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The Effects of an 8-week Walking Program on Serum Lipids, Circulation Matrix Metalloproteinase-9 and Tissue Inhibitor of Metalloproteinase-1 in Post-menopausal Women

[Sekiz Haftalık Yürüme Programlarının Menopoz Sonrası Kadınlarda Serum Lipidleri, Matriks-metalloproteinaz-9 ve Metalloproteinaz Doku Inhibitörü-1 Üzerine Etkileri]

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ABSTRACT

Purpose: To examine the effects of an 8-week walking program on serum lipids, circulation matrix metalloproteinase-9 and tissue inhibitor of metalloproteinase-1 in post-menopausal women.

Method: Body weight, percent body fat, body mass index, estimated maximal oxygen consumption, blood lipids, matrix metalloproteinase-9, tissue inhibitor of metalloproteinase-1 concentrations were assessed and their matrix metalloproteinase-9/tissue inhibitor of metalloproteinase-1 molar ratios were calculated in exercise (n=12) and control (n=12) groups. Exercise group completed an eight-week walking program at moderate (~6.29±0.15km/h; ~62% maximum heart rate reserve) intensity.

Results: Significant changes in estimated maximal oxygen consumption, systolic and diastolic blood pressures, body weight, and body mass index ($p < .05$) were determined in exercise group. However, there were no significant changes in the measured blood lipids (triglyceride, cholesterol, high-density lipoprotein cholesterol, low-density lipoprotein cholesterol), matrix metalloproteinase-9, and tissue inhibitor of metalloproteinase-1 concentrations, and matrix metalloproteinase-9/ tissue inhibitor of metalloproteinase-1 molar ratio of exercise group, except for a nearly significant change in tissue inhibitor of metalloproteinase-1 level ($p = .092$). We determined no significant changes in any of the measured parameters in control group.

Conclusion: Despite its protective effects against coronary heart disease risks, the duration and intensity of this program is not sufficient to cause significant changes in blood lipids, matrix metalloproteinase-9, and tissue inhibitor of metalloproteinase-1 concentrations. A higher-intensity, longer-duration program accompanied with diet is proposed.

Key Words: Walking, Blood Lipids, circulation matrix metalloproteinase-9, tissue inhibitor of metalloproteinase-1, Post-Menopausal Women

ÖZET

Amaç: Sekiz haftalık yürüme programının menopoz sonrası kadınlarda serum lipidleri, matrix metalloproteinase-9 ve metalloproteinase doku inhibitörü-1 üzerine etkisini belirlemek.

Metot: Vücut ağırlığı, vücut yağ yüzdesi, beden kütle indeksi, tahmini maksimal oksijen tüketimi, kan lipidleri, matrix metalloproteinase-9, metalloproteinase doku inhibitörü-1 konsantrasyonları egzersiz (n= 12) ve kontrol (n= 12) gruplarında ölçüldü; matrix metalloproteinase-9/metalloproteinase doku inhibitörü-1 molar oranları hesaplandı. Egzersiz grubuna sekiz hafta orta şiddette (~6.29±0.15km/s; ~%62 maksimum kalp atım sayısı rezervi) bir yürüme programı uygulandı.

Bulgular: Egzersiz grubunda tahmini maksimal oksijen tüketimi, sistolik ve diastolik kan basınçları, vücut ağırlığı, ve beden kütle indeksinde anlamlı değişiklikler tespit edildi ($p < .05$). Fakat, egzersiz grubunun, neredeyse anlamlı metalloproteinase doku inhibitörü-1 düzeyi hariç ($p = .092$), ölçülen kan lipitlerinde (trigliserit, kolesterol, yüksek yoğunluklu lipoprotein kolesterol, düşük yoğunluklu lipoprotein kolesterol), matrix metalloproteinase-9, metalloproteinase doku inhibitörü-1 değerlerinde ve matrix metalloproteinase-9/metalloproteinase doku inhibitörü-1 molar oranında anlamlı değişikler meydana gelmedi.

Sonuç: Kalp hastalıkları riskine karşı koruyucu etkisi hariç, bu programın süre ve şiddeti kan lipidlerinde, matrix metalloproteinase-9 ve metalloproteinase doku inhibitörü-1 değerlerinde anlamlı değişiklikler yaratmak için yeterli olmamıştır. Diyet ile birlikte takip edilecek daha uzun süreli ve şiddeti daha yoğun bir program önerilebilir. .

Anahtar Kelimeler: Yürüyüş, Kan lipidleri, matrix metalloproteinase-9, metalloproteinase doku inhibitörü-1, Menopoz sonrası kadınlar

Introduction

A sedentary lifestyle is a major risk factor for coronary vascular disease (CVD) in the general population. Engaging in any activity such as housework or shopping is better than living a purely sedentary lifestyle, but participating in a planned exercise program will reap even more cardiovascular benefits especially for elders. Walking may be an ideal exercise for older adults because it is safe, cheap and easy to do (1). Regular aerobic exercise has been reported to improve cardiovascular function and assist in the prevention and control of hyperlipidemia, hypertension and diabetes mellitus (2). Hakim et al (3) reported that men who walked more than a mile a day were half as likely to develop CVD than were those who walked less than a quarter mile a day. It has been reported that postmenopausal women walking briskly for at least 1 half-hour five times per week was associated with a 30% reduction in cardiovascular events over 3.2 yrs of follow-up (4). In another 7 year follow-up, women walking at least 1 $h \cdot wk^{-1}$ was associated with a 50% reduction in coronary heart disease (CHD) risk in women reporting no vigorous physical activity (5). Among 1564 middle-aged University of Pennsylvania alumnae followed for 30 yrs, walking 10 or more blocks daily as compared with walking fewer than four blocks daily was associated with a 33% reduction in CVD incidence (6).

Physical exercise can lower the risk of CVD by a number of mechanisms. Exercise alone or in combination with dietary modifications can make lipid disorders, insulin resistance, and inflammation better in high-risk populations (7-10). Research has shown that long-term exercise training improves endothelial function, increases nitric oxide (NO) availability and reduces hypertension in patients with CVD (11,12). The favorable effect of long-term exercise on endothelial function is, in part, due to reduction of reactive oxygen species (ROS) production and thus enhanced NO availability in the cardiovascular tissues (13). To what degree does physical activity reduce the risk of developing CVD due to the anti-inflammatory effects of exercise is not certain. Following acute exercise, there is a transient increase in circulating levels of anti-inflammatory cytokines, whereas chronic exercise reduces basal levels of pro-inflammatory cytokines. Exercise training also induces the expression of antioxidant and anti-inflammatory mediators in the vascular wall that may directly inhibit the development of atherosclerosis (14).

Some researchers share the value that markers of vascular inflammation are new independent early predictors of cardiovascular events (15,16). Of these markers, matrix metalloproteinases (MMPs) are proteinases that participate in extracellular matrix degradation (17,18). Increased activity of MMPs can be the cause of some diseases such as tumor growth, arthritis, and cardiovascular diseases. MMPs predominantly released form macrophages play a key role in the degradation of the fibrous cap surrounding the atherosclerotic plaque (19), thus provoking thrombosis (20). The actual activities of MMPs are regulated by the activation of proMMPs to MMPs by enzymatic cleavage and inhibition by tissue inhibitors of matrix metalloproteinases (TIMPs) (21). Tissue inhibitors of metalloproteinases are specific inhibitors of matrix that participate in controlling the local activities of MMPs in tissues (22). MMPs are inactivated in vivo mainly by specific inhibitors (TIMPs) that bind with high affinity in a molar 1:1 ratio to the catalytic site of MMPs (23).

Studying the response of MMP-9 and TIMP-1 to exercise is interesting as there are limited data on vascular inflammation during training (24-26). The investigation of the role of chronic high-intensity exercise revealed that elevated TIMP-1 levels in athletes with echocardiographic left ventricular hypertrophy (LVH) suggested that fibrosis occurs as part of the hypertrophic process in veteran athletes (24). 12-week endurance training on people with coronary artery disease (CAD) or other cardiovascular risk factors has demonstrated that endurance training decreased MMP-9 levels (25). 3-week diet plus exercise intervention resulted in significant favorable changes in all measured serum lipids and significant reductions in MMP-9 levels (26).

Until recently, CHD was widely perceived to be less of a public health problem for women than for men. However, CHD, in the United States emerges as the leading killer for men by 45 yrs of age and of women by 65 yrs of age. In fact, as soon as overt CHD develops in a woman, she has markedly worse prognosis than a man (27), and these problems are on the increase after menopause. Therefore, studying the risk factors of CHD for post-menopausal women is of great importance. Despite the research indicating the effects of physical activity on blood lipids as the risk factors of CHD, there are limited data on the effect of physical activity on MMPs and TIMPs, the new independent markers of CHD (24-26,28-31). The studies generally investigated the effects of hormone replacement therapy (HRT) on MMPs and TIMPs in post-menopausal women having CHD (20,32,33). To our knowledge, there are no studies investigating the effects of 8-week walking exercises performed with moderate intensity on MMP-9 and TIMP-1 levels together with serum lipids in post-menopausal women. Thus, in our study, we aimed to determine whether 8-week walking exercises of moderate intensity cause changes in blood lipids, MMP-9 and its tissue inhibitor, TIMP-1 in post-menopausal women.

Material And Method

Subject selection: Healthy females (aged between 45-62 years) volunteered for this study. Recruiting criteria were as follows: (1) to be between 45-62 years old, (2) had not experienced any menses in the 12 months preceding their participation in the study, (3) to live in

Manisa for at least 10 years, (4) not planning to leave the area during the experimental period, (5) being a non-smoker, (6) being a sedentary at the beginning of the study (people were accepted as sedentary if they had not performed exercise for 15-minute-duration or longer more than twice per week for the previous 6 months). Exclusion criteria consisted of having a previous history of cardiovascular disease or diagnosed CHD, endocrine or metabolic disorders, resting blood pressure greater than 160/95mmHg, having musculo-skeletal problems, diabetes mellitus, hyperthyroidism, being under HRT, and a ±2 kg change in body weight during the previous year.

Information about the participants was gathered via questionnaires; they were also physically examined thoroughly before the experimental period started; were informed about the study design, and each subject signed the informed consent form. A potential participant meeting the above-mentioned criteria was taken to laboratory screening for electrocardiography and body composition measurements.

We measured the dietary intake of the participants via two questionnaires developed by Dr. Gladys Block (34). We determined that they had a balanced diet and warned them not to change their dietary habits during the intervention period. Participants were not randomized to maintain compliance; they were free to participate either in exercise group (EG) or the control group (CG). 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. The participants were highly motivated so there was a high compliance (about 95%). 27 participants started the intervention, but three of them dropped off due to some personal reasons; 24 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 completion of baseline testing, EG started the 8-week walking program on a 400m outdoor track (5 days per week). With three minute-increments per week they reached 51 minutes at the end of the program. They walked at 60-65% heart rate reserve (HRR) with a speed of ~6.29±0.15km/h. 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.

American College of Sports Medicine (ACSM recommendations were considered in the study design (35). All sessions were supervised and monitored by trained exercise specialists. The exercise intensity was prescribed based on target heart rates (THRs) calculated from the Karvonen equation:

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

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 (36) and noted on training logs together with their total walking distances.

Testing procedures: Pre- and post-study values of resting blood pressure and body composition (Model TBF-300, Tanita Corp., Tokyo, Japan) were taken at 8.00-9.00 a.m, after a 12h fast. Maximal oxygen consumption (VO_{2max}) was estimated via 2km walking test (37,38) by completing five tours on a 400m outdoor track, using the following equation developed for women:

116.2 – 2.98 x duration (min) – 0.11 x Heart Rate – 0.14 x age – 0.39 x Body Mass Index (BMI)

Subjects were warned not to take part in any physical activity within 48h preceding the assessment day.

Blood samples were taken after a 12 h overnight fast between 8.00 and 9.00 a.m. Total cholesterol (TC) concentration was measured by the cholesterol oxidase method; triglyceride (TG) levels were determined by glycerol phosphate oxidase method; high-density lipoprotein cholesterol (HDL-C) was determined enzymatically in the supernatant after precipitation of low-density lipoprotein cholesterol (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 were 1.09, 2.6, and 3.4%, respectively.

Serum concentrations of MMP-9 were measured using ELISA method (Bender Medsystems, Vienna, Austria). The mean intra-assay coefficient of variation (CV) and inter-assay CV for MMP-9 assay were 8.6% and 6.8%, respectively. Serum concentrations of TIMP-1 were measured using ELISA method (BIOSOURCE, California, USA) The intra-assay coefficient of variation (CV) at level 6.3 ng/ml and inter-assay CV at level 6.4 ng/ml for TIMP1 assay were 3.0% and 3.1%, respectively. The lower limit of detection for MMP-9, and TIMP-1 were 0.8 ng/ml and <1 ng/ml, respectively. MMP-9/TIMP-1 molar ratio was calculated by using molecular weight for pro MMP-9 of 92 kDa and molecular weight of TIMP-1 of 28 kDa.

Statistical methods: Data were analyzed using SPSS package program version 10.0 with non-parametric tests because of low numbers of subjects in different groups and lack of homogeneity of variance. Results were presented as median and min-max values. 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 using Wilcoxon Signed Ranks test. Spearman Rank Order correlation coefficient was used to analyze the correlation between the changes in MMP-9, TIMP-1 concentrations and the changes in the other variables measured. Statistical significance was defined at $p< 0.05$ level.

Results

EG members aimed to walk at 60-65% of maximum heart rate reserve (HRR_{max}). The average heart rate (HR) per week during the training for EG was ~130.02±7.20 beat.min^{-1} (corresponding to ~62% of HRR_{max}) and they walked at ~6.29±0.15 km/h, totally ~172123.8±8567.88m; their reported RPE was ~13.95±0.90.

Pre-study evaluations of EG with regard to age, height, body weight, BMI, percent body fat, blood pressures, estimated VO_{2max}, and menopause age were not significantly different from those of CG (Table 1).

There were significant differences between pre- and post-test values in body weight, BMI, estimated VO_{2max}, systolic blood pressure (SBP) and diastolic blood pressures (DBP) ($p< 0.05$ for all) in EG. There were no significant differences in these parameters in CG (Table 2,4).

The increase observed in VO_{2max} of EG was significantly different from that of CG ($p< 0.05$); the reduction determined in the body weight of EG was significantly different from that of CG ($p< 0.05$; Table 3). The reductions in the SBP and DBP values of the EG were a mean of 10.41±7.82 and 2.91±2.57, respectively and were significantly different from those of CG ($p< 0.05$; Table 5).

Eight-week moderate-intensity walking exercises caused no significant changes in the measured blood lipids (triglyceride, total cholesterol, HDL, LDL), MMP-9, TIMP-1 concentrations, and MMP-9/TIMP-1 molar ratios (Table 4), apart from a nearly significant reduction in TIMP-1 ($p= 0.092$). We could not determine any significant changes in these parameters in CG. The changes determined in EG and CG in measured blood lipids, MMP-9, TIMP-1 and MMP-9/TIMP-1 molar ratio were not significantly different from each other (Table 5). No significant correlation was observed between the changes in MMP-9, TIMP-1 concentrations, MMP-9/TIMP-1 molar ratios and the changes in the other variables measured in both groups (details are not shown).

Discussion

To our knowledge, this is the first study determining the changes related to CHD risk factors (blood pressures, blood lipids) together with circulating collagen markers of MMP-9 and its tissue inhibitor TIMP-1 in post-menopausal women as a result of 8-week moderate-intensity walking. This program resulted in significant favorable changes in VO_{2max}, body weight, BMI, SBP and DBP values of post-menopausal women. However, its duration (8 weeks) and intensity seems to be insufficient to alter blood lipids, MMP-9 and TIMP-1 levels.

Blood pressures, VO_{2max} and serum lipids: Exercise has been a widely used method to prevent and cure hypertension (HTN), an important risk factor for CHD (39). Contrary to the reports on the more favorable role of vigorous exercises to lower blood pressures (40), findings from randomized trials indicate that moderate-intensity exercise lowers SBP as or more effectively than does high-intensity exercise; such effects occur in both normotensive and hypertensive individuals and largely are independent of weight change (41). Similar to that, we determined favorable effects on SBP and DBP in post-menopausal women as a result of our moderate-intensity walking exercise program. VO_{2max} decreases 5 to 15% per decade after the age of 25 (42). However, it is now clear that older adults elicit the same 10-30% increases in VO_{2max} with prolonged endurance exercise training as young adults (43,44). Woods et al. stated that 6 months of moderate intensity aerobic exercise (52% VO_{2max}) increases VO_{2max} in elderly post-menopausal women (45). Parallel to the literature, the significant improvement determined in the VO_{2max} values in our EG members is a clear sign of the positive effect of their walking program on their fitness levels to protect them against CHD risks.

Several studies have revealed that athletes engaged in vigorous exercise have more favorable plasma lipid and lipoprotein concentrations than untrained or sedentary subjects (46,47). However, we could not find any statistical changes in any of the measured blood lipids. This might have resulted from the duration and the intensity involved in our study. It has been reported that six months of low-density exercise had no effect on HDL-C concentrations in non-obese older and younger women, but when they continued an additional 6 months of high-intensity training, their HDL-C levels increased by 14% (48). Stein et al. suggest a training intensity threshold, at least 75% of $VO_{2max,}$ at which beneficial changes in HDL-C levels occur (49). Even so, considering the findings of the research showing that more than 1 mg/dl of difference in HDL-C can affect the CHD risk favorably (50,51), the difference we obtained in EG as a result of the walking exercises can be regarded favorable in terms of preventing CHD risks (a difference of 1.16±5.55mg/dl). Research indicated that endurance-trained athletes, compared with sedentary controls, show low levels of TG (52). The unaltered levels of TG in our study might be related with the intensity of the exercise program. A more strenuous program having a longer duration seems likely to cause more favorable effects on both HDL-C and TG levels.

MMP-9 and TIMP-1: Some studies with post-menopausal women investigated the effects HRT on MMPs and TIMPs. In a study conducted with 195 healthy post-menopausal women, the group taking conjugated equine estrogen demonstrated higher levels of MMP-9 compared with healthy women not receiving menopausal therapy or those treated with tibolone (32). Zanger et al. reported significantly raised MMP-9 levels in 10 post-menopausal women receiving oral HRT, who incidentally also had a history of established CAD (20). Wakatsuki et al. found out that transdermal estrogen replacement therapy (ERT) did not change MMP concentrations, but tended to increase TIMP-1 concentrations, suggesting that transdermal ERT may be less likely to

Table 1: Initial physical and physiological characteristics of subjects (median- Min-Max)

Test/unit	**Control Group (n=12) Median (min-max)**	**Exercise Group (n= 12) Median (min-max)**	**p**
Age (yr)	52.5 (46.0-62.0)	53.0 (45.0-62.0)	NS
Height (cm)	152.5 (145.0-163.0)	158.5 (150.0-168.0)	NS
Body Weight (kg)	71.8 (47.8-88.7)	73.9 (62.3-84.8)	NS
BMI ($kg.m^{-2}$)	31.0 (22.7-39.1)	28.9 (24.7-34.4)	NS
Body fat (%)	38.1 (22.8-44.6)	36.4 (32.5-40.6)	NS
VO_{2max} ($ml.kg^{-1}.min^{-1}$)	20.5 (17.4-21.4)	20.4 (18.1-23.1)	NS
Menopause age (y)	3.4 (1.5-6)	3.0 (1.1-6.0)	NS

Group comparisons were made using Mann- Whitney U test; NS= No significant

Table 2: Changes in physical and physiological parameters of subjects

	Control Group (n= 12)			**Exercise Group (n= 12)**		
	Intervention		**Evaluation**	**Intervention**		**Evaluation**
Test/unit	**Pre** median (min-max)	**Post** median (min-max)	**p**	**Pre** median (min-max)	**Post** median (min-max)	**p**
Body Weight (kg)	71.8 (47.8-88.7)	71.9 (47.8-89.6)	NS	73.9 (62.3-84.8)	72.1 (61.1-84.4)	<0.05
BMI ($kg.m^{-2}$)	31.0 (22.7-39.1)	29.8 (22.7-36.4)	NS	28.9 (24.7-34.4)	28.0 (24.0-33.0)	<0.05
Body fat (%)	38.1 (22.8-44.6)	37.1 (22.8-45.1)	NS	36.4 (32.5-40.6)	34.3 (32.6-41.2)	NS
VO_{2max} ($ml.kg^{-1}.min^{-1}$)	20.5 (17.4-21.4)	20.3 (17.4-22.5)	NS	20.4 (18.1-23.1)	26.0 (22.0-28.5)	<0.05

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

	Control Group (n= 12)	**Exercise Group (n= 12)**	**p**
Test/unit	**Median (min-max)**	**Median (min-max)**	
Body Weight (kg)	-0.0 (-2.4- 1.0)	-1.1 (-3.8- 0.0)	<0.05
BMI ($kg.m^{-2}$)	-0.6 (-5.4- 0.4)	-1.3 (-3.2- 0.2)	NS
Body fat (%)	-0.6 (-4.3- 0.8)	-0.4 (-2.4- 1.9)	NS
VO_{2max} ($ml.kg^{-1}.min^{-1}$)	-0.1 (-1.3- 1.0)	-3.9 (-5.7- 0.9)	<0.05

Group comparisons were made using Mann- Whitney U test; NS= No significant

Table 4: Changes in physiological and metabolic parameters of subjects

	Control Group (n= 12)			Exercise Group (n= 12)		
	Intervention		Evaluation	Intervention		Evaluation
Test/unit	Pre median (min-max)	Post median (min-max)	p	Pre median (min-max)	Post median (min-max)	p
SBP (mmHg)	125.0 (110-160)	125.0 (100-150)	NS	120.0 (100.0-130.0)	115.0 (90.0-135.0)	<0.05
DBP (mmHg)	80.0 (70-90)	80.0 (70-95)	NS	80.0 (60.0-90.0)	70.0 (60.0-80.0)	<0.05
TC (mg/dl)	204.5 (145-261)	203.5 (142-252)	NS	237.0 (136.0-298.0)	227.5 (169.0-278.0)	NS
TG (mg/dl)	161.5 (63-405)	139.0 (55-344)	NS	118.5 (64.0-235.0)	110.5 (59.0-275.0)	NS
HDL-C (mg/dl)	39.5 (25-71)	42.0 (25-157)	NS	49.5 (33.0-76.0)	46.5 (35.0-87.0)	NS
LDL-C (mg/dl)	128.5 (73-196)	135.5 (56-176)	NS	145.5 (82.0-207.0)	156.5 (88.0-201.0)	NS
MMP-9 (ng/dl)	374.9 (196.3-469.1)	373.5 (282.0-547.0)	NS	315.8 (198.3-440.9)	329.4 (168.5-485.0)	NS
TIMP-1 (ng/dl)	261.3 (236.5-271.1)	243.7 (216.4-267.9)	NS	258.9 (246.3-271.1)	245.2 (231.3-271.1)	NS
molar ratio MMP-9/TIMP-1	0.42 (0.2-0.6)	0.48 (0.3-0.6)	NS	0.36 (0.2-0.5)	0.40 (0.1-0.6)	NS

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

Table 5: The comparison of the differences obtained in the groups

	Control Group (n= 12)	Exercise Group (n= 12)	p
Test (unit)	Median (min-max)	Median (min-max)	
SBP (mmHg)	-2.0 (-10.0-5.0)	-10.6 (-20.0-0.0)	<0.05
DBP (mmHg)	0.3 (-4.0-3.0)	-2.5 (-6.0-0.0)	<0.05
TC (mg/dl)	-2.0 (-31.0-20.0)	-9.5 (-129.0-76.0)	NS
TG (mg/dl)	-21.5 (-105.0-57.0)	-5,5 (-65.0-89.0)	NS
HDL-C (mg/dl)	1.0 (-9.0-12)	0.5 (-8.0-11.0)	NS
LDL-C (mg/dl)	2.5 (-20.0-25.0)	8.5 (-119.0-60.0)	NS
MMP-9 (ng/dl)	38.2 (-187.06-174.7)	3.6 (-158.2-223.0)	NS
TIMP-1 (ng/dl)	-17.5 (-41.3-31.35)	-13.9 (-36.76-21.33)	NS
molar ratio MMP-9/TIMP-1	0.07 (-0.28-0.25)	0.02 (-0.21-0.30)	NS

Group comparisons were made using Mann- Whitney U test; NS= No significant

promote plaque destabilization and rupture (33). The role of MMP-9/TIMP-1 molar ratio was investigated after myocardial infarction (MI). Bradham et al. reported that MMP-9/TIMP-1 ratio increased significantly post-MI, suggestive of reduced TIMP-1 mediated MMP-9 inhibition, which would potentially enhance extracellular myocardial remodeling. They concluded that induction of a controlled myocardial injury in humans, specifically through alcohol-induced MI, caused species- and time-dependent perturbations of MMP/TIMP stoichiometry that would facilitate myocardial remodeling in the early post-MI setting (53).

However, in none of the aforementioned studies the role of exercise has been investigated. In recent years, the role of physical exercise on MMPs and their tissue inhibitors has gained interest. Several studies demonstrated some acute changes in MMP-9 and TIMP-1 levels due to exercise (28,30,31,54,55). These findings may suggest that there are some changes in myofiber basement membrane via the MMP pathway in response to muscle damaging exercise. The contradiction may result from the modes of the exercise programs since all of the above mentioned studies measured the acute effects; however, we tried to demonstrate the chronic effects of endurance training. In addition, the differences in the exercise intensity and the contraction types of the muscles primarily involved in the studies mentioned may play a role in the differing results (54,55).

There are limited data on the role of MMPs and TIMPs on long-term endurance training in humans (24-26) and the existing studies have contradictory results. It has been reported that diet together with simvastatin caused significant reductions on MMP-9 levels (56). A diet plus exercise program of 21 days led to significant favorable changes in all measured serum lipids and reductions in MMP-9 levels (45). The main reason that we could not find any changes in blood lipids and MMP-9 levels in our subjects might be the lack of a diet program. Researchers determined elevated TIMP-1 levels in veteran athletes with echocardiographic left ventricular hypertrophy (24). Researchers suggested that fibrosis occurs as part of the hypertrophic process in veteran athletes. However, we determined no significant changes except for a nearly significant reduction in TIMP-1 levels in our EG ($p= 0.092$). This discrepancy might be due to the fact that our subjects were sedentary at the start of the study and exercised for recreational purposes for only 8 weeks. However, in the aforementioned study, the participants had been running for about ten years for competitive purposes. In another study, the effects of endurance training on circulating MMP-9 levels in persons at a risk of coronary events were examined and MMP-9 levels were found to be reduced after a 12-week endurance running (41). Unlike their results, our study was not able to alter MMP-9 levels of post-menopausal women, since our program is a milder form of an exercise than running.

Some researchers have reported the benefits of walking exercises on reducing the risk factors for CHD (1-6), however, to our knowledge there are no studies examining the effects of walking exercises on inflammatory markers. With its supervised prospective nature, this is the first study to examine the changes in cardiac risk factors such as serum lipids and MMP-9 and TIMP-1 levels in post-menopausal women following an 8-week moderate-intensity walking program. However, the relatively small sample size of the study is its biggest limitation. We had to form small groups due to the strict inclusion and exclusion criteria and the need for strict supervision. Therefore, a similar program needs to be carried out in longitudinal, well-designed, randomized, more crowded control trials in women with and without HRT. Nevertheless, such an intervention may be useful for those desiring rapid cardiovascular risk reduction because this program was able to lead to some significant improvements in some CHD risk factors such as VO_{2max}, BMI, body weight, and blood pressures.

The beneficial results obtained as a result of moderate intensity walking regarding the increases in VO_{2max}, and the reductions in body weight and BMI in addition to significant decreases in blood pressures can be accepted as the health benefits for post-menopausal women that may enable them to lead a less risky and a more independent life, in addition to some positive contributions to their cardiovascular health; but, this program appears to be ineffective in causing favorable alterations in blood lipids (TG, TC, HDL, and LDL), MMP-9 and TIMP-1 levels. However, it should be noted that more strenuous training programs that cause more beneficial changes in CHD risk factors may not be the most attractive ones for the elderly population because they may pose some risks for the elderly. In order to assure high compliance and attendance, exercise programs should be made attractive for them. We can propose that physical exercise should be made a life-long activity to optimize health-related benefits. Thus, exercising for 30-60 min per day, five days per week, at moderate intensity (60-65% of VO_{2max}) may lead to some health-related benefits, including improved blood pressures, body weight, BMI and cardiorespiratory fitness (VO_{2max}). However, if more favorable changes in most of the CHD risk factors are desired, a higher-intensity, longer-duration walking or running program accompanied with diet is advisable.

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