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Effect of 3-month L-arginine supplementation on insulin resistance and tumor necrosis factor activity in patients with visceral obesity

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Abstract. – *Background:* **The role of tumor necrosis factor alpha (TNF-alpha), one of the adipose tissue products, in the pathogenesis of insulin resistance is well-documented. Many recent studies have shown beneficial influence of L-arginine supplementation on cardiovascular system. However, molecular mechanisms of its positive actions are not fully elucidated.**

Aim: **The aim of the study was to evaluate the influence of L-arginine supplementation on tumor necrosis factor alpha, insulin resistance and selected anthropometric and biochemical parameters in patients with visceral obesity.**

Patients and Methods: **60 patients with visceral obesity were randomly assigned to either receive 9 g of L-arginine or placebo for 3 months. 20 healthy lean subjects were used as control. Selected anthropometrical measurements and blood biochemical analyses were performed at baseline and after 3-months. TNF-alpha and its soluble receptor 2 (sTNFR2) were assessed in both treated groups. Insulin resistance in the participants was evaluated according to the homeostasis model assessment-insulin resistance (HOMA-IR) protocol.**

Results: **The concentration of insulin, TNF-α and sTNFR2 and HOMA-IR level in both obese groups significantly exceeded these observed in the control. Basal TNF-alpha and sTNFR2 concentrations were positively correlated with basal body mass index (BMI), waist circumference, percent of body fat and HOMA-IR. We found that 3-month L-arginine supplementation resulted in significant decrease of HOMA-IR and insulin concentration. Only insignificant tendency to decrease of TNF-alpha and sTNFR2 was observed.**

Conclusions: **Our results confirm TNF-alpha role in the complex pathogenesis of insulin resistance in patients with visceral obesity. 3-months L-arginine supplementation in a dose of 9 g improves insulin sensitivity in patients with visceral obesity with no impact on tumor necrosis factor alpha concentration.**

Key Words:

Obesity, Tumor necrosis factor alpha, Insulin resistance, L-arginine.

Introduction

Obesity prevention, due to its prevalence and significant impact on the health of society, has become one of the major challenges at the beginning of the XXI century[1,2]. Molecular mechanisms involved in the pathogenesis of obesity-related complications have been intensively studied in the recent years. Insulin resistance, common feature of visceral obesity, is a fundamental aspect of the etiology of type 2 diabetes and is also associated with wide array of other pathophysiologic sequelae including hypertension, hyperlipidemia and atherosclerosis[3-5]. One of the important discoveries that have led to a better understanding of impaired insulin response in patients with excessive body weight, was that fat tissue is an active endocrine body[6,7]. Some substances produced by adipocytes were identified, for some of them pathophysiological significance was described. Chronically elevated serum concentration of one of the key adipocytokines – TNF-α, as a consequence of overexpression and overproduction in adipose tissue, was confirmed by many Authors[8-10]. Important role of this pleotropic cytokine in the complex pathogenesis of insulin resistance[11], diabetes[12] and hypertension associated with obesity is suggested[13]. Excessive activity of TNF-α increases impaired endothelial function in obese patients, reduces activity of nitric oxide synthase[14,15] and activates the chronic intravascular inflammatory process[16], which may explain accelerated progression of atherosclerosis in this group of patients. Understanding of the mechanism involved in the development of complications associated with overweight and obesity is crucial to identify new therapeutic options to decrease cardiometabolic risk in these patients.

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L-arginine was first isolated from the sprouts of lupine in 1886, a few years later it was detected in animal tissues. In 1988 it was found that this aminoacid is a substrate for nitric oxide production, which contributed to significant growth of interest in L-arginine in the treatment and prevention of cardiovascular system diseases. An increasing number of evidence points to the potential benefits of the use of L-arginine in patients with hypertension[17], type 2 diabetes[18], atherosclerosis[19] or hypercholesterolaemia[20]. The potential use of L-arginine in patients with obesity seems to be very promising. It is worth noticing that there is a lack of research assessing the potential of L-arginine supplementation in patients with obesity.

The aim of the study was to evaluate the influence of L-arginine supplementation on tumor necrosis factor alpha activity and insulin resistance level in patients with visceral obesity. Moreover, L-arginine impact on blood pressure values and selected anthropometric and biochemical parameters was determined.

Materials and Methods

Selections of Patients

The protocol of the study was approved by Research Ethics Committee of Poznan University of Medical Sciences and registered as no. 221/10. It conformed to all ethical issues included in the Helsinki Declaration.

The study population consisted of 60 subjects with simple obesity. The inclusion criteria were as follows: age 30-60 years, waist circumference > 80 cm for women or > 94 cm for men, stable body weight (less than 3 kg self-reported change during the previous 3 months), no need for use of any pharmacological treatment.

The exclusion criteria were: (1) arterial hypertension, (2) diabetes mellitus, (3) impaired glucose tolerance, (4) history of coronary artery disease, stroke, congestive heart failure or peripheral arterial disease, (5) sleep apnea syndrome, (6) abnormal liver or renal function, (7) clinically significant inflammatory process within respiratory, digestive and genitourinary tract, as well as in oral cavity, pharynx and paranasal sinuses, (8) history of infection within a month before the study, history of use of any dietary supplements within 3 months prior to the study, (9) smoking. Twenty healthy volunteers matched for demographic characteristics were used as the controls.

Study Design

Obese subjects were randomized to L-arginine (Curtis Healthcare, Poland) 9 g tid or placebo (microcrystalline cellulose) for 3 months. 9 g tid was chosen as the most optimal for the patients, as it was nearly twice the amount of arginine in the average food ratio in Poland. The dose appeared to be effective and safe for patients.

All treated subjects underwent dietician consultation and they were instructed to maintain the diet and physical activity through the study. Every 14 days patients were weighted to control their body mass throughout the study. Every 14 days and 3 days before the laboratory tests, compliance to dietary recommendations was determined by obtaining a 24 hr dietary recall from the subjects. The amount of nutrients in the daily diet was processed and evaluated using the dietetic computer software. The intake of protein and arginine during the study was constant. The average daily protein intake was 72.5 g in women and 91.9 g in men, and average arginine intake was 43.3 mg/kg bodyweight in women and 48.6 mg/kg bodyweight in men.

Measurements

All the measurements were obtained at the baseline and after completing a 3-months treatment program. In the control group measurements were taken only at baseline.

Anthropometry

Anthropometric measurements of individuals wearing light clothing and no shoes were carried out. Weight was measured to the nearest 0.1 kg, and height was measured to the nearest 1 cm. Body mass index (BMI) was calculated as weight divided by height squared (kg/m^2). Waist circumference (cm) was measured at the level of the iliac crest at the end of normal expiration. Waist circumference was measured to the nearest 0.5 cm. Visceral obesity was defined as a waist circumference ≥80 cm for women and ≥94 cm for men.

Additionally, percent of body fat (%FAT) was determined by impedance analysis using a Bodystat analyzer (1500 MDD; Bodystat, Isle of Man, UK).

Blood Pressure Measurement

Office blood pressure (BP) was measured using a digital electronic tensiometer (model 705IT, Omron Corporation, Kyoto, Japan). Regular or large adult cuffs were used, depending on patient arm circumference. Hypertension was defined by measurement of arterial blood

pressure as the average of three measurements obtained after 10 min of physical resting by the patients (3 times at 3 different visits within 1 month).

Biochemical Measurements

Blood samples were taken after an overnight fast and after placing each participant in the supine position for 30 minutes.

Serum levels of lipids, including total cholesterol (TCH), high-density lipoprotein cholesterol (HDL-C), and triglyceride were assayed by routine enzymatic method. Low-density lipoprotein cholesterol (LDL-C) was calculated from Friedewald's formula. Level of blood glucose was determined by routine enzymatic method.

Plasma insulin was determined by immunoradiometric assay (DIA Source Immunoassays S.A., Nivelles, Belgium). Insulin resistance in the participants was evaluated according to the homeostasis model assessment–insulin resistance (HOMA-IR) protocol:

- HOMA-IR index = [fasting insulin (mU/l) × fasting glucose (mmol/l)]/22.5
- Serum TNF-α and sTNFR2 were measured by enzyme immunoassay (ELISA) (R&D System, Inc., Minneapolis, MN, USA).

Statistical Analysis

Data are shown as mean±SD. All calculations and statistics were performed with STATISTICA 6.0 program (StatSoft, Krakow, Poland). The comparisons between groups were carried out using the Mann-Whitney U test. Wilcoxon rank sum test was used to analyze the statistical significance between variables before and after 3-month treatment. Simple associations between variables were calculated as the Spearman coefficient of correlation. A p value of <0.05 was regarded as significant.

Results

The baseline characteristics of the patients with visceral obesity randomized to L-arginine (Group 1), placebo (Group 2) and the control group are described in Table I. There was no significant difference in all studied measurements between the L-arginine and placebo group. As expected, obese patients had higher BMI, waist circumference and %FAT as compared to the controls. Concentration of TCH, LDL cholesterol, TG, glucose and insulin in both obese groups (L-arginine and placebo) significantly ex-

Table I. Baseline characteristic of studied groups.

	Control (n = 20)	Group 1 – L-arginine (n = 30)	Group 2 – Placebo (n = 30)
Sex (men/women)	12/8	14/16	17/13
Age (year)	41.4 ± 9,7	43.8 ± 8.2	41.0 ± 8.8
BMI (kg/m^2)	23.2 ± 2.0	39.2 ± 6.0*	37.5 ± 4.8$^{\#}$
Waist circumference (cm)	77.2 ± 11.2	108.2 ± 10.6*	108.3 ± 8.8$^{\#}$
FAT %	22.9 ± 5.4	38.1 ± 5.8*	36.4 ± 4.9$^{\#}$
SBP (mmHg)	130.0 ± 5.3	131.4 ± 5.9	131.7 ± 7.4
DBP (mmHg)	81.8 ± 5.6	83.8 ± 4.1	82.5 ± 4.1
Creatinine (μmol/L)	77.6 ± 9.9	83.2 ± 11.3	81.5 ± 13.2
TCH (mmol/L)	4.1 ± 0.7	5.6 ± 1.2*	5.5 ± 1.1$^{\#}$
LDL (mmol/L)	2.3 ± 0.6	3.6 ± 0.8*	3.5 ± 0.9$^{\#}$
HDL (mmol/L)	1.5 ± 0.4	1.1 ± 0.3*	1.1 ± 0.3$^{\#}$
TG (mmol/L)	0.7 ± 0.3	2.1 ± 1.4*	2.2 ± 0.9$^{\#}$
Glucose (mmol/L)	4.4 ± 0.4	5.2 ± 0.6*	5.0 ± 0.6$^{\#}$
Insulin (μUI/mL)	8.8 ± 1.7	31.0 ± 8.9*	29.2 ± 15.8$^{\#}$
HOMA-IR	1.7 ± 0.4	7.2 ± 2.2*	6.4 ± 3.4$^{\#}$
TNF-α (ng/L)	1.9 ± 0.6	4.6 ± 2.0*	4.6 ±1.8$^{\#}$
sTNFR2 (ng/mL)	1.6 ± 0.5	3.8 ± 1.7*	3.6 ± 1.6$^{\#}$

*$p < 0.05$ L-arginine vs control; # $p < 0.05$ placebo vs control; ** $p < 0.05$ L-arginine vs placebo; BMI – body mass index, FAT – fat content, SBP – systolic blood pressure, DBP – diastolic blood pressure, TCH – total cholesterol concentration, LDL – LDL cholesterol concentration, HDL – HDL cholesterol concentration, TG – triglycerides, HOMA-IR – insulin resistance index, TNF-α – tumor necrosis factor alpha, sTNFR2 – soluble TNF-α receptor 2.

ceeded these observed in the control, whereas HDL concentration was significantly lower. Serum tumor necrosis factor-alpha (TNF-α) and soluble TNF receptor 2 (sTNFR2) levels were significantly higher in patients with obesity comparing to the healthy normal weight controls. Obese patients were significantly more insulin resistant than controls.

Basal sTNFR2 concentration in patients with visceral obesity correlated positively with BMI, waist circumference, %fat, insulin concentration and HOMA-IR. The same positive correlations were observed for TNF-α (Table II).

L-arginine supplementation resulted in significant decrease of insulin concentration. Correspondingly, HOMA-IR decreased from 7.2 ± 2.2 to 5.8 ± 1.9 ($p < 0.001$; Table III).

In contrast, we did not observed any significant changes in both TNF-α and sTNFR2 levels after 3 months of L-arginine treatment. Only insignificant tendency was noticed – $p = 0.09$ for TNF-α and $p = 0.08$ for sTNFR2.

There were no significant changes in the studied parameters in the placebo group.

Discussion

A significant improvement of insulin sensitivity, not related to TNF-α system alternations, in patients with visceral obesity treated with L-arginine for 3 months, whose activity become unchanged during the study, is a new finding demonstrated in our study.

Our research confirmed increased insulin resistance in patients with visceral obesity. Basal HOMA-IR was over 4-times higher as compared to lean control. Similar correlations were shown by other Authors[21]. The evidence confirms the independent contribution of hyperinsulinemia and insulin resistance in the development of cardiovascular complications[22,23]. The results of Insulin Resistance Atherosclerosis Study (IRAS) demonstrated independent (not related to traditional risk factors for cardiovascular disease) relationship between thickness of the intima-media in the carotid artery and a decrease in insulin sensitivity[24]. As a typical consequence of obesity and insulin resistance we found abnormal lipid profile in the studied patients. Critical role of insulin resistance in the pathogenesis of lipid disorders is well-documented[25].

Results of our study are consistent with previous observations, which showed over-activation of TNF-α in patients with obesity[8-11]. We found significantly higher concentrations of both TNF-α and its soluble receptor 2 (sTNFR2). Both level of TNF-α and sTNFR2 correlated positively with BMI, waist circumference and %fat. We did not evaluate concentration of sTNFR1, because of its insignificant association with insulin resistance reported previously[26].

The important role of TNF-α in the pathogenesis of insulin resistance in patients with excessive body weight was shown in animal models[27] and in humans[28,29]. This cytokine inhibits insulin action in adipose tissue, liver and muscle. In the adipose tissue TNF-α inhibits lipogenesis by reducing lipoprotein lipase activity, stimulates

Table II. Correlations between basal TNF-α, sTNFR2 and anthropometrical and biochemical variables in patients with visceral obesity.

	TNF-α		sTNFR2	
	R	*p*-value	R	*p*-value
BMI	0.34	< 0.01	0.28	0.03
Waist circumference	0.42	< 0.01	0.41	< 0.01
FAT %	0.37	< 0.01	0.34	< 0.01
Creatinine	-0.15	NS	-0.15	NS
TCH	-0.06	NS	-0.06	NS
LDL	-0.05	NS	-0.04	NS
HDL	-0.08	NS	0.00	NS
TG	0.00	NS	-0.06	NS
Glucose	-0.09	NS	0.06	NS
Insulin	0.45	< 0.01	0.42	< 0.01
HOMA-IR	0.36	< 0.01	0.40	< 0.01

BMI – body mass index, FAT – fat content, TCH – total cholesterol concentration, LDL – LDL cholesterol concentration, HDL – HDL cholesterol concentration, TG – triglycerides, HOMA-IR – insulin resistance index, TNF-α – tumor necrosis factor alpha, sTNFR2 – soluble TNF-α receptor.

Table III. Studied parameters at baseline and after 3 month treatment in L-arginine and placebo groups.

	L-arginine before treatment	L-arginine after treatment	Placebo before treatment	Placebo after treatment
BMI (kg/m^2)	39.2 ± 6.0	38.9 ± 5.8	37.5 ± 4.8	37.3 ± 4.9
Waist circumference (cm)	108.2 ± 10.6	107.3 ± 9.8	108.3 ± 8.8	107.2 ± 8.0
FAT %	38.1 ± 5.8	37.4 ± 5.3	36.3 ± 4.9	36.1 ± 4.6
SBP (mmHg)	131.4 ± 5.9	130.6 ± 5.8	131.7 ± 7.4	130.3 ± 6.1
DBP (mmHg)	83.8 ± 4.1	83.0 ± 4.3	82.5 ± 4.1	83.5 ± 4.6
TCH (mmol/L)	5.6 ± 1.2	5.5 ± 1.0	5.5 ± 1.1	5.3 ± 0.7
LDL (mmol/L)	3.6 ± 0.8	3.4 ± 0.8	3.5 ± 0.9	3.2 ± 0.9
HDL (mmol/L)	1.1 ± 0.3	1.1 ± 0.3	1.1 ± 0.3	1.2 ± 0.3
TG (mmol/L)	2.1 ± 1.4	2.1 ± 1.3	2.2 ± 0.9	2.1 ± 0.8
Glucose (mmol/L)	5.2 ± 0.6	5.1 ± 0.7	5.0 ± 0.6	5.0 ± 0.5
Insulin (μUI/mL)	31.0 ± 8.9	26.4 ± 7.0*	29.2 ± 15.8	29.8 ± 14.9
HOMA-IR	7.2 ± 2.2	5.8 ± 1.9*	6.4 ± 3.4	6.6 ± 3.3
TNF-α (ng/L)	4.6 ± 2.0	4.3 ± 1.8	4.6 ± 1.8	4.5 ± 1.5
sTNFR2 (ng/mL)	3.8 ± 1.7	3.5 ± 1.4	3.6 ± 1.6	3.7 ± 1.2

* $p < 0.05$ for the difference before and after treatment; Wilcoxon test. BMI – body mass index, FAT – fat content, SBP – systolic blood pressure, DBP – diastolic blood pressure, TCH – total cholesterol concentration, LDL – LDL cholesterol concentration, HDL – HDL cholesterol concentration, TG – triglycerides, HOMA-IR – insulin resistance index, TNF-α – tumor necrosis factor alpha, sTNFR2 – soluble TNF-α receptor 2.

lipolysis by activation of hormone sensitive lipase and increases insulin resistance by inhibiting the phosphorylation of tyrosine and increasing serine phosphorylation in insulin receptor substrate (IRS-1), consequently, leading to decrease of insulin receptor activity. TNF-α also influences the metabolism of glucose directly, reducing the expression of GLUT-4 in muscle cells[30,31]. Our findings – significant correlation between TNF-α, sTNFR2 and insulin concentration and HOMA-IR, confirmed the potential role of TNF-α system in the complex pathogenesis of insulin resistance in obese patients.

Loscalzo[32] defined the mechanisms by which L-arginine improves endothelial function: increased intracellular uptake via the high-affinity cationic transporter; substrate competition with asymmetric dimethylarginine; direct antioxidant activity; stimulated release of histamine from mast cells, which produces a vasodilator response; decreased activity of norepinephrine and increased insulin secretion, which causes vasodilation. Increasing number of evidence confirmed anti-inflammatory and anti-oxidant properties of L-arginine[33-35]. Based on vasoprotective L-arginine action we decided to evaluate its potential benefits in patients with visceral obesity. Endothelial dysfunction and increased cardiometabolic risk are typical features of this group of patients.

3-month L-arginine supplementation did not influence anthropometric parameters and blood pressure values. Similar effect on blood pressure was noticed in our previous study, where healthy normotensives had been treated with L-arginine in a dose of 6 g or 12 g during 4 weeks[36]. In all subjects ambulatory blood pressure measurement (ABPM) was carried out 4 times: before randomization, after 2 and 4 weeks of supplementation and 2 weeks after finishing supplementation. It was found that L-arginine led to non-significant decrease of systolic and diastolic blood pressure. In contrast, we have demonstrated strong association between L-arginine supplementation and blood pressure reduction in patients with essential hypertension[37]. The treatment of hypertensive patients with 12 g of L-arginine for 4 weeks led to lowering of both systolic and diastolic blood pressure in ABPM, with stronger hypotensive effect observed during the night. Schlaich et al[38] reported impaired L-arginine transport in hypertensive individuals and normotensive at high risk for the development of hypertension, which may represent the association between a defective L-arginine/NO pathway and the onset of essential hypertension.

Our results show that L-arginine had no effect on the parameters of lipids. Similar results were obtained by others[39,40]. Contrary, there are reports which show positive influence of L-arginine on lipid metabolism and lipid profile. It was found in experimental and clinical studies that L-arginine supplementation decreased serum total cholesterol, low-density lipoprotein (LDL) and triglycerides[41,42]. It is suggested that hypolipemic effect of L-arginine is at least in part associated

with the increase in NO level in the body and lower fatty acid oxidation[43]. The results of Tan et al[44] indicated that L-arginine regulates the expression of fat-metabolic genes in skeletal muscle and white adipose tissue, therefore, favoring lipogenesis in muscle but lipolysis in adipose tissue, what may influence the lipid profile in serum.

Potential influence of L-arginine on insulin resistance is under discussion. The results of several studies are not uniform and pretty inconclusive[45-48]. The present findings demonstrate beneficial impact of L-arginine supplementation on insulin resistance in patients with visceral obesity. HOMA-IR decreased significantly, although still remained higher than in the healthy control (Table III). Positive influence on insulin resistance was also observed by Piatti et al[18]. In a group of 12 lean type 2 diabetic patients supplementation with L-arginine in a dose of 9 g for 4 weeks resulted in improvement of peripheral and hepatic insulin sensitivity. As the explanation they suggested that insulin resistance is associated with an impairment in the ability of NO to generate its messenger, leading to a decrease in cGMP generation and a relative decline in insulin's ability to produce vasodilatation. The hypothesis was consistent with Petrie at al.'s[49] conclusion that there is a relationship between insulin resistance and endothelial response to inhibition of NO synthesis as well as with evidence that the vasodilatory response is decreased in insulin-resistant individuals. The results obtained in our study were insufficient to determine direct mechanism responsible for favorable effect of L-arginine supplementation on insulin resistance. Body weight, BMI, waist circumference and %fat remained unchanged. We had believed that increase in insulin sensitivity could be related to alternations in TNF-α concentrations. But we found that the increase of insulin sensitivity was unrelated to TNF-α system activity. 3-month L-arginine supplementation in a dose of 9 g influenced neither TNF-α nor sTNFR2. Lack of changes in TNF-α system, although potentially attractive, excluded its potential role in insulin resistance alteration in obese patients treated with L-arginine.

Conclusions

3-month L-arginine supplementation in a dose of 9 g improves insulin sensitivity in visceral obese patients independently of TNF-α activity, which concentration remained unchanged.

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