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Long-Term Callisthenic Exercise–Related Changes in Blood Lipids, Homocysteine, Nitric Oxide Levels and Body Composition in Middle-Aged Healthy Sedentary Women

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Abstract

Regular physical exercise plays an important role in reducing obesity, preventing hyperglycemia, lowering blood lipids and reducing systemic blood pressure. But the question about the nature of the relationship between homocysteine, nitric oxide and physical activity remains unanswered. The aim of this study was to investigate the effects of callisthenic exercises on plasma lipids, homocysteine (Hcy), total nitric oxide (NOx) and body composition in middle-aged healthy sedentary women. Forty-two middle-aged women (ages: 28-49; mean: 41.40 ± 7.3 years) were asked to perform a callisthenic exercise 50 min per session, 3 times per week for 12 weeks in a sports hall. Before and after the exercise, plasma lipids (total cholesterol, high density lipoprotein, low density lipoprotein and triglyceride), Hcy and NO were determined. Body composition, including body mass index, fat percentage, fat free mass, resting systolic and diastolic blood pressures and heart rates were measured. After a 12-week callisthenic exercise program, plasma NOx and Hcy levels were found to be significantly increased ($P < 0.05$). Body composition parameters, lipid profile, resting systolic and diastolic blood pressures and heart rate significantly decreased ($P < 0.05$). Aerobic callisthenic exercises characterized by 50 min/day and 3 days/week resulted in positive changes in important health parameters like reducing obesity, lowering blood lipids and increasing plasma NOx. Cardiovascular improvements might be dependent on the increase of NOx values. But callisthenic exercise in such intensity did not lower the plasma Hcy level. Moreover, Hcy level increased significantly. The result shows that if the Hcy is in the normal levels in healthy subjects, long-term callisthenic exercise do not decrease the Hcy levels despite some beneficial effects on health. On the contrary, the Hcy levels are increased by long-term callisthenic exercises.

Key Words: **exercise, lipids, homocysteine, nitric oxide, body composition, women**

Introduction

During physical exercise, cardiac output increases and blood is widely distributed to the working muscles. This increased tension in the muscle vasculature facilitates the production of nitric oxide (NO) (35). NO is an endothelium-derived relaxing factor and relaxes vascular smooth muscle in the

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systemic and cerebral circulation (8, 16). Jungersten *et al.* (1997) have found that regular exercise upregulates the expression of the endothelial nitric oxide synthase (NOS) gene in vascular tissues and also helps to maintain higher blood NO levels between exercise sessions (28, 46). Increased production of this vasodilating agent might partially explain how physical fitness is beneficial to cardiovascular health (9, 41).

Regulation of vascular NO production may possess therapeutic potential in atherosclerosis and thrombosis for which homocysteine (Hcy) is a risk factor (20, 31). Effects of hyperhomocysteinemia (HHcy) on NO production are controversial. It has been shown that Hcy both increases and suppresses NO. It has been claimed that Hcy does not affect the expression of endothelial nitric oxide synthase (eNOS) but it stimulates formation of superoxide anions (49). These researchers showed that a scavenger of peroxynitrite or cell permeant of superoxide dismutase mimetics reversed the Hcy-induced suppression of NO production by endothelial cells (49). On the other hand, there are some studies which claim that Hcy acts directly on eNOS. Due to these studies, Hcy either upregulates (45) or/and down regulates eNOS (12) and NO production.

Although the exact mechanism is not known, exercise has many beneficial effects on health and cardiovascular diseases and in reducing cardiovascular morbidity and mortality (23). Exercise models that are most suitable for sedentary people in all ages are walking, running, swimming, cycling, aquatic type exercises, yoga type aerobic and dynamic exercises (19, 32, 33).

Callisthenics are aerobic and dynamic exercises and are suitable for sedentary and also for older people. They are rhythmic, smooth, enjoyable exercises that are easy to perform alone or in a group format, and can be modified according to subjects' fitness levels (4). Callisthenics consist of a variety of simple movements that are intended to increase body strength and flexibility using the weight of one's own body for resistance. Callisthenic fitness training can develop both muscle endurance and cardiovascular fitness in addition to improving psychomotor skills such as balance agility and coordination.

The purpose of this study was to evaluate the effects of long-term callisthenic exercise on blood lipids Hcy, NOx and body composition with respect to cardiovascular fitness in middle-aged healthy sedentary women.

Materials and Methods

Subjects

A twelve-week intervention study involved middle-aged sedentary healthy (mean, 41.40 ± 7.3 years; ages from 28-49) women. Subjects were randomly assigned to an exercise program. All were living in the neighborhood locations, and an exercise program was organized with the aid of the Municipality of Kecioren which provided a convenient place. The project was announced on bulletin boards and 60 women applied. Subjects with acute cerebrovascular, cardiovascular, pulmonary, metabolic, psychotic, rheumatic, infectious diseases, cancer and hypertension (blood pressure > 160/95 mmHg), and 10 subjects who were using drugs, cigarettes and alcohol were excluded. A total of 50 subjects met the inclusion criteria. Forty-two women completed to an exercise program. All subjects were instructed about the aims of the study and gave written informed consent which was approved by Gazi University's Health Science Ethics Committee.

Procedures

Blood lipids, Hcy, NOx, body composition, resting blood pressure and heart rate were measured before and after a 12-week exercise program (5). All subjects agreed not to change the diet or lifestyle over the experimental period. Eight subjects broke off the exercise with various reasons in various sessions leaving 42 subjects to finish the study. Subjects performed callisthenic exercise in 50 min/session, 3 times/week for 12 weeks. The exercise program was adjusted every other day of the week to provide the women to have a rest during the day after the trials. Forty-two women participated in the trial at the same time and performed their exercise depending on the commands of a sports trainer under the supervision of a physiotherapist.

The intensity of the callisthenic exercises depended on the numbers of sets and repetitions, and on the length of resting periods. During the initial training sessions, all subjects were instructed how to measure their heart rate. Training heart rate was used to calculate the training intensity and expressed as a percentage of resting heart rate (HR_{res}). The maximum heart rate (HR_{max}) is the highest heart rate an individual can safely achieve through exercise stress, and is dependent on age. This heart rate can be calculated by the formula: 220 - age. The target heart rate or training heart rate (THR) is a desired range of heart rate reached during aerobic exercise which enables one's cardio-respiratory system to receive the highest benefit from a workout. The intensity of this type of exercise can be designed by calculating the 65% and 85% of the maximum heart rate (HR_{max}) (19, 33).

Every training session began at a low level and gradually increased to the training zone. Each workout included warm-up and cool-down phases (5 to 10 min each) during which slow stretching and walking exercises were performed to prevent injury (17, 42). The training program consisted of warming, callisthenic exercises (25 min), exercises for abdomen, hip and leg muscles (10 min) and cooling and stretching exercises (10 min). Callisthenics consisted of spine lateral flexion, spine twisting, neck rotation, neck flexion and extension, trunk lateral flexion and arm circles. Subjects performed the strengthening exercises including sit-ups/crunches and push-ups. Sit-up exercise was started with the back on the floor, knees bent, bottoms of feet against the floor. Shoulders were lifted off the floor by tightening abdominal muscles, bringing the chest closer to the knees to strengthen the abdominal muscles. Push-up exercise was performed with face down on floor, keeping the back straight and palms tight on the back. Then the head and shoulders were raised off the floor. This movement aims at strengthening the back extensor muscles. Each mode of the exercises was repeated ten times.

Blood Samples Analysis

After an overnight fasting, between 8 pm and 10 am the day after, the subjects' venous blood samples (10 ml) were collected from the antecubital vein *via* venipuncture in Vacutainer sodium heparinized tubes by an experienced nurse in Central Laboratories of Gazi Hospital. Tubes were centrifuged right after the sampling at room temperature at 10 min at 3000 × g. The plasma fraction was then transferred to cryotubes. Cryotubes were kept at -80°C until measurement. Plasma levels of total cholesterol, HDL-cholesterol (HDL-C), LDL-cholesterol (LDL-C), triglyceride, Hcy and NOx and blood triglycerides were measured. All measurements were repeated after the exercise treatment.

Blood lipids: Blood samples; plasma total cholesterol (1), HDL-C, LDL-C (27) and triglyceride levels (34) were analyzed using a Hitachi 7250 (Tokyo, Japan) automated analyzer.

Hcy levels: Plasma Hcy was detected with a high-performance liquid chromotography (HPLC) method by ImmuChrom HPLC-application, supplied by ImmuChrom GmbH (Hessen, Germany). The method was used in accordance with the instruction of the kit. The kits included all reagents for preparation and separation of the samples with the exception of the columns (IC2801rp) and the controls (IC2801ko). Both were also supplied by ImmuChrom GmbH. For the determination of Hcy the sample is reduced and derivatised in one step. The albumin bound and the oxidized Hcy is reduced and converted into a fluorescent probe. During a precipitation step, high molecular substances were removed. The sample was cooled (2-8°C), centrifuged and injected into the HPLC system. The isocratic separation *via* HPLC at 30°C was performed using a reversed phase column. One run lasted 5 min. The chromatograms were recorded by a fluorescence detector. The quantification was performed by the delivered plasma calibrator. The concentration was calculated *via* the "internal standard method" by an integration of the peak areas respectively peak heights.

Plasma total NO (NOx) levels: NOx levels were obtained from an ELISA reader by the vanadium chloride (VCl_3)/Griess assay. After the centrifugation of blood samples, the plasma was separated. Samples were deproteinised with 0.3 M NaOH and 5% (w/v) $ZnSO_4$ centrifugated at 14,000 rpm for 5 min and supernatants were used for the assays. Nitrate standard solution was serially diluted. After loading the plate with the samples (100 µl), the addition of vanadium III chloride (VCl_3) (100 µl) to each well was rapidly followed by the addition of Griess reagents, sulphanilamide (SULF) (50 µl) and N-(1-naphtyl) ethylenediamide dihyrochloride (NEDD) (50 µl). After incubation, usually for 30-45 min in 37°C, samples were measured at 540 nm using an ELISA reader (7).

Determination of Height and Weight

Height and weight were measured in light clothing without shoes. Height and weight were both measured standing. Height was measured with a Harpenden stadiometer (Critikon Service Center, Reading, UK) to 0.1 cm and weight with a Soehnle digital electronic scale to 0.1 kg.

Determination of Body Fat

Skinfold thickness was measured at two sites (triceps and suprailiac) using Cliffon N. J. calipers. Percentage body fat and subsequent fat-free mass were estimated using Sloan and Weir's equation (30).

Determination of Body Mass Index (BMI)

BMI was calculated as weight in kilogram divided by height in meters squared (BMI = kg/m^2)*.

Blood Pressure

Resting systolic and diastolic blood pressures were measured as mmHg by using a stethoscope and a sphygmomanometer in sitting position. The blood pressure was determined as the mean from three

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ACSM: *ACSM's guidelines for exercise testing and prescription*. 5. ed. Baltimore: Williams and Wilkins, 2008.

Table 1. Demographic characteristics of subjects

N	Age (years)	Height (cm)	Body Weight (kg)
42	41.40 ± 7.3	156.8 ± 4.9	76.1 ± 11.5

Table 2. Body mass index, percentage body fat, rest heart rate and blood pressure of subjects

Variables	N	Pre Exercise	Post Exercise	*t*	*P*
Body Mass Index (kg/m^2)	42	30.9 ± 4.3	29.5 ± 3.7	8.6	0.000**
Body Fat %	42	27.1 ± 4.1	23.5 ± 3.2	13.0	0.000**
Resting Heart Rate (pulse/min)	42	74.3 ± 7.5	70.6 ± 6.7	2.5	0.016*
Systolic Blood Pressure (mmHg)	42	120.3 ± 10.2	111.8 ± 11.4	7.8	0.000**
Diastolic Blood Pressure (mmHg)	42	74.9 ± 8.3	68.6 ± 5.9	4.7	0.000**

$^*P < 0.05$, $^{**}P < 0.01$.

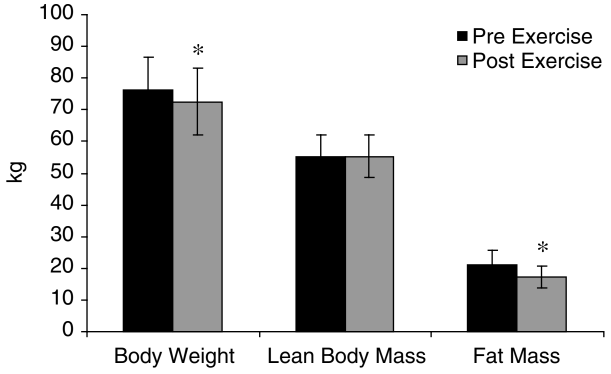

Fig. 1. Significant differences in body weight and fat mass values after treatment ($P < 0.05$). No significant change was observed in lean body mass ($P > 0.05$).

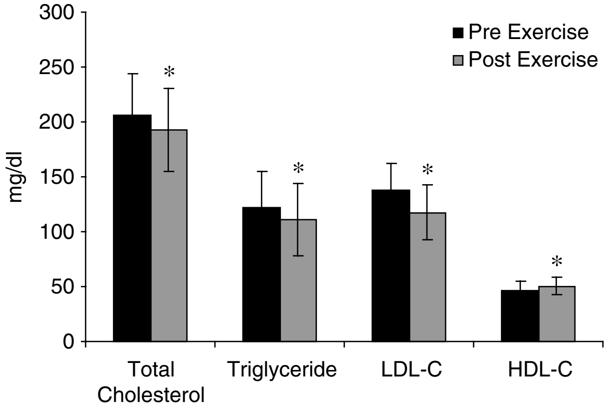

Fig. 2. Significant reductions were observed in total cholesterol, triglyceride, LDL-C and increase HDL-C after exercise ($P < 0.01$).

separate measurements.

Statistical Analysis

SPSS (statistical package for the social sciences) version 11.0 was used in data analysis. Data normality was tested using the Kolmogorov-Smirnow test. Descriptive statistics were calculated for all variables and are reported as means ± standard deviation (SD) Differences in body composition and biochemical parameters before and after the treatment were tested using the Student's paired *t*-test. Pearson's rank correlation coefficients were performed to examine the relationship between variables. A *P* value less than 0.05 was considered as statistically significant.

Results

The demographic and clinical data of the studied groups are shown in Tables 1 and 2. The body compositions and lipid profiles of the subjects are shown in Figs. 1 and 2. There were significant decreases in all body composition parameters ($P < 0.05$) except lean body mass values after the exercise treatment. Resting systolic and diastolic blood pressures ($P < 0.01$) and heart rate ($P < 0.05$) of the subjects decreased significantly after a 12-week exercise program.

The lipid profiles of the women are shown in Fig. 2. There were significant decreases in total cholesterol, triglyceride, LDL-C and an increase in HDL-C after treatment ($P < 0.01$).

Although Hcy levels were expected to decrease due to long-term exercise as claimed by some literature, the mean value of all the samples increased significantly ($P < 0.05$). However, none of the samples exceeded the normal limits of 5-15 μM of Hcy. Also, there were significant increases in NOx levels after treatment ($P < 0.05$) (Fig. 3).

The correlations of Hcy and NO are shown in

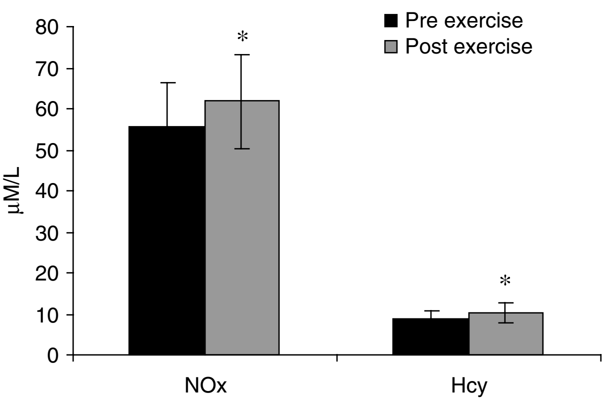

Fig. 3. Significant increases in NOx and Hcy levels after exercise ($P < 0.05$).

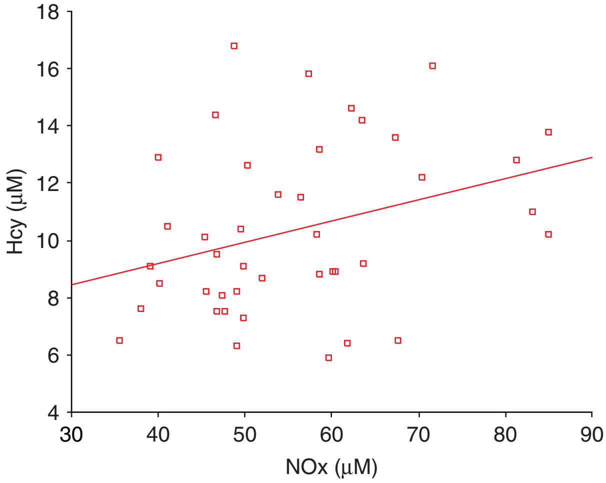

Fig. 4. Positive correlation between Hcy and NOx levels (r = 0.42, $P < 0.05$).

Fig. 4. Hcy concentration was positively correlated with NOx levels (r = 0.42, $P < 0.05$). An inverse correlation was found between Hcy and total cholesterol levels (r = -0.22, $P > 0.05$) (Fig. 5).

Discussion

When performed regularly, callisthenics can be beneficial for both muscular and cardiovascular fitness. We applied callisthenics to our group of middle-aged women to allow them to perform a leader-directed group exercise as a form of synchronized physical training to increase group cohesion.

Our subjects performed long-term (12 weeks) regular aerobic callisthenic exercises characterized by 50 min/sessions and 3 days/week. On some occasions, some of the subjects could not participate in some of the sessions. We tried to avoid this irregularity to occur repeatedly and no more than once a week. The most important findings of long-term callisthenic exercise in the middle-aged women were significant elevation in plasma NOx and Hcy levels and significant decrease in body composition parameters, lipid profiles (total cholesterol, HDL-C, LDL-C and triglyceride levels), resting heart rate and decrease in systolic and diastolic blood pressures.

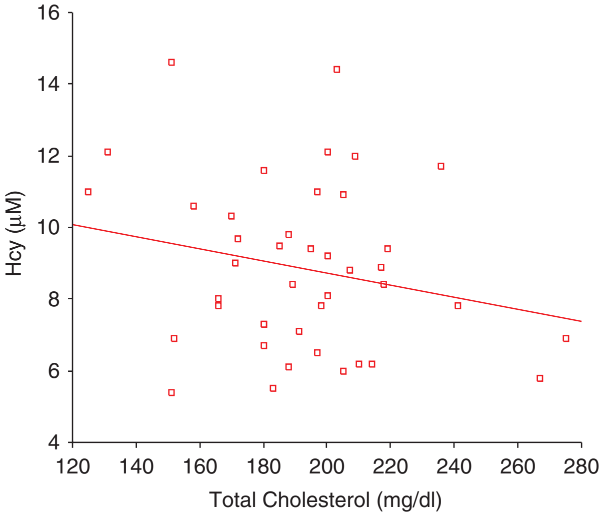

Fig. 5. Negative correlation between Hcy and total cholesterol levels (r = -0.22, $P > 0.05$).

Regular physical exercise plays an important role in reducing obesity, preventing hyperglycemia, lowering blood lipids and reducing systemic blood pressure (15). In considering of all the 42 women, both systolic and diastolic pressures were decreased significantly (mean: 120.26/74.90 mmHg and 111.85/68.61 mmHg, respectively) probably due to significant increases of the blood endothelial NOx and its' vasodilatation effect on the vessels. Regular exercise is a key component of cardiovascular risk prevention strategies. It is shown that long-term physical exercise improved endothelium-dependent vasorelaxation through an increase in the release of NO in normotensive as well as hypertensive subjects (26). The possible mechanism of reduction of blood pressure is an increase in vascular shear stress resulting from increased blood flow by exercise, promoting the enhanced formation of NO (36) and that chronic exercise upregulates the expression of the endothelial NO synthase gene and can conduct the lower level of blood pressure (41). For instance, resistant training exercise combined with aerobic exercise is recommended for hypertensive patients to lower blood pressure (6).

We evaluated plasma Hcy levels and plasma lipids before and after a 12-week exercise trial to investigate the cardiovascular risk factors for the women because some studies claim that elevated plasma concentration of Hcy is an independent risk factor for cardiovascular disease (13). While Randeva *et al*. found reduced Hcy and increased NO in their exercised women (38), Hcy levels in our subjects significantly increased after treatment program. At the base line, Hcy levels of all our subjects were within normal limits (mean = 8.86 ± 2.34 μM) and none of them had HHcy. This result might be because of our criteria for the selection of healthy women for the study. Also, at the end of the exercise trial when all the subjects were considered as a group, there was a slight increase in Hcy levels and the changes were significant. But these elevations have not been considered within the HHcy limits (mean = 10.36 ± 2.94 μM). Normal Hcy levels range from 5 to 15 μM (12 μM being the upper reference limit for populations on a folic acid-fortified diet) with elevations of 16 to 30 μM, 30 to 100 μM and >100 μM classified as mild, moderate and severe HHcy, respectively (39).

Generally, the effects of exercise on blood Hcy levels are found to be inconsistent. Some researches highlighted an exercise-induced fall in Hcy concentrations (38, 44) but there are many reports proving that the physical exercise does not contribute to depressing plasma Hcy levels and/or that in some instances it would even produce an increase (25). As a result, the relationship between the Hcy level and physical activity remains unclear. However, acute exercise has been reported in several studies to produce modest increases in Hcy with longer term exercises programs having no effects, or beneficial effects. In addition, improvements in Hcy levels have most commonly been reported in subjects with raised baseline Hcy or poor fitness (5). In contrast with these studies, Wright *et al*. found no effects of acute exercise on plasma Hcy levels (48). It is possible that exercise intensity may influence Hcy responses. Vincent *et al*. suggested in a subgroup analysis of six months that high intensity, resistance training programs produced lesser reduction on Hcy levels than less intense programs (47). As a result, the question on the relationship between Hcy levels and physical activity remained unanswered. It sets forward that regular, non-intensive exercises or training could decrease plasma Hcy concentrations, but prolonged intensive exercise has the contrary effect (21). Endurance exercise may induce a considerable Hcy increase which is most probably determined by the duration of the exercise (24).

There is a broad agreement that physical activity is independently associated with reduced risk of coronary heart diseases and that at least a part of this reduction may be due to favorable changes in circulating lipids induced by regular physical exercises (14). At the end of the 12-week callisthenic exercise trial, total cholesterol, LDL-C and triglyceride levels decreased and HDL-C increased significantly. The effect of exercise training on LDL-C has usually been rather small typically in the range of 5-10% and highly variable. However, a number of suitably designed and executed training studies have reported lower plasma LDL-C concentrations after training on a regular basis (22). HDL-C level has been known to play a role in protecting blood vessels from atherogenesis by preventing the generation of oxidatively modified low-density lipoprotein cholesterol (18, 37).

Many studies showed that in a long-term and moderate intensity aerobic exercise program done by sedentary women, body composition was dramatically changed (2, 10, 40, 43). In general, the ability of exercising to induce changes in body weight, fat free mass and fat mass is affected by various factors, including the type, intensity, frequency and duration of exercise (3). Moderate intensity, long-term and regular aerobic exercises are effective in burning fat. Hence, it can be thought that this kind of exercise caused a decrease in body weight, the percentage of body fat and body mass index (11).

In this study, aerobic callisthenic exercises characterized by 50 min/day and 3 days/week resulted in positive changes in important health parameters like reducing obesity, lowering blood lipids and increasing plasma NOx. Cardiovascular improvements might be dependent on the increase of NOx values. But callisthenic exercise in such intensity did not lower the plasma Hcy level. Moreover, Hcy level increased significantly. Such a result shows that if the Hcy is in the normal levels in healthy subjects, long-term callisthenic exercise does not decrease the Hcy levels despite some beneficial effects on health; on the contrary long-term callisthenic exercise increases the Hcy levels.

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