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CONTROLLED AEROBIC TRAINING IMPROVES ENDOTHELIAL FUNCTION AND MODIFIES VASCULAR REMODELING IN HEALTHY ADULTS WITH HIGH NORMAL BLOOD PRESSURE

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The aim of the study was to assess endothelial function in adults with high normal blood pressure (HNBP) undergoing controlled aerobic training. The study was conducted among 31 volunteers with HNBP. Subjects underwent supervised cycle ergometer training for 12 weeks. Exercise intensity was assessed by monitoring the pulse with intention to keep the heart rate increase within the range of 40% to 65% of the heart rate reserve. The control group consisted of 14 healthy adults, not subjected to any intervention. The control group was examined twice at 12-week intervals (non-exercising time control). Vascular endothelial function was determined by flow-mediated dilation (FMD) and by measuring total nitric oxide products (NOx). The measurement of carotid intima-media complex thickness (IMT) was an indirect method of assessing vascular remodeling. Blood pressure (ABPM method), anthropological parameters and lipid profile were also assessed. There was a significant change in FMD after 3-month training in the study group: the average FMD training was 5.21 ± 2.17%, while after the program FMD increased to 9.46 ± 3.69% ($P < 0.001$). After training, the NO_x also increased from 1.01 ± 0.38 μmol/L to 1.27 ± 0.48 μmol/L ($P < 0.001$). Effects were observed irrespective of participants' sex. Interestingly, a modest but significant reduction of IMT was also observed, from 0.5 ± 0.06 mm to 0.46 ± 0.10 mm ($P = 0.04$). There was also a reduction in the percentage of body fat content from 25.01 ± 8.77% to 22.31 ± 8.79% ($P < 0.001$). No statistically significant changes were noted after 12 weeks of training in the blood pressure and lipid profile. In the control group no statistically significant changes of any parameter were observed. Regular aerobic exercise improves nitric oxide-dependent endothelial function of the vessels and can initiate regression of atherosclerosis in people with HNBP.

Key words: *endothelial function, high normal blood pressure, exercise, nitric oxide, prehypertension, flow-mediated dilation, anthropometrics*

INTRODUCTION

Hypertension is a major medical and social problem for the current world population. People with high-normal blood pressure (HNBP) are particularly prone to the development of hypertension. In the guidelines published in 2013 by the European Society of Hypertension (ESH) and the European Society of Cardiology (ESC) high-normal blood pressure (HNBP) was defined as a systolic blood pressure of 130 – 139 mmHg and/or diastolic of 85 – 89 mmHg in office measurements (1). Although not accepted by all international guidelines, this classification led to some considerations regarding the functional and structural changes presented by these individuals. People with HNBP are characterized not only by a greater risk of progression to persistent hypertension (2), but also by increased risk of cardiovascular events (3). This population deserves more attention than usually ascribed to intervene and prevent complications, as endothelial dysfunction (ED) may represent an early change in those who develop hypertension later in life. An important element of prevention in this group of persons is implementation of non-pharmacological methods influencing the lifestyle. In this regard, ESH and ESC recommend among other things, regular physical activity (1). While the effect of regular physical activity on cardiovascular risk is well defined it may be related to a number of possible mechanisms for the normalization of blood pressure.

Studies suggest that regular physical activity can prevent ED, probably by the restoration of nitric oxide (NO) availability. Regular physical activity increases blood flow and linear shear forces, resulting in increased nitric oxide formation and bioavailability. Moreover, physical exercise can activate processes related to angiogenesis and chronic anti-inflammatory effect, which leads to modification of endothelial function (EF) (4). Regular exercise can result in improvement of vascular ED by reducing concentration of asymmetric dimethylarginine (ADMA) (5), causing increased availability of NO. Elevated ADMA concentrations were observed in patients with hypertension (6) and healthy people who were diagnosed with thickened intima-media thickness (IMT) (7). The studies also confirmed increased ADMA levels in patients with newly diagnosed, previously untreated pharmacologically hypertension (8). Furthermore, regular physical activity can increase the activity of nitric oxide synthase 3 - an enzyme found in endothelial cells, responsible for NO synthesis (9). A meta-analysis of randomized controlled trials also showed that aerobic endurance training reduced systolic and diastolic blood pressure in healthy subjects (10) and in those with hypertension by reducing vascular resistance, suppressing the sympathetic nervous system and the renin-angiotensin system (11), that impairs microvascular and metabolic insulin sensitivity in skeletal muscle (12). Exercises are also associated with unequivocal health benefits and results in many structural and functional changes of the myocardium that enhance performance and prevent heart failure (13). It is also worth to emphasize the impact of physical exercise on potassium homeostasis. It is known that physical exercise can facilitate the release of potassium from skeletal muscle, which can lead to hyperkalemia. Also, commonly used drugs in hypertensive patients, including angiotensin converting enzyme inhibitors (ACEI) and statins, increase the risk of hyperkalemia. However, studies have shown that physical exercise in these patients can be safely used because they only cause short-term, but within the normal range, increase in potassium which returns to baseline after rest (14). In recent years attention has also been paid to the role of the immune system in ED, including monocytes, which can be activated by mechanical stretching, which may be due to the loss of NO signaling (15), macrophages that play an important role in the regulation of the nitric oxide synthase function (16) and a pro-inflammatory status leading to the deposition of low density lipoproteins (LDL) and subsequent infiltration of circulating leukocytes in the intima (17). However, there are no studies that explicitly confirm the beneficial effect of exercise on the immune system in the context of improving EF. Despite this, it is believed that regular physical activity play a very important role in improving endothelial dysfunction, although the optimal regimens for exercise remain unclear and have not been addressed in the context of vascular remodeling. Modulation of EF and processes responsible for developing atherosclerosis by regular exercise may be an important element in the prevention of hypertension.

The aim of the study was to assess the change of endothelial function in patients with HNBP undergoing controlled, moderate intensity (40 – 65% of heart rate reserve (HRR)) aerobic training.

MATERIALS AND METHODS

Subjects

The study was conducted among 31 healthy adults (23 male and 8 female; mean ± SD: age, 44.3 ± 5.57 years; body mass, 83.19 ± 15.22 kg; body height, 1.72 ± 0.09 m; body mass index, 27.79 ± 3.38 kg m^{-2}], who took part in the training program. The control group consisted of 14 healthy adults (8 male and 6 female; mean ± SD: age, 45.0 ± 3.41 years; body mass, 83.10 ± 12.16 kg; body height, 1.74 ± 0.08 m; body mass index, 27.43 ± 2.54 kg m^{-2}], not subjected to any intervention (non-exercising time control). The inclusion criteria for the study were low level physical activity and the presence of HNBP. All subjects were non-smokers and were not taking any medications at the time of enrollment. The research was carried out in the urban population in Poland. The study complied with the World Medical Association Declaration of Helsinki regarding ethical conduct of research involving human subjects. The study project received approval from the Commission for Bioethics at the Regional Medical Chamber in Cracow, Poland (opinion No. 71/KBL/OIL/2010). Study participants were informed about the aim of the study, laboratory conditions, equipment and research procedures. The subjects in the studied group provided written informed consent to participate in the study.

Study design

Before entering the study, the subjects underwent a standard medical evaluation, ECG and blood tests (blood count, glucose, C-reactive protein) to eliminate medical contraindications to exercise. After qualifying to the study the subjects performed an electrocardiographic exercise stress test, followed by 12 weeks of an aerobic moderate intensity training program. All planned measurements from the study were taken up to 5 days before and 5 days after the end of the training program. The control group was examined twice at 12-week intervals. During study, all persons were advised not to change their diet and not to receive dietary supplements or alcohol.

Physical activity

One of the criteria for the inclusion of volunteers in the study was physical activity at a low level (inadequate and sedentary), which was examined using the International Physical Activity Questionnaire, which is a well validated measure of physical activity (18). A short version of the IPAQ adapted to the Polish conditions was used for the study (19).

Blood pressure measurements

In order to participate in the project, HNBP had to be found in at least 2 measurements during 2 different/separate visits to the supervising physician. HNBP was defined as systolic blood pressure of 130 – 139 or diastolic BP of 85 – 89. Measurements were made using the oscillatory method on the left shoulder, in line with accepted rules (20), using the upper arm device Omron M10-IT (Omron, Japan). HNBP was confirmed by 24-hour ambulatory blood pressure monitoring (ABPM) using The 90217A Ultralite Ambulatory Blood Pressure System (Spacelabs Healthcar, USA) (21). For statistical analysis, the mean systolic (SBP) and diastolic blood pressure (DBP) were used.

Anthropometrics and body composition

Body mass (BM) and percentage of body fat (BF) were measured using a body composition analyzer BC-418 (Tanita, Japan). Body height (BH) was measured using an anthropometric set (Sieber Hegner Machines, Switzerland). Based on these measurements, the body mass index (BMI) was calculated.

Vascular function and structure assessments

Vascular endothelial function was determined by assessing flow-mediated dilation (FMD). FMD is defined as the percentage change in brachial artery diameter in relation to the diameter prior to ischemia. This non-invasive method developed by Celermajer *et al*. (22) remains the most widely used technique for evaluating mediation of endothelial mediators in vasodilation. FMD method uses ultrasound to measure changes in brachial artery diameter in response to increased blood flow induced by transient ischemia and subsequent reactive forearm hyperemia. The study was conducted with GE Vivid 3 Ultrasound (GE Medical System, USA) with a 10 MHz linear ultrasonic transducer, in accordance with the recommendations of the International Brachial Artery Reactivity Task Force (23). FMD measurements were carried out in accordance with the protocol set out in the recommendations of the ESC international working group (24). Studies demonstrate a relationship between peak FMD in the brachial artery and plasma levels of nitrite. The FMD test is a sensitive method for evaluating the effects of standard risk factors for atherosclerosis on vascular EF (21, 25, 26). This relationship shows that brachial FMD may be used as markers of peripheral EF (27). A meta-analysis demonstrated that impairment of brachial artery FMD is significantly associated with future cardiac morbidity and mortality.

An indirect method of assessing endothelial function was the measurement of common carotid artery intima-media thickness (IMT), which is a validated surrogate marker for atherosclerosis (28). The study was conducted in accordance with the methodology based on Mannheim consensus recommendations (29), using GE Vivid 3 Ultrasound (GE Medical System, USA) with a 10 MHz linear ultrasonic transducer. The intima-media complex was evaluated on a wall (distal to the head) of the common carotid artery at one centimeter before bifurcation. A fragment of the wall with a well-visible intima-media complex, devoid of atherosclerotic plaques, was analyzed. The image was evaluated using the original GE Vivid 3 Ultrasound software. The visualization of the vessels was done on both sides and the final value was the mean of measurements on both arteries. Despite the fact that this study is illustrative and evaluates structures much larger than the layer of endothelial cells, it was proven that the outcome correlates with EF (30). Similarly to FMD, assessment of carotid IMT correlates with the presence of risk factors for coronary artery disease (CAD) (31), and is recognized as a prognostic factor in hypertension and in assessing the overall risk of cardiovascular events (32).

Plasma nitric oxide products levels and lipid profiles

The plasma nitric oxide (NO) was indirectly examined by the determination of its products. NO undergoes a series of reaction with several molecules present in biological fluids. The final products of NO are nitrite and nitrate (NO_x). The concentration of NO_x was determined spectrophotometrically using a microplate reader ChroMate (Awareness Technology, USA) with a commercially available Nitrate/Nitrite Colorimetric Assay Kit, No780001 (Cayman Chemical, USA) (33).

The lipid profile was analysed using Cobas 600 with Cobas C 501 chemistry module analyser (Roche Diagnostics, Germany). Total cholesterol, high-density lipoproteins (HDL) and triglycerides (TG) were determined using enzymatic method and level of LDL were calculated from the formula: LDL = Cholesterol-HDL-(TG/2,19) (34).

Supervised training procedure

Before training, subjects underwent electrocardiographic exercise stress test restricted to symptoms, performed on cycle ergometer to check their health and to determine the pulse peak. Then they were included in cycle ergometer training. The subjects participated in the 12 week moderate intensity supervised aerobic (endurance) training program on a cycle ergometer CRG 200, working in Beta AsTER Rehabilitation System (Aspel, Poland). Training included 3 sessions per week, Mondays, Wednesdays and Fridays. The intensity of exercise was 40 – 65% HRR (35, 36). Each training unit consisted of warm up (5 – 10 minutes of omni-directional movements, stretching, and cycling with a slow increase in load to 50% of training intensity), main exercise (30 – 40 minutes of cycling with a range of training pulse) and cool down exercises (5 – 10 minutes of cycling with low load and then stretching). The time of the workout and then its intensity increased during the training program (*Table 1*). The subjects had a pedaling rate at which they felt most comfortable (60 – 80 revolutions per minute) and the training system automatically dosed the load according to the training heart rate. All subjects tolerated intensity of exercise well and their subjective fatigue during exercise ranged from 12 to 13 points in a Borg's 6 – 20 rating, which is well correlated with the heart rate (37). The training was always conducted at regular times of day, in the afternoon, in an air-conditioned room with a temperature of 18 – 20°C. A physician supervised each training session.

Statistical analysis

Results are presented as mean and standard deviation (SD) or as median and interquartile range according to the distribution of the data. The Shapiro-Wolf test was used to evaluate the normal distribution. The obtained variables were subjected to a statistical analysis with the use of the Student's t-test or Wilcoxon test, respectively. The significance of the observed changes was ascertained at $P < 0.05$. Statistica v. 12.0 (Stat-Soft, USA) was used to calculate the test results.

RESULTS

Vascular function and structure assessments

After the training program there was a significant change in FMD in the research group: the FMD before exercising was 5.21 ± 2.17%, while after the program it increased by 4.25 percentage points to 9.46 ± 3.69% (P = 0.000001). Similar values and changes were obtained in the subgroup of men, where the median increased by 3.81 percentage points from 5.22 ± 2.18% to 9.03 ± 3.17% (P < 0.000027). In women, there was an even higher increase in FMD, by 4.88 percentage points, from mean value of 5.11 ± 4.38% to 9.99 ± 6.67% (P = 0.011719) (*Table 2*). After 12-week training, a statistically significant increase in NO_x in blood plasma was achieved by 0.26 µmol/L (25.7%) in the whole group (from 1.01 ± 0.38 µmol/L to 1.27 ± 0.48 µmol/L, P < 0.000001), by 0.21 µmol/L (18.6%) in the subgroup of men (from 1.13 ± 0.34 µmol/L to 1.34 ± 0.31 µmol/L, P < 0.000034) and by 0.25 µmol/L (26.3%) in the subgroup of women (from 0.95 ± 0.15 µmol/L to 1.20 ± 0.25 µmol/L, P = 0.001904) (*Table 2*). It also showed a statistically significant change of IMT in the total group: initially median IMT was 0.5 ± 0.06 mm and after the training program it decreased by 8% (0.04 mm) to 0.46 ± 0.10

Table 1. Duration and intensity of the main part training unit in the following weeks.

Week of training	1	2	3	4	5	6 – 12
Time [min]	30	35	40	40	40	40
Intensity [% HRR]	40 – 50	40 – 50	40 - 50	45 – 55	50 – 60	55 – 65

Table 2. Mean values (± SD) or median values (interquartile ranges) of flow-mediated dilation (FMD), total nitrate/nitrite levels in blood plasma (NO_x), intima-media thickness (IMT), systolic blood pressure (SBP) and diastolic blood pressure (DBP) in the study group.

Parameter	Before training	After training	P
	Men and women (n = 31)		
FMD [%]	5.21 ± 2.17	9.46 ± 3.69	0.000001**
NO_x [µmol/L]	1.01 ± 0.38	1.27 ± 0.48	0.000001**
IMT [mm]	0.50 ± 0.06	0.46 ± 0.10	0.039626*
SBP [mmHg]	131.65 ± 3.57	130.74 ± 4.00	0.066187
DBP [mmHg]	80.65 ± 5.21	79.71 ± 5.03	0.051512
	Men (n = 23)		
FMD [%]	5.22 ± 2.18	9.03 ± 3.17	0.000027**
NO_x [µmol/L]	1.13 ± 0.34	1.34 ± 0.31	0.000034**
IMT [mm]	0.51 ± 0.06	0.46 ± 0.11	0.038620*
SBP [mmHg]	132.39 ± 2.79	131.61 ± 3.26	0.145262
DBP [mmHg]	79.87 ± 5.22	79.17 ± 5.26	0.145262
	Women (n = 8)		
FMD [%]	5.11 ± 4.38	9.99 ± 6.67	0.011719*
NO_x [µmol/L]	0.95 ± 0.15	1.20 ± 0.25	0.001904*
IMT [mm]	0.49 ± 0.13	0.48 ± 0.09	0.888637
SBP [mmHg]	129.50 ± 4.81	128.25 ± 5.06	0.305184
DBP [mmHg]	82.88 ± 4.76	81.25 ± 4.23	0.177738

* Significantly different P < 0.05; **significantly different P < 0.001

Table 3. Mean values (± SD) or median values (interquartile ranges) of flow-mediated dilation (FMD), total nitrate/nitrite levels in blood plasma (NO_x), intima-media thickness (IMT), systolic blood pressure (SBP) and diastolic blood pressure (DBP) in the control group.

Parameter	Baseline	After 12 weeks	P
	Men and women (n = 14)		
FMD [%]	8.04 ± 1.53	8.03 ± 1.95	0.974216
NO_x [µmol/L]	1.14 ± 0.33	1.23 ± 0.29	0.109224
IMT [mm]	0.49 ± 0.07	0.50 ± 0.07	0.502203
SBP [mmHg]	131.66 ± 5.74	133.00 ± 6.5	0.610120
DBP [mmHg]	83.00 ± 12.50	81.50 ± 3.88	0.556299
	Men (n = 8)		
FMD [%]	7.93 ± 1.14	7.90 ± 1.12	0.933383
NO_x [µmol/L]	1.16 ± 0.40	1.22 ± 0.32	0.077371
IMT [mm]	0.50 ± 0.085	0.52 ± 0.07	0.325263
SBP [mmHg]	135.00 ± 5.00	134.00 ± 6.5	0.326990
DBP [mmHg]	79.87 ± 5.79	80.75 ± 3.69	0.328530
	Women (n = 6)		
FMD [%]	8.27 ± 2.33	8.29 ± 3.31	0.976409
NO_x [µmol/L]	1.10 ± 0.17	1.19 ± 0.32	0.574137
IMT [mm]	0.47 ± 0.03	0.46 ± 0.03	0.839901
SBP [mmHg]	128.25 ± 5.12	129.00 ± 5.66	0.623838
DBP [mmHg]	82.25 ± 7.09	81.50 ± 4.79	0.608358

mm (P = 0.039626). In the group of men, median IMT decreased by 8% (0.05 mm) from 0.51 ± 0.06 mm to 0.46 ± 0.11 mm, and this change was also statistically significant (P = 0.038620). In women, the median IMT decreased only by 2% (0.01 mm) and it was not statistically significant (*Table 2*). After the training program, the minimum lower values of the average SBP and DBP were obtained in the whole research group and in the subgroup of men and women; however, these changes were not statistically significant (*Table 2*). In the control group, without intervention, after 12 weeks no statistically significant changes were observed in FMD, NO_x, IMT, SBP and DBP (*Table 3*).

Anthropometrics, body composition and lipid profiles

It is worth noting that after the training program there was a statistically significant reduction in BF in the whole group by 2.7 percentage points (10.8%) from 25.01 ± 8.77% to 22.31 ± 8.79%, P < 0.000089 and by 2.4 percentage points (10.2%) in the

Table 4. Mean values (± SD) or median values (interquartile ranges) of body height (BH), body weight (BW), body mass index (BMI) and body fat (BF) in the study group.

Parameter	Before training	After training	P
Men and women (n = 31)			
BH [m]	1.724 ± 0.09	–	–
BW [kg]	83.19 ± 15.22	82.66 ± 14.94	0.022561*
BMI [kg m^{-2}]	27.79 ± 3.38	27.62 ± 3.31	0.030670*
BF [%]	25.01 ± 8.77	22.31 ± 8.79	0.000089**
Men (n = 23)			
BH [m]	1.752 ± 0.075	–	–
BW [kg]	86.64 ± 15.16	86.04 ± 14.84	0.034658*
BMI [kg m^{-2}]	28.06 ± 3.32	27.87 ± 3.24	0.050951
BF [%]	23.54 ± 5.68	21.14 ± 4.85	0.000589**
Women (n = 8)			
BH [m]	1.635 ± 0.052		
BW [kg]	71.84 ± 8.97	71.56 ± 9.21	0.443969
BMI [kg m^{-2}]	26.91 ± 3.69	25.37 ± 5.28	0.398025
BF [%]	33.29 ± 6.18	32.11 ± 6.97	0.038619*

* Significantly different P < 0.05; ** significantly different P < 0.001

Table 5. Mean values (± SD) or median values (interquartile ranges) of body height (BH), body weight (BW), body mass index (BMI) and body fat (BF) in the control group.

Parameter	Baseline	After 12 weeks	P
Men and women (n = 14)			
BH [m]	1.736 ± 0.08	–	–
BW [kg]	83.10 ± 12.16	83.28 ± 12.20	0.442698
BMI [kg/m^2]	27.43 ± 2.54	27.53 ± 2.56	0.236722
BF [%]	26.50 ± 5.82	26.39 ± 5.76	0.342479
Men (n = 8)			
BH [m]	1.787 ± 0.032	–	–
BW [kg]	89.77 ± 8.11	89.98 ± 8.17	0.557518
BMI [kg/m^2]	28.04 ± 2.27	28.16 ± 2.38	0.315920
BF [%]	22.92 ± 2.84	22.85 ± 2.38	0.6782730
Women (n = 6)			
BH [m]	1.633 ± 0.221	–	–
BW [kg]	69.75 ± 5.65	69.87 ± 5.52	0.491832
BMI [kg/m^2]	26.22 ± 2.83	26.27 ± 2.76	0.561823
BF [%]	33.67 ± 2.69	33.49 ± 2.79	0.115493

subgroup of men from 23.54 ± 5.68% to 21.14 ± 4.85%, P < 0.000589. In women, the average reduction in BF was only 3.5%, however this change was statistically significant (P = 0.038619). In addition, a slight but significant reduction in BW (in the whole group and subgroup of men) and BMI (in the whole study group) were observed (*Table 4*). In the control group, after 12 weeks no statistically significant changes were observed in BW, BMI and percentage BF (*Table 5*).

The lipid profile was examined, which did not show statistically significant changes in the study group (*Table 6*) and in the control group (*Table 7*).

DISCUSSION

The results of our research confirm that ED is present in people with HNBP, and that regular physical activity leads to a beneficial modification of EF. This is confirmed by the improvement of EF indices after the completion of 12-week moderate-intensity supervised aerobic training program. An increase in the NO_x level was observed, indicating an increase in NO, which resulted in the improvement of FMD. A slight but statistically significant decrease in IMT thickness was also shown (changes have not been observed in women), which is an indirect method of endothelial evaluation. These changes were caused by physical training, as no other lifestyle factors were modified (diet, for example), and changes in the above parameters were not observed in the control group - not participating in the exercises. This study provides additional evidence that moderate intensity aerobic exercises have a beneficial effect on EF in sedentary adults with HNBP and are an important element in preventing hypertension and its dangerous complications in this population. Despite the changes in NO_x and FMD, there was no significant reduction in SBP and DBP. The results of this study partly agree with other studies conducted with people with HNBP or pre-hypertension (SBP 120 – 139 and / or DBP 80 – 89). Differences

Table 6. Mean values (± SD) of total cholesterol (Chol), high-density lipoproteins (HDL), low-density lipoproteins (LDL) and triglycerides (TG) in the study group.

Parameter	Before training	After training	P
Men and women (n = 31)			
Chol [mmol/l]	5.48 ± 1.10	5.41 ± 1.04	0.376859
HDL [mmol/l]	1.30 ± 0.36	1.28 ± 0.31	0.649238
LDL [mmol/l]	3.41 ± 0.94	3.39 ± 0.90	0.810697
TG [mmol/l]	1.68 ± 0.93	1.61 ± 0.88	0.474386
Men (n = 23)			
Chol [mmol/l]	5.50 ± 1.15	5.47 ± 1.07	0.883335
HDL [mmol/l]	1.18 ± 0.25	1.20 ± 0.25	0.411356
LDL [mmol/l]	3.44 ± 0.97	3.46 ± 0.91	0.842192
TG [mmol/l]	1.93 ± 0.94	1.81 ± 0.93	0.393578
Women (n = 8)			
Chol [mmol/l]	5.41 ± 0.98	5.20 ± 0.99	0.092909
HDL [mmol/l]	1.64 ± 0.44	1.53 ± 0.36	0.245698
LDL [mmol/l]	3.32 ± 0.88	3.21 ± 0.89	0.288235
TG [mmol/l]	0.98 ± 0.38	1.02 ± 0.26	0.760225

Table 7. Mean values (± SD) of total cholesterol (Chol), high-density lipoproteins (HDL), low-density lipoproteins (LDL) and triglycerides (TG) in in the control group.

Parameter	Baseline	After 12 weeks	P
Men and women (n = 14)			
Chol [mmol/l]	5.11 ± 0.93	5.07 ± 0.84	0.421813
HDL [mmol/l]	1.39 ± 0.48	1.43 ± 0.45	0.057123
LDL [mmol/l]	2.87 ± 0.77	2.76 ± 0.65	0.094577
TG [mmol/l]	1.86 ± 1.55	1.89 ± 1.45	0.438206
Men (n = 8)			
Chol [mmol/l]	5.19 ± 0.98	5.16 ± 0.90	0.649120
HDL [mmol/l]	1.30 ± 0.54	1.36 ± 0.51	0.059645
LDL [mmol/l]	2.82 ± 0.82	2.72 ± 0.67	0.167457
TG [mmol/l]	2.34 ± 1.71	2.37 ± 1.58	0.742780
Women (n = 6)			
Chol [mmol/l]	4.95 ± 0.91	4.88 ± 0.97	0.570656
HDL [mmol/l]	1.59 ± 0.32	1.60 ± 0.33	0.760820
LDL [mmol/l]	2.95 ± 0.72	2.84 ± 0.74	0.447165
TG [mmol/l]	0.88 ± 0.30	0.94 ± 0.27	0.086594

in the results of different authors may also be caused by demographic differences of the studied populations, such as diet, climate and somatic constitution.

Beck *et al*. examined the effect of an 8-week training program conducted 3 times a week on EF in adults (18 – 35 years) with prehypertension. They observed in a group of 13 people (9 men and 4 women) participating in endurance exercise training, improvement of FMD by 63%, increase in NO_x by 23%, and reduction of resting SBP and DBP. The regimen consisted of walking/running on the treadmill with an interval to keep the heart rate in the range of 65% to 85% of the predetermined maximum heart rate. Interestingly, they also noted an improvement in FMD by 34%, an increase in NO_x by 19% and a decrease in resting SBP by 9.6 mm Hg and DBP by 8 mm Hg in a 15-person group (11 men and 4 women) participating in resistance exercise training (38). Higashi *et al*. studied the effect of aerobic training in normotensive people with mild untreated spontaneous hypertension who did not have the habit of exercising. The authors suggest an improvement in endothelium-dependent vasodilation of the forearm mediated by NO. A 10-person group (7 men and 3 women) with hypertension and a group of 7 normotensive people took a 30-minute fast march 5 to 7 times a week for 12 weeks. The authors measured vascular reactions of the forearm with acetylcholine (endothelium-dependent vasodilator) using a plethysmograph before and after exercise treatment. In the group of patients with hypertension after 12 weeks of training the forearm blood flow response to acetylcholine significantly increased, in addition a decrease in forearm vascular resistance, SBP, DBP, total cholesterol, LDL and an increase in HDL were observed. Interestingly, there has been no increase in NO_x. In the normotensive group the training resulted in a smaller but statistically significant forearm blood flow response to acetylcholine, lowering the forearm vascular resistance, the increase in HDL and LDL reduction. Changes in the above parameters were not recorded in 7 adults (6 men and 1 woman) in the control group of patients with hypertension and normotensive group (5 men), who were subjected to 12-week observation without lifestyle modification (39). Whereas

Tsukiyama *et al*. did not obtain any changes in NOx, SBP and DBP after aerobic training in a group of 41 people (19 men and 22 women), who had no history of serious disease. Continuous exercise training was performed using a stationary bicycle for one hour a day, five times per week, for 4 weeks. The exercise intensity was set to 60% of maximum heart rate (calculated by the Karvonen formula). Interestingly, there was an increase in the level of L-arginine, a NO substrate (40). Similarly, Casey *et al*. in their studies did not observe changes in NO_x as well as in FMD after applying resistance training to young healthy people (41). This was probably due to normal endothelial function of the normotensive subjects, which did not cause any changes in post-workout functioning. In turn, Podgorska *et al*. studied the effect of regular aerobic exercise on EF by comparing the values of FMD, L-arginine and ADMA in young healthy males (players from football and handball team) and untrained people. The study showed increased FMD in athletes in comparison to untrained persons and there were no statistical differences in L-arginine and ADMA (42). It seems that the benefits of exercise on endothelial function in asymptomatic subjects are less consistent than in people who show reduced endothelial function before exercise treatment (43). The increase of EF indices after physical exercise was also observed in the elderly, in whom the reduced bioavailability of NO synthesized is a key mechanism of ED (44). Filar-Mierzwa *et al*. showed an increase in NO_x by 7.3% after applying dance exercises in a group of 20 women, aged from 61 to 82 years (45). In turn Martel *et al*. demonstrated that 6-month strength training normalizes resting BP in older healthy people with high normal blood pressure. They subjected group of 21 persons (11 men and 10 women) aged 65 – 73 years. The strength training program led to reductions in both SBP and DBP (46). Nualnim *et al*. examined the effect of 12-week swimming exercises on EF in 19 adults (men and women) from 50 to 80 years old with prehypertension or stage 1 hypertension. They observed that swimming training resulted in an increase of previously reduced FMD by 118% (from 3.3 to 7.2%) and a significant reduction of SBP, a decrease in DBP was not statistically significant (47). The important role of regular exercise, through the NO mechanism, has also been demonstrated in the treatment of secondary, induced renovascular hypertension in experimental studies with animals. Research by Kumat *et al*. confirmed that renovascular hypertension-induced oxidative injury can be alleviated by regular exercise through modulation of oxidant-antioxidant balance *via* the involvement of the endothelial NO system. In the study group of rats, regular exercise has been shown to increase immunohistochemical staining of aortic endothelial NO synthase, reduce the staining of NO-induced synthetase, and also reverse alterations in echocardiographic and oxidative parameters, which was not observed in the groups of sedentary rats which, interestingly, shown increased pro-inflammatory cytokines, lipid peroxidation and neutrophil infiltration with reductions in antioxidant glutathione and catalase levels in cardiac tissues (48).

Analysis of changes in IMT under the influence of the applied physical exercises should be very careful and requires further studies as scientific reports on this subject are inconclusive. In one of the first studies to examine the effect of exercise training on IMT, 137 healthy men (aged 18 – 77 years) who were either sedentary or endurance trained were studied. In both groups carotid IMT were progressively higher with age. There were no significant differences in measures of carotid IMT between sedentary and endurance-trained men at any age. In addition, no change in IMT has been demonstrated in the group of 18 healthy sedentary subjects after 3 months of endurance training (49). Subsequent studies performed in healthy postmenopausal women (50) and in 20- to 40-year-old men and women (51) also showed no difference in carotid atherosclerosis between endurance-trained subjects and sedentary controls. However other cross-sectional studies predominantly performed in middle-aged and older humans have reported that low fitness correlates independently with increased carotid IMT (52, 53). Lakka *et al*. examined the 4-year change in carotid IMT and found that lower fitness in middle-aged men was the strongest independent predictor of a 4-year increase in carotid IMT (54). On the other hand, Rowley *et al*. found a significantly lower carotid artery IMT in elite squash players compared with less active controls. The difference in training intensity and load may explain these disparate results, as elite squash players exercised more than 22 hours per week at high intensity (55), whereas in other study intensity and load of exercises were lower (56). Scientific reports also reveal an increase in the thickness of the complex in persons with HNBP and prehypertension people. Ma *et al*. have shown that people with HNBP have an increased IMT compared to normal blood pressure group. High normal blood pressure is related to higher risk of carotid artery atherosclerosis in this cohort (57). Manios *et al*. showed that patients with office blood pressure levels in the prehypertension range, who also had elevated daytime ambulatory blood pressure levels, had higher IMT values than patients with prehypertension with normal daytime ambulatory blood pressure values and normotensive individuals (58). Thijssen *et al*. studied the effect of 8-week aerobic training on wall thickness of femoral and carotid artery in a group of 8 healthy young individuals. They showed that cycle exercise training was associated with modest (but significant) decreases in wall thickness in the superficial femoral and carotid arteries. These findings suggest that exercise training causes systemic adaptation of the arterial wall in healthy young subjects (59). Moreover, data derived from the Tromso Study, a large population-based trial, identified sex and age as modulators of the inverse relationship between physical activity and carotid atherosclerosis. More specifically, the inverse relationship between physical activity and carotid artery atherosclerosis was observed in men, but not in women (60). This may explain the lack of IMT changes in the group of women in own research.

In summary, this study provides additional evidence that moderate-intensity aerobic exercise have beneficial effects on EF in people with HNBP.

The limitation of the current study was a small research group, especially women, which resulted from the difficulty to find volunteers with HNBP who would commit to 3-month training, although the recruitment was conducted in a large, almost 1-million city, lasted several months and was propagated in local media (TV and radio), on websites and through advertisements in health care facilities. Lack of time proved to be a barrier to participation in training.

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