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Effects of brisk walking program on plasma homocysteine level and lipid profile in sedentary young subjects

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Research Report

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Purpose: The purpose of this study was to examine the effects of 6-week brisk walking training on plasma homocysteine levels and lipid profiles in sedentary young subjects. **Materials and methods:** Fourteen female and 15 male physiotherapy students were included voluntarily in this study. The subjects performed six weeks of brisk walking program, with a speed of 6.4 km/h and over, three times a week. Plasma homocysteine, total cholesterol triglyceride, high density lipoprotein-cholesterol (HDL-c) and low density lipoprotein-cholesterol (LDL-c) levels were measured before and after the exercise training. **Results:** After the training, plasma homocysteine levels increased whereas HDL-c, and triglyceride levels decreased. These differences were not statistically significant ($p>0.05$). On the contrary, total cholesterol and LDL-c decreased significantly ($p<0.05$). In addition, plasma homocysteine levels were higher in males as compared to females before and after training. **Conclusions:** The results suggest that brisk walking training for 18 sessions, as an aerobic exercise, could be effective on the lipid profile, but this training program does not change the levels of plasma homocysteine.

Key words: Walking; training, Homocysteine, Healthy subjects.

Sedanter genç bireylerde tempolu yürüme programının plazma homosistin düzeyine ve lipid profiline etkileri

Amaç: Bu çalışmanın amacı 6 haftalık tempolu yürüme eğitiminin plazma homosistin düzeyi ve lipid profiline etkilerini belirlemektir. **Gereç ve yöntem:** Çalışmaya gönüllü, 14 kadın ve 15 erkek fizyoterapi öğrencisi dahil edildi. Bireyler haftada üç gün ve altı hafta boyunca, 6.4 km/h ve üzeri hızda tempolu yürüme programını tamamladı. Eğitim öncesi ve eğitim sonrası bireylerin plazma homosistin, total kolesterol, trigliserid, yüksek dansiteli lipoprotein-kolesterol (HDL-c) ve düşük dansiteli lipoprotein-kolesterol (LDL-c) düzeyleri analiz edildi. **Sonuçlar:** Eğitim sonrası, plazma homosistin düzeyi arttı, HDL-c ve trigliserid düzeyi azaldı. Ancak bu azalmalar istatistiksel olarak anlamlı değildi ($p>0.05$). Bunun aksine, total kolesterol ve LDL-c kolesterol düzeyinin anlamlı olarak azaldığı bulundu ($p<0.05$). **Tartışma:** Bu çalışmanın sonucu, aerobik bir egzersiz olan 18 seanslık tempolu yürüme eğitiminin lipid profili üzerinde etkili olabildiği, ancak plazma homosistin düzeyini değiştirmediğini desteklemiştir.

Anahtar kelimeler: Yürüme; eğitim, Homosistin, Sağlıklı birey.

Homocysteine is a non-essential sulphur-containing amino acid formed from the demethylation of an essential amino-acid methionine.[1-3] High homocysteine level is a risk factor for cardiovascular disease.[4-6] Several mechanisms that may underlie the positive association between homocysteine concentration and risk for cardiovascular disease include oxidation of low density lipoprotein-cholesterol (LDL-c), toxic effects on endothelial cell, impaired platelet activity, and increased smooth cell proliferation.[2,7]

Plasma homocysteine level is also positively related to total-cholesterol level, blood pressure, and heart rate, and inversely related to physical activity.[1,8,9]

Regarding a possible link between homocysteine metabolism and physical activity, an important pathway is the vitamin B12, folic acid, and vitamin B6 dependent formation of the universal methyl group donating substance S-adenosylmethionine for proper energy production as it converts guanidinoasetate to creatine. Creatine phosphate is utilized within skeletal muscles for storing high-energy phosphate bonds. It has been speculated that alterations in the creatine synthesis may affect the homocysteine levels, since the formation of S-adenosylhomocysteine (precursor of homocysteine) from adenosylemethionine is thought to be enhanced by high creatine synthesis.[10-12]

During the anaerobic alactacide metabolism, creatine turnover is elevated. Physical activity influences plasma protein metabolism and turnover and thus the concentration of certain aminoacids, including methionine.[10,12]

Other possible mechanism between exercise and homocysteine is that exercise effects vasoregulation, increases nitric oxide production in endothelial cells, and causes a decrease in homocysteine.[13]

In respect to cardiovascular disease, effect of physical activity on homocysteine levels depends on the intensity and frequency of the exercise, gender, age, nutrition, and other risk factors.[1,10-12] However, there is no consensus on the type and frequency of exercise that reduces the homocysteine levels.

The purpose of this study was to investigate the effect of brisk walking training on plasma homocysteine level in young, sedentary, and healthy subjects.

Material and methods

Subjects:

Subjects were healthy young volunteer students from School of Physical Therapy and Rehabilitation, Dokuz Eylül University, İzmir, Turkey. The mean age of 14 females and 15 male students was 20.02±2.12 years.

Subjects who were smokers, had homocysteine levels higher than 20 micromol/L, total-cholesterol levels higher than 200 mg/dL, systolic blood pressure (SBP) and diastolic blood pressure (DBP) higher than 140 and 100 mmHg, respectively, a history of surgery related to lower extremities and who had performed regular exercise for the last six months were excluded from the study.

Approval was obtained from Dokuz Eylül University Human Ethics Committee before commencing the study, and written consent was taken from all subjects.

Subjects were asked to maintain their normal, regular diet without any changes throughout the study period, and they were not allowed to take any vitamin supplements especially vitamin B6, vitamin B12 or folate.

Subjects underwent a submaximal cycling test to determine their aerobic capacity. A computerized Monark 839 cycle ergometer with its own heart rate monitor (Monark Exercise AB, Vansbro, Sweden) was used for the tests. Aerobic capacities of all subjects were described as "low" according to Astrand's classification.[14]

Laboratory assessments:

All the measurements were performed the day before and after the training programs. Blood samples were obtained between 9 and 10 am following a 12-h overnight fasting. Blood specimens in the tubes with EDTA were put in ice-water immediately after collection and centrifuged at 4°C within 30 minutes after sampling. Plasma aliquotes were stored at -70°C until analysis. Plasma homocysteine levels were measured by the competitive immunoassay method (DPC Immulite, Los Angeles, USA). After manual sample pre-treatment step, homocysteine was released from its binding proteins and converted to S-adenosyl-homocysteine by an off-line 30-minute incubation at 37°C in the presence of S-adenosyl homocysteine hydrolase and dithiothreitol. Homocysteine levels in treated samples were determined by typical immunoassay method.

Total-cholesterol, triglyceride, glucose, high density lipoprotein-cholesterol (HDL-c), and LDL-c levels were measured by Roche kits at chemistry otoanalyser (Roche, DP Moduler System, Tokyo, Japan). Fasting homocysteine samples and blood samples were measured by the same procedure after the exercise training (6 weeks later).

Exercise Training:

Brisk walking (6.4 km/h–7.0 km/h) was used for exercise training.[15] Each period of exercise included a 5-minute warm-up, 30-minute exercise training, a 5-minute cool-down. The warm-up and cool-down periods included flexibility exercises for hamstrings, quadriceps, gastrocnemius and lumbar extensors, and slow walking (approximately 100-150 meter).

Heart rate was measured using manual palpation on the left radial artery for 30 seconds, first by a physiotherapist, and then by subjects themselves.

Blood Pressure was measured on the upper right arm (over the brachial artery) using a standard mercury sphygmomanometer.

Study design:

Demographic variables and laboratory analyses were completed and type and intensity of the training was explained to subjects by a physiotherapist. Subjects walked around a large garden of their campus under the supervision of a physiotherapist.

Statistical analysis:

Variables were analyzed using SPSS 11.0 data managements system. Values are presented as mean ± standard deviation (X±SD). Changes within subjects at different points of examination were investigated by Wilcoxon Signed Rank test. Mann-Whitney U test was used to compare homocysteine levels of males and females. A $p<0.05$ was regarded as significant.

Results

All of the subjects completed exercise training and each subject performed brisk walking program for 18 sessions, three times per week. Then their laboratory tests were repeated six weeks later. The characteristics of 29 subjects are outlined in Table 1.

Resting SBP and resting DBP decreased after brisk walking training, but decrease was not significant ($p>0.05$). At the end of training, resting heart rate was significantly lower compared with initial values ($p<0.05$) (Table 1).

Table 1. Characteristics of the subjects before and after brisk walking training.

	Before training X±SD	After training X±SD	
Age (years)	20.0±2.1		
Height (cm)	167.5±5.3		
Body mass (kg)	58.5±3.7	58.3±1.4	*
Resting SBP (mmHg)	110±8.3	105±8.5	*
Resting DBP (mmHg)	74±6.4	72±8.7	*
Resting HR (beats/min)	78.6±6.8	73.8±6.2	**

* p>0.05, ** p<0.05.

After 18 sessions of brisk walking training, mean plasma homocysteine level increased from 10.81±2.76 micromol/L to 11.37±2.97 micromol /L. Similarly, after training, fasting glucose, HDL-c, and triglyceride levels were lower compared with before training values. These differences were not significant ($p>0.05$) (Table 2). Even though HDL-c decreased after training, HDL-c/total cholesterol ratio increased after training from 0.31±0.14 mg/dL to 0.35±0.34 mg/dL ($p<0.05$).

It was found that total-cholesterol level decreased from 144.16±17.82 mg/dL to 132.46±23.29 mg/dL, LDL-c level decreased from 79.91±17.62 mg/dL to 72.45±17.12 mg/dL. These decreases were statistically significant ($p<0.05$) (Table 2).

Mann Whitney U test was used to compare homocysteine levels of females and males. It was found that plasma homocysteine level was higher in males as compared to females before (p= 0.005) and after training (p=0.029). When mean differences of homocysteine levels were compared between genders, the values of females (mean difference=0.62±1.54 micromol/L) were higher than males' (mean difference=0.45±2.42 micromol/L) and the difference was statistically significant (p=0.014).

Table 2. Homocysteine and lipid levels of the subjects before and after the training.

	Before training X±SD	After training X±SD	
Hcy (micromol/L)	10.8±2.8	11.4±3.0	*
TG (mg/dL)	72.3±14.4	69.8±21.1	*
Total-c (mg/dL)	144.2±17.8	132.5±23.3	**
HDL-c (mg/dL)	48.7±11.2	46.1±9.3	*
LDL-c (mg/dL)	79.9±17.6	72.5±17.1	**

Hcy: Homocysteine, TG: Triglyceride, c: Cholesterol.
* $p>0.05$, ** $p<0.05$.

Discussion

At the beginning of the study, we expected that plasma homocysteine level would decrease with aerobic exercise training in healthy young subjects who had low aerobic capacity according to Astrand's classification. Instead, we found that plasma homocysteine levels increased after exercise training consisting of brisk walking, but this increase was not statistically significant.

In the literature, there were few studies investigating the effects of exercise on plasma homocysteine level.[1,10-12,16-19] Some of the studies were related to acute effects of exercise,[10,20,21] and other studies were related to chronic effects of exercise training.[10-12,17,18] It seemed to be that the effect of exercise on the level of homocysteine could change depending on intensity and frequency of the training, age, gender and present risk factors such as heredity, and poor nutrition with B6, B12 and folate.[10-12,16,17]

Hordaland reported that there was an inverse relation between mean plasma homocysteine level and the level of activity. They found that plasma homocysteine level was lower in the subjects who performed moderate and heavy training, when compared to values of homocysteine levels in the sedentary subjects.[1]

Konig et al. investigated plasma homocysteine level in athletes who performed moderate (9.1 h/week) or high exercise training (14.9 h/week). They found that after eight weeks, plasma homocysteine levels decreased only in the high training group only.[10]

Some researchers proposed that resistance training tended to decrease plasma homocysteine concentration related to increased muscle protein synthesis,[12,18] and other researches proposed that aerobic training tended to decrease plasma homocysteine level if it is high intensity.[1,10,17]

Herrmann et al. reported that homocysteine level increased progressively after three weeks of volume-oriented training in young swimmers.[17] In our study, brisk walking training as an aerobic exercise for six weeks caused decreasing the total-cholesterol and LDL-c, but it caused a minimal increase in plasma homocysteine level. Either the intensity or the frequency of training was not sufficiently qualified in reducing plasma homocysteine.

In our study, there was a significant reduction in total-cholesterol and LDL-c, independent from the plasma homocysteine. The HDL-c decreased, but the part of HDL-c in total cholesterol increased after the training program (HDL-c/total cholesterol; before the training: 0.31 and after the training: 0.35). This result was similar to the findings stating a decrease in lipid profile and raising HDL-c after aerobic exercise training.[22] Homocysteine levels of males were higher than those of females, and the mean difference in females was higher than that of males after the training.

At the end of this study, the results suggest that brisk walking training applied three days a week, a total of 18 sessions, could have positive effects on the lipid profile in healthy young subjects. On the other hand, this training program did not change level of the plasma homocysteine.

The limitation of this study was that we could include a control group because of financial difficulties. Further studies are needed to ensure a comparison between control and training groups. In addition, further study investigating training responses with various exercise intensity, frequency and duration in healthy young subjects warranted.

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