity on OPG and RANKL levels has taken considerable attention in recent years. In a study, no change has been found in serum OPG and sRANKL levels of athletes (cross-country skiers and biathlon athletes) and controls (7). In contrast, some increases in OPG levels of the participants have been detected (11) and the researcher stated that their finding was confirmed with the results of DXA measurements. Nevertheless, serum OPG increases do not always reflect the increase in bone density since some inverse correlations have been determined between serum OC and OPG, and lumbar spine BMD and serum OPG (15).

Observing some increases in OPG levels seems quite possible as a result of high-intensity strength training, because it has been widely emphasized that mechanical loads such as high intensity resistance exercises, which produce dynamic strains within bones, may play an important role in controlling bone mass and strength (18,20). However, high-impact and weight-bearing exercise may impose injury risks to the participants. Life-long exercise programs on the other hand are more preferable because they may protect against multiple-risks such as poor muscle strength, flexibility, balance and coordination, all of which facilitate falls that may result in fractures. The exercise programs applied in this study may be life-long recommended, and may provide optimum health benefits together with some protective effects against bone resorption and some vascular diseases.

It has been proposed that an increase in OPG levels by training could lead to a decrease of sRANKL and consequently an inactivation of osteoclasts resulting in a reduction of bone resorption, and an increase in bone mass (23), with a reference to the literature indicating that this may lead to a decrease in fracture incidence. Contrary to this suggestion, we found OPG levels significantly decreased in both walking groups although there was not a significant change in their sRANKL values as indicated in literature, suggesting that serum RANKL levels did not show a significant correlation with BMD measured in humans (22). Although RANKL is an essential factor for osteoclasts' activity, it is somewhat difficult to explain our results that serum levels of RANKL did not change, but OPG levels were significantly reduced as a result of physical activity.

Even though we may conclude that continuous walking activity of eight weeks causes a reduction in OPG levels that might reduce bone resorption, independent of intensity, it is not possible to draw a final

conclusion from this present study, since it has some limitations. Due to the strict participation criteria, the number of participants recruited was limited. In addition, the comparability of present data with other studies is also greatly limited, as no study investigating the effects of walking exercises with different intensities on OPG/RANKL system existed to date. Therefore, whether walking exercises are related to any RANKL and OPG level changes in pre-menopausal women needs to be further studied in different populations, especially including different exercise programs such as high-impact or resistance training programs, with more participants, in a longer period of time.

The results of this study may still be accepted aas interesting, since data reveals for the first time that OPG levels may be reduced as a result of walking exercises, which in turn may have protecting effects against osteoporosis and some types of vascular diseases.

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