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# Do Walking Programs Affect C-Reactive Protein, Osteoprotegerin and Soluble Receptor Activator of Nuclear Factor-Kappaβ Ligand?

## [Yürüyüş Programları C-Reaktif Protein, Osteoprotegerin ve Soluble Reseptör Aktivatör Nükleer Faktör-Kappaβ Ligand Düzeylerini Etkiler mi?]

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**ABSTRACT**

**Aim:** To examine the effects of 10-week walking programs on maximal oxygen consumption, body composition, serum lipids, highly-sensitive C-reactive protein, osteoprotegerin and soluble receptor activator of nuclear factor-kappaβ ligand.
**Method:** Twenty-seven middle-aged men (40-60years) walked for ten weeks, five days per week, 40-52min per day at either moderate or high intensity (~5.95±0.26km/h and ~7.64±0.36km/h; 50-55% and 70-75% maximum heart rate reserve, respectively). Non-walking, sedentary men served as controls (n=13). Estimated maximal oxygen consumption, body composition, lipid profile, highly-sensitive C-reactive protein, osteoprotegerin and soluble receptor activator of nuclear factor-kappaβ ligand were determined before and after the study.
**Results:** After 10 weeks, estimated maximal oxygen consumption improved in both exercise groups ($p<.05$), favoring high-intensity group ($p<.05$). Body weight, percent body fat, and body mass index reduced significantly in both exercise groups ($p<.05$). Walking programs did not cause any significant changes in blood lipids, highly-sensitive C-reactive protein, and osteoprotegerin levels; however, soluble receptor activator of nuclear factor-kappaβ ligand levels were found to decrease in high-intensity group ($p<.05$) and the change observed in both exercise groups was different from the change in control group ($p<.05$).
**Conclusion:** For protective effects against cardiac risk factors and arterial calcification, high-intensity walking programs are advisable due to the greater improvements in soluble receptor activator of nuclear factor-kappaβ ligand and estimated maximal oxygen consumption.
**Key words:** Walking exercises, middle-aged men, estimated maximal oxygen consumption, lipid profile, highly-sensitive C-reactive protein, soluble receptor activator of nuclear factor-kappaβ ligand

**ÖZET**

**Amaç:** On haftalık yürüyüş programlarının maksimal oksijen tüketimi, vücut kompozisyonu, serum lipidleri, yüksek sensitiviteli C-reaktif protein, osteoprotegerin ve soluble reseptör aktivatör nükleer faktör-kappaβ ligandı üzerine etkilerini incelemek.
**Yöntem:** Yirmi yedi orta yaşlı (40-60yaş) erkek on hafta, haftada beş gün, günde 40-52 dakika orta veya yüksek şiddette (sırasıyla ~5.95±0.26km/s ve ~7.64±0.36km/s; maksimum kalp atım hızı rezervinin %50-55 ve %70-75'inde) yürüdüler. Yürüyüş yapmayanlar kontrol grubunu oluşturdu (n=13). Egzersiz programı öncesi ve sonrasında, tahmini maksimal oksijen tüketimi, vücut kompozisyonu, lipid profili, yüksek sensitiviteli C-reaktif protein, osteoprotegerin ve soluble reseptör aktivatör nükleer faktör-kappaβ ligandı düzeyleri belirlendi.
**Bulgular:** On haftanın sonunda tahmini maksimal oksijen tüketimi yüksek-şiddet grubunun lehine ($p<.05$) her iki egzersiz grubunda arttı ($p<.05$). Vücut ağırlığı, vücut yağ yüzdesi ve beden kitle indeksi değerlerinde egzersiz gruplarında anlamlı azalmalar meydana geldi ($p<.05$). Yürüyüş antrenmanları kan lipit değerleri, yüksek sensitiviteli C-reaktif protein ve osteoprotegerin düzeyleri üzerinde etkili bulunmadı, ancak soluble reseptör aktivatör nükleer faktör-kappaβ ligand yüksek şiddet grubunda anlamlı olarak azaldı ($p<.05$); egzersiz gruplarında belirlenen değişim kontrol grubundaki değişimden farklıydı ($p<.05$).
**Sonuç:** Soluble reseptör aktivatör nükleer faktör-kappaβ ligand ve tahmini maksimal oksijen tüketiminde meydana gelen gelişmelerden dolayı kardiyak risk faktörlerine ve damarsal kalsifikasyona karşı koruyucu etkiler yaratabilmek için yüksek şiddet yürüme programları önerilebilir.
**Anahtar Kelimeler:** Yürüyüş egzersizi, orta yaşlı erkek, tahmini maksimal oksijen tüketimi, lipit profili, yüksek sensitiviteli C-reaktif protein, osteoprotegerin, soluble reseptör aktivatör nükleer faktör-kappaβ ligandı

## Introduction

Coronary heart disease (CHD) has been one of the major causes of morbidity and mortality in recent years. (1). Poor blood lipid profile and inactive life-style are the factors known to affect CHD. Physical activity is associated with a lower risk of cardiovascular disease (CVD) (2). Physical exercise has been found to decrease plasma triglyceride (TG) and low-density lipoprotein cholesterol (LDL-C); increase plasma high-density lipoprotein cholesterol (HDL-C) levels (3), which are very closely related to CHD.

Preventing the development and advancement of CVD by controlling the risk factors is important. Previous research has indicated that inflammation in coronary artery is associated with the formation and advance of plaque (4). Therefore, in recent years, there is growing evidence for highly-sensitive C-reactive protein (hs-CRP) that it is a much better predictor of CHD than usual known cardiovascular risk factors alone (5) and chronically elevated levels contribute independently to later risk of CHD (6). It has been reported that the initial development of myocardial infarction, stroke, and obstructive arteriosclerosis can be predicted by hs-CRP in healthy subjects (7). The role of physical activity on CRP levels is not clear. It was hypothesized that the baseline CRP concentration is affected by two antagonistic influences. While intense physical exercise produces micro injuries and a local inflammatory reaction in the musculature causing a delayed increase of the CRP concentration in the blood, regular physical training generates an anti-inflammatory reaction with a lowering effect on the CRP level (8). This might result from the enhanced antioxidative mechanisms after regular physical exercise (9,10). Therefore, many researchers have attempted to investigate the relationship between physical exercise and CRP concentrations. Some favorable changes in hs-CRP levels due to physical activity were determined (8,11-13). In two recent studies, an inverse association of hs-CRP levels and cardiorespiratory fitness levels were observed (14,15). Since CRP is an inflammatory marker, these findings suggest that the association between exercise and reduced cardiovascular risk may be mediated by anti-inflammatory effects of regular physical activity (16).

Very recently, osteoprotegerin (OPG), a key factor in bone remodeling (17), a member of the tumor necrosis factor receptor family, and a decoy receptor for the receptor activator of nuclear factor-kappaβ ligand (RANKL) (18), has been implicated in human atherogenesis. Cross-sectional studies demonstrate a relationship between OPG levels and the severity of coronary atherosclerosis (19,20). In the light of these data, OPG seems to play an important role in atherosclerosis and could be a marker of atherosclerotic lesions. In addition, myocardial protein levels of OPG, RANK, and RANKL levels were found to be increased after human heart failure (21). There are very few studies on the role of physical activity on OPG-RANKL system, and the existing studies mainly examined the role of physical activity on bone remodeling process and revealed conflicting results (22-24). To our knowledge there are no studies on the role of physical activity on OPG-sRANKL system as CHD risk determinants. Therefore, this present study aims to examine the effect of different-intensity walking programs on maximal oxygen consumption, body composition, serum lipids, hs-CRP, OPG, and sRANKL levels in the middle-aged men.

## Material and Method

### *Subject selection*

Healthy males (aged between 40-60 years) volunteered for this study. Recruiting criteria were as follows: (1) to live in Manisa for at least 10 years and not planning to leave the area during the experimental period, (2) being a non-smoker, (3) having BMI between 25-30kg/$m^2$, (4) not participating in a regular exercise program in the previous six months. Exclusion criteria consisted of having a previous history of cardiovascular disease or diagnosed CHD, endocrine or metabolic disorders, resting blood pressure greater than 140/90mmHg, having musculo-skeletal problems, diabetes mellitus, hyperthyroidism, taking antidepressants or anti-inflammatory drugs, and a ±5 kg change in body weight during the previous year.

We gathered the information about the participants via questionnaires, informed them about the study design, and obtained a signed consent form from each participant. They were physically examined thoroughly before the experimental period started. The participant who met the above-mentioned criteria was taken to laboratory screening for electrocardiography and body composition measurements. We measured the dietary intake of the participants via the "Healthy Lifestyle Behavior Scale" (25). The analysis of the scale revealed that the participants had balanced diatery intake and they were warned not to change their dietary habits throughout the study period.

In order to supply compliance, the participants were free to choose to participate either in exercise group (EG) or the control group (CG). After that, the EG volunteers were randomly classified as either moderate intensity walking group (MIWG; n= 13) or high intensity walking group (HIWG; n= 14). EG 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. Of the 55 volunteers, 10 of them could not meet the criteria. Five of the 45 men who started the intervention dropped off due to some personal or health reasons; 40 of them completed the intervention period and were taken to final evaluation. The ethical council of Celal Bayar University, Faculty of Medicine, approved the study.

### *Study protocol*

After the initial testing and measurements, EG started the 10-week walking program on a 400m outdoor track

(5 days per week). They started to walk 40 minutes a day, and with three minute-increments in every two weeks, they reached 52 minutes at the end of the program. MIWG walked at 50-55% maximum heart rate reserve ($HRR_{max}$) with a speed of ~5.95±0.26km/h and HIWG walked at 70-75% $HRR_{max}$ with a speed of ~7.64±0.36km/h. Five-minute warm-up and 5-minute cool-down activities were performed in each walking session.

The program was designed based on the American College of Sports Medicine recommendations (26) and supervised and monitored by trained exercise specialists. The exercise intensity was prescribed based on target heart rates (THRs) calculated from the Karvonen equation:

[$Heart\ Rate_{maximum}$ – $Heart\ Rate_{rest}$) x (0.50-0.55) + $Heart\ Rate_{rest}$] for MIWG and

[$Heart\ Rate_{maximum}$ – $Heart\ Rate_{rest}$) x (0.70-0.75) + $Heart\ Rate_{rest}$] for HIWG.

$HR_{maximum}$ was predicted via 220 – age formula.

Heart rates of the participants were taken through use of Polar Pacer heart rate monitors (Polar Vantage, Kempele, Finland). Their Rate of Perceived Exertion (RPE) was also taken using a 15-point RPE scale and noted on training logs together with their total walking distances.

### *Testing procedures*

Pre- and post-study values of body weight, body mass index (BMI), and percent body fat were taken through body composition analyzer (Model TBF-300, Tanita Corp., Tokyo, Japan) at 8.00-9.00 a.m, after a 12h fast. Astrand - Ryhming test, for estimating maximal oxygen consumption ($VO_{2max}$) from submaximum workloads, was performed on a calibrated bicycle ergometer (Monark 860, Varberg, Sweden) and $VO_{2max}$ was predicted using Astrand-Ryhming nomogram. Subjects were informed not to take part in any physical activity within 48h before the assessment day.

Blood samples were taken after a 12 h overnight fast between 8.00 and 9.00 a.m. TC concentration was measured by the cholesterol oxidase method; TG levels were determined by glycerol phosphate oxidase method; HDL-C was determined enzymatically in the supernatant after precipitation of LDL-C with Beckman Coulter kits at BECKMAN COULTER Unicel D×C 800 analyzer (SYNCRON LX Systems, Beckman Coulter, Ireland). LDL-C concentration was calculated using the Friedwald formula. In TC, TG, and HDL-C analyses, within run coefficients of variation (CV) were 1.09, 2.6, and 3.4%, respectively. Hs-CRP levels were determined by chemiluminescent immunometric assay with Siemens Medical Diagnostics Limited kits at IMMULITE 2000 analyzer (Immulite 2000, Los Angeles, CA, USA). For hs-CRP analysis intra assay CV at level 2.8 mg/L was 3.4%, and inter-assay CV at level 2.8mg/L was 3.8%. Serum concentrations of OPG were measured using ELISA method (BioVendor Research and Diagnostic Products, Modrice, Czech Republic). The intra-assay CV at level 5.41 pmol/L and inter-assay CV at level 5.59 pmol/L for OPG assay were 2.4% and 4.2%, respectively. The lower limit of detection for OPG was 0.4 pmol/L. Serum concentrations of sRANKL were measured using ELISA method (BioVendor Research and Diagnostic Products, Modrice, Czech Republic). The intra-assay CV at level 96 pmol/L and inter-assay CV at level 88 pmol/L for sRANKL assay were 7.9% and 8.3%, respectively. The lower limit of detection for sRANKL was 10 pmol/L.

***Statistical methods:*** Data were analyzed using SPSS package program version 15.0. Since a one-sample Kolmogorov-Smirnov test indicated that the variables were not normally distributed, nonparametric tests were used and the descriptive statistics were given as median-min-max values. Kruskall-Wallis test was used to compare changes among the study groups. Bonferroni-corrected Mann-Whitney U test was used to determine the difference between the two groups. The differences between pre-training and post-training values were determined by using Wilcoxon Signed Ranks test. Statistical significance was defined at $p< 0.05$ level.

## Results

MIWG members aimed to walk at 50-55% of maximum heart rate reserve ($HRR_{max}$). The average heart rate (HR) per week during the training for MIWG was ~127.46±5.02 $beat.min^{-1}$ and they walked at ~5.95±0.26 km/h, totally 253509.6±16870.16m; their reported RPE was 11.9±0.49. HIWG aimed to walk at 70-75% of $HRR_{max}$. The HR per week during the training for HIWG was ~148.64±2.84 $beat.min^{-1}$ and they walked at ~7.64±0.36km/h, totally 292089.32±13922.73m; their reported RPE was 14.71±0.64.

Pre-study evaluations of study groups were not significantly different from each other, except for the significant difference in their sRANKL values ($p<.05$; Table 1). Ten-week walking programs caused significant differences in body weight, BMI, percent body fat, and estimated $VO_{2max}$ in HIWG and MIWG ($p<.05$). There were no significant changes in any of the measured physical and physiological parameters in CG (Table 2). Walking programs of different intensity appears to have similar effects since there were no significant changes between the two exercise groups in terms of measured physical and physiological parameters apart from a significant difference in the estimated $VO_{2max}$ levels, favoring HIWG ($p<.05$). However, the two exercise groups revealed significant differences from the CG in all measured physical and physiological parameters ($p<.05$; Table 3).

We determined no significant changes in the measured blood lipids (TC, TG, HDL-C, LDL-C), hs-CRP, and OPG levels of the study groups apart from a significant reduction in HIWG and a significant increase in sRANKL values in CG ($p<.05$; Table 4); the changes observed in sRANKL in both exercise groups were different from the change of CG ($p<.0.5$; Table 5).

**Table 1.** Initial physical and physiological parameters of the groups (median-min-max)

| Test/unit | CG (n=13) Median Min-Max | HIWG (n= 14) Median Min-Max | MIWG (n= 13) Median Min-Max | p |
|---|---|---|---|---|
| Age (year) | 52.0<br>45.0-60.0 | 51.5<br>38.0-59.0 | 52.0<br>45.0-55.0 | NS |
| Height (cm) | 173.0<br>160.0-182.0 | 176.0<br>165.0 -190.0 | 173.0<br>163.0-188.0 | NS |
| Body weight (kg) | 79.8<br>58.9-99.3 | 86.1<br>68.6-104.5 | 80.2<br>69.1-91.0 | NS |
| BMI ($kg/m^2$) | 26.1<br>21.1-31.4 | 28.1<br>22.6-32.3 | 26.8<br>22.3-29.9 | NS |
| Percent body fat (%) | 27.8<br>20.5-33.8 | 26.8<br>22.69-33.08 | 29.4<br>22.7-34.6 | NS |
| $VO_{2max}$ ($ml \cdot kg^{-1} \cdot min^{-1}$) | 26.1<br>20.4-38.5 | 26.3<br>16.9-35.5 | 27.6<br>16.0-37.7 | NS |
| TG (mg/dL) | 93.0<br>62.0-290.0 | 79.5<br>32.0-180.0 | 109.0<br>41.0-216.0 | NS |
| TC (mg/dL) | 215.0<br>137.0-248.0 | 191.0<br>143.0-230.0 | 200.0<br>146.0-302.0 | NS |
| HDL-C (mg/dL) | 38.0<br>31.0-53.0 | 35.0<br>28.0-49.0 | 38.0<br>28.0-59.0 | NS |
| LDL-C (mg/dL) | 137.0<br>87.0-181.0 | 136.0<br>85.0-158.0 | 143.0<br>82.0-221.0 | NS |
| hs-CRP (mg/L) | 1.9<br>0.3-5.26<br>(n= 11) | 2.0<br>0.6-5.6<br>(n= 12) | 3.4<br>0.5-6.4 | NS |
| OPG (pmol/L) | 4.9<br>3.0-6.6 | 4.1<br>1.9-7.4 | 5.1<br>3.2-15.5 | NS |
| sRANKL (pmol/L) | 197.20<br>76.90-500.60 | 458.75*<br>133.90-1849.80 | 210.55<br>88.60-488.10<br>(n= 12) | <.05 |

CG= Control Group; HIWG= High-intensity Walking Group; MIWG= Moderate-intensity Walking Group

Group comparisons were made using Kruskall-Wallis and Bonferroni-corrected Mann-Whitney U tests; NS= No significant; *p< .0167 different from MIWG and CG.

**Table 2.** Changes in the physical and physiological parameters following 10-week walking programs

| | CG (n=13) | | | HIWG (n= 14) | | | MIWG (n= 13) | | |
|---|---|---|---|---|---|---|---|---|---|
| Test/unit | Pre Median Min-Max | Post Median Min-Max | p | Pre Median Min-Max | Post Median Min-Max | p | Pre Median Min-Max | Post Median Min-Max | p |
| Body weight (kg) | 79.8<br>58.9-99.3 | 80.0<br>60.8-100.9 | NS | 86.1<br>68.6-104.5 | 83.9<br>68.0-100.0 | <.05 | 80.2<br>69.1-91.0 | 78.0<br>68.0-90.8 | <.05 |
| BMI ($kg/m^2$) | 26.1<br>21.1-31.4 | 26.7<br>21.8-31.3 | NS | 28.1<br>22.6-32.3 | 27.0<br>22.5-30.0 | <.05 | 26.8<br>22.3-29.9 | 25.5<br>22.1-29.5 | <.05 |
| Percent body fat (%) | 27.8<br>20.5-33.8 | 27.3<br>21.0-37.3 | NS | 26.8<br>22.69-33.08 | 26.2<br>21.8-32.2 | <.05 | 29.4<br>22.7-34.6 | 28.3<br>22.7-34.3 | <.05 |
| $VO_{2max}$ ($ml \cdot kg^{-1} \cdot min^{-1}$) | 26.1<br>20.4-38.5 | 26.4<br>20.1-36.2 | NS | 26.3<br>16.9-35.5 | 32.7<br>26.0-44.4 | <.05 | 27.6<br>16.0-37.7 | 29.6<br>18.0-39.0 | <.05 |

CG= Control Group; HIWG= High-intensity Walking Group; MIWG= Moderate-intensity Walking Group

Within group comparisons were made using Wilcoxon Signed Ranks test; Intervention data are presented as median-min-max; NS= No significant

**Table 3.** The comparison of the differences obtained in the groups

| | CG (n=13) | HIWG (n= 14) | MIWG (n= 13) | |
|---|---|---|---|---|
| Test/unit | Median<br>Min-Max | Median<br>Min-Max | Median<br>Min-Max | p |
| Body weight (kg) | 0.3<br>-1.3-3.5 | -1.9*<br>-5.7-0.3 | -1.3*<br>-2.7-(-0.2) | <.05 |
| BMI (kg/m$^2$) | 0.5<br>-0.4-0.7 | -0.9*<br>-2.3-0.0 | -0.6*<br>-1.8-(-0.2) | <.05 |
| Body fat (%) | 0.00<br>-3.0-3.6 | -0.9*<br>-2.1-(-0.3) | -0.9*<br>-2.6-(-0.7) | <.05 |
| $VO_{2max}$ (ml·kg$^{-1}$·min$^{-1}$) | -0.2<br>-2.3-0.6 | 5.4*,a<br>3.7-9.6 | 3.6*<br>1.3-6.6 | <.05 |

CG= Control Group; HIWG= High-intensity Walking Group; MIWG= Moderate-intensity Walking Group

Group comparisons were made using Kruskall-Wallis and Bonferroni-corrected Mann-Whitney U tests; Intervention data are presented as median-min-max; NS= No significant; *p< .0167 different from CG; ap<.0167 different from MIWG

**Table 4.** Changes in the blood lipids and inflammation markers following 10-week walking exercises

| | CG (n=13) | | | HIWG (n= 14) | | | MIWG (n= 13) | | |
|---|---|---|---|---|---|---|---|---|---|
| Test/unit | Pre<br>Median<br>Min-Max | Post<br>Median<br>Min-Max | p | Pre<br>Median<br>Min-Max | Post<br>Median<br>Min-Max | p | Pre<br>Median<br>Min-Max | Post<br>Median<br>Min-Max | p |
| TG (mg/dL) | 93.0<br>62.0-290.0 | 69.9<br>80.0-245.0 | NS | 79.5<br>32.0-180.0 | 92.0<br>28.0-168.0 | NS | 109.0<br>41.0-216.0 | 110.0<br>41.0-214.0 | NS |
| TC (mg/dL) | 215.0<br>137.0-248.0 | 192.0<br>140.0-256.0 | NS | 191.0<br>143.0-230.0 | 173.5<br>130.0-221.0 | NS | 200.0<br>146.0-302.0 | 204.0<br>119.0-274.0 | NS |
| HDL-C (mg/dL) | 38.0<br>31.0-53.0 | 37.0<br>26.0-50.0 | NS | 35.0<br>28.0-49.0 | 34.0<br>28.0-47.0 | NS | 38.0<br>28.0-59.0 | 36.0<br>30.0-49.0 | NS |
| LDL-C (mg/dL) | 137.0<br>87.0-181.0 | 139.0<br>81.0-200.0 | NS | 136.0<br>85.0-158.0 | 125.5<br>73.0-158.0 | NS | 143.0<br>82.0-221.0 | 144.0<br>65.0-199.0 | NS |
| hs-CRP (mg/L) | 1.9<br>0.3-5.26<br>(n= 11) | 1.4<br>0.2-3.2<br>(n= 11) | NS | 2.0<br>0.6-5.6<br>(n= 12) | 1.2<br>0.6-3.4<br>(n= 12) | NS | 3.4<br>0.5-6.4 | 1.0<br>0.3-5.9 | NS |
| OPG (pmol/L) | 4.9<br>3.0-6.6 | 5.4<br>3.5-9.9 | NS | 4.1<br>1.9-7.4 | 4.7<br>2.9-9.8 | NS | 5.1<br>3.2-15.5 | 5.3<br>3.1-10.4 | NS |
| sRANKL (pmol/L) | 197.20<br>76.90-500.60 | 241.00<br>115.90-495.90 | <.05 | 458.75<br>133.90-<br>1849.80 | 357.15<br>131.60-1381.60 | <.05 | 210.55<br>88.60-488.10<br>(n= 12) | 205.85<br>50.30-<br>362.20<br>(n= 12) | NS |

CG= Control Group; HIWG= High-intensity Walking Group; MIWG= Moderate-intensity Walking Group

Within group comparisons were made using Wilcoxon Signed Ranks test; Intervention data are presented as median-min-max; NS= No significant

## Discussion

To our knowledge, this is the first study examining the effects of different-intensity walking programs on OPG-sRANKL system as predictors of cardiovascular risk factors. The most outstanding finding is the significant reduction in sRANKL concentrations by means of high intensity walking. In addition, the significant gains obtained in exercise groups in $VO_{2max}$, favoring high-intensity group, and significant differences in BMI, body weight, and percent body fat in both exercise groups, which were not present in CG, may be the indicators of beneficial effects of walking programs on reducing risk factors against CHD.

### *Blood lipids, Body Composition, and $VO_{2max}$*

Studies have revealed that regular physical activity is related with lower TC, LDL-C, TG and higher HDL-C levels (27,28,29). However, we found no changes in any of the measured blood lipids, which is parallel to the results of Murphy et al, who determined no significant changes in any of the measured blood lipids as a result of walking programs (30). These results may be related to the exercise intensities since walking exercises are not as effective as competitive training programs or marathon run to cause significant changes in blood lipids. Longer duration, higher intensity training programs preferably together with a low-calorie diet are necessitated for significant blood lipid changes. We detected significant changes in both exercise groups in terms of body weight, body fat and BMI. The changes observed in the body weight of the exercise groups were found to be more

**Table 5.** The comparison of the differences obtained in the groups

| | CG (n=13) | HIWG (n= 14) | MIWG (n= 13) | |
|---|---|---|---|---|
| Test/unit | Median<br>Min-Max | Median<br>Min-Max | Median<br>Min-Max | p |
| TG (mg/dL) | -18.0<br>-87.0-76.0 | 1.5<br>-64.0-85.0 | -6.0<br>-96.0-105.0 | NS |
| TC (mg/dL) | -4.0<br>-36.0-8.0 | -4.5<br>-35.0-28.0 | -4.0<br>-35.0-40.0 | NS |
| HDL-C (mg/dL) | -1.0<br>-12.0-5.0 | -2.0<br>-12.0-6.0 | -2.0<br>-10.0-10.0 | NS |
| LDL-C (mg/dL) | -8.0<br>-28.0-27.0 | -5.0<br>-32.0-28.0 | 8.0<br>-89.0-28.0 | NS |
| hs-CRP (mg/L) | -0.4<br>-2.1-2.7<br>(n= 11) | -0.5<br>-4.1-0.7<br>(n= 12) | -0.8<br>-3.2-1.9 | NS |
| OPG (pmol/L) | 0.8<br>-2.2-4.3 | 0.1<br>-1.2-3.0 | -1.0<br>-6.9-3.5 | NS |
| sRANKL (pmol/L) | 37.50<br>-32.00-153.30 | -118.05*<br>-468.20-348.70 | -44.50*<br>-290.80-211.10<br>(n= 12) | <.05 |

CG= Control Group; HIWG= High-intensity Walking Group; MIWG= Moderate-intensity Walking Group

Group comparisons were made using Kruskall-Wallis and Bonferroni-corrected Mann-Whitney U tests; Intervention data are presented as median-min-max; NS= No significant; *p< .0167 different from CG

than the changes in their body fat and BMI. This might have resulted from the water and mineral loss during exercise. It is known that people lose not only fat but also water and minerals during exercise and diet. In addition, a calorie-restriction diet may be more effective to cause more reductions in the fat mass (31). According to Blair et al. (32) a target of a "good enough" level of fitness for people in the age range of 40-60 yrs, $VO_{2max}$ of 35 $ml.kg^{-1}.min^{-1}$ for men can be accepted as the optimal functional capacity to confer a decrease in the risk of CHD. With an average $VO_{2max}$ of 33.23±5.21 $ml.kg^{-1}min^{-1}$, the HIWG members seem to reach a better fitness level than MIWG (30.34±4.96 $ml.kg^{-1}min^{-1}$); therefore, walking programs of high-intensity may be followed by middle-aged men since they resulted in more increases in $VO_{2max}$ –an important determinant of cardiovascular health.

## *Hs-CRP*

Resent research has shown that the development of the atherosclerotic plaque is associated with inflammation (33,34). Research has suggested that hs-CRP, an inflammation marker, is a strong and independent index for CHD (35). Auer et al determined high CRP levels in patients after unstable angina and after acute myocardial infarction (36). Physical activity may be effective in modifying the inflammatory process. Thus, research has indicated that physical activity and physical fitness have inverse associations with the levels of CRP (37). Geffken et al determined that physical activity is in inverse correlation with CRP levels in physically active elderly population (13). hs-CRP levels were found to be decreased with a 9-week endurance training (8). Some researchers indicated that lower hs-CRP levels were related to the reduction in body weight and generally moderate intensity endurance training is effective in reducing hs-CRP levels (8, 11, 13). We could not determine any significant reductions in hs-CRP levels in our study; yet the nearly significant reduction observed in MIWG (p=0.087) may be accepted important and is parallel to the literature (8, 11, 13). Previous studies suggested that longer duration endurance training causing weight loss after the subjects undergo weight-loss programs is effective in lowering hs-CRP levels (31, 38). Despite significant weight reductions in our exercise groups, we could not observe any reductions in their hs-CRP levels. Weight loss can be accelerated in individuals who start exercise for the first time, but longer duration and higher intensity exercise programs may be necessary to cause changes in inflammatory markers. Therefore, this marker needs further investigation with more crowded groups following longer-period training programs with an accompanying calorie-restriction diet.

## *OPG-RANKL*

OPG is known to be secreted by bone (17), but it could also be produced by a variety of cells including coronary artery smooth muscle cells and endothelial cells of the cardiovascular system (19,39). In recent years, research has suggested that OPG, which has an important role in bone remodeling, is also effective in human atherogenesis (18). Various studies have shown that OPG may have important roles in the vasculature. Genetically modified *OPG−/−* mice with deficient OPG production exhibit a postnatal decrease in total bone density and unexpectedly develop medial calcification of the aorta and renal arteries, suggesting that regulation

of OPG, its signaling pathway, or its ligand(s) may play a role in the long observed association between osteoporosis and vascular calcification. The arteries exhibiting calcification in *OPG−/−* mice are sites of endogenous OPG expression, suggesting that OPG may have a role in protecting these arteries from pathological calcification (40). Increased OPG was observed in subgroups of Type 1 diabetic patients with neuropathy and with signs of CVD (41). A prospective study in elderly women pointed out an association between high levels of OPG with all cause mortality and cardiovascular mortality (42). Rhee et al in their study reported that mean OPG levels increased significantly as the number of stenotic vessels increased, and OPG levels were related to the severity of stenotic coronary arteries. Therefore OPG was found to be a risk factor for progressive atherosclerosis (20). However, Gannage-Yared did not find a statistical difference in OPG values between men with or without coronary artery disease. They suggested that OPG may have a link with fat mass and glucose homeostasis independently of its association with atherosclerosis (43). Ueland et al hypothesized that the OPG/RANK/RANKL axis could be involved in the pathogenesis of heart failure (HF). In their clinical and experimental studies, they found that in a rat model of post-infarction HF, OPG, RANK, and RANKL gene expression was increased in the ischemic part of the left ventricle. Enhanced myocardial protein levels of OPG, RANK, and RANKL were also seen in human HF (21). Therefore, the increases in OPG and RANKL levels may be interpreted as the vascular calcification and/or the heart failure. In order to protect from atherosclerosis or HF, it is important that OPG and RANKL levels should be low. The significant reduction we observed in sRANKL levels in our HIWG is therefore considerable.

In recent years, the role of physical activity on OPG-sRANKL system has taken attraction; however, the majority of the studies aimed to point out the role of physical activity on OPG-RANKL system as bone remodeling determinants (22-24). To us, examining the role of physical activity on OPG-sRANKL system is considerably important since the studies revealed associations between OPG-sRANKL pathway, vascular calcification, atherosclerosis and HF (21,39-41,43). However, we could not find studies examining the response of the OPG-RANKL system as cardiovascular risk determinants on physical activity. Therefore, this study is of great importance since it is the first one trying to look at exercise and OPG-RANKL system from this point of view. As the aforementioned studies have confirmed the relationship between increased levels of OPG – RANKL system on CHD (21,40-42), the unchanged levels of OPG in our exercise groups and the reduction observed in sRANKL levels in the HIWG may be interpreted as the beneficial effects of physical activity, particularly high-intensity walking, as a protective mechanism.

Despite being the first trial examining the effects of different-intensity walking programs on hs-CRP –an important inflammation marker, and OPG-RANKL pathway as CHD determinants, our study has some limitations. Its small sample size and relatively short period make it difficult to generalize the findings. In addition, lack of similar studies examining the effects of different type of exercise on OPG-sRANKL system as CHD determinants prevents us from comparing our findings with the previous research. Even so, our findings may be beneficial for future researchers; however, the results need further investigation in more crowded groups, with participants from different age groups, considering the biological variance of the OPG-RANKL system and preferably with an accompanying diet program of low-calorie food.

## Conclusion

The significant improvements we obtained in $VO_{2max}$ levels, body weight, BMI, and percent body fat support the current recommendations to increase physical activity to reduce the risk of cardiovascular diseases. However, we found no significant changes in serum lipids, hs-CRP and OPG levels. Even so, it is possible to consider high-intensity walking programs more beneficial for protective effects against cardiac risk factors and arterial calcification due to the higher increases in $VO_{2max}$ and significant reductions in sRANKL levels as a result of high-intensity walking.

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