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Effect of L-Arginine on Some Biochemical Markers of Metabolic Syndrome Associated with Brain Function in Female Wistar Rats

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Abstract: Metabolic syndrome (Mes) was associated with insulin resistance and endothelial dysfunction. In particular, endothelial dysfunction was associated with a significant reduction in nitric oxide, a metabolite of L-arginine (Arg). Insulin resistance occurs following the failure of insulin to maintain glucose balance or regulate appetite via signaling in the brain. Thus, this study investigated the influence of Arg on some biochemical markers of Mes associated with the brain function and on the brain histology of female Wistar rats. Two groups of rats (n = 8) were exposed to a single dose of 60 mg kg^{-1} b.wt. of Arg and 3 ml kg^{-1} b.wt. of distilled water respectively as treated and control groups. Exposure was per oral (p.o) for twenty eight consecutive days. Exposure to Arg evoked a significant increase ($p<0.01$) in aspartate amino transferase (AST) activity (24.95±0.10 IU L^{-1}) and ammonium ion (NH_4^+) concentration (39.58±0.16 μg mL^{-1}) in the rats' serum. It increased ($p<0.01$) the aspartate amino transferase to alanine amino transferase (AST:ALT) ratio (0.37±0.01) of the rats. Brain section of Arg-treated rats revealed degeneration, characterized by necrosis, oedema and reduction of astrocytes. AST:ALT ratio had a significant positive correlation (r = 0.01) with AST activity and NH_4^+ concentration. The results suggest Arg-induced adverse influence on the studied markers of Mes associated with the brain function. Hence, exposure to Arg may impair the brain function and possibly, worsen Mes related to brain function of the rats.

Key words: Metabolic syndrome, brain histology, ammonium ion, oedema, female rats, L-arginine

INTRODUCTION

Metabolic syndrome (Mes) is a cluster of medical disorders. The syndrome is characterized by obesity, insulin resistance (type 2 diabetes mellitus), atherogenic dyslipidemia and hypertension (Deedwania and Gupta, 2006; Gallagher *et al*., 2010; Mahajan *et al*., 2010). Mes was associated further health risks in animals (Azhar, 2010; Siddiqui, 2011; Pelucchi *et al*., 2010; Rosato *et al*., 2011; Capasso *et al*., 2010; Mugnai, 2010). The prevalence of Mes is on the increase and the pattern cuts across every age, location and gender (Gotto *et al*., 2006; Grundy, 2008; Ijeh *et al*., 2010; Bakoma *et al*., 2011). However, female gender is an independent risk factor for the development of Mes (Ravikiran *et al*., 2010). In addition, in the females, Mes could result to polycystic ovary syndrome (Mathur, 2010) that may worsen infertility.

The pathophysiology of Mes was associated with endothelial dysfunction following a significant reduction in nitric oxide, a metabolite of L-arginine (Garlichs *et al*., 2000). Abnormal concentration of nitric oxide, NO, a vasodilator molecule could result in pathologicl conditions (Lokhande *et al*., 2006; McGrowder and Brown, 2007). In addition, a decrease in ARG availability resulted in the reduction of the biological activity of NO (Harisa, 2011) and in the conversion of NO into peroxynitrites that could mediate cell damage (Subratty *et al*., 2007). Furthermore, Mes was associated with insulin resistance, the failure of insulin to maintain glucose balance that may result to type 2 diabetes and obesity (Ezeanyika and Egbuonu, 2011). Insulin is the key hormone in the regulation of glucose homeostasis (Cohn *et al*., 2005; Lann and LeRoith, 2007) and appetite via. signaling in the brain (Gallagher *et al*., 2010). Moreover, Arg could enhance the production and release of insulin (Egbuonu, 2012). These suggest that Arg, a precursor of Nitric Oxide (NO) (Moncada *et al*., 1991), may affect Mes in animals via. possible alteration of their brain function.

L-Arg is commonly used in diets and drugs owing to its possible benefits in animals (Egbuonu *et al*., 2012). However, Arg-induced adverse response in rats was reported (Lokhande *et al*., 2006; Nematbakhsh *et al*., 2008). Thus, this study investigated the influence of L-arginine on some biochemical markers of Mes associated

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with brain function in female Wistar albino rats. The study also examined the effect of L-arginine on the brain histology of the rats. The choice of female rats in this study derived from report that the female gender is an independent risk factor for incidence of Mes (Ravikiran *et al*., 2010) and that the prevalence of Mes is higher in the females (Mangat *et al*., 2010; Kilic *et al*., 2010; Titty *et al*., 2008) where it could result to polycystic ovary syndrome (Mathur, 2010).

MATERIALS AND METHODS

Chemicals and reagents: The animal study was carried out between August and September, 2010. Chemicals used in this study were procured from reputable dealers in Nsukka, a University town in Enugu State, Nigeria. L-arginine is a product of Sigma Chemical Company, St. Louis, U.S.A.

Concentration determination/justification: The test concentration, ARG (60 mg kg^{-1} b.wt.), was based on the concentration used in earlier studies (Alexander *et al*., 2004; Egbuonu *et al*., 2010a, b,c) in line with WHO reported daily oral intake of Arg (Marshal, 1994).

Animals and treatment: The female Wistar rats used in this study were obtained from the animal house of the Faculty of Biological Sciences University of Nigeria, Nsukka. The rats weighed 60-80 g, similar to weight range of rats used by Amin and Nagy (2009). The animal study was according to International, National and institutional guidelines for the care and use of laboratory animals in Biomedical Research (CCAC, 1985; WMA/APS, 2002) as approved by the Departmental adhoc Ethical Committee, Department of Biochemistry University of Nigeria Nsukka, Nigeria.

The rats acclimatized for a week and thereafter were randomized into two groups with sample size of eight rats each. Two groups of female Wistar albino rats (n = 8) were exposed to a single dose of 60 mg kg^{-1} body weight (b.wt.) of Arg and 3 mL kg^{-1} b.wt. of distilled water respectively as treated and control groups. Exposure was per oral (p.o) for twenty eight consecutive days.

The rats were housed in a well-ventilated stainless steel cages at room temperature (28±2°C) and tropical humid condition. They were maintained under standard natural photoperiodic condition of twelve hours of light alternating with twelve hours of darkness (i.e., a normal daylight/dark cycle). 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: Collection of the respective blood sample of animals, sacrificed 24 h after the 28 days oral exposure, 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 to out flow of blood into clean non-anticoagulated tube. Centrifugation of clotted blood at 3000 rpm for 10 min yielded the serum. Thereafter, the serum (aspirated separately into stoppered polystyrene tubes) was stored in a deep freezer for subsequent use in determining the aspartate amino transferase activity and ammonium ion concentration. Organ specimen (brain) excised from the sacrificed rats for histology was fixed in 10% formaldehyde buffered saline (formal saline) until used.

Determination of parameters

Serum aspartate aminotransferase (AST) activity: The serum aspartate aminotransferase (AST) activity assay was by the method of Reitman and Frankel (1957). The method was based on the coupling of oxaloacetate (oxaloacetic acid) formed from the aspartate aminotransferase catalysed reaction with chromogen (2, 4-dinitrophenyl hydrazine) in alkaline medium to yield colored hydrazone that was measured colorimetrically at 540 nm.

Serum ammonium ion (NH_4^+) concentration: Determination of ammonium ion concentration was by colorimetric method as described in AOAC (2005). This based on the principle of colorimetric estimation at 480 nm of ammonia, distilled after alkalinization, by nesslerizaton or titrimetry.

Calculation of the diagnostic ratio: AST:ALT ratio was calculated from the corresponding parameters obtained in the same study. However, alanine amino transfrase activity was not reported here.

Organ histology: Organ specimen (brain) promptly excised from the sacrificed rats for histological examination were fixed in 10% formaldehyde buffered saline (formal saline) until used as reported (Egbuonu *et al*., 2010c). In brief, after dehydration (in graded levels (70-100%) of alcohol), clearing (in xylene impregnated with paraffin wax) and sectioning (at 5 microns thickness using rotary microtone) the sections were floated on a water bath maintained at a temperature of 2-3°C below melting point of the paraffin wax. Thereafter, drying of the sections was performed on a hot

plate maintained at a temperature of 2-3°C above the melting point of the paraffin followed by staining and mounting of the sections using haematoxylin and eosin.

Statistical analysis: Analysis of data to determine significant difference in mean 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 ($r = 0.01$).

RESULTS

Serum aspartate aminotransferase (AST) activity: A significant increase ($p<0.01$) in the serum AST activity was recorded in the Arg-treated rats (24.95±0.10 IU L^{-1}). This represents an increase by 8.95 % (Fig. 1).

Serum aspartate aminotransferase to alanine aminotransferase (AST:ALT) ratio: The computed serum AST:ALT ratio increased ($p<0.01$) in Arg-fed rats (0.37±0.01) relative to control, representing an increase by 32.14 % relative to control (Fig. 2).

Serum ammonium ion (NH_4^+) concentration: As depicted in Fig. 3, the serum NH_4^+ ion concentration in Arg-treated rats increased ($p<0.01$) (39.58±0.16 μg mL^{-1}) above that of the control rats. This represents an increase of 8.05% relative to control.

Table 1: Correlations of AST:ALT ratio, serum AST activity and ammonium ion concentration

	Serum Serum AST	Serum AST:ALT ratio	Ammonium ION
Serum AST			
Pearson Correlation	1	0.980**	0.946**
Sig. (2-tailed)		0.000	0.000
N	16	16	16
Serum AST:ALT ratio			
Pearson Correlation	0.980**	1	0.957**
Sig. (2-tailed)	0.000		0.000
N	16	16	16
Serum ammonium ION			
Pearson Correlation	0.946**	0.957**	1
Sig. (2-tailed)	0.000	0.000	
N	16	16	16

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

Correlation outcome: As revealed in Table 1, AST:ALT ratio had a significant positive correlation ($r = 0.01$) with AST activity (0.980) and ammonium ion concentration (0.957).

Histomorphology of the brain: Brain sections of the control rats showed typical brain histology, with population of normal cells (Fig. 4). Sections collected from

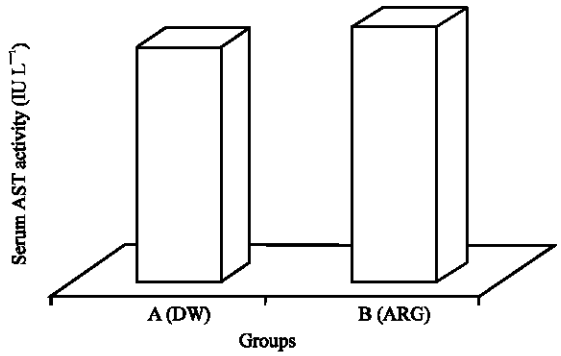

Fig. 1: Influence of distilled water (DW) and ARG on serum AST activity of rats

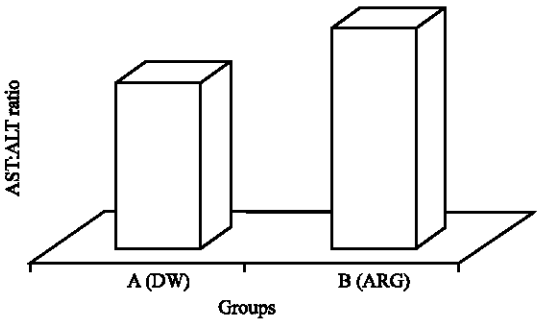

Fig. 2: Effect of distilled water (DW) and ARG on serum AST:ALT ratio of rats

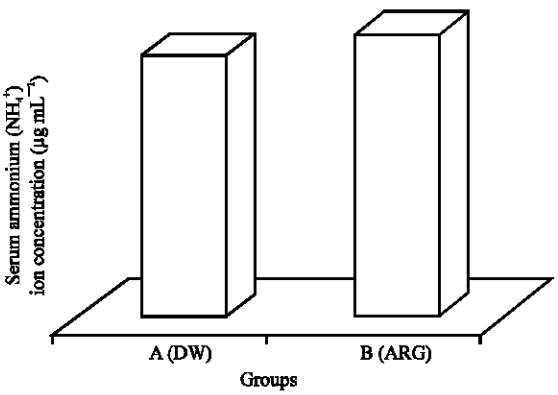

Fig. 3: Effect of distilled water (DW) and ARG on serum ammonium ion concentration of rats

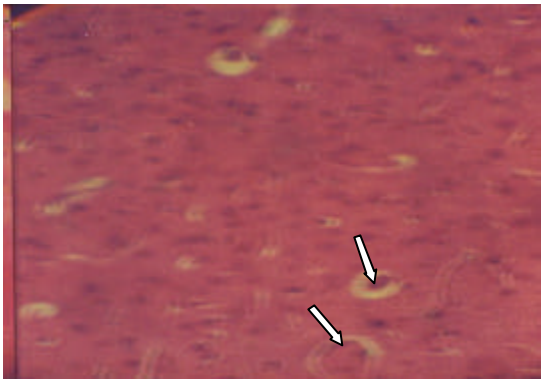

Fig. 4: Brain section of control (Group A) rat showing typical histology, with lots of Astrocytes (arrow heads). H and E stains, ×400

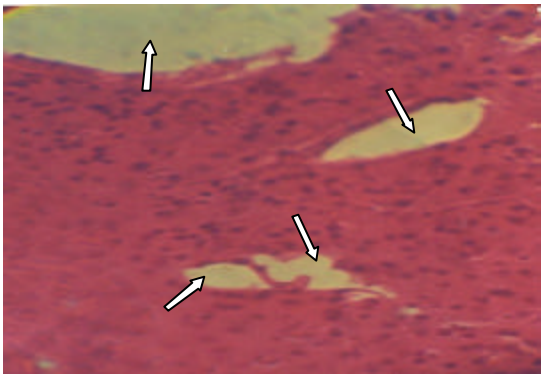

Fig. 5: Brain section of rats exposed to Arg (Group B) showing moderate necrosis, oedema and reduction of astrocytes (arrow heads). H and E stains ×400

rats treated with Arg (Group B) showed moderate oedema. The population of astrocytes was moserately reduced (Fig. 5).

DISCUSSION

Metabolic syndrome (Mes) 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 Mes pathogenesis with a significant reduction in NO availability (Garlichs *et al*., 2000) suggested that Arg, through its major precursor role in NO synthesis (Moncada *et al*., 1991), may improve Mes. Indeed, Arg improved the renal function markers of metabolic syndrome (Egbuonu and Ezeanyika, 2013). However, Arg worsened the markers of Mes related to lipid metabolism (Egbuonu and Ezeanyika, 2012), warranting this study to ascertain the effect of Arg on some biochemical markers of metabolic syndrome associated with the brain function of female rats.

Elevated serum AST activity may indicate damage to other high metabolic organs besides the liver (Bush 1991; Egbuonu *et al*., 2012). Arg ingestion to rats elicited a significant increase ($p<0.01$) in AST activity of the rats' serum, indicating altered function of high metabolic organs. The observation is consistent with that of earlier study in male rats (Egbuonu *et al*., 2010c), adduced to adverse influence on high metabolic organs including the brain (Egbuonu *et al*., 2010b).

A reduced serum aspartate aminotransferase to alanine aminotransferase (AST:ALT) ratio was associated with enhanced insulin resistance (Hanley *et al*., 2005) and incidence of Mes (Tzima *et al*., 2009; Sidorenkov *et al*., 2010). Consistent with earlier study in male rats (Egbuonu *et al*., 2010c), exposing the female rats to Arg increased their computed AST:ALT ratio, precluding liver damage as the source of increased AST activity in this study and suggesting suppressed insulin resistance or Mes. Women with Mes had lower AST:ALT ratios than those without (Tzima *et al*., 2009). Thus, exposeing female rats to Arg may improve Mes related to reduced AST:ALT ratio in the female rats.

By contrast with control, Arg ingestion to the rats increased ($p<0.01$) the serum ammonium (NH_4^+) ion concentration, indicating adverse influence on the brain (Lichter-Konecki *et al*., 2008). Ammonium ion build up may result in oedema, increased extracellular concentration of glutamate and energy depletion in the brain (Rodrigo *et al*., 2009). With buildup in the blood, ammonium ion may traverse the blood brain barrier and in the brain, its conversion to glutamate via. glutamate dehydrogenase depletes α-ketoglutarate ultimately halting citric acid cycle activity resulting to reduced energy production and brain damage. Furthermore, NH_4^+ could combine with glutamate in a glutamine synthetase catalyzed reaction to produce glutamine, which as an osmolyte could elicit direct osmotic effect on the brain resulting to brain swelling or oedema. Consistent with this study (Zhao *et al*., 2003) arginine exacerbated oedema in mice. However, contrary to this study, Kondoh *et al*. (2010) demonstrated the protective effect of arginine against cerebral oedema. The observed increase in ammonium ion concentration in the ARG-treated group is a significant shortcoming of possible Arg benefit on MES hence, deserves follow up.

Histomorphologic alterations in organs were the most consistent treatment-related changes and in concert with anthropometric and biochemical results may give a clear picture of physiological function of animals (Egbuonu *et al*., 2010c). Agent-induced physiological and biochemical disturbances (Adeniran *et al*., 2006), as well as alterations in liver and kidney histology (Farrag and Shalby, 2007; Egbuonu *et al*., 2010c) have been reported. In support of this study, the brain section of female rats exposed to Arg showed degenerative changes characterized by necrosis and oedema. Pearson's correlation analysis indicated that AST:ALT ratio correlated positively (r = 0.01) with AST activity and ammonium ion. This may suggest concerted Arg-induced adverse response on these markers in the female rats (Egbuonu and Ezeanyika, 2013).

CONCLUSION

In conclusion, the results suggest Arg-induced adverse influence on the studied markers of Mes associated with brain function. Hence, exposure to Arg may impair the brain function and possibly, worsen Mes related to brain function of the rats. The finding of this study may limit the possible potential use of Arg in managing MES in animals hence, warrants further investigation.

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