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Effects of two different exercise trainings on plasma homocysteine levels and other cardiovascular disease risks

[Farklı iki egzersiz eğitiminin plazma homosistein düzeyi ve diğer kardiyovasküler hastalık riskleri üzerine etkileri]

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ABSTRACT

Purpose: To investigate the influences of regular sub-maximal aerobic and resistance exercise trainings for 3 months on plasma homocystenie level and other CVD risks (lipid profile, body mass index, etc.) and compare the effects of these two different types of exercise training on these parameters.

Method: Thirty-eight individuals mean aged 21.68±1.38 years were included. Participants were divided into three groups as aerobic exercise (AE) (n=13), resistance exercise (RE) (n=13) and control group (CT) (n=12). Exercises were done four times a week during 12 weeks. CT (n=12) didn't do any exercise. Blood analyses (plasma homocystenie, vitamin and lipid levels) and anthropometric measurements were performed, maximum aerobic capacity was assessed.

Results: It was found that plasma homocystenie level and lipid profile didn't change in exercise groups and CT group after 12 weeks ($p>0.05$). Body mass index and waist circumference decreased in both exercise groups ($p<0.05$), maximal aerobic capacity didn't change ($p>0.05$). Homocystenie level has positive correlation with waist circumference (r=0,392,p=0.22) and waist-to-hip ratio (r=0,501,p=0.02),and has negative correlation with folic acid level (r=-0,447,p=0.01). Folic acid level was the essential factor for homocystenie level respect to multiple regression analysis (p=0.03,R^2=0.60).

Conclusion: Sub-maximal aerobic or resistance exercise training didn't change homocysteine level when folic acid level at normal values in blood. Independent from the type of exercise, chronic exercise didn't considerably change lipid profiles in normolipidemic individuals. Aerobic capacity didn't change after 12 weeks exercise training whereas body composition improved.

Key Words: Homocysteine, lipids, exercise

Conflict of interest: There's no conflict of interest of the authors.

ÖZET

Amaç: 3 aylık düzenli sub-maksimal aerobik ve dirençli egzersiz eğitiminin plazma homosistein düzeyi ve diğer kardiyovasküler hastalık riskleri üzerindeki (lipid profili, beden kütle indeksi,v.s) etkilerini araştırmak ve bu parametreler üzerindeki etkilerini karşılaştırmaktı.

Gereç ve Yöntemler: Yaş ortalaması 21.68±1.38 yıl olan 38 birey çalışmaya alındı. Bireyler dirençli egzersiz grubu (DE) (n=13), aerobik egzersiz grubu (AE) (n=13) ve kontrol grubu (KT) olmak üzere 3 gruba ayrıldı. Egzersizler haftada 4 gün 12 hafta süreyle yapıldı. Kontrol grubu (n=12) egzersiz yapmadı. Egzersiz öncesinde ve 12.haftanın sonunda; kan analizleri (plazma homosistein, vitamin ve lipit düzeyleri), antropometrik ölçümler yapıldı ve maksimum aerobik kapasite değerlendirildi.

Bulgular: Egzersiz ve kontrol gruplarında 12 hafta sonunda plazma homosistein düzeyinin ve lipit profilinin anlamlı olarak değişmediği bulundu ($p>0.05$). Her iki egzersiz grubunun beden kütle indeksi, bel çevresi ve bel-kalça oranları azaldı ($p<0.05$), maksimal aerobik kapasite değişmedi ($p>0.05$). Homosistein ile bel çevresi (r=0,392,p=0.22) ve bel-kalça oranı (r=0,501,p=0.02) ile olumlu yönde, folik asit düzeyi ile olumsuz yönde (r= -0,447,p=0.01) korelasyon bulundu. Yapılan çoklu regresyon çözümlemesi sonucunda homosistein düzeyi üzerinde etkili temel faktörün folik asit düzeyi olduğu belirlendi (p=0.03,R^2=0.60).

Sonuçlar: Folik asit miktarı kanda normal sınırlarda iken sub-maksimal aerobik ve dirençli egzersiz eğitimi homosistein düzeyini değiştirmedi. Kronik egzersiz, yapılan egzersizin tipinden bağımsız olarak, lipit profilini, normal değerlere sahip bireylerde değiştirmedi. 12 haftalık egzersiz eğitimi sonrasında aerobik kapasite değişmezken; beden kompozisyonu gelişti.

Anahtar kelimeler: Homosistein, lipitler, egzersiz.

Çıkar Çatışması: Yazarların çıkar çatışması bulunmamaktadır.

Introduction

Regular exercise decreases resting hearth rate, blood pressure, low density lipoprotein (LDL) cholesterol level, plasma insulin level, improves physical fitness [1-5] and minimize the risk factors of the cardiovascular diseases (CVD) [1-3].

Homocysteine (Hcy) is a sulfur-containing nonprotein-forming amino acid and intermediate in the metabolic pathway of the dietary essential amino-acid methionine [6]. Homocysteine is a metabolic product of methionine [5,7-10] and its metabolism has two pathways which requires folate+vitamin B_{12} and vitamin B_6 [11]. For the last 20 years hyperhomocysteinemia (blood Hcy concentration equal or higher than 16 μmol/L) is a commonly accepted independent risk factor for cardiovascular diseases [5,8,12-18]. It's thought that the increase in Hcy levels causes atherosclerosis and thrombosis through several ways [1,10,11,18-20].

Physical activity, folic acid and/or vitamin B_6-B_{12} levels can influence Hcy methabolism [11,13,21]. Either an inefficiency of certain enzymes or a nutritional deficiency of the vitamins can lead to hyperhomocysteinemia [20]. The correlations between Hcy and gender, genetic conditions, age, medication and lifestyle factors such as nutritional deficiencies (folic acid), smoking, nutrition, alcohol intake and physical activity were determined in several studies [15,19,22]. The Hordaland Homocysteine Study is the first large population based study that examines the relationship between physical activity and blood Hcy concentrations [6]. They found that physical activity was negatively associated with blood Hcy concentration whereas some researchers found no association between physical activity level and Hcy [23-25].

In chronic exercise studies it was determined that Hcy level decreased in patients with high Hcy concentrations [26] or low physical fitness levels [10,27]. There's still equivocal results for effects of sub-maximal aerobic exercise training [5,9,26,28] and limited information about the effect of resistance exercises [10,27] on Hcy levels in sedentary individuals. For the last two decades, the American College of Sports Medicine recognized resistance training as a significant component of a comprehensive fitness program to improve cardiovascular functions in adults [29]. Resistance exercise training would contribute to the reduction in Hcy levels, although the mechanisms of the effect of chronic exercise on Hcy levels, particularly resistance exercise is not clear. It's showed that exercise affect amino acid and protein metabolism and uptake/production of Hcy is influenced especially in exercises which increase metabolic demands and may necessitate the conversion of Hcy to succinyl-coenzyme A as a means of increasing energy production from the trichloroacetic acid cycle [27]. From a different point of view, it's reported that aerobic exercises may not improve people's perception that they can undertake heavy work, whereas resistance training has been found to improve self efficacy for strength related tasks [30]. Furthermore, Hcy levels correlate with anger whereas resistance exercise is associated with decreased anxiety and improved emotional health [27].

There are plenty of studies investigate the effect of physical activity on CVD risk factors (high triglyceride (TG) levels, total cholesterol (Total-c) levels and blood pressure) in sedentary individuals. It's accepted that physical activity lowers these parameters [3,31].

The results of the studies investigating the effects of chronic exercises on plasma Hcy level are inconsistent and there's still no clear consensus [5,8,10,22,23,26,32-34]. In our knowledge there's no study that investigated the effects of two different types of exercises on Hcy levels and compared them.

A 5 μmol/L increment increase in total plasma Hcy levels was found related with a 60% to 80% increased risk for coronary heart disease. American Heart Association stated that Hcy lowering treatments such as vitamin B supplement may be inefficient to prevent recurrent heart attacks and stroke [18]. In order to elicit lower Hcy levels; changing the lifestyle of the people with proper diet and exercise programs are thought to be more effective solution. We consider that determining the most effective exercise program which can influence Hcy levels would considerably help hyperhomocysteinemia treatment and it would be helpful for health professionals to encourage variety in the prescription of exercise and development of effective intervention programs, as well.

According to this literature knowledge the aims of this study were investigating the influences of sub-maximal aerobic and resistance exercise trainings on plasma Hcy level and other CVD risks (lipid profile, body mass index, ect.) and compare the effects of these exercise trainings on these parameters.

Material and Method

Participants

Thirty-eight healthy participants (30 Female-8 Male), mean aged 21.68±1.38 years were included the study.

Approval was obtained from University of Dokuz Eylul, Human Ethics Committee (Date-number:06.09.2005-190) before commencing this study, and the written consent was taken from all participants.

Individuals who aren't smokers and have Hcy level lower than 15 μmol/L, Total-c level lower than 200 mg/dL, systolic blood pressure (SBP) and diastolic blood pressure (DBP) lower than 140 and 90 mmHg respectively, no history of injury related to extremities, no history of cardiovascular disease and performed no regular exercise for the last 6 months were included the study.

Study design

Forty-eight healthy individuals included the study. Participants who didn't match with inclusion criteria were excluded following initial blood analysis. Thirty-eight participants were randomized into three groups as resistance exercise (RE) (n1=13), aerobic exercise (AE) (n2=13) and control (CT) (n3=12) groups. Table of random numbers was used for randomization. CTs didn't do any exercise. Maintaining of habitual dietary intake and weekly physical activity of all groups were controlled by phone interview at the end of each week [26]. All assessments were done in the beginning and at the end of 12 weeks. This study was conducted in School of Physiotherapy and Rehabilitation, Dokuz Eylul University.

Measurements

Demographic information

Age, gender, weight and height, body mass index (BMI) of the participants were recorded.

Anthropometry [35]

Waist circumference; was measured with the tape placed perpendicular to the long axis of the body above umbilicus and horizontal to the floor and applied with tension without putting pressure on the abdominal wall and recorded as centimeters.

Waist-to-hip ratio; was calculated by the waist circumference divided by the hip circumference. The measuring tape placed horizontally around the maximum circumference of the buttocks. Measurement was read at the level of the tape to nearest 0.1 cm and recorded as centimeters.

Maximum Aerobic Capacity

The maximum aerobic capacity was measured with UKK 2 km Walk-test (Urho Kaleva **Kekkonen Institute** for Health Promotion Research (**UKK**), Tampere, Finland) [36].

Blood pressure (mmHg) and heart rate (beats per minute [bpm]) was measured on the right upper arm (over the brachial artery) [13]. Heart rate was monitored with Polar Heart Rate Monitor (S610i,Polar Electro Oy,Finland). The following formula was used to predict estimated $V0_{2max}$ (ml/kg/min) [36];

Women

116.2-2.98×Time for 2 km in seconds–0.11×Heart rate at the end of the test in bpm–0.14×Age in years−0.39×BMI (Weight in kg/[Height in m]^2)

Men

184.9-4.65×Time for 2 km in seconds–0.22×Heart rate at the end of the test in bpm–0.26×Age in years−1.05×BMI (Weight in kg/[Height in m]^2)

Fitness categories according to VO_{2max} for both genders (aged between 20-24 years) are defined by UKK Institute [36].

Blood analyses

Blood analyses were evaluated in main laboratory of Faculty of Medicine in Dokuz Eylul University. To eliminate the possible effect of the hormonal status on Hcy, blood samples of female participants were collected during the first 10 days of their menstrual cycle.

The blood samples were collected between 8-9 a.m. following a 12 hours overnight fasting. For Hcy measurement, blood samples were collected in EDTA-containing tubes and they were centrifuged within the 10-20 min. at 4ºC, 4000 ×g for 10 min. Blood samples were stored on ice until centrifugation. The plasma fractions were kept at -20ºC until measurements. Plasma Hcy levels were measured by the competitive immunoassay method (Intra-assay CV % = 0.98 %, Inter-assay CV % = 1.60 %) (DPC Immulite,Los Angeles,USA) [8,23]. Lipid analyses were done immediately after blood collecting. Total-c, triglyceride (TG), HDL-cholesterol (HDL-c), LDL-cholesterol (LDL-c) levels were measured with colorimetric/enzymatic method by a chemistry auto analyzer (Roche,DP Modular System,Tokyo,Japan). When the TG level was lower than 400mg/dL, LDL-c calculated using the Friedewald formulae: {Total-c}-{(TG/5) + HDL-c }[28]). All methods were daily controlled by internal quality system and were certified by NEQAS external quality control system.

Exercise training

12 weeks exercise program was done for 4 times/week, under physiotherapist supervision.

Aerobic training included warm-up (10 minutes [min]) period, sub-maximal aerobic exercise (25 to 55 min) and cool-down (10 min) period. Warm-up and cool-down periods consisted of stretching exercises for musculoskeletal system and low-intensity exercises for cardiopulmonary system. Aerobic exercise program performed on treadmill (Advance 4200,USA). Exercise training started with the intensity of 55% Target Heart Rate (THR) for 25 min in the first week and finished with 75% THR for 55 min [26]. Heart rate was monitored with Polar Heart Rate Monitor and also checked from the monitor on treadmill. The progression model for aerobic exercise training is illustrated in Table 1.

Resistance training included 10 min warm-up, 45 to 55 min exercise and 10 min cool-down. One-repetition maximum (1RM) was determined for Flexor muscles of the hip, Gluteus medius muscle, Quadriceps femoris muscle, Hamstring muscles, Rectus abdominis muscle, Latissimus dorsi muscle, Deltoid muscle (middle part), Biceps brachii muscle of each participant in the beginning of the study. Resistance exercise program consisted of the completion of three sets of 8 repetitions, with 10 s of rest between each exercise and 60 s of rest between each set, performed at 70% of each participant's predetermined 1RM in the first week then 10 repetitions were done in the second week. Then the same procedure was performed for 2 weeks using the new 1RM, and so on [10,37]. Free weights and exercise stations were used for resistance [10].

Statistical methods

Variables were analyzed with SPSS 15.0 data management system. All variables were normally distributed. Therefore, percentages, arithmetic means and standard deviations were used for descriptive statistics.

Potential baseline differences among groups were analyzed with One Way analysis of variance (ANOVA) test.

Two way ANOVA with repeated measures with time (within) and treatment groups (between) as factors was used to test for time by exercise effects on participants' anthropometrics, maximum aerobic capacity, and the results of the blood analyses. When significant changes were observed in ANOVA tests, Tukey's post hoc test was applied to locate the source of significant difference [38]. Absolute changes in the variables from baseline to second measurements (Δ) were calculated.

The correlation between Hcy level and anthropometry, vitamin levels, maximum aerobic capacity was determined by using Pearson's correlation analysis [38]. Then, multiple linear regression analyses were used to determine the essential factor related with Hcy level [38]. An extended-model approach for covariate adjustment was set up with these models:

Model 1= BMI + Waist circumference + Waist-to-hip ratio

Model 2= Model 1 + Vitamin B_{12} level + Folic acid level

Model 3= Model 2 + Maximum aerobic capacity

Model 4= Model 3 + Group (Both exercise training and non-exercise)

The level of significance was set at p= 0.05.

Results

There were 9 females-4 males in AE group, 11 females-2 males in RE group, 10 females-2 males in CT group. There was no significant difference for gender distribution among groups (χ^2=1.10,S=2,p=0.58).

All groups were similar respect to their descriptive and anthropometric variables at the baseline (Table 2) (p>0.05).

Table 1. Progression of aerobic exercise program

Week	1.	2.	3.	4.	5.	6.	7.	8.	9.	10.	11.	12.
Intensity (%)	55	55	55	55	65	65	65	65	75	75	75	75
Time (minute)	25	35	45	55	25	35	45	55	25	35	45	55

Table 2. The descriptive and anthropometric characteristics of the participants

	AE group n=13	RE group n=13	CT group n=12	p
	$\bar{X} \pm S$ (Min-Max)	$\bar{X} \pm S$ (Min-Max)	$\bar{X} \pm S$ (Min-Max)	
Age, years	22.31 ± 1.80 (20-26)	21.00 ± 1.08 (19-23)	21.75 ± 0.75 (21-23)	0.48
Height, cm	168.23 ± 9.23 (149-180)	169.23 ± 6.77 (157-182)	163.25 ± 6.11 (157-177)	0.24
Body mass, kg	62.62 ±10.03 (44-74)	61.92 ± 10.94 (53-94)	56.33 ± 8.72 (42-75)	0.64
BMI, kg/m^2	22.05 ± 2.62 (16.96-27.34)	21.55 ± 2.81 (18.34-28.38)	21.06 ± 2.37 (16.41-25.35)	0.12

Values are means ± standard deviations, p <0.05
AE : Aerobic exercise, RE: Resistance exercise, CT :Control, BMI: Body mass index

Blood analyses

The repeated measures ANOVA test, which was used to determine the group and time effect for biochemical analyses, didn't yield significant group or time interaction for Hcy levels, lipid profiles and folic acid levels in AE group ($p>0.05$) (Table 3). Only vitamin B_{12} levels was significantly different among groups after 12 weeks ($F=7.03, p= 0.00$). There was a time effect for changing in vitamin B_{12} levels ($F=35.41, p=0.00$). Vitamin B_{12} significantly decreased ($p1=p2=0.00$) in both RE group and CT group whereas other variables didn't change ($p>0.05$) (Table 3) (Figure 1). Changes in B_{12} levels were similar in both groups ($p=0.77$). The interaction of time and group wasn't significant ($p>0.05$). These reductions of vitamin B_{12} levels were higher than the percentage of biological variation of vitamin B_{12} in both groups (http://www.westgard.com/biodatabase1.htm).

There were no significant differences among three groups with regard to Hcy (Figure 2) and all other biochemical variables ($p>0.05$) (Table 3), there were no time effect for any of those variables ($p>0.05$), and the interaction of time and group wasn't significant ($p>0.05$). Table 3 shows the biochemical variables at baseline and second measurements with absolute changes and percentages.

Anthropometry

The BMI changes in each group were significantly different ($F=7.78, p=0.01$). The interaction of time and group was significant ($p<0.05$). It's found that BMI values decreased in RE group ($p=0.01$) and AE group ($p=0.02$) whereas increased in CT after 12 weeks ($p=0.04$) (Table 4) (Figure 3). Changes in BMI were similar in RE and AE groups ($p>0.05$). There was no time effect for changing in BMI ($p>0.05$).

Changes for waist circumferences among groups were significantly different ($F=5.30, p=0.01$). The interaction of time and group was significant ($p<0.05$). Waist circumference decreased in both two exercise training group (RE;p=0,02,AE;p2=0.01) after 12 weeks exercise but didn't change in CT group ($p=0.36$) (Table 4) (Figure 4). Changes in waist circumferences were similar in RE and AE groups ($p>0.05$). The main effect of time was not significant for waist circumference ($p>0.05$).

Waist-to-hip ratio didn't significantly change in any groups ($F=1.17, p=0.32$) (Table 4).

Maximum Aerobic Capacity

Estimated VO_{2max} changes were calculated for all groups and no difference was found ($p=0.61$). Although estimated VO_{2max} seemed improved in AE group after 12 weeks exercise training, further statistical analyses showed that this was due to time effect ($F=5.93, p=0.02$). The interaction of time and group wasn't significant ($p>0.05$).

The correlation between Hcy level and anthropometry, vitamin levels, maximum aerobic capacity, physical activity

Total Hcy level had moderate positive correlation with waist circumference ($r=0{,}392, p=0.02$) and strong positive correlation waist-to-hip ratio ($r=0{,}501, p=0.00$) and moderate negative correlation with folic acid level ($r=-0{,}447, p=0.01$). No correlation between Hcy level and physical activity was found ($p>0.05$) (Table 5). BMI, waist circumference and waist-to-hip ratio have positive correlations with each other ($p<0.01$) (Table 5).

After correlation analyses, multiple linear regression analyses were used to determine the essential factor related with Hcy level. It was determined that the folic acid level was the essential factor for Hcy level ($p=0.03, R^2=0.60$).

Discussion

We investigated the influences of sub-maximal aerobic and resistance exercise trainings on plasma Hcy level and other CVD risks and found that these exercise trainings didn't change the plasma Hcy levels, lipid profile and maximum aerobic capacity but improved body composition.

It's well known that sedentary life style increases CVD risks whereas regular exercise plays an important role in decreasing these risks [1,2,4,5]. Although the mechanism of relationship between Hcy level and CVD risks is still not clear, hyperhomocysteinemia is accepted as inducing atherosclerotic and thrombotic events due to endothelial cell injury and dysfunction, increased platelet adhesiveness, enhance LDL oxidation and deposition in the arterial wall, and direct activation of the coagulation process [1,10,11,18,19]. Endothelial dysfunction occurs in reduction of the bioavailability of endothelial nitric oxide (NO) and high Hcy decreases the availability of endothelial-derived NO [1,11,20]. Preventive effect of exercise is told to be related to increase in NO production (increase in both activity and protein content) by vascular endothelial cells, therefore increasing endothelial dysfunction [1]. It is reported that endothelium-dependent vasodilatation occur as early as 4 weeks exercise training depending on increased availability of NO. Another possible mechanism might be improvement of the antioxidant capacity of the vasculature by exercise training [1]. However, we found no change in Hcy levels after exercise training. It's accepted that the Hcy levels can change depend on type and frequency of the exercise training [8,34]. Cooper et al. found no change for Hcy level with 6 weeks aerobic exercise program [39]. 6 weeks exercise duration seems too short even for showing a change in body composition, cardiorespiratory system and other systems. In another study no change was reported in Hcy levels after 17 weeks aerobic training with the frequency of 2 days/week [40]. This frequency doesn't

Table 3. Biochemical variables of the groups

	AE group $\overline{X} \pm S$	RE group $\overline{X} \pm S$	CT group $\overline{X} \pm S$
Hcy (µmol/L)			
Pre	10.42±3.29	8.59±2.41	8.63±1.63
Post	10.61±3.01	8.94±2.32	8.86 ±1.78
Δ	0.18±1.28 (% 2.94±12.05)	0.35±1.40 (% 6.22±16.30)	0.28±0.97 (% 3.39±11.00)
Vitamin B_{12} (pg/mL)			
Pre	262.92±76.05	332.15±106.45	286.42±111.33
Post	245.92±97.04	224.77±77.94	231.83±98.62
Δ	-17.00±59.55 (% 6.81±22.77)	-107.46±72.98 (% 30.60±15.68) §	-54.58±49.67 (% 18.75±14,17) §
Folic acid (ng/mL)			
Pre	6.73±2.38	7.19±1.70	9.74±3.47
Post	6.70±1.19	7.16±1.69	8.29±3.01
Δ	-0.03±1.12 (% 0.31± 14.38)	-0.03±0.11 (% 16.20±31.31)	-1,45±2.51 (% 12.43±18.23)
Trigliserid (mg/dL)			
Pre	64.00±20.12	67.85±24.30	79.08±29.81
Post	76.46±46.85	66.08±26.20	98.75±64.89
Δ	12.46±38.90 (% 17.71±54.49)	-1.77± 10.30 (% 2.91±11.78)	19.67±45.85 (% 18.93±45.55)
Total-cholesterol (mg/dL)			
Pre	158.08±24.91	164.70±25.87	162.83±19.40
Post	161.61±36.74	159.00±28.70	166.92±21.26
Δ	3.54±19.93 (% 1.63±11.70)	-5.69±12.44 (% 3.48± 7.18)	4.08±16.52 (% 2.85±10.26)
HDL-cholesterol (mg/dL)			
Pre	58.76±19.01	60.31±17.28	54.08±7.69
Post	59.69±17.99	59.23±17.00	54.08±9.94
Δ	0.92±4.94 (% 2.42±8.16)	-1.08±5.65 (% 1.27±10.50)	0.00±7.30 (% 0.24±13.17)
LDL-cholesterol (mg/dL)			
Pre	87.15±20.87	91.76±23.06	92.92±17.92
Post	86.53±27.94	86.54±23.29	93.08±14.97
Δ	-0.61±16.17 (% 1.37±15.89)	-5.23±11.67 (% 5.25±11.05)	0.17±0.97 (% 1.54±13.83)

Δ, absolute changes in the variables from baseline to second measurements. Percentages were showed in the parenthesis
§ Different from the first measurement to second measurement at $p < 0.05$
Values are means ± standard deviations
AE : Aerobic exercise, RE: Resistance exercise, CT :Control

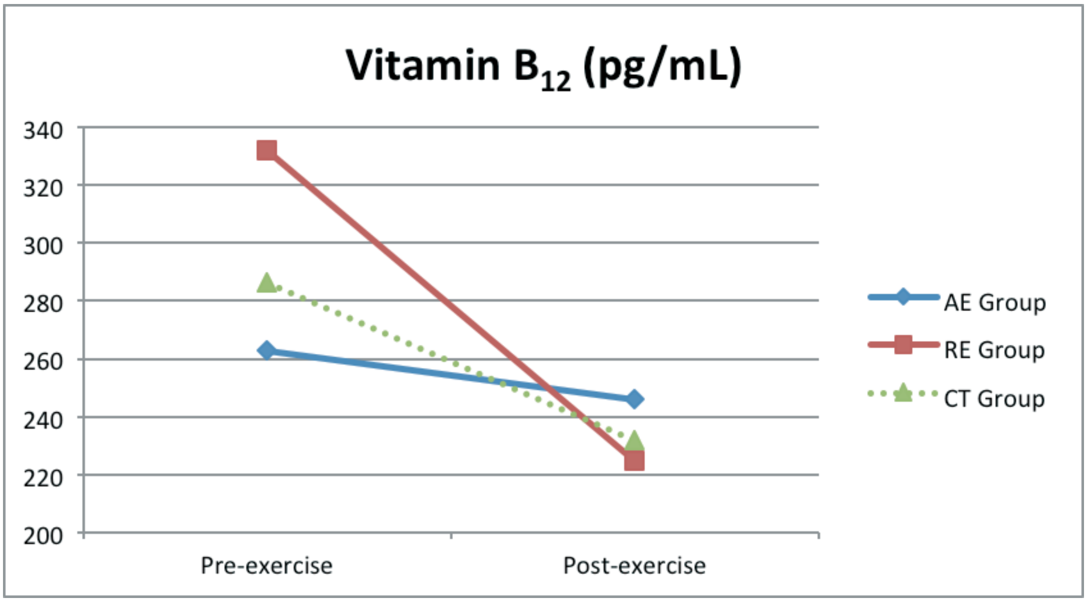

Figure 1. Vitamin B12 alterations in groups (mean). AE : Aerobic exercise, RE: Resistance exercise, CT :Control

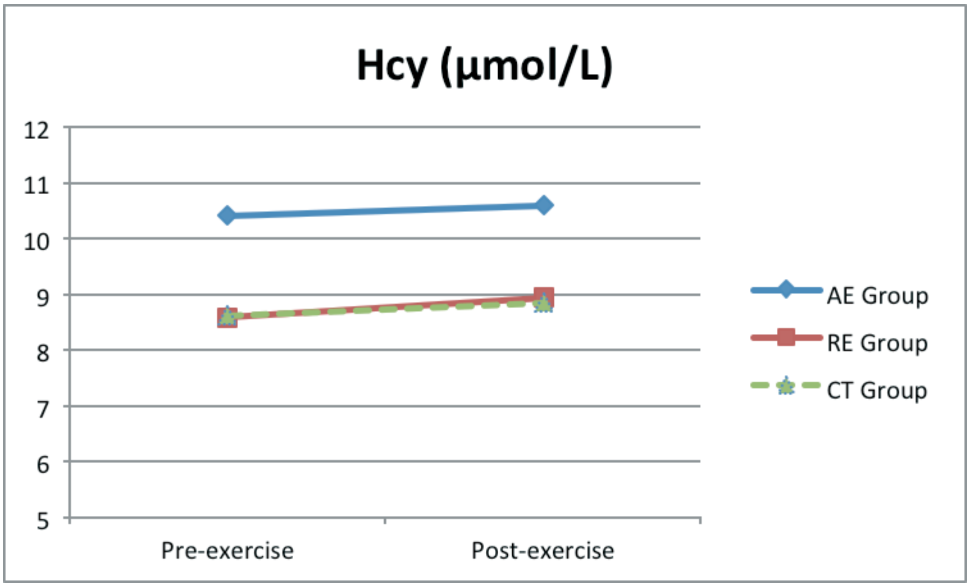

Figure 2. Hcy alterations in groups (mean). AE : Aerobic exercise, RE: Resistance exercise, CT :Control, Hcy: Homocysteine

seem enough to elicit Hcy changes. Therefore, we carefully designed our exercise trainings with suggested intensity, frequency and duration [29]. Researchers found that aerobic exercise training lowered Hcy levels in patients with hyperomocysteinemia [32,34] and it's stated that Hcy levels can be lowered by some interventions in people with hyperhomocysteinemia rather than people with normal Hcy levels [1,26,32]. In our study, the reason of ending up with no change in Hcy levels might be related with the normal Hcy levels of our participants. There are only two studies investigated the effects of resistance exercise training on Hcy levels in sedentary people [10,27]. Vincent et al. used low and high intensity resistance exercise for 6 months in older adults and found 5,30% and 5,34% reduce in Hcy levels, respectively [27]. In their second study, they explored the effect of resistance exercises on systemic oxidative stress, Hcy levels and lipoproteins. They found that Hcy levels and oxidative stress decreased but Total-c, HDL-c and lipoprotein-a levels didn't change after 6 months training [10]. All these studies were investigating the chronic effects of aerobic or resistance exercises alone. To the best of our knowledge; there's only one study that compares the acute effects of two types of exercises on Hcy levels in sedentary people [41] and our study is the first research that compares the chronic effects of these

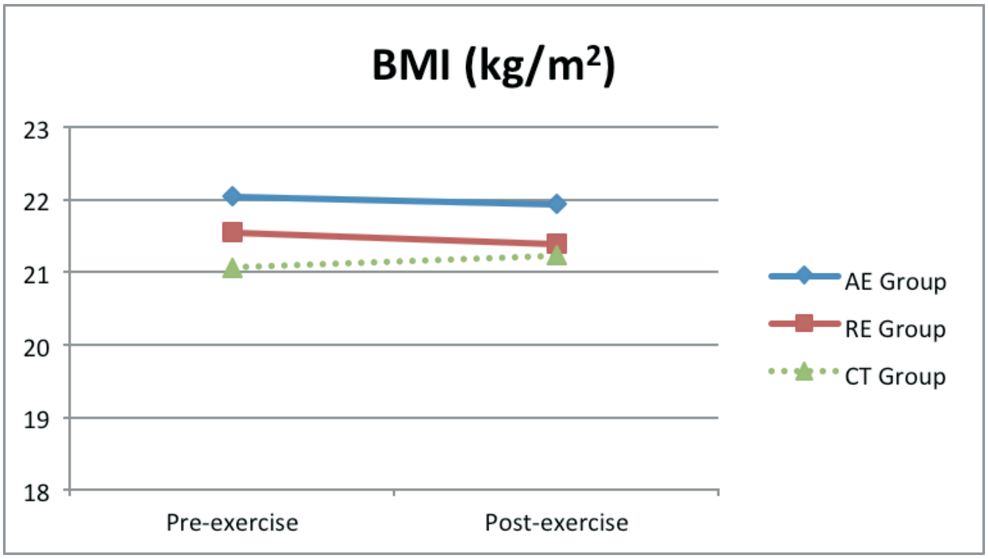

Figure 3. Changing of BMI in groups (mean). AE : Aerobic exercise, RE: Resistance exercise, CT :Control, BMI: Body mass index

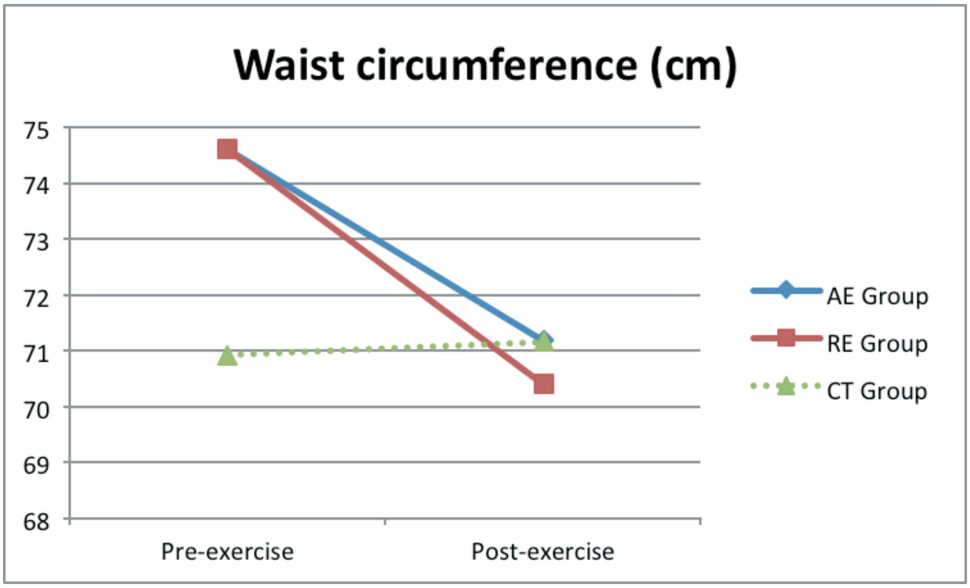

Figure 4. Changing of waist circumference in groups (mean). AE : Aerobic exercise, RE: Resistance exercise, CT :Control

exercises on Hcy. Although, our aerobic and resistance exercise trainings were intense enough with proper frequency and duration to elicit blood Hcy changes in sedentary young individuals, Hcy levels didn't change possibly due to normal Hcy levels of the participants.

We found that maximum aerobic capacities of the participants were below average according to estimated VO_{2max} measurements. Some of the researchers found lower Hcy levels in people with high physical activity levels [6,14] whereas others found no association [12,23,25]. The relationship between physical activity and Hcy levels is explained by the vitamin B_{12}, folic acid and vitamin B_6 dependent pathway: where s-adenosyl-methionine donates its methyl group to guanidinoacetate to form creatine and s-adenosyl-homocysteine in order to produce energy. Creatine phosphate is utilized within skeletal muscles to store high-energy phosphate bonds. During the high intensity exercise that is predominated by anaerobic system, the creatine turnover is elevated. It was thought that the alterations of the creatine synthesis might change the Hcy levels due to formation of S-adenosylhomocysteine (the precursor of Hcy) from S-adenosylmethionine enhanced by high creatine synthesis [11]. Further; physical activity influences plasma protein metabolism and turnover and the concentration of amino acids

Table 4. Anthropometric variables and aerobic capacity of the groups

	AE group $\bar{X} \pm S$	RE group $\bar{X} \pm S$	CT group $\bar{X} \pm S$	p
BMI (kg/m²)				
Pre	22.05±2.62	21.55±2.81	21.06±2.37	
Post	21.94±2.75	21.39±2.85	21.23±2.43	
Δ	-0.11±0.22 †	-0.16±0.18 †	0.17±0.26	0.01*
Waist circumference (cm)				
Pre	74.61±8.51	74.61±9.30	70.92±7.37	
Post	71.19±7.42	70.42±9.20	71.17±5.45	
Δ	-3.42±2.78 †	-4.19±3.21 †	0.25±3.02	0.01*
Waist-to-hip ratio				
Pre	0.76±0.07	0.75±0.07	0.74±0.04	
Post	0.74±0.07	0.72±0.07	0.74±0.04	
Δ	-0.02±0.02	-0.03±0.03	0	0.32
Maximum aerobic capacity (ml/kg/min)				
Pre	44.84±10.66	36.33±3.14	42.89±4.84	
Post	47.81±15.16	38.80±4.22	43.69±4.73	
Δ	2.98±6.40	2.47±4.86	0.80±4.44	**0.61**

Δ, absolute changes in the variables from baseline to second measurements.
Values are means ± standard deviations
* ,3 groups were significantly different at $p < 0.05$.
† Different from control group at $p < 0.05$.
AE : Aerobic exercise, RE: Resistance exercise, CT :Control, BMI: Body mass index

Table 5. The relationship of Hcy level and anthropometry, vitamin levels, maximum aerobic capacity, physical activity

		Hcy	BMI	WC	WHR	B_{12}	FA	Estimated $VO_{2\ max}$
Hcy	r		0,15	0,39	0,50	-0,30	-0,45	0,30
	p		0,37	*0,02	*0,00	0,07	*0,01	0,07
BMI	r	0,15		0,79	0,48	-0,28	-0,28	0,04
	p	0,37		*0,00	*0,00	0,10	0,09	0,83
WC	r	0,39	0,79		0,86	-0,31	-0,24	0,28
	p	*0,02	*0,00		*0,00	0,06	0,15	0,09
WHR	r	0,50	0,48	0,86		-0,41	-0,29	0,50
	p	*0,00	*0,00	*0,00		*0,01	0,08	*0,00
B_{12}	r	-0,30	-0,28	-0,31	-0,41		0,24	-0,19
	p	0,07	0,10	0,06	*0,01		0,16	0,24
FA	r	-0,45	-0,28	-0,24	-0,29	0,24		-0,16
	p	*0,01	0,09	0,15	0,08	0,16		0,33
Estimated $VO_{2\ max}$	r	0,30	0,04	0,28	0,50	-0,19	-0,16	
	p	0,07	0,83	0,09	*0,00	0,24	0,33	

Hcy; homocysteine, BMI; body mass index, WC; waist circumference, WHR; waist-to-hip ratio, $B_{12;}$ vitamin B_{12} FA; folic acid, Estimated VO_{2max}; maximum oxygen consumption.
* $p<0.05$

such as methionine [1,16]. An increase in methyl group turnover increases Hcy production. In most researches there's a lack of investigating the vitamin B_{12}/folic acid levels or lack of carefully controlling and measuring (objective measurements such as blood analysis) of these parameters which alter Hcy metabolism. In our study we measured these parameters by blood analysis and controlled these parameters to avoid interfering the results of exercise trainings. We didn't find a correlation between aerobic capacity and Hcy levels, but further statistical analysis showed that the main factor for Hcy levels was blood folic acid levels. We might not have showed the effect of activity levels on Hcy level since the folic acid levels of the participants were normal. Additionally, vitamin B_{12} levels decreased in RE and CT group at the end of 12 weeks study period and these reductions were higher than the percentage of biological variation (15%) [42] of vitamin B_{12}. This might be indicating a change of dietary status. However; the folic acid level is declared as the main factor that can affect Hcy metabolism in great number of the researches [11,15-17,19,21,23]. Our results supported these findings. Furthermore; folic acid levels among all three groups remained same during the study. For this reason, the reduction of vitamin B_{12} levels shouldn't be considered as responsible of unchanged Hcy levels.

Several researchers investigated the effects of exercise training on lipid profiles and body composition which are the factors for developing CVD in sedentary people [3,28,31]. After long term exercise training skeletal muscle lipoprotein lipase activity and mass increases, capillary density increases, removal and use of fatty acids improves (31). In Halbert et al.'s meta-analysis, it's found that the aerobic exercises decrease Total-c, TG and LDL-c while resistance exercises increase HDL-c and decrease LDL-c [3]. In another meta-analysis it's stated that aerobic exercises increase HDL-c along with decreasing Total-c and LDL-c [31]. Okura et al. reported that diet plus aerobic exercise improve lipid profile and decrease Hcy levels in obese individuals [22]. Researchers pointed out that regular exercise seems more effective in hyperlipidemic people whereas it's difficult to make a significant change in people with normal lipid profiles [3,31]. The reason of our failure to show a change in lipid profile after aerobic and resistance trainings in our study might be normal lipid levels of the participants.

Obesity, especially android-type, is associated several major disease risks [22]. One of the epidemic researches showed that BMI and WHR have positive correlation with Hcy levels [43]. Similarly, we found positive correlations between Hcy-WC and Hcy-WHR. BMI, WC and WHR correlated with each other but there was no relationship between Hcy levels an d BMI. Body composition can change according to exercise type. Although moderate intensity aerobic exercise training is mostly used to regulate total body composition and body fat [35], resistance exercises improve muscle strength which would be helpful to accomplish activities of daily living and tasks that require strength [29, 30]. In our study both resistance and aerobic exercise training improved BMI, WC and WHR. However; Hcy levels didn't change although there's a positive relationship between Hcy and WC-WHR.

55 min sub-maximal aerobic and resistance exercises for 4 days/week during 12 weeks didn't change Hcy levels. Although, our exercise training programs seemed suitable to elicit blood Hcy changes in sedentary young individuals, it can be said that these exercise trainings may not be eligible to change Hcy levels when folic acid levels and/or Hcy levels are at normal values. The Hcy and folic acid levels of the participants were at normal values in the beginning and at the end of the study, and this result supports our explanation. Even though aerobic and/or resistance exercises doesn't change Hcy levels, it's determined that other CVD risk factors can be controlled by increasing the physical activity.

Conclusion

Sub-maximal aerobic and resistance exercise training didn't change Hcy levels in young individuals with normal Hcy and folic acid levels. Hcy level has positive correlation with waist circumference and waist hip ratio and negative correlation with folic acid level. Folic acid is the essential factor that influences Hcy levels and if folic acid levels are at normal values changing Hcy levels with exercise training is difficult. Carefully controlling of the factors that possibly alter Hcy metabolism (vitamin B_{12}, folic acid, age) while designing researches examining the effect of exercise on Hcy is highly important. Both aerobic and resistance exercise training didn't change lipid profile in young individuals with normal lipid profile. Neither aerobic nor resistance exercise trainings improved aerobic capacity but improved body composition. In the future; conducting research on effects of exercise training in people with hyperhomocysteinemia and/or low folic acid levels, and people with cardiac risks is suggested.

Ethical issues: Approval was obtained from University of Dokuz Eylul, Human Ethics Committee (Date and number: 06.09.2005-190) before commencing this study.

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References

[1] Hayward R, Ruangthai R, Schneider CM, Karnilaw P, Chicco A, et al. Attenuation of homocysteine-induced endothelial dysfunc-

tion by exercise training. Pathophysiology 2003; 9:207-14.

[2] Mora S, Cook N, Buring JE, Ridker PM, Lee IM. Physical activity and reduced risk of cardiovascular events: potential mediating mechanisms. Circulation 2007; 116:2110-8.

[3] Halbert JA, Silagy CA, Finucane P, Withers RT, Hamdorf PA. Exercise training and blood lipids in hyperlipidemic and normolipidemic adults: a meta-analysis of randomized, controlled trials. Eur J Clin Nutr 1999; 53:514-22.

[4] Donnelly JE, Blair SN, Jakicic JM, Manore MM, Rankin JW, *et al.* American College of Sports Medicine Position Stand. Appropriate physical activity intervention strategies for weight loss and prevention of weight regain for adults. Med Sci Sports Exerc 2009; 41:459-71.

[5] Gaume V, Mougin F, Figard H, Simon-Rigaud ML, N'Guyen UN, *et al.* Physical training decreases total plasma homocysteine and cysteine in middle-aged subjects. Ann Nutr Metab 2005; 49:125-31.

[6] Nygard O, Vollset SE, Refsum H, Stensvold I, Tverdal A, *et al.* Total plasma homocysteine and cardiovascular risk profile. The Hordaland Homocysteine Study. JAMA 1995; 274:1526-33.

[7] De Cree C, Malinow MR, van Kranenburg GP, Geurten PG, Longford NT, *et al.* Influence of exercise and menstrual cycle phase on plasma homocysteine levels in young women- a prospective study. Scand J Med Sci Sports 1999; 9:272-8.

[8] Herrmann M, Wilkinson J, Schorr H, Obeid R, Georg T, *et al.* Comparison of the influence of volume-oriented training and high-intensity interval training on serum homocysteine and its cofactors in young, healthy swimmers. Clin Chem Lab Med 2003; 41:1525-31.

[9] Gelecek N, Teoman N, Ozdirenc M, Pınar L, Akan P, *et al.* Influences of acute and chronic aerobic exercise on the plasma homocysteine level. Ann Nutr Metab 2007; 51:53–8.

[10] Vincent HK, Bourguignon C, Vincent KR. Resistance training lowers exercise-induced oxidative stress and homocysteine levels in overweight and obese older adults. Obesity (Silver Spring) 2006; 14:1921-30.

[11] Selhub J. Homocysteine metabolism. Annu Rev Nutr 1999; 19:217-46.

[12] Saw SM, Yuan JM, Ong CN, Arakawa K, Lee HP, *et al.* Genetic, dietary and other lifestyle determinants of plasma homocysteine concentrations in middle-aged and older Chinese men and women in Singapore. Am J Clin Nutr 2001; 73:232-9.

[13] Nygard O, Refsum H, Ueland PM, Vollset SE. Major lifestyle determinants of plasma total homocysteine distribution. Am J Clin Nutr 1998; 67: 263-70.

[14] Kuo HK, Yen CJ, Bean JF. Levels of homocysteine are inversely associated with cardiovascular fitness in women, but not in men: data from the National Health and Nutrition Examination Survey 1999-2002. J Intern Med 2005; 258:328-35.

[15] Stanger O, Herrmann W, Pietrzik K, Fowler B, Geisel J, *et al*. Clinical use and rational management of homocysteine, folic acid, and B vitamins in cardiovascular and thrombotic diseases. Z Kardiol 2004; 93:439-53.

[16] Di Santolo M, Banfi G, Stel G, Cauci S. Association of recreational physical activity with homocysteine, folate and lipid markers in young women. Eur J Appl Physiol 2009; 105:111-8.

[17] Stanger O, Herrmann W, Pietrzik K, Fowler B, Geisel J, *et al.* DACH-LIGA homocystein (German, Austrian and Swiss homocysteine society): consensus paper on the rational clinical use of homocysteine, folic acid and B-vitamins in cardiovascular and thrombotic diseases: guidelines and recommendations. Clin Chem Lab Med 2003; 41:1392-403.

[18] Malinow M, Bostom R, Krauss M. Homocyst(e)ine, diet, and cardiovascular diseases: a statement for healthcare professionals from the nutrition committee, American Heart Association. Circulation 1999; 99:178-82.

[19] Refsum H, Nurk E, Smith AD, Ueland PM, Gjesdal CG, *et al.* The Hordaland Homocysteine Study: a community-based study of homocysteine, its determinants, and associations with disease. J Nutr 2006; 136(6 Suppl):1731S-40S.

[20] Kothekar MA. Homocysteine in cardiovascular disease: a culprit or an innocent bystander? Indian J Med Sci 2007; 61:361-71.

[21] Sheu WH, Chin HM, Lee WJ, Wan CJ, Su HY, *et al.* Prospective evaluation of folic acid supplementation on plasma homocysteine concentrations during weight reduction: a randomized, double-blinded, placebo-controlled study in obese women. Life Sci 2005; 76:2137-45.

[22] Okura T, Nakata Y, Ohkawara K, Numao S, Katayama Y, *et al***.** Effect of weight reduction on concentration of plasma total homocysteine in obese Japanese men. Obes Res Clin Pract 2007; 1:213-21.

[23] Rousseau AS, Robin S, Roussel AM, Ducros V, Margaritis I. Plasma homocysteine is related to folate intake but not training status. Nutr Metab Cardiovasc Dis 2005; 15:125-33.

[24] Ruiz JR, Hurtig-Wennlof A, Ortega FB, Patterson E, Nilsson TK, *et al.* Homocysteine levels in children and adolescents are associated with the methylenetetrahydrofolate reductase 677C>T genotype, but not with physical activity, fitness or fatness: the European Youth Heart Study. Br J Nutr 2007; 97:255-62.

[25] Husemoen LL, Thomsen TF, Fenger M, Jorgensen T. Effect of lifestyle factors on plasma total homocysteine concentrations in relation to MTHFR (C677T) genotype. Inter99 (7). Eur J Clin Nutr 2004; 58:1142-50.

[26] Okura T, Rankinen T, Gagnon J, Lussier-Cacan S, Davignon J, *et al.* Effect of regular exercise on homocysteine concentrations: the HERITAGE Family Study. Eur J Appl Physiol 2006; 98:394-401

[27] Vincent KR, Braith RW, Bottiglieri T, Vincent HK, Lowenthal DT. Homocysteine and lipoprotein levels following resistance training in older adults. Prev Cardiol 2003; 6:197-203.

[28] Boreham CA, Kennedy RA, Murphy MH, Tully M, Wallace WF, *et al.* Training effects of short bouts of stair climbing on cardiorespiratory fitness, blood lipids, and homocysteine in sedentary young women. Br J Sports Med 2005; 39:590-3.

[29] Pollock ML, Gaesser GA, Butcher JD, Després JP, Dishman RK, *et al.* (American College of Sports Medicine) American College of Sports Medicine Position Stand. The recommended quantity and quality of exercise for developing and maintaining cardiorespiratory and muscular fitness, and flexibility in healthy adults. Med Sci Sports Exerc 1998; 30:975-91.

[30] Adams J, Cline MS, Reed M, Masters A, Ehlke K, Hartman J. Importance of resistance training for patients after cardiac event. Proc (Bayl Univ Med Cent) 2006; 19: 246-48.

[31] Kelley GA, Kelley KS, Tran ZV. Aerobic exercise and lipids and lipoproteins in women: a meta-analysis of randomized controlled trials. J Womens Health (Larchmt) 2004; 13:1148–64.

[32] Ali A, Mehra MR, Lavie CJ, Malik FS, Murgo JP, *et al.* Modulatory impact of cardiac rehabilitation on hyperhomocysteinemia in patients with coronary artery disease and "normal" lipid levels. Am J Cardiol 1998; 82:1543-5, A8.

[33] Steenge GR, Verhoef P, Greenhaff PL. Effect of creating and resistance training on plasma homocysteine concentration in healthy volunteers. Arch Intern Med 2001; 161:1455-56.

[34] Randeva HS, Lewandowski KC, Drzewoski J, Brooke-Wavell K, O'Callaghan C, *et al.* Exercise decreases plasma total homocysteine in overweight young women with polycystic ovary syndrome. J Clin Endocrinol Metab 2002; 87:4496-501.

[35] Slentz C, Duscha Bi, Johnson JL, Ketchum J, Aiken LB. Ef-

fects of the amount of exercise on body weight, body composition, and measures of central obesity. : STRRIDE--a randomized controlled study. Arch Intern Med 2004; 164:31-9.

[36] Guide for The UKK Institute 2-km Walking Test. UKK Institute, 1993.

[37] Ratamess N, Alvar BA, Evetoch TK, Housh TJ, Kibler WB, *et al*. (American College of Sports Medicine) American College of Sports Medicine position stand. Progression models in resistance training for healthy adults. Med Sci Sports Exerc 2009; 41:687-708.

[38] Aksakoğlu G. Sağlıkta Araştırma ve Çözümleme, İkinci yazım, 2006; pp:215-229, 283-299,300-309, DEÜ Rektörlük Basımevi, İzmir.

[39] Cooper A, Kendrick A, Stansbie D, Sargent D, West J. Plasma homocysteine in sedentary men: influence of moderately intense exercise. Cardiovasc Rev Rep 2000; 21:371-4.

[40] De Jong N, Chin A Paw MJ, De Groot LC, Rutten RA, Swinkels DW, *et al*. Nutrient-dense foods and exercise in frail elderly: effects on B vitamins, homocysteine, methylmalonic acid, and neuropsychological functioning. Am J Clin Nutr 2001; 73:338-46.

[41] Subaşı SS, Gelecek N, Özdemir N, Örmen M. Influences of acute resistance and aerobic exercises on plasma homocysteine level and lipid profiles. Turk J Biochem 2009; 34(1):9–14.

[42] Westgard QC. Biological Variation Database Specifications. http://www.westgard.com/biodatabase1.htm (Last accessed: January 2011)

[43] Rasmussen LB, Ovesen L, Bulow I, Knudsen N, Laurberg P, *et al*. Folate intake, lifestyle factors, and homocysteine concentrations in younger and older women. Am J Clin Nutr 2000; 72:1156-63.