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THE EFFECT OF SHORT – TERM EXERCISE ON NITRIC OXIDE (NO) SERUM CONCENTRATIONS IN OVERWEIGHT AND OBESE WOMEN

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Abstract. Objective*:* The aims of the present study was to examine the effect of overweight and obesity on serum concentrations of nitric oxide metabolites and evaluate the differences of exercise induced NO production in obese and lean women. Materials and Methods*:* The study groups consisted of 154 women including 102 obese and 24 overweight patients and 28 lean controls. Serum concentrations of nitric oxide metabolites were measured before and after exercise with the use of ELISA kits. 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 triglicerydes were determined by enzymatic procedure. Impedance analysis (Bodystat) was used to determine body composition. Results*:* Serum concentration of NO in overweight group and obese group was significantly higher when compared to controls, $p<0.05$ and $p<0.01$, respectively. There was no difference in levels of NO between overweight and obese groups .During exercise NO concentrations increased significantly in all groups and the post- exercise levels did not differ statistically in overweight and obese groups from that in controls. The value of ΔNO was the lowest in obese group but there were no significant differences between obese, overweight and control groups. Conclusions: Obesity may attenuate the exercise - induced endothelial NO release. ***(Biol.Sport 25:125-134, 2008)***

Key words: Nitric oxide – Exercise - Obesity

Introduction

Obesity is a well documented cardiovascular disease risk factor. One of the possible physiopathological mechanisms responsible for development of cardiovascular damage is the development of endothelial dysfunction. So far the

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mechanism underlying endothelial dysfunction in obese humans remains unclear and incompletely explored [14].

It seems that the development of endothelial dysfunction partially results from insulin resistance which is associated with obesity. This endothelial dysfunction is characterized by decreased production of the nitric oxide relaxing factor, which protects the vessels wall against the development of arteriosclerosis and thrombosis [11]. However, recent studies showed increased basal serum concentration of NO in obese subjects when compared to lean controls [2,15].

It seems that the above phenomenon may result from the overproduction of NO in adipose tissue, since some studies revealed presence of endothelial nitric oxide synthase (eNOS) and inducible nitric oxide synthase iNOS in both rats and human white adipose tissue and increased activity of NOS in human adipose tissue in obesity [3,9,10].

So far a number of studies in animals have revealed endothelial dysfunction in sedentary rats when compared to trained [6,7].

Since low physical activity level is one of the factors responsible for development of obesity and since most obese people lead sedentary life style, the aim of present study was to compare the exercise - induced NO production in obese and overweight women without others cardiovascular risk factors with exercise induced NO production in lean subjects.

Materials and Methods

The study group consisted of 24 overweight women (age 32.9±11.3 yrs, body mass 75.4±6.8 kg, BMI 28.1±1.6 kg/m^2) and 102 obese women (age 40.7±10.8 yrs, body mass 96.0±15.8 kg and BMI 36.6±5.1 kg/m^2). The control group included 28 lean volunteers (age 29.4±9.4 yrs, body mass 59.4±7.7 kg and BMI of 21.8±2.0 kg/m^2). The study and control groups characteristics are presented in table 1.

All patients had simple obesity without additional diseases. Subjects with evidence of acute or chronic inflammatory diseases were excluded from the study. Other exclusion criteria were: smoking, use of any drugs, fragment use of 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.

Majority of obese and overweight patients led sedentary live while the control subjects declared regular physical activity at least three times a week.

Table 1
Subjects' characteristics

		Obese	Overweight	Controls
N		102	24	28
Age	(years)	40.7±10.8	$32.9\pm11.3^{++}$	$29.4\pm9.4^{\#\#\#}$
Weight	(kg)	96.0±15.8	$75.4\pm6.8^{++++}$	$59.4\pm7.7^{\#\#\#\#}$ ****
BMI	(kg/m^2)	36.6±5.1	$28.1\pm1.6^{++++}$	$21.8\pm2.0^{\#\#\#\#}$ ****
Fat – free mass	(%)	56.2±7.0	$64.8\pm3.8^{++++}$	$77.5\pm4.9^{\#\#\#\#}$ ****
Body fat	(kg)	42.8±12.8	$26.6\pm4.0^{++++}$	$13.6\pm4.1^{\#\#\#\#}$ ****
Body fat	(%)	43.8±7.0	$35.3\pm3.8^{++++}$	$22.4\pm4.8^{\#\#\#\#}$ ****

$^{\#\#\#}$ $p<0.001$; $^{\#\#\#\#}$ $p<0.001$ obese vs. control groups
*$p<0.05$; **** $p<0.0000001$ overweight vs. control groups
$^{++}$ $p<0.005$; $^{++++}$ $p<0.0000001$ overweight vs. obese groups

The study was conducted after obtaining informed consent from all the subjects. The study was approved by the local Ethical Committee.

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, triglicerydes and glucose were measured by enzymatic procedure. LDL cholesterol concentration was calculated with Fridewald 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 ELISA 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 the 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.$NO+O_2^- \rightarrow ONO_2^{-H+} \rightarrow NO_3^- + H^+$ 2. $2NO+O_2 \rightarrow N_2O_4 \xrightarrow{H2O} NO_2^- + NO_3^- + 2H^+$ 3. $NO+NO_2^- \rightarrow N_2O_3 \xrightarrow{H2O} 2NO_2^- + 2H^+$). 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.

R&D Systems' Total Nitric Oxide Assay involves the conversion of nitrate to nitrite by the enzyme nitrate reductase. 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 μmol/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 concentrations lactate serum were measured with the strip test (Boehringer Mannheim, Germany) using ACCUSPORT analyzer before and after exercise.

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

Results

The time of exercise test did not differ statistically in overweight and obese groups when compared to controls.

Maximum heart rate obtained during exercise test was statistically lower in obese group when compared to controls ($p<0.0005$), but there was no difference between overweight and control groups (Table 2).

Resting concentrations of lactate did not differ statistically in overweight and obese groups when compared to controls.

Table 2
Duration of exercise and heart rate achieved during exercise test

		Obese	Overweight	Controls
Time	(s)	393.7±85.7	407.1±88.7	418.2±88.8
Post–exercise heart rate (min^{-1})		158.8±17.1	164.0±15.2	173.0±16.4 ###

p<0.0005 obese vs. control groups

Post-exercise lactate concentrations was significantly lower in both overweight and obese groups than in controls (p<0.001 and p<0.001 respectively). There was no difference in post-exercise lactate concentrations between overweight and obese groups.

The values of Δlactate were also significantly lower in both overweight and obese groups than in controls. (p<0.0005 and p<0.0005 respectively) (Table 3).

Table 3
Serum lactate concentrations

		Obese	Overweight	Controls
Resting lactate	(mmol/l)	1.3±0.6 +++++	1.3±0.7 +++++	1.2±0.6 +++++
Post – exercise lactate	(mmol/l)	3.6±1.4	3.4±1.5	5.4±2.4## **
Δ lactate		2.3±1.5	2.1±1.1	4.2±2.3### ***

p<0.001; ### p<0.0005 obese vs. control groups
** p<0.001; *** p<0.0005 overweight vs. control groups
+++++ p<0.0000001 before vs. after exercise

Resting NO serum concentrations in overweight and obese group were significantly higher when compared to controls, p<0.05 and p<0.01, respectively. There was no difference in levels of NO between overweight and obese groups (Table 4).

During exercise NO concentration increased significantly in all groups and the post-exercise levels did not differ statistically in overweight and obese groups from that obtained in controls. The value of ΔNO was the lowest in obese group, but

there were no differences between obese or overweight group and control group (Table 4).

Table 4
Serum concentrations of nitric oxide

	Obese	Overweight	Controls
Resting NO (μmol/l)	32.9±9.1 $^{+++++}$	35.1±12.1 $^{+++++}$	28.2±8.1 $^{\#\# *}$ $^{+++++}$
Post- exercise NO (μmol/l)	41.6±12.0	46.5±13.2	40.2±13.1
Δ NO	8.7±8.7	11.3±10.7	12.0±10.6
% increase of post- exercise NO	26.4	32.5	42.5

$^{\#\#}$ p<0.01 obese vs. control groups
* p<0.05 overweight vs. control groups
$^{+++++}$ p<0.0000001 before vs. after exercise

There was no correlation between post-exercise NO concentrations or ΔNO with age, body mass, BMI, body fat mass.

Concentration of total cholesterol did not differ between overweight and obese group and between overweight and control group, but it was statistically higher in obese group when compared to controls (p<0.005).

Concentration of HDL cholesterol was significantly higher in overweight group, when compared to obese group (p<0.05) and controls (p<0.05). However, levels of total cholesterol and HDL cholesterol in all groups were in the normal reference range.

Concentration of LDL cholesterol did not differ statistically in overweight and obese groups when compared to controls.

Concentration of triglicerydes in all groups were in normal reference range and did not differ statistically in overweight and obese groups from that in controls. It was statistically lower in overweight than in obese group (p<0.05).

Blood concentration of glucose did not differ between groups and in all of them was within normal reference range.

Serum concentration of insulin in overweight and obese groups were statistically higher than in controls (p<0.05 and p<0.00005, respectively). Its

concentration was also statistically higher in obese subjects than in overweight patients ($p<0.01$). However, all insulin levels were with in normal reference range (Table 5).

Table 5
Plasma lipids, glucose and insulin

	Obese	Overweight	Controls
Total cholesterol (mmol/l)	5.44±0.97	5.19±0.65	4.86±0.82$^{\#\#}$
HDL cholesterol (mmol/l)	1.35±0.27	1.50±0.32$^{\times}$	1.33±0.25*
LDL cholesterol (mmol/l)	3.47±0.83	3.26±0.74	3.09±0.76
Triglicerides (mmol/l)	1.33±0.80	1.01±0.51$^{\times}$	1.05±0.40
Glucose (mmol/l)	5.04±0.99	4.67±0.49	4.72±0.50
Insulin (μ IU/ml)	19.1±11.4	13.0±6.0$^{\times}$	9.9±3.9$^{\#\#}$*

$^{\#\#}$ $p<0.005$;$^{\#\#\#\#}$ $p<0.00005$; obese vs. control groups
* $p<0.05$; overweight vs. control groups
$^{\times}p<0.05$; overweight vs. obese groups

We did not observe associations between resting and post-exercise concentrations of NO and serum concentrations of lipids, glucose and insulin. We did not also ascertain correlations between ΔNO and serum levels of lipids, glucose or insulin.

Discussion

Our results revealing increased serum concentrations of NO in overweight and obese women when compared to controls are in accordance with our preliminary reports [15], which also showed increased resting serum concentrations of NO in obese subjects when compared to lean controls. Similar results received also Choi *et al.* [2], who showed that obesity leads to increased NO production in human and

that this increase begins from BMI level >25kg/m^2 in both males and females. Despite the fact that Choi *et al.* investigated adolescents (male and female) aged between 14-19 and we evaluated overweight and obese women aged between 17-64, both Choi's *et al.* and our findings show increased serum concentrations of NO in subjects with BMI over 25kg/m^2. We did not find association between serum concentrations of NO and age. Therefore, it seems that increased serum concentrations of NO may be a result of its overproduction in adipose tissue, but further studies are required to examine if, and to what extent, fat cells are able to release nitric oxide. Ferlito and Gallina [4] reported different results to ours. They observed an insignificant increase of NO concentrations in diabetics, being overweight and having increased blood pressure when compared to healthy controls. However Ferlito and Gallina measured plasma nitrite in patients with type 1 and 2 diabetes and we measured NO in overweight and obese women with serum concentrations of glucose and insulin with in the normal reference range.

We did not observe differences in mean time of exercise test and maximum heart rate between study groups. The above findings are surprising, because lean subjects declared regular physical activity, at least three times a week while majority of obese and overweight patients declared low or very low physical activity.

Despite the lack of significant differences in time of exercise test, we showed significantly lower serum concentrations of lactate in overweight and obese group when compared to controls. These differences did not result from resting serum concentrations of lactate, which did not differ between study groups. This observation is difficult to clarification and requires further study.

After exercise test, despite the short exercise duration in all study groups, we observed significant increase in serum concentrations of NO. But we did not reveal differences in post-exercise concentration of NO between overweight, obese subjects and controls.

The results concerning exercise-induced production of NO are in accordance with observations done by Bode–Boger *et al.* [1], who assessed nitrate excretion in urine in trained and sedentary men. These investigators showed, that the rate of nitrate excretion with urine was similar in both group and after 30 minutes of exercise increased almost twice in both group. Therefore Bode–Boger *et al.* suggested that strenuous submaximal exercise resulted in increased production of NO, irrespectively of the training status.

However, Poveda *et al.* [8], who assessed serum levels of nitrate and nitrite in serum in athletes and sedentary persons observed higher basal concentrations of these compounds in athletes. However, in the latter group there were no exercise-

related changes in the nitrate concentration. Therefore, these investigators concluded, that NO release did not increase during exercise in trained athletes.

Participants of the present study differed significantly as regards to the level and regularity of physical activity, which probably explains the difference in obtained results.

Despite comparable range of exercise load and duration in all groups, we observed the lowest ΔNO in the group of obese patients.

In none of the study groups significant correlation was ascertained between age and serum concentrations of resting NO, post-exercise serum concentrations of NO or ΔNO.

These results are contradictory to those obtained by Tadei *et al.* [13] and Gerhard *et al.* [5], who showed a correlation between age and endothelial dysfunction in humans.

We did not find any correlation between post-exercise serum concentration of NO or ΔNO and serum concentrations of lipids, glucose or insulin.

On the basis of the results obtained in this study it may be assumed, that there is a tendency towards worsening of endothelial function in obese subjects and that the increase in resting serum concentrations of NO may be a results of its production in other tissue than endothelial cells.

This hypothesis is in accordance with results of some studies. Steinberg *et al.* [12] demonstrated that the increase in blood flow into the leg in response to methacholine, a muscarine agents, is blunted in obese humans, and the degree of dilatation was inversely related to the degree of obesity. These authors also observed that the increment in blood flow in response to sodium nitroprusside – exogenous donor NO was no different between obese and lean subjects.

Conclusions: Obesity may attenuate the exercise–induced endothelial NO release.

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