Research Article

Exogenous L-Arginine attenuates asymmetrical dimethylarginine elevation in experimental diabetes

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ABSTRACT

Diabetes mellitus is characterized by impaired endothelial cell production of the vasodilator and anti platelet adhesion factor nitric oxide (NO) .This study aimed to evaluate the role of exogenous L-arginine in diabetic rats in context of regulation of Asymmetrical Dimethylarginine (ADMA) and NO levels. Seventy five male albino rats were used in this study and divided into five groups ; Group I (Control group): healthy rats received a vehicle , Group II (L-arginine group): healthy rats received L-arginine (10 mM /Kg b.w./day) orally for eight weeks, Group III (Diabetic group): diabetic rats received a vehicle, Group IV (Treated group): diabetic rats received L-arginine (10 mM /Kg b.w./day)orally for eight weeks, Group V (Prophylactic group): healthy rats received L-arginine (10 mM /Kg b.w./day) orally before(two weeks) and after (eight weeks) induction of diabetes. Fasting blood sugar, insulin, blood urea, serum arginase and nitric oxide were determined. ADMA was determined by HPLC; separation was achieved on reversed phase column (150 X 4.6 mm C18).Mobile phase was eluted by gradient method; flow-rate was 1.0 ml/min. The wavelengths of fluorescence detector were set at 338 and 425 nm. In diabetic rats, serum ADMA was significantly increased along with the reduction of NO; however, L-arginine supplementation significantly decreased serum ADMA and increased NO in both prophylactic and treated groups. In conclusion, supplementation of L-arginine is a potentially novel mean to prevent diabetes-associated endothelial dysfunction through the reduction of asymmetrical dimethylarginine and the elevation of nitric oxide.

Key words: diabetes mellitus, endothelial dysfunction, L-arginine, ADMA, nitric oxide.
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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 urine [1].This process is characterized by impaired endothelial cell production of the vasodilator and antiplatelet adhesion factor nitric oxide (NO) and decreased NO bioavailability [2].

Nitric oxide is a vasodilator and inhibitor of platelet aggregation, leucocytes migration, cellular adhesion and vascular smooth muscle proliferation [3, 4]. The main function of NO is to provide the vascular homeostasis. When the NO levels is decreased, endothelial homeostasis is impaired in the direction of vasoconstriction and endothelial dysfunction begins [5].

ADMA is synthesized and released by endothelial cells and present in human plasma in amounts that are sufficient to inhibit NO production [6, 7]. Thus, ADMA might be thought as a key molecule that contributes to endothelial dysfunction. Elevated plasma concentrations of ADMA are also present in hypercholesterolemic and hypertensive

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patients, in patients with chronic heart failure, and in other patient groups at high risk of developing cardiovascular disease.

It is clear that, in insulin release, several molecules are involved, such as glucose [8], L-arginine [9], and polyamines [10]. The action of L-arginine on insulin release has been demonstrated [8]. L-arginine is the precursor of nitric oxide which has relevant action on insulin release from pancreatic β cells in the presence of D-glucose [11].

From this point of view, we aimed to study the role of exogenous L-arginine in diabetic rats in context of regulation of ADMA and NO levels.

EXPERIMENTAL SECTION

Materials
Chemicals
Streptozotocin ,L-arginine , Asymmetric dimethylarginine (HPLC standard), O-Phthaldialdehyde (OPA), mercaptoethanol and boric acid were purchased from Sigma Aldrich Medical Company St.Louis USA.

Methanol (HPLC grade), Tetrahydrofuran (THF), sodium acetate, 5-sulfosalicylic acid (5-SSA) and N-(1-naphthyl) ethylenediamine were purchased from Merck (Merck, Germany).

Experimental animals
Male albino rats 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. The animals had free access to water and standard rodent chow diet. All animals received human care in compliance with guidelines of the Ethical Committee of National Research Centre, Egypt and followed the recommendations of the National Institutes of Health Guide for Care and Use of Laboratory Animals.

Methods
Induction of diabetes
Streptozotocin (STZ) was dissolved in 50 mM sodium citrate (pH 4.5) solution containing 150 mM NaCl. The solution containing (6.0 mg/100g body weight) was subcutaneously administrated in rats; fasting blood sugar was estimated after 3 days to confirm the development of diabetes mellitus [12].

Experimental design
Seventy five male albino rats were used in this study and divided into five groups (15 rats each) as follow:
Group I (Control group): healthy rats received a vehicle , Group II (L-arginine group): healthy rats received L-arginine (10 mM /Kg b.w./day)orally for eight weeks, Group III (Diabetic group): diabetic rats received a vehicle, Group IV (Treated group): diabetic rats received L-arginine (10 mM /Kg b.w./day)orally for eight weeks, Group V (Prophylactic group): healthy rats received L-arginine (10 mM /Kg b.w./day) orally before(two weeks) and after (eight weeks) induction of diabetes [13].

After the experimental period, animals were kept fasting for 12 hours before blood sampling. Blood was withdrawn from the retro-orbital venous plexus of the eye using capillary tubes and the blood of each rat was collected into two tubes; one contains sodium floride for blood glucose estimation and the other contains heparin for other biochemical parameters.

Biochemical assay:
Fasting blood sugar was determined using enzymatic colorimetric method. Centronic, Germany [14]. Plasma insulin level was estimated by ELISA using BioSoure INS-EASIA Kit [15].

Insulin resistance was calculated from the equation:

Insulin resistance = fasting glucose (mg dl^{-1}) X fasting insulin (μIU ml^{-1})/405 [16].

Serum nitrite/nitrate (NO) was measured using ELISA micro plate reader [17], while serum arginase and blood urea were determined by colorimetric method using spectro UV-vis double beam (UVD-3500), Labomed,Inc. [18, 19].

Determination of serum Asymmetric dimethylarginine by HPLC:
ADMA was determined by HPLC method modified from the method described previously [20],

Sample preparation
25 mg 5-sulfosalicylic acid (5-SSA) were added to 1 mL plasma , mix well and left in an ice-bath for 10 min.; the precipitated protein was removed by centrifugation at 2000 g for 10 min., the supernatant was filtered through a 0.2 μ m filter, then mix 10 µL of sample and 100 µL of OPA solution and left to react for 3 min. before injecting onto HPLC.

HPLC condition:
10 µl of sample-OPA were injected in HPLC; separation was achieved on reversed phase column (150 X 4.6 mm C18). The mobile phase consisted of sodium acetate buffer, methanol and THF (A, 82:17:1; B, 22:77:1, % v, respectively) and eluted by gradient method as described in table 1. Column temperature adjusted at 37°C and flow-rate was 1.0 ml/min. The wavelengths of fluorescence detector were set at 338 and 425 nm. A linear standard curve was constructed by plotting peak areas versus the corresponding concentrations. The concentration in samples was obtained from the curve.

Table (1): Gradient method for ADMA analysis

Time (min.)	Mobile phase A (% v)	Mobile phase B (% v)
0	100	0
5	90	10
15	60	40
28	25	75
32	0	100
35	100	0

Statistical analysis:
Results were expressed as mean ± standard error. Data were analyzed by independent sample t test (SPSS) version 15 followed by (LSD) test to compare significance between groups. Difference was considered significant when P value <0.05.

RESULTS AND DISCUSSION

Endothelial dysfunction is a major factor contributing to morbidity and mortality in diabetes mellitus**,** NO may be the most important factor responsible for diabetes associated cardiovascular complications. A deficiency of arginine could impair the endothelium-dependent relaxation. A marked decrease in plasma concentration of arginine in diabetic rats provides a basis for dietary supplementation of arginine as a potentially novel mean to prevent diabetes-associated endothelial dysfunction [21].

In the present study, the elevation in blood glucose level and decline in serum insulin level of diabetic group (table 2) may be attributed to the specific destruction of ß-cells by STZ [22], thus, in STZ induced diabetes, cells fail to produce insulin which causes excess glucose accumulation in the blood instead of being utilized or stored.

Table (2): Blood glucose, insulin and insulin resistance levels in different studied groups

Groups	Parameters	Glucose (mg/dl)	Insulin (µIU/ml)	Insulin resistance (mgdl[-1] µIU ml [-1])
Control	Mean ± S.E	79.7±1.1	11.7±0.4	2.3±0.1
L-arginine	Mean ± S.E	79.2±1.4[b]	11.4±0.4[b]	2.2±0.1[b]
Diabetic	Mean ± S.E	243.2±2.7 [a]	8.5±0.2 [a]	5.1±0.9 [a]
	% change [a]	205.1%	-27.4%	121.7%
Prophylactic	Mean ± S.E	180.3±1.6 [a,b]	9.5±0.3 [a,b]	4.2±0.1 [a,b]
	% change [a]	126.2%	-18.8%	82.6%
	% change [b]	-25.9%	11.8%	-17.6%
Treated	Mean ± S.E	203.5±1.7 [a,b]	9.0±0.3 [a]	4.5±0.1 [a,b]
	% change [a]	155.3%	-23.1%	95.7%
	% change [b]	-16.3%	5.9%	-11.8%

Significant p value ≤ 0.05
a = significant difference compared to control group
b = significant difference compared to diabetic group
%change [a]: percent of change from control group
%change [b]: percent of change from diabetic group

The elevation of ADMA in diabetic rats compared to those of control group in our study (table 3) may be attributed to the oxidative stress that decreased the activity of dimethylarginine dimethylaminohydrolase (DDAH) [23], the enzyme that degrades ADMA, and contributes to its elevation in the endothelial cells [24]. In addition,

hyperglycemic may be associated with the enzyme arginine methyltransferase, which synthesizes ADMA, because hyperglycemia-induced oxidative stress up-regulates the expression of arginine methyltransferases [25, 26].

Table (3): ADMA and nitric oxide levels in different studied groups

Groups	Parameters	ADMA (μmol/L)	NO (μmol/L)
Control	Mean ± S.E	0.40±0.1	133.5±1.6
L-arginine	Mean ± S.E	0.21±0.2[b]	168.3±1.4[b]
Diabetic	Mean ± S.E	1.01±0.1[a]	74.3±3.1[a]
	% change [a]	152.5%	-44.3%
Prophylactic	Mean ± S.E	0.45±0.1[a,b]	128.6±1.8[b]
	% change [a]	12.5%	-3.7%
	% change [b]	-55.4%	73.1%
Treated	Mean ± S.E	0.71±0.1[a,b]	104.3±1.9[a,b]
	% change [a]	77.5%	-21.9%
	% change [b]	-29.7%	40.4%

Significant p value ≤ 0.05
a = significant difference compared to control group
b = significant difference compared to diabetic group
%change [a]: percent of change from Control group
%change [b]: percent of change from Diabetic group

Moreover, hyperglycemia-induced oxidative stress is an important factor for the reduction of NO as was found in this study ; this may be due to the elevation of superoxide anion production from glucose autoxidation and accumulation of advanced glycation end product due to nonenzymatic cross-linking of proteins via oxidative stress which can reduce the bioavailability of NO and activation of the polyol pathway, that increases the use of nicotinamide adenine dinucleotide phosphate and the reduction of NO biosynthesis [27]. Blood urea and serum arginase were significantly increased in diabetic group compared to control, the percent of changes were 92.3 and 153.6% respectively in diabetic group from control (table 4). The elevation of blood urea and plasma arginase may be a contributing factor in the elevation of ADMA and the reduction of NO. In agreement, the previous studies indicated that, up regulation of arginase inhibits endothelial nitric oxide synthase (NOS) -mediated NO synthesis and may contribute to endothelial dysfunction in diabetes [28,29]**.**

Table (4): Blood urea and plasma Arginase levels in different groups

Groups	Parameters	Arginase (U/L)	Urea (mg/dl)
Control	Mean ± S.E	41.4±0.8	28.4±1.5
L-arginine	Mean ± S.E	36.9±1.0[b]	26.7±1.8[b]
Diabetic	Mean ± S.E	105.0±2.8[a]	54.6±1.1 [a]
	% change [a]	153.6%	92.3%
Prophylactic	Mean ± S.E	63.6±1.7[a,b]	40.4±1.6[a,b]
	% change [a]	53.6%	42.3%
	% change [b]	-39.4%	-26%
Treated	Mean ± S.E	82.8±1.5[a,b]	46.0±1.2[a,b]
	% change [a]	100%	62%
	% change [b]	-21.1%	-15.8%

Significant p value ≤ 0.05
a = significant difference compared to control group
b = significant difference compared to diabetic group
%change [a]: percent of change from Control group
%change [b]: percent of change from Diabetic group

L-arginine is a versatile amino acid in animal and human cells, serving as a precursor for the synthesis not only of proteins but also of nitric oxide, urea, glutamate, creatine, polyamines and other molecules involved in regulating cellular homeostasis [30].

L-arginine was supplemented to diabetic rats in order to improve the ability of the endothelium to counteract offenses to the vascular wall by circulating blood cell vasoconstrictors, and oxygen-derived free radicals.

Supplementation of L-arginine in this study increased insulin level in treated and prophylactic groups by 5.9 % and 11.8% respectively from the diabetic group (table 2). Thus, L-arginine administration induces increased insulin secretion from β cells; insulin was shown to have vasodilatory effects, as well as antiinflammatory properties [31]. The insulin signaling cascade mediates insulin action in insulin responsive tissues and has been shown to stimulate NO generation in vascular smooth muscle and skeletal muscle.

In addition, L-arginine administration increases NO production, which induces secondary vasodilation. The resultant increase in blood flow enhances glucose uptake. It was found that, NO stimulates glucose transport in isolated skeletal muscles by increasing levels of glucose transporter 4 (GLUT4) at the cell surface. As a result, insulin resistance improved as was found in our study [32].

The reduction of plasma arginase and blood urea in both treated and prophylactic groups in this study (table 4) was confirmed by Kashyap et al. [33], who indicated that ,during the insulin clamp, the blood glucose concentration is allowed to decline to baseline, thereby removing the stimulatory effect of hyperglycemia on plasma arginase activity. In addition to restoration to normal of some other metabolic factor(s) (i.e., elevated plasma free fatty acid levels) resulting in reduced plasma arginase activity.
We concluded that: supplementation of L- arginine is a potentially novel mean to prevent diabetes-associated endothelial dysfunction through the reduction of asymmetrical dimethylarginine and the elevation of nitric oxide.

Acknowledgement

<div align="center">REFERENCES</div>

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