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Full length Research Paper

Effect of L-arginine on selected markers of metabolic syndrome related to oxidative stress, glucose metabolism and nitric oxide synthesis in female Wistar albino rats

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Female gender is an independent risk factor for the development of metabolic syndrome (MES) (a cluster of features indicating metabolic disorders), that is associated with oxidative stress, insulin resistance and a significant reduction in nitric oxide (NO), a major metabolite of L-arginine (ARG). This study aimed to ascertain the effect of ARG on selected markers of MES related to glucose metabolism, oxidative stress and nitric oxide synthesis, in female Wistar albino rats. Two groups of rats were given 3 ml/kg body weight (bw) of distilled water, DW and 60 mg/kg bw of ARG, respectively as control and treated groups. Exposing ARG to female rats evoked a significant reduction ($p<0.01$) in fasting blood glucose (20.13±2.35 mg/ 100 ml), malondialdehyde (MDA) (6.42±0.29 mg/ 100 ml) and uric acid (7.96±0.23 mg/ 100 ml) concentrations but a significant increase ($p<0.01$) in calcium ion (Ca^{2+}) (77.04±1.19 mmol/l) concentration in the rats' serum. MDA, uric acid and fasting blood glucose concentrations correlated positively ($\rho = 0.01$), but negatively ($\rho = 0.01$) with Ca^{2+} concentration, indicating association of enhanced antioxidant activity and glucose metabolism with elevated nitric oxide synthesis. Thus, ARG elicited hypoglycaemic and antioxidant activity in the female rats probably *via* enhanced nitric oxide synthesis.

Keywords: Metabolic syndrome, nitric oxide, malondialdehyde, uric acid, calcium ion, insulin resistance, antioxidant, glucose metabolism.

INTRODUCTION

Metabolic syndrome (MES) is a cluster of cardiovascular risk factors that is characterized by obesity, atherogenic dyslipidemia, and hypertension (Deedwania and Gupta, 2006; Gallagher *et al.,* 2010). It is not a disease entity. MES is a cluster of medical disorders in an individual that could predispose animals to further health challenges. These include type 2 diabetes mellitus (Wilson *et al.,* 2005; Azhar, 2010), cancer (Siddiqui, 2011; Pelucchi *et al.,* 2010; Rosato *et al.,* 2011; Capasso *et al.,* 2011) and obstructive sleep apnea (Mugnai, 2010).

Therefore, MES could contribute to significant premature mortality (Kozumplik *et al.,* 2010). Its scourge is pandemic, and the pattern cuts across every age, location and gender hence is of global public health concern (Gotto *et al.,* 2006; Grundy, 2008). However, MES is more prevalent in developed nations, urban areas, female gender and with increasing age than otherwise (Mangat *et al.,* 2010; Kilic *et al.,* 2010).

The association of a significant reduction in NO with the pathophysiology of MES (Garlichs, *et al.,* 2000) suggested that L-arginine (ARG), a major precursor in the synthesis of NO (Moncada *et al.,* 1991), may improve MES in animals. Insulin, the key hormone in the regulation of glucose homeostasis, is the main pathogenic factor of type 2 diabetes mellitus and the main feature of obesity which are major components of MES (Lann and LeRoith, 2007). In a normal condition, insulin stimulates glucose transport, uptake and utilization by cells, inhibits hepatic gluconeogenesis, and decreases adipose-tissue lipolysis and hepatic production of very-low-density lipoproteins (Gallagher *et al.,* 2010).

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ARG could enhance the production and release of insulin (Harold, 2006). It could reduce hypertension (Alexander *et al.*, 2004), probably by its potential to induce vasodilatation (Rang *et al.*, 2003). Previous studies revealed that ARG administration improved endothelium function in animal models of diabetes mellitus (Pieper *et al.*, 1997), and hypercholesterolaemia (Kawano *et al.*, 2002). Furthermore, ARG increased polyamines production (Mendez and Arreola, 1992) and decreased oxidative stress in diabetic rat models (El-Missiry, *et al.,* 2004).

The present study aimed to ascertain the effect of ARG on selected markers of MES related to glucose metabolism, oxidative stress and nitric oxide synthesis. This study sought to achieve the stated aim through the specific objectives of studying the effect of ARG on the concentration of uric acid, malondialdehyde calcium ion and fasting blood glucose, using female rats as model.

MATERIALS AND METHODS

Chemicals and reagents

The chemicals used in this study were of analytical grade and were products of reputable companies based in Europe and America.

Concentration determination/justification

The test concentration, ARG (60 mg/kg body weight, bw) was calculated and adjusted based on the WHO reported daily ARG oral intake (Marshal, 1994) and the concentration used in earlier studies (Olney, 1969; Alexander *et al.,* 2004; Egbuonu *et al.,* 2010a,b,c).

Equipment/instruments

The Department of Biochemistry, University of Nigeria, Nsukka and Bishop Shanahan Memorial Hospital, Nsukka provided standard equipment and instruments used in the course of this study. These include the following, Bench centrifuge (Wisterfuge model 1384), UV/Vis Spectrophotometer (JENWAY 6205), Spectrophotometer (NOVASPEC LKB Biochrome, model 4049, Germany), Accu-Chek® glucometer (Roche Diagnostic, GmbH, Germany).

Experimental design

Animals and treatment

Procurement of female weanling Wistar rats used in this study was from the animal house of the Faculty of Biological Sciences University of Nigeria, Nsukka. The i.e, rats weighed 60-80g.The animal study was according to International guidelines for the care and use of lab animals in Biomedical Research (APS, 2002).

The rats acclimatized for a week and immediately thereafter were randomized into two groups with sample size of eight rats each. Group B rats were exposed to ARG (60 mg/kg bw) whereas Group A rats were given distilled water (DW) (3 ml/kg bw).Exposure route was by oral intubation, which was consecutive for 28 days.

The rats, housed in a well-ventilated stainless steel cages at room temperature (28±2^{o}C) and tropical humid condition, were maintained under standard natural photoperiodic condition of twelve hours of light alternating with twelve hours of darkness. In compliance with the ethical guidelines for treating laboratory animals, the rats were allowed unrestricted access to tap water and standard rat chow (Grand Cereals and Oil Mills Limited, Jos, Nigeria) for the experimental period.

Sample collection and preparation

The animals were fasted overnight before sacrifice after 28 days. Collection of the respective blood samples of animals was by ophthalmic venous plexus or retro orbital sinus venipuncture. This involved inserting a sterile capillary tube into the medial canthus of the eye of the rat to puncture the retro-bulbar plexus resulting in out flow of blood into clean glass sample tubes: (i) non-anticoagulated tube for serum tests and (ii) anticoagulated tubes for whole blood test (fasting blood glucose concentration).

Centrifugation of clotted blood at 3000 rpm for 10 minutes yielded the serum. Thereafter, the serum (aspirated separately into stoppered polystyrene tubes) was stored in a deep freezer for subsequent use in determining the selected serum biochemical markers of metabolic syndrome (MDA, uric acid and calcium ion concentrations).

Parameters determined

Fasting blood glucose

Measurement of the fasting blood glucose was on the whole blood samples collected from each rat. The measurement was with Accu-Chek® glucometre (Roche Diagnostic, Germany). Calibration of the glucometre was with the standard test strips supplied by the manufacturer.

Serum malondialdehyde (MDA) concentration

The malondialdehyde concentration determination in

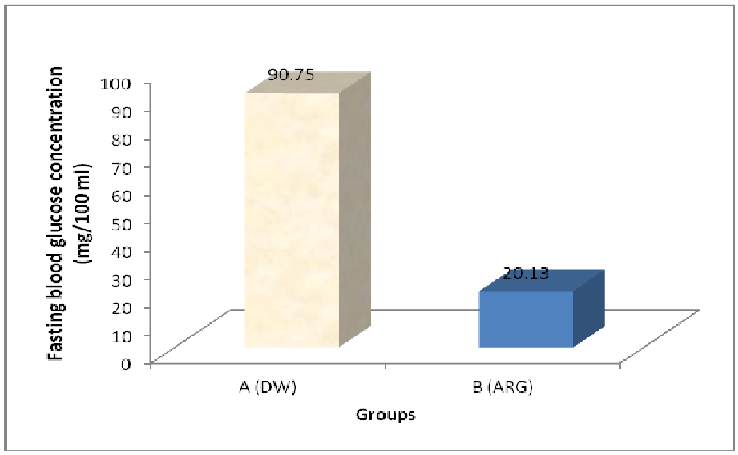

Figure I. Influence of DW and ARG on fasting blood glucose concentration of rats

serum was by the method of Wallin *et al.* (1993). The method was based on the principle that malondialdehyde, a thiobarbituric acid reacting substances (TBARS), reacts with thiobarbituric acid (TBA) to give a red or pink colour which absorbs maximally at 532 nm.

Serum uric acid

The uric acid concentration determination in serum was by the Caraway method as described (Ochei and Kolhatkar, 2008). The method was based on the principle that uric acid is readily oxidized to allantoin and so can function as a reducing agent in many chemical reactions forming colored product that is measureable at 650-700 nm.

Serum calcium ion (Ca^{2+}) concentration

The measurement of calcium ion was by a standard method described earlier (Ochei and Kolhatkar, 2008). This method based on the principle of colorimetric estimation at 612 nm of deep blue color formed by calcium ions complex with methylthymmol under acidic conditions.

Statistical Analysis

Analysis of data to determine the significant differences in means was by Student's t-test, using the Statistical Package for the Social Sciences (SPSS) for Windows version 16.0 (SPSS Inc., Chicago, IL., USA). Results were expressed as mean and standard deviation (Mean±SD) of eight rats per group at significance level of $p<0.01$. Furthermore, correlation of the results for possible association among the studied parameters was by Pearson's bivarate method ($\rho = 0.01$).

RESULTS

Fasting blood glucose concentration

Administration of ARG to rats reduced the fasting blood glucose concentration of the rats (20.13±2.35 mg/100 ml) in contrast with the control animals (90.75±3.28 mg/100 ml). This observation represented a decrease of 77.82 % and was statistically significant ($p<0.01$) (Figure I).

Serum malondialdehyde (MDA) concentration

The serum MDA concentration decreased in the ARG-treated rats (6.42±0.29 mg/100 ml) compared with the control rats (14.57±0.46 mg/100 ml). This is a decrease of 55.94 % relative to the control and was significant at 0.01 probability level (Figure II).

Serum uric acid concentration

As shown in Figure III, the serum uric acid concentration of rats exposed to high concentration of ARG (7.96±0.23 mg/100 ml) significantly decreased ($p<0.01$) in contrast to the control rats (10.37±0.37 mg/100 ml). This represents a decrease of 23.24 % relative to the control.

Serum calcium ion (Ca^{2+}) concentration

The results of this study presented in Figure IV reveal that the serum calcium ion in the ARG-treated rats

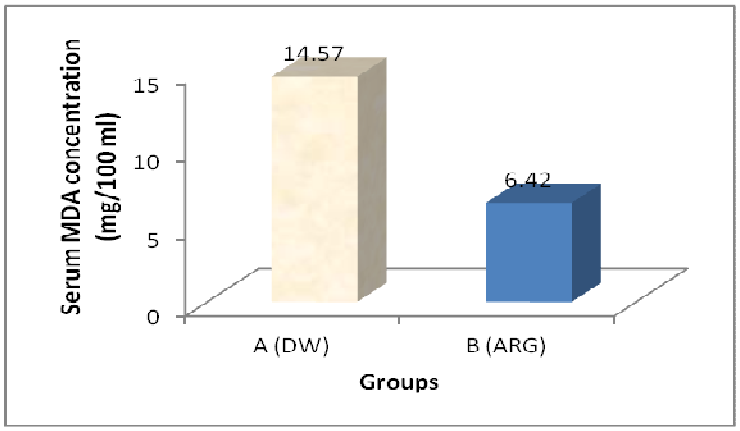

Figure II. Influence of DW and ARG on serum MDA concentration of rats

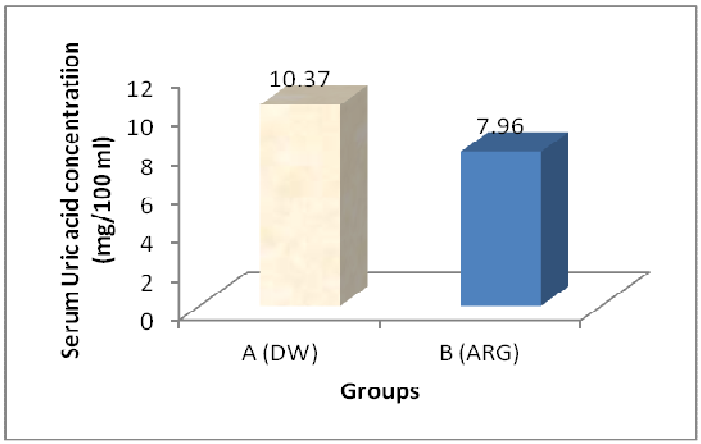

Figure III. Effect of DW and ARG on serum uric acid concentration of rats

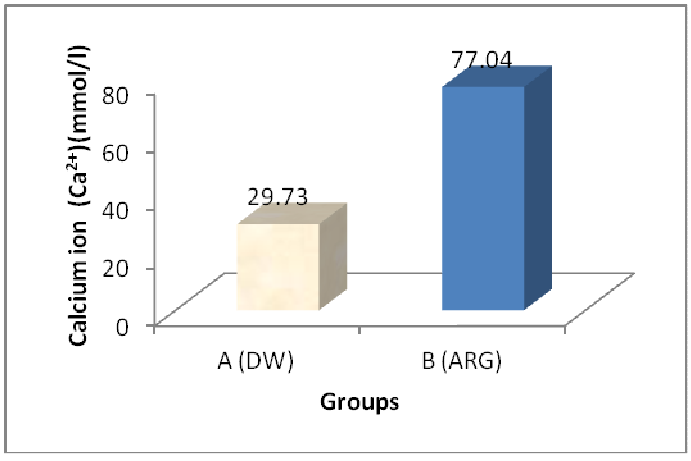

Figure IV. Influence of DW and ARG on serum calcium ion (Ca^{2+}) concentration of rats

Table I. Spread sheet showing Pearson two-tailed correlation analysis of MDA, Calcium ion, Uric acid and Fasting blood glucose concentrations

		MDA Concentration	CALCIUM ION Concentration	URIC ACID Concentration	FASTING BLOOD GLUCOSE Concentration
MDA Concentration	Pearson Correlation	1	-.991**	.967**	.993**
	Sig. (2-tailed)		.000	.000	.000
	N	16	16	16	16
CALCIUM ION Concentration	Pearson Correlation	-.991**	1	-.965**	-.997**
	Sig. (2-tailed)	.000		.000	.000
	N	16	16	16	16
URIC ACID Concentration	Pearson Correlation	.967**	-.965**	1	.958**
	Sig. (2-tailed)	.000	.000		.000
	N	16	16	16	16
FASTING BLOOD GLUCOSE Concentration	Pearson Correlation	.993**	-.997**	.958**	1
	Sig. (2-tailed)	.000	.000	.000	
	N	16	16	16	16

**. Correlation is significant at the 0.01 level (2- tailed).

(77.04±1.19 mmol/l) increased above that of the control rats (29.73±1.79 mmol/l). The observed increase of 159.13 % was significant at 0.01 probability level.

Correlation of the paramaters

As presented in Table I, calcium ion concentration correlated negatively (ρ = 0.01) with MDA, uric acid and fasting blood glucose concentrations. However, MDA, uric acid and fasting blood glucose concentrations correlated positively (ρ = 0.01).

DISCUSSION

The female gender is an independent risk factor for the development of MES (Kilic *et al.,* 2010; Ravikiran *et al*., 2010).The syndrome predisposes animals to further health risks (Lerman-Garber, *et al.,* 2010; Szosland, 2010; De Flines and Scheen, 2010; Brietzke 2010; Zambon *et al.,* 2010). The association of pathogenesis of MES with insulin resistance (Ezeanyika and Egbuonu, 2011) and significant reduction in NO availability (Garlichs *et al.,* 2000) suggested that ARG, a precursor in the synthesis of NO (Moncada *et al.,* 1991) and insulin (Harlod, 2006), may improve MES in animals. Therefore, the aim of this study is to ascertain the effect of ARG on selected markers of MES related to glucose metabolism, oxidative stress and nitric oxide synthesis, using female albino rats as model.

Elevated fasting blood glucose predicted MES in animals (Tang *et al.,* 2010). Consistent with previous report (Vasilijevi´c *et al.,* 2007), exposing ARG to the rats reduced their fasting blood glucose concentration, indicating enhanced glucose metabolism that could protect against diabetes and obesity which are major components and risk factors of MES. The result was as expected since ARG enhanced the synthesis of insulin (Harold, 2006) which perhaps facilitated efficient glucose transport, uptake and utilization by cells resulting to reduced blood glucose concentration as observed in this study. Thus, ARG may improve MES related to hyperglycaemia in the female rats.

Calcium ion concentration in serum had a direct realationship with NO synthesis (Garlichs *et al.,* 2000). Thus, in this study, NO synthesis was assessed *in vivo* by measuring the calcium ion (Ca^{2+}) concentration in serum. ARG is the major precursor in the synthesis of NO (Moncada *et al*., 1991), thus, as would be expected, ARG ingestion by rats increased ($p<0.01$) the serum Ca^{2+} concentration, implying enhanced NO synthesis. The pathophysiology of MES is associated with a significant reduction in NO by way of endothelial dysfunction (Ezeanyika and Egbuonu, 2011), a situation of impaired function of the endothelium in maintaining balance between vascular dilatation and vascular

constriction, where increased vascular constriction results to high blood pressure. Thus ARG may be useful in the management of MES related to reduced NO synthesis perhaps *via* enhanced NO synthesis.

Stress was a factor in the development of insulin resistance *via* increased oxidative stress (Rayssiguier *et al.,* 2010). It has been reported to increase MES components, including type 2 diabetes (Bonora, 2006; Kyrou and Tsigos, 2007). In particular, increased oxidative stress was associated with physiological dysfunctions in animals (Mezzetti *et al.,* 2000; Rayssiguier *et al.,* 2010), including MES (Holvoet, 2008). Consistent with earlier reports that arginine attenuated oxidative stress in animal models (Kawano *et al.,* 2002; El-missiry *et al.,* 2004; Dasgupta, *et al.,* 2006), the serum MDA concentration in the present study decreased ($p<0.01$) in the ARG-treated group relative to the control. This suggests antioxidant or free radical scavenging potential of ARG in the female rats.

Furthermore, uric acid, a product of purine metabolism, and a strong reducing agent with over half the antioxidant capacity of blood plasma in humans (Baillie *et al.,* 2005) is excreted *via* the kidney thus, may serve as an antioxidant and renal function markers. In line with the present study on MDA, ARG ingestion by rats decreased ($p<0.01$) uric acid concentration in serum relative to the control, further suggesting antioxidant or free radical scavenging potential of ARG and absence of varied pathologies in the female rats. Uric acid is a marker for varied biochemical disorders. For instance, elevated serum uric acid concentration indicated renal insufficiency (Ochie and Kolhatkar, 2008; Ghazavi *et al.,* 2008; See *et al*., 2011), obesity (Gil-Campos, *et al.,* 2009), cardiovascular diseases (Cai *et al.,* 2009; Borges *et al*., 2010; Kuo *et al*., 2010; Saito *et al.,* 2010) and MES in females (Ogbera and Azenabor, 2010).

The observation on these markers of oxidative stress is quite remarkable because NO, the major metabolite of ARG, that is associated with the pathophysiology of MES (Garlichs *et al.,* 2000), is an inorganic free radical that could, depending on concentration, exert both antioxidant and prooxidant activities in animals. Thus, it appears that ARG as used in this study enhanced the synthesis of optimal concentration of NO, warranting the expression of its antioxidant effect in the rats.

Generally, Pearson's correlation analysis showed that MDA, uric acid and fasting blood glucose concentrations correlated positively ($\rho = 0.01$), but negatively ($\rho = 0.01$) with Ca^{2+} concentration, indicating association of enhanced antioxidant activity and glucose metabolism with elevated nitric oxide synthesis.

In conclusion, this study suggests that ARG elicited hypoglycaemic and antioxidant activity in the female rats probably *via* enhanced nitric oxide synthesis. Results of this study highlighted possible effect of L-arginine on selected markers of metabolic syndrome related to glucose metabolism, oxidative stress and nitric oxide synthesis in female Wistar rats. Harnessing insights gained from this study in dietary food choice or in nutraceutical or pharmafood formulations may improve MES in especially female animals.

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