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Effects of two different eight-week walking programs on insulin resistance and ER stress-related markers in pre-menopausal women

Premenopozal kadınlarda 8 haftalık farklı şiddetteki yürüyüş antrenmanlarının insülin direnci ve ER stresiyle ilişkili markerler üzerine etkileri

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Abstract: Objective: The relationship between exercise and endoplasmic reticulum (ER) stress, insulin resistance, and obesity is a new field of research. To our knowledge, there are no studies examining the effects of walking exercises on ER stress related markers and insulin resistance in pre-menopausal women. To examine the effect of two different eight-week walking programs on the tumor necrosis factor-alpha (TNF-α), Fetuin-A (α2 - Heremans Schmid glycoprotein), c-Jun N-terminal kinase-1 (JNK-1) and retinol-binding protein-4 (RBP-4), the related markers of ER stress and insulin resistance in pre-menopausal women.

Methods: Exercise groups (moderate tempo walking group; MTWG; n=11; brisk walking group BWG; n=12) walked five days a week, starting from 30 minutes, gradually increasing up to 51 minutes. BWG walked at ~70–75% and MTWG at ~50–55 of HRRmax. Body mass index (BMI), body fat percentage (%), VO_{2max}, serum TNF-α, Fetuin-A, JNK-1, RPB-4, blood lipids, and insulin resistance levels were determined before and after the intervention.

Results: VO_{2max} increased in both exercise groups favoring BWG; RBP-4 decreased in both exercise groups ($p<0.05$). Serum TNF-α and, TG ($p<0.05$), BMI and percent body fat ($p<0.01$), and insulin levels reduced significantly in BWG ($p<0.05$). The reductions observed in both exercise groups in insulin resistance, and the increases determined in the negative levels of JNK-1 in BWG may be clinically important.

Conclusion: Both type of walking resulted in similar positive effects on RBP-4. The reduction observed in TNF-α, RBP-4, and the increases in the negative levels of JNK-1 in BWG show the positive effects of brisk walking on ER stress. The reduction in insulin resistance in relation to the possible reductions in ER stress and apoptosis in BWG may be more effective to prevent metabolic diseases.

Keywords: Walking exercises, Premenopausal women, Estimated VO_{2max}, Endoplasmic reticulum stress, Insulin resistance, Obesity

Özet: Amaç: Egzersiz ile Endoplazmik Retikulum (ER) stresi, insülin direnci ve obezite arasındaki ilişki yeni araştırılan bir alandır. Bilgimiz dahilinde literatürde premenopozal kadınlarda yürüyüş egzersizlerinin, ER stresiyle ilişkili markerler ve insülin direnci ile ilişkisini inceleyen çalışma bulunmamaktadır. Pre-menopozal dönemdeki kadınlarda 8 haftalık farklı şiddette yapılan yürüyüş egzersizlerinin tumor necrosis factor-alpha (TNF-α), Fetuin-A (α2 - Heremans Schmid glycoprotein), c-Jun

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N-terminal kinase-1 (JNK-1) ve retinol-binding protein-4 (RBP-4) gibi ER stresiyle ilgili olabilecek markerler ile insülin direnci üzerine etkisini incelemektir.

Metod: Egzersiz grupları (hızlı tempo yürüyüş grubu: HTYG; n=12; orta tempo yürüyüş grubu: OTYG; n=11), haftada beş gün, günde 30 dakikadan başlayarak 51 dakikaya kadar sabit olarak artan sürelerde yürüdüler. HTYG kalp atım sayısı yedeğinin yaklaşık ~%70–75 şiddetinde (hızında), OTYG ise ~%50–55 şiddetinde yürüdü. Egzersiz periyodundan önce ve sonra beden kütle indeksi (BKI), vücut yağ oranı (%), indirekt maksimal oksijen tüketimi (VO_{2max}), serum TNF-α, Fetuin-A, JNK-1, RBP-4, kan lipidleri ve insülin direnci düzeyleri belirlendi.

Bulgular: VO_{2max} HTYG'de daha belirgin olmak üzere her iki egzersiz grubunda artarken, RBP-4 düzeyleri her iki egzersiz grubunda anlamlı olarak azaldı ($p<0.05$). Serum TNF- α ve TG ($p<0.05$). BKİ, vücut yağ oranı ($p<0.01$), ve insulin seviyeleri ($p<0.05$) HTYG'de anlamlı olarak azaldı. Her iki egzersiz grubunun insulin direnç seviyesinde gözlenen azalmalar ve HTYG'nin JNK-1'in negatif değerlerindeki artışlar klinik olarak anlamlı olabilir.

Sonuç: Her iki tip yürüyüş RBP-4 üzerinde benzer oranda olumlu etki yapmıştır. HTYG'de TNF-α, RBP-4 seviyelerindeki azalma ve JNK-1'in negatif değerlerindeki artışlar hızlı tempo yürüyüşlerin ER stresi üzerinde olumlu etkisini düşündürmektedir. ER stresi ve sonrasında apoptozisde meydana gelen azalma ile ilişkili olarak insülin direncinin azalması metabolik hastalıkları önlemede hızlı tempo yürüyüşün daha etkili olduğunu gösterebilir.

Anahtar Kelimeler: Yürüyüş egzersizi, Premenopozal kadın, İndirek VO_{2max}, Endoplazmik retikulum stres, Insulin direnci, Obezite

1 Introduction

Obesity increases the risk of a wide range of diseases, but its underlying mechanisms have not been understood thoroughly, yet [1]. However, there is evidence that endoplasmic reticulum (ER) stress plays an important role in the development of obesity-related insulin resistance and inflammation [2]. ER is an intracellular organelle in which proteins have their specific structures by folding. The cellular response which occurs as a result of the accumulation of the unfolded or misfolded proteins and the deformation of homeostasis of ER in ER is defined as ER stress [3]. A cell needs the activation of a signaling pathway called unfolded protein response to overcome ER stress [4]. ER increases its capacity in order to fold the unfolded proteins accumulated in its lumen. The synthesis of helper molecules which support the folding is increased [5]. Adipose tissue is an important inflammatory source in obesity and type-2 diabetes (DM) [6]. The inflammatory role of adiposities is related to their expansion, hyperplasia and hypertrophy and they involve in ER stress [7]. Recent research has revealed that in visceral obesity, ER stress created by increased lipid load is related to insulin resistance and Type-2 DM occurrence [8].

Tumor Necrosis Factor-α (TNF-α) is a cytokine released from fatty cells. c-jun NH2-terminal kinase (JNK) is a protein having a function in signaling in the apoptotic pathway. JNK regulates gene expression and enables the determination between life and death against stress [9]. JNK can interfere with insulin action in cultured cells and are activated by inflammatory cytokines and free faty acids (FFA) [10]. JNK and TNF-α signaling plays a crucial role in the development of obesity-associated insulin resistance and ER stress [10]. Abnormal production of inflammatory cytokines such as TNF-α and increased concentrations of FFA are crucial players in obesity-induced insulin resistance. Recent studies have revealed that exercise alone can reduce inflammatory markers and improve insulin sensitivity [11–14]. There are studies revealing increased TNF-α levels after a single bout of exercise [15] and other studies showing no significant changes in TNF-α levels as a result of combined aerobic and strength training [16]. M. vastus lateralis protein pJNK activity assessed by western blot have been reported to be significantly decreased and heat shock protein 70 (HSP70) expression to be significantly increased in insulin resistant obese persons after a single bout of 60min aerobic exercise [17]; however, vastus lateralis pJNK activity assessed by immunoblotting showed 1.4-fold increase following maximal concentric or eccentric knee extensions [18].

Retinol-binding protein 4 (RBP-4) is largely produced in the liver and released by the fatty cells [19]. RBP-4 influences metabolic processes such as food intake, glucose and lipid metabolism, inflammation, and insulin resistance and is found to be elevated in insulin resistant states and obesity [20]. Research examining the effects of exercise on RBP-4 levels has led to contradictory results. While some studies determined reductions in RBP-4 levels as a result of training programs of different kind [21–23]; some of them reported an increasing trend [24] and no changes [25].

Fetuin-A (2 Heremans schmid glicoprotein, Ahsg), is a protein-like molecule secreted mainly from the liver in adults and has been proposed as a link between obesity, fatty liver, and insulin resistance [26] and its high levels

Table 1: General characteristics of participants at baseline.

Parameters	BWG (n =12)		MTWG (n=11)		CG (n=12)	
	Median	Min Max	Median	Min Max	Median	Min Max
Age (yr)	44.0	35–45	38.0	35–45	38.5	35–45
Height (cm)	163.5	156–173	160.0	155–175	163.5	157–175
Body weight (kg)	87.2	78.1–105.5	85.4	78–98.2	84.6	81–97.7
BMI (kg/m^2)	31.9	30.1–36.5	32.0	30.3–36.4	31.6	29.5–38.4
Percent body fat (%)	40.4	34–46.1	44.0	37.4–52.4	40.2	34.4–50
VO_{2max} ($mL.kg^{-1}.min^{-1}$)	23.3	18.8–38.3	23.2	16.4–35.2	22.4	13.2–35.3

BWG: Brisk walking group; MTWG: Moderate tempo walking group; CG: Control group. SI Units=centi- (c, 10^{-2}), kilo- (k, 10^3).

have been correlated with insulin resistance. It is thought that it has a role in a new mechanism of type-2 diabetes phatophysiology [27] and metabolic disorders [28]. It has been explained that ER stress induced by high glucose and palmitate increased the expression of fetuin-A and further contributed to the development of insulin resistance [28]. Studies examining the link between exercise and Fetuin-A levels also have contradictory results. Some of them reported no changes in Fetuin-A levels [29,30]; however, some of them determined reductions in Fetuin-A levels following an exercise program [31,32].

Intervention studies aiming to highlight the effect of exercise on ER stress related markers have revealed contradictory results. In addition, to our knowledge, there are no studies examining the effects of walking exercises on serum JNK-1 levels and insulin resistance in pre-menopausal women. We hypothesize that in pre-menopausal women, serum TNF-α, RBP-4, Fetuin-A and JNK-1 levels, possible markers of ER stress, can be decreased with our brisk walking program. Therefore, the aim of the present study is to point out the changes in serum TNF-α, RBP-4, Fetuin-A and JNK-1 levels in pre-menopausal women following an 8-week, two different-intensity walking programs.

2 Materials and Methods

2.1 Subject Selection

Female volunteers (35–45 yrs) were recruited through public announcement methods. Recruiting criteria were as follows: (1) having regular menses within the 12 months preceding the study, (2) living in İzmir for at least 10 years, (3) promising to stay in the area during the study, (4) being a non-smoker, (5) being sedentary at baseline. Participants having a history or diagnosed cardiovascular disease, endocrine or metabolic disorders, musculo-skeletal problems, having diabetes mellitus and hyperthyroidism were excluded. Questionnaires were used to gather information; the subjects underwent a thorough physical examination before the experimental period and were given information about the study design. Their signed consent forms were taken; their electrocardiography and body compositions were measured in the laboratory.

Upon deciding to participate in the exercise (n=23) or the control group (n=12), the exercise group members were randomly assigned to the brisk walking (BWG; n=12) or moderate tempo walking (MTWG; n=11, Table 1) group. Their eating habits were determined by the related section of "The health-profile lifestyle profile" [33]. The groups were found to be homogenous in terms of their eating habits.

Participants were warned not to take any form of physical exercise and not to change their eating habits during the intervention. The study was approved by the Ethical Council of the Celal Bayar University, Faculty of Medicine and conducted in accordance with the principles of Helsinki Declaration.

2.2 Study design

Body composition was measured using bioelectrical impedance analyzer (Model TBF-300, Tanita Corp., Tokyo, Japan). 2 km walking test was used to estimate maximal oxygen consumption (VO_{2max}) [34]. The following equation developed for women was used to estimate VO_{2max} [34]:

116.2-2.98 x duration (min)-0.11 x HR-0.14 x age-0.39 x BMI

Blood samples were collected at rest. Subjects were warned not to perform any physical activity within 48 hours preceding the assessment day.

2.3 Exercise program

Under the control of exercise specialists, all exercise group members walked on an outdoor track (400 m) for 8 weeks, 5 days per week in accordance with the principles of the American College of Sports Medicine (ACSM) recommendations [35]. The exercise intensity was determined using the Karvonen equation:

$[(HR_{maximum} - HRrest) \times (0.60–0.65) + HRrest]$

BMG members started to walk 30 minutes, and with three-minute increments they reached 39 minutes at the end of the first four weeks, at 70% heart rate reserve (HRR). On the next four weeks, they walked up to 51 minutes at 75%HRR. MTWG walked in the same duration, but at 50% HRR for the first four weeks and 55% HRR for the second four weeks. Their heart rate readings were taken by Polar Pacer heart rate monitors (Polar Vantage, Kempele, Finland) at least three times in order to ensure compliance with the training intensity (walking speed), and their Rate of Perceived Exertion (RPE) was also taken using a 15-point RPE scale and was noted on training logs and the total walking distances were also recorded. The subjects also underwent a 5-minute warm-up and 5-minute cool-down sessions.

2.4 Blood analysis

Following a 12 h overnight fast, venous blood samples were collected from an antecubital vein (9 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 two months) in all samples. Serum triglyceride (TG), and glucose assays were performed by enzymatic spectrophotometric methods with Advia 1800 autoanalyzer (Siemens Medical Solutions Diagnostics Limited, NY, USA). Follicle stimulating hormone (FSH), luteinizing hormone (LH) and insulin, levels were determined in Advia centour XP analyzer two-site sandwich immuno chemiluminescance assay (Siemens Medical Solutions Diagnostics Limited, NY, USA) Glucose, TG, FSH, LH, insulin and analyses, within-run coefficients of variation were 0.5%, 0.6%, 2.9%, 2.3%, and 4.6%, respectively.

Serum TNF-α levels, serum RBP-4 levels, total serum JNK- 1 levels and serum Fetuin-A levels were analyzed by Enzyme-Linked ImmunoSorbent Assay (ELISA) method by commercial reagents eBioscience, CA 92121 USA, Millipore. Billerica, MA 01821, USA, Cusabio Research.430223 China and Biovendor Research.62100 Czech Republic respectively. The inter and intra-assay coefficient of variation (CV) for TNF- α at level 5.0 pg/ml were 8.1% and 7.7%, RBP-4 at level 1.5 pg/ml were 4-8% and 3–8%, for Fetuin-A at level 6.2 ng/ml were 2.2% and 3.1%, for respectively.

Homeostasis model assessment-estimated insulin resistance (HOMA-IR) was computed with the formula: fasting plasma glucose (mmol/l) times fasting serum insulin (mU/l) divided by 22.5.

2.5 Statistical analysis

The Kruskall-Wallis test was used to compare changes among the study groups; Mann-Whitney U test was used to determine the difference between the two groups. Bonferroni correction was made following Mann-Whitney U test. The differences between pre and post data of the intervention period were determined by using the Wilcoxon test. All comparisons were considered statistically significant at $p<0.05$, except the difference between two groups. Statistically significant level between the two group was considered at $p<0.0167$ owing to Bonferroni correction ($0.05/3= p<0.0167$).

3 Results

All members of our study had serum FSH levels below 50 or FSH/LH ratio <1 therefore considerered as premenapousal (data not shown). BWG members had an average heart rate (HR) of ~149.25±2.22 $beat.min^{-1}$ (corresponding to ~71% of HRR); MTWG members had an average HR of ~132.63±2.80 $beat.min^{-1}$, (corresponding to ~51% of HRR). Mean walking speed for the whole program for BWG was ~6.49±0.24 km/h; and it was ~5.21±0.17 km/h for MTGW. The RPE reported by BWG was 14.16±1.19 and it was 12.36±1.12 for MTWG. Total distance walked for BWG was ~184571±3152 m and it was ~156046±1851m for MTWG.

At the pre-study evaluation, the subjects were not significantly different with regard to age, body weight, body fat, and VO_{2max}. After 8 weeks, VO_{2max} increased favoring BWG (BWG $p<0.01$, MTWG $p<0.05$); body weight, percent body fat, BMI decreased ($p<0.01$) in BWG. However, no significant differences were detected in MTWG and CG (Table 2).

Among the measured ER stress markers, we detected a significant decrease in RBP-4 of the exercise groups ($p<0.05$). Insulin decreased in BWG significantly ($p<0.05$). TNF-α and triglyceride concentrations decreased significantly in BWG ($p<0.05$); however, no significant change was observed in Fetuin-A. We did not determine any significant changes in any of the markers of the CG except

Table 2: Changes in physical and physiological characteristics of subjects following the intervention .

Variable	n	Pre-intervention Median (min/max)	Post-intervention Median (min/max)	Differences Median (min/max)
Body weight (kg)				
BWG	12	87.2 (78.1/105.5)	84.6 (77.7/104)[b]	-1.35 (-7.9/-0.1)[d]
MTWG	11	85.4 (78/98.2)	82 (77.4/95)	-1.00 (-5.2/-0.2)[d]
CG	12	84.6 (81/97.7)	85.7 (75/98.6)	0.95 (-7/3.7)
Percent body fat (%)				
BWG	12	40.4 (34/46.1)	38.8 (33/44.7)[b]	-2.10 (-6.1/-0.9)
MTWG	11	44 (37.4/52.4)	43 (36.2/53)	-0.50 (-3.1/7.0)
CG	12	40.2 (34.4/50)	45.2 (39/54.4)	1.00 (-2.0/3.1)
BMI (kg/m^2)				
BWG	12	31.9 (30.1/36.5)	31.4 (29.5/36)[b]	-0.55 (-2.9/0.00)[d]
MTWG	11	32 (30.3/36.4)	32 (30/36)	-0.39 (-3.0/0.9)
CG	12	31.6 (29.5/38.4)	31.8 (29/38.7)	0.30 (-2.8/2.1)
VO_{2max} ($mL.kg^{-1}.min^{-1}$)				
BWG	12	23.3 (18.8/38.3)	28.3 (23/39.1)[b]	4.75 (0.11/9.5)[d]
MTWG	11	23.2 (16.4/35.2)	25.4 (22.2/36.2)[a]	1.66 (-2.4/9.9)[d]
CG	12	22.4 (13.2/35.3)	22.6 (11/32.2)	-0.52 (-5.3/1.1)

[a]$p<0.05$ change from baseline; [b]$p<0.01$ change from baseline; [d]$p<0.0167$ BWG and MTWG vs CG. SI Units=centi- (c, 10^{-2}), kilo- (k, 10^3).

for a significant increase in TNF-α levels ($p<0.05$; Table 3). Although there was a significant reduction in insulin levels of BWG ($p<0.05$), we couldn't determine any significant change in homeostasis model assessment-estimated insulin resistance (HOMA-IR) index, in exercise groups; however, there was an insignificant reduction in glucose and HOMA-IR levels in all groups.

After 8 weeks, the difference obtained in VO_{2max}, Body weight, BMI, TNF-α, TG, Fetuin-A. by intervention in favoring BWG was different that of CG ($p<0.0167$; Table 2, Table 3).

According to the suggestion of the manufacturing company, JNK-1 analysis results are interpreted as "positive-intermediate-negative" The post-exercise frequency distribution of the exercise groups revealed an increase in negative values in the BWG. The increase in negative values obtained in BWG could be an indicator that the walking exercises applied in this study are not active on JNK-1 markers on the path to ER stress.

4 Discussion

The present study investigating the effects of two different 8-week walking programs on the possible markers of ER stress and insulin resistance in pre-menopausal women revealed beneficial effects on cardiorespiratory function (VO_{2max}) and some physical characteristics of the BWG subjects. In addition, we found significant decreases in TNF-α and RBP-4 levels in exercising groups, favoring BWG; however, walking exercises did not cause a significant change in fetuin-A levels.

It is known that exercise alone can reduce inflammatory markers and improve insulin sensitivity [11]. Ko *et al*. observed lower TNF-α levels together with changes in obesity factors and body composition as a result of regular exercise in subjects performing aerobic (60% HRRmax) and resistance exercise [12]. TNF-α levels of obese women decreased following 12 weeks of aerobic exercise (at 70% of VO_{2max}) [13]. However, TNF-α values of post-menopausal women have been shown to increase after a single bout of 60-min resistance training [15]. Others found TNF-α levels to show no changes as a result of combined aerobic and strength training [16]. Aerobic exercise however performed at 50–70% of HRR resulted in a decrease in serum TNF-α levels [14]. The results obtained in our study are concordant with the results of the studies in which participants performed exercise at similar intensities [12–14]. However, our results are contradictory with those of the studies examining the acute response of TNF-α to exercise [15] and combining aerobic and resistance training [16]. This discrepancy may be the result of the different mechanisms involved in resistance training and the participants with Type 2 diabetes [16].

The increase in the amount of adipose tissue leads to obesity and it causes the synthesis and secretion of obesity-related factors such as TNF-α [12]. The hyperglycemia that typically accompanies obesity and insulin resistance is a driving force behind elevated cytokine production [11].

Table 3: Changes in biochemical parameters of all subjects following the intervention.

Variable	n	Pre-intervention Median (min/max)	Post-intervention Median (min/max)	Differences Median (min/max)
Insulin (mg/dl)				
BWG	12	8.3 (4.2/21.3)	7 (3.3/22.1)[a]	-1.23 (-7.1/0.8)
MTWG	11	5.6 (3/11.2)	5.9 (2.6/11.8)	-0.20 (-3.2/2.0)
CG	12	6.4 (2.7/43)	5.1 (2.2/24.1)	-0.82 (-18.9/2.11)
Glukoz (mg/dl)				
BWG	12	87 (70/221)	88.5 (74/110)	-1.00 (-122/28)
MTWG	11	81 (77/99)	82 (60/97)	-2.00 (-21/9.0)
CG	12	84.5 (70/116)	83 (66/106)	-4.00 (-10.0/7.0)
Rbp-4 (ng/ml)				
BWG	12	65.4 (47.6/307.1)	51.2 (64/136)[a]	-18.3 (-170.2/17.9)
MTWG	11	61.4 (34.7/169)	49.5 (20.4/107)[a]	-27.4 (-88.0/11.0)
CG	12	55.4 (15/307)	59 (26.3/307)	11.2 (-17.1/25.4)
TNF- α (pg/ml)				
BWG	12	9.8 (6.5/18.5)	7.2 (6.1/9.7)[a]	-2.34 (-11.0/2.3)[c]
MTWG	11	7.6 (6.4/13.9)	8.3 (6.4/11.1)	0.47 (-5.1/3.5)
CG	12	7 (5.9/13.5)	10.5 (6.4/14.6)[a]	3.16 (3.5/7.0)
Fetuin-A (ng/ml)				
BWG	12	567.3 (510.2/651.9)	579.2 (444.4/645.8)	-52.3 (-107.3/52.1)[c]
MTWG	11	570.8 (513.5/655.2)	556.8 (485.3/608.9)	-10.4 (-116.2/90.5)
CG	12	546.9 (411.6/635.9)	570.6 (504.6/649.1)	23.9 (-54.9/103.5)
Triglyceride (mg/dl)				
BWG	12	137.5 (97/215)	114 (69/257)[a]	-18.0 (-139.0/97.0)[c]
MTWG	11	93 (48/239)	90 (50/239)	-1.00 (-25.0/27.0)
CG	12	55 (36/172)	56 (29/151)	-1.00 (-37.0/21.0)
HOMA - IR (mg/dl)				
BWG	12	2.01 (0.9/4.4)	1.56 (0.7/4.9)	-90.0 (-119/-30.0)
MTWG	11	1.2 (0.5/2.7)	1.1 (0.5/2.8)	-0.07 (-0.36/0.92)
CG	12	1.28 (0.4/12.3)	1 (0.4/6.3)	0.20 (-0.44/6.02)

[a]$p<0.05$ change from baseline; [c]$p<0.0167$ BWG vs. CG; SI units: milli- (m, 10^{-3}), micro- (μ, 10^{-6}), nano- (n, 10^{-9}), pico- (p, 10^{-12}).

Studies carried out following a regular moderate intensity have revealed that the reduction in TNF-α levels is related to the losses in body weight, BMI and body fat [36,37]. Observing more favorable results on obesity markers, insulin sensivity and ER stress; a significant reduction in TNF-α levels as a result of brisk walking in this present study, may lead us to think that only brisk walking may be effective in preventing metabolic diseases. In addition, the reduction in TNF-α and insulin levels may lead to a reduction in cytokine production, which in turn reduces the inflammatory effect of obesity [11]. Moreover, the decrease in TNF-α secretion is associated with the improvement in glucose tolerance. Therefore, the decrease of glycemia via exercise regulates proinflammatory cytokine release and control of hyperglycemia. When plasma glucose is in normal levels, stimulation of TNF-α production is decreased. Our findings and interpretations are concordant with the findings of both in vivo [38] and in vitro [39] studies.

RBP-4 is an adipokine recently discovered to be elevated in insulin-resistant individuals and obese people [20]. RBP-4 levels of obese men decreased following 12 weeks of aerobic exercise (brisk walking, mild jogging at 50–70% of VO_{2max}) [26]. The researchers suggested that exercise training without calorie restriction improves several cardiovascular disease risk factors and circulating RBP-4 and adipokine concentrations. As a result of 10-week stepping exercise (at 70% of VO_{2max}) RBP-4 levels reduced [22]. A 12-week aerobic and resistance training resulted in decreases in RBP-4, without a significant change in the aerobic group [23]; but, with high intensity exercises (75-80% VO_{2max}), an increasing trend in RBP-4 levels in female athletes was found at the end of 8 weeks [24]; however, no changes in RBP-4 levels were found

in female athletes following 8 weeks of aerobic exercise (50-60% VO_{2max}). The researchers concluded that RBP-4 levels are associated with obesity profiles and insulin resistance; thus, RBP-4 levels were not affected in female athletes [25]. The significant reductions in RBP-4 levels both in BWG and MTWG in the present study may enable us to suggest brisk walking or moderate tempo walking as beneficial exercise regimens for pre-menopausal women as a protective intervention. Despite the similarity between our study and the one carried out by Ahmedi *et al.* [25] the findings revealed some differences in body fat percentage, fasting glucose, insulin, insulin resistance and RBP-4 levels after 8 weeks training. This discrepency may have resulted from the frequency of the exercise programs (3 vs. 5 days a week) and the baseline percent body fat of the participants (18% vs. 40–43%).

Fetuin-A, an abundant serum protein, has recently been proposed as a link between obesity, fatty liver, and insulin resistance [26,40] and has a diagnostic potential as a biomarker for protecting cardiovascular diseases and disorders associated with metabolic syndrome. Neither a 3-month combined aerobic plus resistance exercise programme with obese women [29] nor a 6-week aerobic program with non-diabetic obese women caused changes on fetuin-A levels [30]. However, 7 days supervised exercise training (60 min/day; 85% VO_{2max}) [31] and 12 weeks of aerobic exercise (at 85% of VO_{2max}) caused significant reductions on circulating fetuin-A and insulin resistance in obese adults [32]. The researchers suggest that lower Fetuin-A after exercise correlated with lower insulin resistance. Therefore, the non-significant reduction we observed in Fetuin-A levels in our exercising groups may be clinically important in preventing cardiovascular and metabolic diseases. It is clear from the findings of the previous studies that more strenuous programs combined with weight training and energy restriction are required to cause significant reductions in Fetuin-A levels.

The JNK can interfere with insulin action in cultured cells and are activated by inflammatory cytokines and FFAs, molecules that have been implicated in the development of type 2 diabetes [10]. JNK activity of insulin resistant obese subjects decreased after a single bout of 60 min aerobic exercise. In addition, a significant positive correlation was observed between plasma insulin concentration and JNK levels in the skeletal muscle, a result that supports the participation of this stress kinase in the development of insulin resistance [17]. Although we have assessed the serum levels of JNK by ELISA method, the increase in the negative values of JNK of our BWG group could be discussed as similar to the aforementioned study [17]. However, maximal concentric or eccentric knee extensions (a total of 20 sets of 10 repetitions) increased JNK activity levels of healthy sedantary people [18]. Thus, we can conclude that brisk walking is more effective to cause reductions in JNK levels.

5 Conclusion

In our study, aiming to examine the effects of two different 8-week walking programs on the ER related stress markers and insulin resistance in premenopausal women, both type of walking programs resulted in similar positive effects on RBP-4; however, significant reductions were observed in TNF-α, RBP-4, insulin parameters and the increases in the negative levels of JNK-1 only in BWG, could point out the positive effects of brisk walking on ER stress. The reduction in insulin resistance in relation to the possible reductions in ER stress and apoptosis may be of great importance to prevent metabolic diseases. Therefore, brisk walking is advisable to increase aerobic capacity, to prevent metabolic disorders as well as cardiyovascular diseases.

Despite these favorable findings, studies including larger number of subjects, performing different types, volume, longer duration and intensity of exercises accompanied with a diet program, with immunoblooting or western blotting techniques using muscle biopsies should be carried out to draw firmer conclusions on the effects of exercise on ER stress-related markers and insulin resistance.

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6 References

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