

THE EFFECT OF WEIGHT LOSS ON SERUM CONCENTRATIONS OF NITRIC OXIDE INDUCED BY SHORT - TERM EXERCISE IN OBESE WOMEN

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Abstract. Objective: The aim of present study was to examine the effect of weight loss comprising regular moderate physical activity on resting serum concentrations of nitric oxide metabolites and exercise induced NO release. Materials and Methods: The study was carried out in 43 obese women without additional diseases (age 41.8 ± 11.9 y, body weight 94.5 ± 15.1 kg, BMI 36.5 ± 4.6 kg/m²). All obese patients participated in a 3-month weight reduction programme that consisted of 1) a group instruction in behavioural and dietary methods of weight control every two weeks; 2) 1000-1400kcal/day balanced diet, and 3) moderate physical exercises (30 minutes, 3 times a week). Before and after treatment body mass and height were measured, body mass index (BMI) was calculated. Body composition was determined by impedance analysis using a Bodystat analyser. The serum concentration of nitric oxide metabolites before and after exercise was measured using spectrophotometry method by Griess. The serum concentrations of lactate before and after exercise were measured with the use of strip test (ACCUSPORT analyzer). Serum concentration of insulin was measured with the use of RIA. Plasma glucose, cholesterol, HDL cholesterol and triglycerides were determined by enzymatic procedure. Results: The mean weight loss during treatment was 8.3 ± 4.3 kg. We did not observe differences between resting serum concentrations of NO and lactate before and after weight loss. During exercise serum NO concentrations increased significantly both before and after weight loss treatment. After the weight reduction treatment, the time of exercise test increased significantly $P < 0.005$, but there were no significant differences between the value of NO before and after weight loss. Conclusion: 3 – month regular physical activity and weight loss did not influence exercise-induced nitric oxide production.

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Key words : Nitric oxide - Exercise - Weight loss

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Introduction

One of the potential mechanism involved in the development of cardiovascular disease is endothelial dysfunction and decreased endothelial nitric oxide production. Previous study showed that body mass reduction decreased the risk of development of cardiovascular disease [17]. Recent studies showed that NO and the sympathetic nervous system mediate cardiovascular effects of insulin. Evidence indicates that sympathetic overactivity and impaired vasodilatation in insulin-resistant states result from the defect in nitric oxide production [14]. However, in obesity, a common state connected with insulin resistance, we observed increased basal serum concentration of nitric oxide [10]. Similar results were revealed by Choi *et al.* [2]. It may be a consequence of the presence of endothelial nitric oxide synthase (eNOS) and inducible nitric oxide synthase (iNOS) in human subcutaneous adipose tissue [2,3,13].

Our recent study also revealed that obesity may attenuate the endothelial NO release [11]. So far some studies suggest that exercise increases systemic nitric oxide production in men, which may contribute to beneficial effects of physical training in subjects with cardiovascular disease [1]. Since low physical activity is one of the risk factors of obesity and may be one of the causes of impairment in endothelial NO release, it seems that weight loss and regular physical activity may increase endothelial NO release.

The aim of present study was to examine the effect of weight loss comprising regular moderate physical activity on resting serum concentrations of nitric oxide metabolites and exercise induced NO release.

Materials and Methods

The study was carried out in 43 obese women (age 41.8 ± 11.9 y, body weight 94.5 ± 15.1 kg, BMI 36.5 ± 4.6 kg/m²). The study group characteristic is presented in Table 1.

All patients had simple obesity without additional diseases. The exclusion criteria were: evidence of present or recent (preceding 3 months) infectious disease, fever, and drug therapy, smoking, use alcohol more than two drinks monthly.

To avoid diet effect on serum concentrations of NO metabolites, the subjects were given a list of foods potentially rich in nitrate and were requested to abstain from these foods for three days before sample collection. Specifically herbal or black teas, beer, wine, preserved meat, fish and cheese were excluded from the diet.



All obese patients participated in a 3-month weight reduction programme that consisted of 1) a group instruction in behavioural and dietary methods of weight control meetings conducted every two weeks; 2) a 1000-1400 kcal/day balanced diet, and 3) physical exercises (30 minutes 3 times a week – walking, cycling and swimming).

The study was approved by the local Ethics Committee. All subjects gave their informed consent for the study.

The measurements were performed at the baseline and after the 3-month treatment. The body weight and height were measured and body mass index (BMI) was calculated as weight in kilograms divided by square of the height in meters.

Body composition was determined by impedance method using Bodystat analyzer. Blood samples were collected in the morning after an overnight fast and immediately after exercise performed on cycle ergometer.

Serum concentrations of total cholesterol, HDL – cholesterol, triglycerides and glucose were measured by enzymatic procedure. LDL cholesterol concentration was calculated with Friedewald formula. Serum concentration of insulin was measured with the use of RIA.

The blood serum concentration of nitric oxide metabolites were measured using a commercially available highly sensitive spectrophotometry method by Griess kits (Genzyme Diagnostics, Cambridge, USA, R&D Systems' Total Nitric Oxide Assay). The transient and volatile nature of NO makes it unsuitable for most convenient detection methods. However, since most of NO is oxidized to nitrite (NO_2^-) and nitrate (NO_3^-), the concentration of these anions have been used as quantitative measure of NO production. After the conversion of NO_3^- to NO_2^- , the spectrophotometric measurement of NO_2^- is accomplished by using the Griess Reaction ($1.\text{NO}+\text{O}_2^- \rightarrow \text{ONO}_2^- \xrightarrow{\text{H}^+} \text{NO}_3^-+\text{H}^+$ $2.2\text{NO}+\text{O}_2 \rightarrow \text{N}_2\text{O}_4 \xrightarrow{\text{H}_2\text{O}} \text{NO}_2^-+\text{NO}_3^-+2\text{H}^+$ $3.\text{NO}+\text{NO}_2^- \rightarrow \text{N}_2\text{O}_3 \xrightarrow{\text{H}_2\text{O}} 2\text{NO}_2^-+2\text{H}^+$). The conversion of NO into nitrate and nitrite by these reactions varies in each system. The interaction of NO in a system is measured by the determination of total nitrate and nitrite concentrations in the sample.

&D Systems' Total Nitric Oxide Assay involves the conversion of nitrate to nitrite by the nitrate reductase enzyme. The detection of total nitrite is then determined as a colored azo – dye product of the Griess Reaction that absorbs visible light at 540 nm.

The sensitivity of the Total Nitric Oxide Assay is typically less than 1.35 $\mu\text{mol/L}$. Mean intra – assay coefficient of variance was 3.1%, range: 1.2-5.3% and mean inter – assay coefficient of variance was 4.1%, range 3.3-7.0%.



The serum concentrations of lactate before and post – exercise were measured with the strip test (Boehringer Mannheim, Germany) using ACCUSPORT analyzer before and after exercise.

All subjects performed the exercise on cycle ergometer (Kettler computer software), the exercise load was increased every 3 minutes (50,100,150 W), The total duration of exercise did not exceed 9 minutes.

The heart rate (HR) value was monitored with the use of pulsimeter during the test.

The test was terminated when patients reached 85% of HR_{max} (HR_{max} was calculated with formula $HR_{max} = 220 - age$) or when they requested to stop the exercise due to fatigue, pain in joints etc.

The collected data was subjected to statistical analysis using t-test and Pearson's correlation analysis. The level of statistical significance was set at $P < 0.05$. Values are expressed as mean \pm SD.

Results

Mean weight loss was 8.3 ± 4.3 kg. BMI decreased from 36.5 ± 4.6 at the baseline to 33.4 ± 4.6 after treatment. The body weight reduction treatment led to significant decrease in body fat absolute and percentage ($P < 0.05$ and $P < 0.000001$ respectively), it was accompanied by moderate decrease in free fat mass absolute $P < 0.05$ and increase in free fat mass percentage $P < 0.05$ (Table 1).

Table 1

The characteristics of patients and the effects of treatment

		before	after	Δ
Weight	(kg)	94.5 ± 15.1	$86.2 \pm 13.7^{****}$	-8.2 ± 4.3
BMI	(kg/m^2)	36.5 ± 4.6	$33.4 \pm 4.6^{****}$	-3.1 ± 1.7
Fat – free mass	(kg)	53.0 ± 7.5	$50.8 \pm 6.0^*$	-2.2 ± 6.2
Fat – free mass	(%)	56.6 ± 8.3	$59.3 \pm 5.8^*$	3.7 ± 6.6
Body fat	(kg)	41.5 ± 12.1	$35.4 \pm 9.0^{***}$	-6.1 ± 6.4
Body fat	(%)	43.8 ± 7.0	40.7 ± 5.7	-3.1 ± 6.1

* $P < 0.05$; *** $P < 0.000001$; **** $P < 0.0000001$



After the weight loss treatment, the time of exercise test increased significantly $P < 0.005$ without increase in maximum heart rate during exercise test (Table 2).

Table 2

The exercise test's characteristics

		before	after
Time	(s)	381.0±74.5	417.8±91.4*
Maximum heart rate	(1/min)	158.5±17.7	160.6±14.3

* $P < 0.005$

Post – exercise serum concentrations of lactate increased significantly before and after treatment. However, resting and post - exercise concentrations of lactate did not differ between before and after weight reduction. We did not observe differences between post – exercise increase of lactate before and after weight loss treatment too (Table 3).

Table 3

Concentrations of lactate

		before	after
Resting lactate	(mmol/l)	1.4±0.8 ++++	1.2±0.6 ++++
Post – exercise lactate	(mmol/l)	3.5±1.5	3.4±1.0
Δ lactate		2.1±1.3	2.2±1.1

++++ $P < 0.0000$

During exercise NO concentration increased significantly both before and after weight loss treatment. However, resting and during exercise NO concentrations before and after weight loss did not differ statistically; there was also no difference between increase of NO before and after treatment (Table 4).



Table 4

Serum concentrations of nitric oxide

		before	after
Resting NO	($\mu\text{mol/l}$)	33.5 \pm 10.1 +++++	35.6 \pm 13.5 +++++
Post- exercise NO	($\mu\text{mol/l}$)	40.9 \pm 13.5	42.8 \pm 14.3
Δ NO		7.4 \pm 8.1	7.2 \pm 7.1

+++++P<0.0000001 before and after exercise

We did not observe significant differences between serum concentrations of total cholesterol, LDL cholesterol, triglycerides and insulin after weight loss.

Serum concentration of HDL cholesterol significantly decreased and serum concentration of glucose significantly increased after treatment (P<0.05 and P<0.005 respectively). However HDL cholesterol and glucose levels remained in the normal references range (Table 5).

Table 5

Plasma lipids. glucose and insulin

		before	after
Total cholesterol	(mg/dl)	207.2 \pm 33.7	206.3 \pm 23.8
HDL cholesterol	(mg/dl)	53.9 \pm 10.6	51.1 \pm 8.1*
LDL cholesterol	(mg/dl)	132.1 \pm 34.0	131.6 \pm 33.0
Triglycerides	(mg/dl)	106.5 \pm 46.5	98.0 \pm 39.1
Glucose	(mg/dl)	89.9 \pm 10.6	95.4 \pm 13.9**
Insulin	(μ IU/ml)	17.0 \pm 8.0	14.8 \pm 8.7

*P<0.05;**P<0.005

There were no correlations between post - exercise NO concentration and increase NO during exercise with age, body mass, BMI, body fat mass absolute and percentage, and time of exercise before as well as after treatment. We also did not observe association between increase of NO and decrease of body mass, BMI, body fat mass kg and % and increase time of exercise.



We did not observe association between resting and during exercise NO concentration and serum concentrations of lipids, glucose and insulin both before and after treatment. There was also no correlations between Δ NO and these factors. There were also no correlations between Δ NO and Δ lipids, glucose and insulin.

Discussion

Obesity is one of the risk factors of the development of cardiovascular disease, probably, at least partially, due to impairment in endothelial function as a consequence of insulin resistance, a common state associated with obesity. Moreover, some studies show that dysfunction of endothelium is related to age and regular activity can prevent age-dependent impairment in endothelial function. A preserved endothelial function can protect the vessel wall from the development of atherosclerosis and thrombosis, whereas a dysfunctional endothelium can negatively act as a promoter of atherosclerotic vascular damage. Therapeutic intervention that improves endothelial function could therefore have a beneficial impact on cardiovascular disease. Therefore, it is tempting to speculate that the part of the beneficial effect of physical training program on development of cardiovascular disease could be related to an improvement in endothelial function [15,16,18].

In present study 3-month weight reduction treatment comprising a balanced diet and regular 30 minutes daily exercise resulted in decrease in body fat absolute and percentage. However, we did not reveal differences between resting concentration of NO before and after weight loss, it may suggest that after weight loss basal endothelial NO production increased. It is in accordance with previous studies, which showed overexpression of inducible and endothelial NO synthases in adipose tissue [2,3] and with our results, which revealed increased basal concentration of nitric oxide in overweight and obese women [10]. This hypothesis is also in accordance with observations made by Kingwell *et al.* [8], who observed that four weeks of cycle training increases basal production of nitric oxide from forearm, as well as with results obtained by Levis *et al.* [9], who showed that regular, 4-week, home-based cycle training resulted in increased basal concentration of NO.

We observed increase in serum concentration of NO during exercise both before and after weight reduction. Even though there was a significant increase in the time of exercise after weight loss, we did not observe differences between NO concentration after exercise before and after treatment. It is contrary to results obtained by Higashi *et al.* [6], who revealed that long-term physical training improves endothelium-dependent vasorelaxation through an increase in the release



of nitric oxide in normotensive as well as hypertensive lean subjects. However Higashi *et al.* examined lean subjects and we examined obese patients, who remained obese after 3-month weight reduction treatment.

On the other hand, Jodoin *et al.* [7]. showed that in asymptotic subjects with risk factors for atherosclerosis including sedentary lifestyle and dyslipidemia three-month lifestyle intervention with a modified diet and regular exercise did not improve vascular endothelial function. Our observations are also in accordance with results obtained by Green *et al.* [4], who observed that exercise training enhances the peak vasodilatory capacity of the vasculature without influencing basal or stimulated activity of the nitric oxide dilator system *in vivo*. It is also in accordance with our previous results, which revealed the attenuation of exercise – induced endothelial NO release in overweight and obese women [11], yet study patients remained obese after 3-month weight reduction treatment.

Similar results were also received by Poveda *et al.* [12], who showed that regular physical activity resulted in higher basal nitric oxide concentration in a group of trained athletes, compared to a group of untrained people. However, short-term activity produced similar increments in nitric oxide metabolites in both group, and did not show exercise-related changes.

Basal and post treatment Δ NO was irrespective of the time of exercise, the age, body mass, BMI, body fat, serum lipids and insulin levels. These results are contradictory to these obtained by Tadei *et al.* [16] and Gerhard *et al.* [5], who showed a correlation between age and endothelial dysfunction in humans. Tadei *et al.* [16] also observed that regular physical activity prevents age – related impairment in nitric oxide availability, but they examined elderly athletes, whereas we middle – aged obese women.

We observed a significant decrease in HDL cholesterol after weight loss, but HDL concentration remained in the normal reference range. It was revealed that regular, 4-week, home-based cycle training (3 x 30 minutes/week at 65% maximum oxygen consumption [V_{O_2} max]) activity in hypercholesterolemic patients did not exert any influence on lipid profile [9]. It seems that increased glucose level may be a result of decrease in insulin level.

On the basis of our obtained results it seems that there may exist more important factors, which influenced exercise – induced endothelial NO release than these described above and further studies are necessary to clarify this problem.

Conclusion

3 – month regular physical activity and weight loss did not influence exercise-induced nitric oxide production.



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