Regulation of Hyperglycemia and Dyslipidemia by Exogenous L-Arginine in Streptozotocin-induced Diabetic Rats

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Abstract: L-arginine is a conditionally essential amino acid in human diet that serves as the substrate for nitric oxide synthases (NOS) enzymes that generate NO, a key chemical involved in normal endothelial function. The study evaluate role of L-arginine in improvement of insulin resistance and lipid profile in diabetic rats. Seventy five male albino rats weighting 180-200 g were divided into five groups including; Control group, L-arginine group, Diabetic group, Treated group and Prophylactic group. Fasting blood samples were collected from all groups for determination of fasting blood glucose, insulin, insulin resistance, HDL, LDL, cholesterol and triglyceride. The results showed that groups received L-arginine have a significant increase in insulin and HDL. While they showed significant decrease in glucose, insulin resistance LDL, cholesterol and triglyceride. In conclusion L-arginine may be a novel nutrient, which has important implications for the prevention and treatment of diabetic patients.

Key words: Diabetes Mellitus • L-Arginine • Endothelial Function And Nitric Oxide

INTRODUCTION

Diabetes mellitus is a complex of metabolic disease characterized by hyperglycemia, diminished insulin production, impaired insulin action, or a combination of both resulting in the inability of glucose to be transported from the blood stream into the tissues, which in turn results in high blood glucose levels and excretion of glucose in the urine [1].

Diabetes is associated with reduced plasma concentrations of arginine. Thus, dietary supplementation of L-arginine could be beneficial for the treatment of endothelial dysfunction in diabetic patients. L-arginine supplementation normalized the endothelium-dependent relaxation in diabetic aorta by enhancing NO availability and restoring the acetylcholine-stimulated cGMP generation [2].

In the diabetic state, it is well known that oxidative stress is increased due to excessive production of oxygen free radicals and impaired antioxidant defense mechanisms. Increasing arginine supply to diabetic rats improved vascular reactivity, reduced blood pressure and normalized lipid peroxidation. Further, concentrations of malondialdehyde, a product of lipid peroxidation, may be reduced by arginine in diabetic patients and diabetic rats [3].

L-arginine is engaged in several metabolic pathways within the human body. It serves as a precursor for the synthesis not only of proteins but also of urea, polyamines, proline, glutamate, creatine and agmatine. L-arginine is an essential component of the urea cycle, the only pathway in mammals that allows the elimination of toxic ammonia from the body. Ornithine, the by-product of this reaction, is a precursor for the synthesis of polyamines, molecules essential for cell proliferation and differentiation [4]. L-arginine is also required for the synthesis of creatine, an essential energy source for muscle contraction. Agmatine, which has a clonidine-like action on blood pressure, is also formed from L-arginine, though its physiological function is not yet fully understood. However, current interest in L-arginine is focused mainly on its close relationship with the
important signal molecule nitric oxide. L-Arginine is the only substrate in the biosynthesis of NO, which plays critical roles in diverse physiological processes in the human body including neurotransmission, vasorelaxation, cytotoxicity and immunity [4].

L-arginine is reported to have beneficial effects on several complications including pulmonary hypertension, type-1 diabetes, β cell neogenesis, insulin sensitivity and improvement of endothelial function and reduction of fat mass in diabetic rats [5]. This work aims to evaluate L-arginine supplementations will improve insulin resistance and serum lipid levels in streptozotocin induced diabetic rats.

**MATERIALS AND METHODS**

**Material:** L-arginine and streptozotocin were purchased from Sigma Aldrich Medical Company St.Louis USA.

**Experimental Animals:** Male albino rats (Sprague Dawely strain) weighting 180-200 g were obtained from the animal house of National Research Center, Giza, Egypt. The animals were housed in individual suspended stainless steel cages in a controlled environment (22-25°C) and 12 hour light, 12 hour dark with food and water freely available.

**Methods**

**Induction of Diabetes:** Streptozotocin (STZ) was dissolved in 50 mM sodium citrate (pH 4.5) solution containing 150 mM NaCl. The final concentration of the injectable solution was containing (6.0 mg/100g body weight) was subcutaneously administrated in rats; fasting blood sugar was estimated after 3 days to confirm the induction of diabetes mellitus according to Uchiyama and Yamaguchi [6].

**Experimental Design:** Seventy five male albino rats were divided into five groups (15 rats each).

**Group I:** (Control group): healthy rats.

**Group II:** (L-arginine group): healthy rats received (10 mM L-arginine/Kg b.w./day orally).

**Group III:** (Diabetic group): diabetic rats.

**Group IV:** (Treated group): diabetic rats received (10 mM L-arginine /Kg b.w./day orally)

**Group V:** (Prophylactic group): healthy rats received L-arginine before and after induction of diabetes (10 mM L-arginine /Kg b.w./day orally) according to Méndez and Balderas [7].

After 8 weeks, animals were kept fasting for 12 hours before blood sampling, blood was withdrawn from the retro-orbital venous plexus of the eye using a capillary tube, blood collected in tubes contain sodium florid for blood glucose and insulin estimation. The remaining part of blood then left to clot and centrifuge at 3000 r.p.m. using cooling centrifuge for 15 minutes, serum was separated and divided into aliquots and stored at -20°C until assayed.

**Biochemical Assay:** Fasting glucose in serum was performed according to the method of Passing and Bablok [8] the kit was supplied by Biocon Diagnostic, Germany. Serum insulin was performed according to Judzewitsch et al. [9] the kit was provided from DRG, USA. Insulin resistance was calculated from the equation according to Mathews et al. [10].

Insulin resistance = fasting glucose (mg dl⁻¹) x fasting insulin (µIU ml⁻¹)/405.

Serum HDL-cholesterol was performed according to the method of Lopez-Virella et al. [11], the kit was supplied from Biocon Diagnostic, Germany. Serum LDL-cholesterol was calculated from equation developed by Friedewald et al. [12]. Serum triglycerides were determined using Kit from Centronic, Germany, according to Fossati [13]. Cholesterol in serum was performed according to the method of Allain et al. [14].The kit was supplied from Biocon Diagnostic, Germany.

**Statistical Analysis:** Statistical analysis using SPSS (Statistical package for social science) version 12, software package for data analysis was done [15].

The quantitative data were presented in the form of mean and standard error (SE) and the following tests were used:

**Test of Significance:** One way ANOVA was used to compare between the means. P value < 0.05 is considered to be significant.

**Correlation Coefficient:** Pearson's correlation coefficient was done between each two variables to study the relation between them.
RESULTS

In the present study, fasting blood glucose and insulin resistance levels were significantly increased in diabetic group compared to control group, while this value was improved by L-arginine administration in treated groups compared to diabetic group. The insulin level was significantly decreased in diabetic group compared to control group while this value increased by L-arginine administration in treated groups compared to diabetic group (Table 1).

The LDL, cholesterol and triglycerides levels were elevated in diabetic group compared to control group, while these values decreased by L-arginine administration in treated groups compared to diabetic group. The HDL level was significantly decreased in diabetic group compared to control group while this value increased by L-arginine administration in treated groups compared to diabetic group as shown (Table 2).

In the present study, Insulin resistance is directly proportional with glucose, LDL, cholesterol and triglyceride but it inversely proportional with insulin and HDL.

Pearson's correlation was calculated with insulin resistance and the other evaluated parameters. It reveals a negative correlation with insulin and HDL as shown in Figures 2, 3, however a positive correlation was demonstrated with glucose, LDL, cholesterol and triglyceride as presented in Figures 1, 4, 5, 6.
Table 1: Blood glucose, insulin and insulin resistance levels in different groups.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Control</th>
<th>L-arginine</th>
<th>Diabetic</th>
<th>Prophylactic</th>
<th>Treated</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glucose (mg/dl)</td>
<td>Mean ± S.E</td>
<td>79.7±1.1</td>
<td>79.2±1.4</td>
<td>243.2±2.7</td>
<td>180.3±1.6</td>
</tr>
<tr>
<td>Insulin (µIU/ml)</td>
<td>Mean ± S.E</td>
<td>11.7±0.4</td>
<td>11.4±0.4</td>
<td>8.5±0.2</td>
<td>9.5±0.3</td>
</tr>
<tr>
<td>Insulin resistance (mgdL⁻¹ µIU mL⁻¹)</td>
<td>Mean ± S.E</td>
<td>2.3±0.1</td>
<td>2.2±0.1</td>
<td>5.1±0.9</td>
<td>4.2±0.1</td>
</tr>
</tbody>
</table>

Significant p value < 0.05

a = significant difference compared to control group
b = significant difference compared to diabetic group

Table 2: Serum Lipid profile in different studied groups

<table>
<thead>
<tr>
<th>Parameters</th>
<th>HDL- cholesterol (mg/dl)</th>
<th>LDL- cholesterol (mg/dl)</th>
<th>Cholesterol (mg/dl)</th>
<th>Triglyceride (mg/dl)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>Mean ± S.E</td>
<td>55.7±1.3</td>
<td>55.3±0.9</td>
<td>73.5±2.9</td>
</tr>
<tr>
<td>L-arginine</td>
<td>Mean ± S.E</td>
<td>59.8±1.5</td>
<td>52.8±1.0</td>
<td>70.2±2.3</td>
</tr>
<tr>
<td>Diabetic</td>
<td>Mean ± S.E</td>
<td>39.0±1.4</td>
<td>119.9±1.0</td>
<td>150.7±3.2</td>
</tr>
<tr>
<td>Prophylactic</td>
<td>Mean ± S.E</td>
<td>49.4±1.0</td>
<td>70.2±0.9</td>
<td>107.3±3.9</td>
</tr>
<tr>
<td>Treated</td>
<td>Mean ± S.E</td>
<td>45.5±1.0</td>
<td>80.9±1.1</td>
<td>120.8±2.8</td>
</tr>
</tbody>
</table>

Significant p value < 0.05

a = significant difference compared to control group
b = significant difference compared to diabetic group

**DISCUSSION**

L-arginine is a basic natural amino acid. It is engaged in several metabolic pathways within the human body. It serves as a precursor for the synthesis not only of proteins but also of urea, polyamines, proline, glutamate, creatine and agmatine [4].

In the present study, the elevation in the serum glucose level and decline in serum insulin level of diabetic group may be attributed to the specific destruction of β-cells by STZ which produces the hormone insulin for normal glucose homeostasis [16,17].
Insulin enables the cells to absorb glucose from the blood and also helps in the utilization of the glucose in the cells by glycolysis, tricarboxylic acid cycle, hexose monophosphate shunt and glycogenesis. In STZ induced diabetes, cells fail to produce insulin which causes excess glucose accumulation in the blood instead of being utilized or stored. The decline in the mean insulin values observed in the present study has also been reported by many earlier workers [18,19].

In the present study, it was found that the treated groups received L-arginine (Prophylactic and treated groups) showed significant decrease in glucose and insulin resistance levels as compared to diabetic group and increased significantly in insulin compared to diabetic group these results were in agreement with Young et al. [20], Flynn et al. [21] and Salt et al.[22]. Also this group showed significantly increase in glucose and insulin resistance compared to control group.

L-Arginine is known to stimulate the secretion of insulin from beta cells of the pancreas [21]. Dietary supplementation of L-arginine resulted in an increase in the plasma insulin levels in both STZ-induced diabetic and non-diabetic rats. Note that in the STZ-diabetic rat model, not all the ß-cells are destroyed and the remaining cells can secret a physiologically significant quantity of sufficient insulin to keep the animals alive for up to 2 months and also would promote net protein synthesis and glucose utilization in skeletal muscle. The available evidence suggests that the action of insulin and arginine involves the following mechanisms. First, both insulin and arginine stimulate NO production by endothelial cells, which would contribute to increase in blood flow and, therefore, glucose and amino acid uptake by skeletal muscle in vivo [22]. Second, NO itself stimulates glucose transport and oxidation by skeletal muscle [20]. Third, physiological concentrations of NO may inhibit muscle proteolysis [21].

In the present study, it was found that the group received L-arginine showed significant increased in HDL and also showed significant decrease in LDL, cholesterol and triglyceride compared to control group. These results were in agreement with Gad [4], Méndez and Balderas [7] and Méndez and Zarzoza [23] who stated that L-arginine could be explained not only by its possible participation as insulin secretagogue, but also, by antilipolytic action of polyamines formed from L-arginine. The effects of polyamines have been observed for spermidine and spermine, which enhance glucose oxidation and inhibit lipolysis by suppressing endogenous cyclic AMP levels in a manner similar to insulin in isolated rat fat cells.

In the present study, it was found that the diabetic group showed significant decreased in HDL and also showed significant increased in LDL, cholesterol and triglyceride compared to control group. These results were in agreement with the previous studies which suggest that lipoprotein abnormalities are higher in diabetics than in non-diabetics [24-26].

Veiraiah [27] suggested that hyperglycemia leads to an increase in LDL cholesterol by reducing the ability of the body to remove cholesterol. When blood sugars are too high, LDL cholesterol and the receptors for LDL in the liver become coated with sugar (Glycosylated), impairing...
the liver's ability to remove cholesterol from the bloodstream. In addition, hyperglycemia also leads to inhibition of lipoprotein lipase and further aggravating hyperlipidemia.

When diabetes is not under good control, there are high levels of glucose in the body, so, the conversion of glucose into glycogen increased and stored in the liver, when the liver becomes too saturated with glycogen, though, glucose is instead used to synthesized fatty acids that are released into the bloodstream; these fatty acids are used to produce triglycerides, which build up in fat cells and contribute to body fat [28].

Hyperlipidemia is a recognized complication of DM characterized by elevated levels of cholesterol, triglycerides and LDL. One of the major pathogenesis of lipid metabolism disturbances in diabetes is the increased mobilization of free fatty acids from adipose tissue and secondary elevation of free fatty acid level in the blood due to insulin deficiency or insulin resistance. The excessive lipolysis in diabetic adipose tissue may lead to increased free fatty acids in circulation which enter the liver and are esterified to form triglycerides. The fatty acid compositions of various tissues are altered in both experimental and human diabetes. The finding in the present study is in correlation with the findings of Krishna et al. [17] and Sharma et al. [29].

In the present study, it was found that the treated groups received L-arginine (Prophylactic and treated groups) decreased significantly in HDL and also showed significant increase in LDL, cholesterol and triglyceride compared to control group these results were in agreement with Méndez and Balderas [7]. Also, this group showed significant increase in HDL and significant decreased in LDL, cholesterol and triglyceride compared to diabetic group.

In this study, the tendency to normalization of lipid and lipoprotein, levels in diabetic rats treated with L-arginine could be explained according to Kawano et al. [30] who stated that its accelerated formation from L-arginine in the pancreas of diabetic rats could, together with spermidine and spermine, not only stimulate glucose uptake but also inhibit lipolysis in diabetic rats these results were in agreement with.

CONCLUSION

L-Arginine may be a novel nutrient, which improves insulin resistance and lipid metabolism. Also has an important implication for the prevention and treatment of diabetic patients.

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REFERENCES