

The effect of L-arginine supplementation on serum resistin concentration in insulin resistance in animal models

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Abstract. – **BACKGROUND:** Understanding the mechanism of development of the insulin resistance associated with obesity is crucial in identifying new therapeutic options for obese patients.

AIM: The aim of this study was to examine the effects of L-arginine on the secretion of resistin in the context of insulin resistance in animal models.

MATERIALS AND METHODS: 30 male and female Wistar rats were assigned to three equal groups: the standard diet group, the high-fat diet group, and the high-fat diet supplemented with L-arg group. After 6 weeks, the weight of the rats was measured. The animals were euthanized. The relative weight of the perirenal fat was determined and blood samples were taken for serum glucose, insulin, NO, and resistin. Insulin resistance was estimated using homeostasis model assessment (HOMA).

RESULTS: It was found that the absolute and relative masses of the perirenal fat were significantly higher in rats fed the high-fat diet than in the control group. In rats on the high-fat diet supplemented with L-arginine, a tendency for perirenal fat to decrease was observed.

The high-fat diet resulted in significant increases in glucose and insulin concentrations, and L-arginine supplementation significantly ameliorated the increase in both glucose and insulin. Moreover, significant decreases in NO concentration were seen in rats fed the high-fat diet. L-arginine supplementation protected significantly against increased NO concentration.

Increases in HOMA-IR level and in resistin concentrations were observed in rats fed the high-fat diet. L-arginine supplementation partially restored HOMA-IR levels to those of the control group and did not influence resistin concentration.

CONCLUSIONS: L-arginine supplementation improves insulin sensitivity in rats fed a high-fat diet, independently of resistin activity.

Key Words:

L-arginine, Insulin resistance, Resistin, Rats.

Introduction

The incidence of obesity and associated diseases is increasing at alarming rates almost over the entire planet. Nearly 1 billion adults worldwide are overweight, and at least 300 million are obese^{1,2}.

Although obesity is recognized as a leading risk factor for insulin resistance, type-2 diabetes, hypertension, atherosclerosis, stroke, and some types of cancer, pharmacological treatments for this chronic disease are limited³.

Understanding the mechanism involved in the development of complications associated with overweight and obesity is crucial for identifying new therapeutic options to decrease insulin resistance and other cardiometabolic risk in these patients.

L-arginine is a conditionally essential amino acid that is a natural constituent of dietary proteins. Besides its role in protein metabolism, L-arginine is involved in the production of nitric oxide and in the synthesis of creatine, L-ornithine, L-glutamate, and polyamines⁴. Moreover, L-arginine is a potential factor affecting the endocrine system, as it induces the secretion of insulin and glucagon from the pancreas⁵. Growing evidence indicates that dietary L-arginine supplementation can reduce adiposity and improve insulin sensitivity in some animal models, as well as in obese humans; this has also been confirmed in our earlier studies⁶. However, the mechanisms behind the potential metabolic benefits of L-arginine are yet to be discovered.

Excess body fat is stored primarily in white adipose tissue, which is an active endocrine organ⁷. It releases a variety of factors, the so-called adipocytokines. One of these, resistin – whose serum levels are elevated in both genetic and diet-induced models of obesity and insulin-resis-

tance – was recently added to the list of adipose tissue products potentially involved in the pathogenesis of insulin resistance^{8,9}.

The effects of resistin secretion on animal models consuming L-arginine have, to the best of our knowledge, not yet been sufficiently studied. The present study was designed to examine the effects of L-arginine on the secretion of resistin in the context of insulin resistance in animal models.

Materials and Methods

The protocol of the study was approved by the local Bioethical Commission in Poznan (approval no. 20/2011).

Experimental Design

Eight-week-old male and female Wistar rats were purchased from the Department of Toxicology, Medical University of Poznan, Poland. All rats were housed individually in polycarbonate cages obtained for the purpose of the study on a 12-h light:12-h dark cycle. The indoor animal house temperature was $21 \pm 2^\circ\text{C}$, relative humidity was $60 \pm 5\%$, and subsequent light-dark cycles lasted 12 hours. All rats were provided ad libitum diet and distilled water for 42 days. Feed intake was measured daily, while body weight gain was monitored weekly. Over the whole course of the experiment, animals were under veterinary supervision.

After a five-day period of adaptation to the laboratory conditions, the rats were randomly divided into three equal groups. In the control group (CON), 12 rats (6 male and 6 female) were allowed free access to standard diet. In the high-fat diet group (FAT), 12 rats received a high-fat diet. In the arginine group (ARG), 12 rats received a high-fat diet containing L-arginine (Curtis Healthcare, Warsaw, Poland) at 20 g/kg diet. The animals were fed a standard semisynthetic diet based on AIN-93M (35) or a high-fat diet modified with amounts of fat and sodium chloride. The full composition of the diets is presented in Table I.

At the end of the experiment, following 16 hours of starvation, the animals were weighed and euthanized by intraperitoneal injection of thiopental (40 mg/kg body mass) and killed by cardiac puncture. The blood samples were collected in serum-separated tubes for biochemical studies. The coagulated blood was left to clot at

Table I. Ingredient and nutrient composition of the diets (grams per kilogram diet).

Ingredient	Standard diet	High fat diet
Casein	140	140
Wheat starch	625	430
Sucrose	100	100
Potato starch	50	50
Vitamin mixture	10	10
Mineral mixture	35	35
Sunflower oil	40	40
Lard	–	160
Sodium chloride	–	35
Total energy (kcal/100 g diet)	420	515
Total protein (% of energy)	18	18
Total fat (% of energy)	9	39

room temperature for 30 min, and then centrifuged for 15 min at 2500 r.p.m. at 4°C . The serum was then separated and stored at -70°C until analyzed. Visceral perirenal fat was dissected and weighted.

Examined Parameters

Selected nutrition parameters in rats

In all animals, weight gain, and absolute and relative weight of visceral fat was determined. The relative weight of visceral fat was defined as the percentage of body weight.

Laboratory Measurements

Serum glucose concentration was assayed using an enzymatic method involving hexokinase and glucose-6-phosphate dehydrogenase (Siemens Healthcare Diagnostics, Erlangen, Germany).

Nitric oxide concentration was determined by means of the spectrophotometric method in serum using a testing set by Oxis (Oxis International, Foster City, CA, USA).

The plasma level of resistin was determined by an enzyme-linked immunosorbent assay, following the manufacturer's instructions strictly (BioVendor, Heidelberg, Germany).

The plasma concentration of insulin was determined by enzyme-linked immunosorbent assay, following the manufacturer's instructions strictly (Demeditec Diagnostic, Kiel-Wellsee, Germany).

Insulin resistance was estimated by homeostasis model assessment (HOMA) according to the formula insulin resistance index = fasting insulin (g/L) \times fasting glucose (mg/dl)/405.

Table II. Effects of dietary L-arginine on initial body mass, weight gain and visceral fat tissue.

Groups	n	Parameters			
		Diet intake (g/day)	Initial body mass (g)	Weight gain (g)	Visceral body fat (g)/percentage of body mass (%)
CON	12	21.5 ± 1.8	190.1 ± 12.2	153.5 ± 17.8	3.0 ± 0.2/0.84 ^a
FAT	12	20.5 ± 1.2	188.8 ± 12.8	157.2 ± 19.2	3.5 ± 0.2/1.05 ^b
ARG	12	20.8 ± 1.5	190.7 ± 12.5	142.5 ± 20.1	3.1 ± 0.3/0.9 ^{ab}

Values are means ± SEM for twelve rats. Means in the same column with different superscripts are significantly different ($p < 0.05$); CON-control group, FAT-group with high fat diet, ARG-group with high fat diet and L-arginine.

Statistical Analysis

Data are presented as means ± SEM. Statistical significance was determined by ANOVA followed by Tukey's post hoc test. A value of $p < 0.05$ was regarded as a significant difference. All calculations and statistics were performed with Statistica for Windows, version 6.0.

Results

It was found that the average intake of diet, initial body weight, and weight gain were comparable across all groups (Table II). The absolute and relative mass of visceral fat (as a percentage of body mass) was significantly higher in rats fed the high-fat diet than in the control group. In rats on the high-fat diet supplemented with L-arginine, a tendency for perirenal fat to decrease was observed.

The results presented in Table III demonstrate that six weeks of the high-fat diet resulted in a significant increase ($p < 0.05$) in glucose and insulin concentrations. L-arginine supplementation significantly ameliorated the increase in both

glucose and insulin. Moreover, significant decreases in NO concentrations were seen in rats fed the high-fat diet. L-arginine supplementation significantly protected against increased NO concentration.

From the results in Figures 1-2, it is clear that a significant increase ($p < 0.05$) in HOMA-IR levels and in resistin concentrations occurred in rats fed the high-fat diet. L-arginine supplementation partially restored HOMA-IR to that of control levels, and did not influence resistin concentration.

Discussion

Obesity is a chronic and costly condition that is increasing rapidly throughout most of the world¹⁰. Obesity has become one of the major public health concerns of the twenty-first century¹¹. Substances that reduce body weight, and thus insulin resistance, and the other risk factors for cardiovascular diseases are continuously being sought.

A significant improvement in insulin sensitivity, not related to resistin, in rats fed the high-fat

Table III. Effects of dietary L-arginine on serum glucose (Glu) concentration, insulin concentration and nitric oxide (NO) in high fat diet rats.

Groups		Parameters		
		Glu (mg/dL)	Insulin (µg/L)	NO [µM]
CON	102.2 ± 13.0	65.2 ± 5.7 ^a	0.31 ± 0.05 ^a	8.08 ± 1.46 ^b
FAT	106.0 ± 13.5	73.6 ± 8.0 ^b	0.62 ± 0.07 ^c	4.568 ± 1.60 ^a
ARG	100.7 ± 10.9	67.2 ± 9.8 ^{ab}	0.46 ± 0.08 ^b	14.50 ± 2.60 ^c

Values are means ± SEM for twelve rats. Means in the same column with different superscripts are significantly different ($p < 0.05$); NO – nitric oxide; CON-control group, FAT-group with high fat diet, ARG-group with high fat diet and L-arginine.

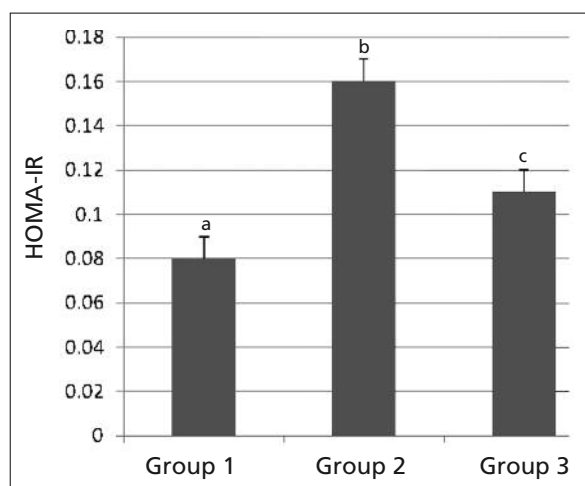


Figure 1. Effects of dietary L-arginine on insulin resistance index. Group 1-control group, group (CON), 2-group with high fat diet (FAT), group 3-group with high fat diet and L-arginine (ARG); a, b, c: Significant differences between groups.

diet treated with L-arginine is a new finding demonstrated in our study. Our research has confirmed increased insulin resistance in rats fed the high-fat diet. HOMA-IR was significantly higher than in the control group. Similar correlations have been shown by other authors^{12,13}. The evidence confirms the independent contribution of hyperinsulinemia and insulin resistance in the development of cardiovascular complications^{14,15}.

Resistin has been found to be an *in vitro* antagonist of insulin in human preadipocytes¹⁶⁻¹⁸. The results of our study are consistent with previous observations that have shown higher concentrations of resistin in obese rats¹⁹. We found significantly higher concentrations of resistin than in the control. The significant role of resistin in the pathogenesis of insulin resistance has been shown in animal models and in humans^{20,21}. Animal models confirm the influence of resistin on impaired glucose uptake, leading to balance disturbances. The group of obese rats was characterized by small activation of AMP kinase in the liver and muscles²¹ which could lead to insulin resistance.

Human hepatic cells overexpressing resistin have been found to show impaired glucose uptake and glycogen synthesis²². The correlation with insulin resistance was definitely confirmed in 2008 by Rabe et al²³.

The potential influence of L-arginine on insulin resistance is under discussion. The results of several studies are not uniform and are rather

inconclusive²⁴⁻²⁷. The present findings demonstrate a beneficial impact of L-arginine supplementation on insulin resistance in rats fed a high-fat diet. In our study, HOMA-IR significantly decreased in rats treated with L-arginine, although it still remained higher than in the control group (Figure 1). L-arginine supplementation increases lipolysis and inhibits lipogenesis by modulating the expression and function of key enzymes involved in antioxidative responses and fat metabolism in insulin-sensitive tissue. Nitric oxide (NO), which is synthesized from L-arginine by NO synthase (NOS), participates in multiple cell-signaling pathways²⁸. The results presented in Table III demonstrate significant decreases in NO concentrations in rats fed the high-fat diet. L-arginine supplementation protected significantly against increased NO concentration.

In view of the significant progress of L-arginine research, the objectives of this report were to highlight recent major findings from animal models and human subjects regarding the anti-obesity effects of L-arginine^{29,30}. One such anti-obesity effect was observed by Flu et al³¹, beginning at week four of supplementation, when the treated rats began to lose white fat mass and the control group did not. At the end of a ten-week period of supplementation, epididymal and retroperitoneal fat weights in the L-arginine treated rats were 28% lower than in the case of the controls. In our study, the absolute and relative masses of the visceral fat (as a percentage of body mass) was significantly higher in rats fed the high-fat diet than in the control. In rats on the high-fat diet supplemented with L-arginine, tendency for visceral fat to decrease was observed.

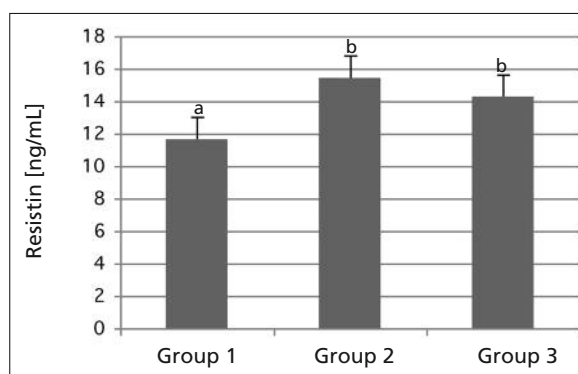


Figure 2. Effects of dietary L-arginine on serum resistin. Group 1-control group (CON), group 2-group with high fat diet (FAT), group 3-group with high fat diet and L-arginine (ARG); a, b: Significant differences between groups.

The results of both animal and human studies indicate that L-arginine supplementation may be a novel therapy for obesity and metabolic syndrome, acting via decreased plasma levels of glucose, increased levels of NO, and decreased insulin resistance.

The results obtained in our study were insufficient to determine the direct mechanism responsible for the favorable effect of L-arginine supplementation on insulin resistance. It cannot be excluded that the increase in insulin sensitivity could be related to resistin concentrations. However, we did find that the increase in insulin sensitivity was unrelated to resistin activity. The lack of changes in resistin concentration, though potentially attractive, excluded its potential role in insulin resistance alteration in rats fed the high-fat diet with L-arginine.

Conclusions

We showed that L-arginine supplementation improves insulin sensitivity in rats fed a high-fat diet independently of resistin activity, the concentration of which remained unchanged.

Conflict of Interest

The Authors declare that there are no conflicts of interest.

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