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Sub-chronic Concomitant Ingestion of L-arginine and Monosodium Glutamate Improves Feed Efficiency, Lipid Metabolism and Antioxidant Capacity in Male Wistar Rats

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Abstract: The use of L-arginine (ARG) is common in supplements, whereas, Monosodium Glutamate (MSG) is widely used as flavor enhancing food additive. Thus, ARG and MSG may be present together in human diets, warranting this study aimed at investigating the effect of concomitant ingestion of ARG and MSG on some biochemical indices in male rats. Twelve male albino rats were grouped into three (n = 4) and concomitantly exposed to 0:0, 20:5 and 60:15 mg kg^{-1} of ARG:MSG. Exposure was peroral and every twenty four h for 28 days. ARG plus MSG treatment caused a significant ($p \leq 0.05$) increase in Feed Efficiency (FE) (Low dose: 5.23±22%; High dose: 5.60±11%), whereas, it decreased ($p \leq 0.05$) the serum Total Cholesterol (T-Chol) (low dose: 80.83±0.11 mg/100 mL, high dose: 92.55±0.14 mg/100 mL), triacylglycerol (TAG) (low dose: 179.91±0.09 mg/100 mL, high dose: 119.77±0.32 mg/100 mL) and malondialdehyde (MDA) (low dose: 5.00±0.07 mg/100 mL, high dose: 24.36±0.10 mg/100 mL) concentrations of the rats in a dose dependent manner. However, (at the high dose) the increase in Body Weight (BW) (0.08±0.07 kg), Feed Intake (FI) (0.40±0.03 kg) and Water Intake (WI) (0.65±0.18 L) induced by ARG plus MSG exposure was not significant ($p \geq 0.05$), suggesting non treatment related effect on these routine parameters. However, exposure to ARG plus MSG may significantly improve feed efficiency, lipid metabolism and antioxidant capacity in the male rats.

Key words: Serum, food additive, supplement, metabolic syndrome, antioxidant capacity

INTRODUCTION

L-arginine (ARG) is a basic amino acid present in nuts and commonly used in supplements, whereas Monosodium Glutamate (MSG), a salt of an acidic amino acid, glutamate (Furst and Stehle, 2004), is a food additive added intentionally, usually in small quantities, to enhance flavor (Omer *et al*., 2008). Thus, ARG and MSG may be present together in human diets.

Although MSG is generally accepted as a safe food additive requiring no specific average daily or upper limit intakes (Samuels, 1999), inadvertent abuse of MSG may occur owing to its abundance, mostly without labeling, in many food condiments (Egbuonu *et al*., 2009). Furthermore, possible MSG-induced adverse influences have been reported in experimental animal models (Igwebuike *et al*., 2011; Waggas, 2009; Egbuonu *et al*., 2010a, b; He *et al*., 2008), including induction of obesity via increased lipid profiles and development of leptin resistance (Afifi and Abbas, 2011).

On the other hand, there is increasing use of L-arginine in diets and drugs. This may be related to its reported multiple physiological benefits in animals (Jobgen *et al*., 2006; Wang *et al*., 2009; Wu *et al*., 2007), including attenuation of the stress response (Hamasu *et al*., 2009; Suenaga *et al*., 2008a, b), immune function enhancement (Li *et al*., 2007; Tan *et al*., 2009), treatment of chronic anal fissure (Eshghi, 2007) and recently, apparent improvement of renal function markers of metabolic syndrome in female rats (Egbuonu and Ezeanyika, 2012). Nevertheless, reported ARG-induced pathologies (via excessive production of nitric oxide) in rats abound (Lokhande *et al*., 2006; Nematbakhsh *et al*., 2008).

The substances (ARG and MSG) may be present together in human diets and drugs. However, data on the effect of simultaneous ingestion of ARG and MSG in animal, to the knowledge of the author, are not much. Thus, the present preliminary study aimed at investigating the effect of concomitant ingestion of ARG and MSG on some biochemical indices in male rats.

MATERIALS AND METHODS

Chemicals: The study was conducted between June 2005 to June 2006. ARG, MSG and other chemicals were purchased from a reputable dealer in Nsukka, a university town in Enugu State, Eastern Nigeria. The chemicals were used without further purification.

Animals and treatment: To achieve the stated objectives, adult male rats were exposed to 0:0, 20:5 and 60:15 mg kg^{-1} body weight ARG:MSG, peroral, for 28 consecutive days. The control rats were exposed to 3 body weight of the vehicle, Distilled Water (DW), in accordance with ethical guidelines for animal welfare as approved by Biochemistry Department, Faculty of Biological Sciences, University of Nigeria Nsukka, Enugu State, Nigeria.

Twelve adult male Wistar rats (average weight, 76.66 g) were kept in clean stainless steel cages in a well ventilated facility at a temperature of 28±2°C with a 12 h daylight/night cycle under humid tropical conditions. The rats were allowed free access to standard rat feed and tap water. After a week acclimatization, the rats were randomly assigned to groups A, B and C (n = 4) just enough to justify the importance of the study in relation with the number of animals used (PHS, 1996).

Group A (the control) rats were exposed to 3 mL kg^{-1} b.wt. of the vehicle and distilled water. Whereas, groups B and C (the treated) rats were simultaneously exposed to 20:5 and 60:15 mg kg^{-1} b.wt. ARG:MSG diet, respectively. The exposure was *per os* and consecutive for 28 days.

Blood sample collection for clinical chemistry determination: At the end of experiment (day 28), the animals were deprived of feed and drinking water to ensure overnight fasting before their sacrifice by retro orbital sinus venipuncture. Blood sample of the respective rats was collected into dry clean sample vial using sterile capillary tubes (containing no anticoagulant) as described by Egbuonu *et al.* (2009). Thereafter, the blood sample was centrifuged for 10 min at 3,000 x, room temperature and the serum was stored in deep freezer for serum assays.

Parameters determined

Serum malondialdehyde (MDA) concentration: The malondialdehyde concentration determination in 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 total cholesterol (T-Chol) concentration: The serum total cholesterol concentration determination was by the method of Zlatkis *et al.* (1953). This method based on the principle that the reaction of both cholesterol and cholesterol esters with ethanol and the Hydrochloric Acid (HCl) in chromogen could yield color of equal absorbance measured at 550 nm.

Serum triacylglycerol (TAG) concentration: The serum TAG concentration determination was by the method of Carlson (1963). This method based on the principle of colorimetric determination of glycerol (at 570 nm) after alkaline hydrolysis of glycerides.

Body weight change, feed intake (FI), water intake (WI) and feed efficiency (FE): Determination of the b.wt. kg of the rats was on the first day and on the last day. The difference in the b.wt. of the rats measured on the first day and the last day of the treatment was the total body weight change.

Measurement of the feed intake and water intake, calculated as the total daily feed and water consumed relative to b.wt. change, was by this relation:

$$\text{Individual rat feed or water consumed} = \frac{\text{Total feed (or total water) consumed}}{\text{Total b.wt. change} \times \text{Individual rat b.wt. change}}$$

Feed efficiency, the body mass gain in grams per kilocalories consumed, calculation was with the relationship below:

$$\text{Feed efficiency (FE)} = \frac{\text{Weight gained (g)}}{\text{(g kcal) consumed}} \times 100$$

where, the caloric content of feed in kcal g^{-1} was based on 3.573 kcal g^{-1} value for standard chow diet (Fraulob *et al.*, 2010).

Statistical analysis: Differences in mean were checked by Student's t-test using Statistical Package of Social Sciences (SPSS) version 11.0 (SPSS Inc., USA) as earlier described (Egbuonu *et al.*, 2010a). Significance was accepted at $p \leq 0.05$ probability level. Data in the text and tables were presented as means and standard error of the Mean (SEM).

RESULTS

Serum T-Chol, TAG and MDA concentrations: Contrary to control, ARG plus MSG treatment caused a significant ($p \leq 0.05$) decrease in the serum total cholesterol (T-Chol) (low dose: 80.83±0.11 mg/100 mL, high dose: 92.55±0.14 mg/100 mL), triacylglycerol (TAG) (low dose: 179.91±0.09 mg/100 mL, high dose: 119.77±0.32 mg/100 mL) and malondialdehyde (MDA) (low dose: 5.00±0.07 mg/100 mL, high dose: 24.36±0.10 mg/100 mL) concentrations of the rats in a dose dependent manner (Table 1).

Feed intake (FI) and water intake (WI): Data revealed that ARG plus MSG exposure had a significant ($p \leq 0.05$) increase in the rats' FI (0.59±0.09 kg) and

Table 1: Effect of ARG plus MSG on serum malondialdehyde (MDA), triacylglycerol (TAG) and total cholesterol (T-Chol) concentrations

Groups	ARG:MSG (mg kg^{-1})	MDA (mg/100 mL)	TAG (mg/100 mL)	T-Chol (mg/100 mL)
A: Control	0:0	41.08±0.13	183.82±0.18	103.50±0.10
B: Low dose	20:5	5.00±0.07[a]	179.91±0.09[a]	80.83±0.11[a#]
C: High dose	60:15	24.36±0.10[a]	119.77±0.32[a]	92.55±0.14[a]

Sample number, n = 4, [a]Mean difference is significant from control ($p \leq 0.05$), [#]Egbuonu *et al.* (2010b), Values are expressed as Mean±SEM

Table 2: Effect of ARG plus MSG on intake of water (WI) and feed (FI)

Groups	ARG:MSG (mg kg^{-1})	WI (L)	FI (kg)
A: Control	0:0	0.64±0.10	0.37±0.04
B: Low dose	20:5	0.74±0.06[a]	0.59±0.09[a]
C: High dose	60:15	0.65±0.18	0.40±0.03

Sample number, n≥4, [a]Mean difference is significant from control ($p \leq 0.05$), Values are Mean±SEM

Table 3: Effect of ARG plus MSG on body weight change (kg) and feed efficiency (%)

Groups	ARG:MSG (mg kg^{-1})	Feed efficiency (%)	Body weight (kg)
A: Control	0:0	4.54±19	0.06±0.02
B: Low dose	20:5	5.23±22[a]	0.11±0.01[a#]
C: High dose	60:15	5.60±11[a]	0.08±0.07

Sample number, n = 4, [a]Mean difference is significant from control ($p \leq 0.05$), [#]Egbuonu *et al.* (2010b), Values are Mean±SEM

WI (0.74±0.06 L) at the low dose. However, the increase in FI (0.40±0.03 kg) and WI (0.65±0.18 L) observed at the high dose was not significant ($p \geq 0.05$) (Table 2).

Body weight and feed efficiency: On comparison with control, data presented in Table 3 revealed that ARG plus MSG-induced increase in Body Weight (BW) of the rats was significant ($p \leq 0.05$) at the low dose (0.11±0.01 kg), but non significant ($p \geq 0.05$) at the high dose (0.08±0.07 kg). However, ARG plus MSG treatment significantly ($p \leq 0.05$) increased the computed Feed Efficiency (FE) (low dose: 5.23±22%, high dose: (5.60±11%) of the rats irrespective of dose.

DISCUSSIONS

ARG and MSG may be present together in human diets and drugs. However, data on the effect of simultaneous ingestion of ARG and MSG in animal, to the knowledge of the author, are not much. Thus, the effect of concomitant ingestion of ARG and MSG on some biochemical indices in male rats was investigated further in the present study.

On comparison with control, exposure to ARG plus MSG increased body weight, indicating caloric excess and storage, probably due to improved lipid metabolism via diminished lipogenesis and/or enhanced lipolysis as suggested by Afifi and Abbas (2011). Consistent with the present result, MSG alone (Afifi and Abbas, 2011; He *et al.*, 2008) and together with ARG (Egbuonu *et al.*, 2010a) enhanced body weight in rats. However, the body weight increase was not significant at the high dose, hence may not be treatment related.

Increased feed intake observed in this study is suggestive of orexigenic potential of ARG plus MSG in rats. Previously, MSG alone enhanced feed intake in experimental animal (Diniz *et al.*, 2005). Increased feed intake may enhance energy balance or caloric storage, hence increased body weight observed in the rats. However, BW gain may occur independent of increased feed intake (Von Diemen *et al.*, 2006).

Water intake increased in ARG plus MSG-treated rats, indicating suppressed caloric storage. Water is an electronegative enhancer (Batmanghelidj, 2010), thus increase in water intake could enhance fat emulsification and lipolysis resulting in decreased fat (calorie) storage. This may be supported by the hypothesis of improved lipid metabolism following decreased serum T-Chol and TAG concentrations reported in this study.

Furthermore, exposure to ARG plus MSG increased the computed feed efficiency, an indicator of efficient energy utilization. Efficient energy utilization connotes zero net energy balance, hence no storable energy. Thus, this could be pointing to the potential of ARG plus MSG to suppress caloric storage in the rats.

Substance-induced metabolic disorder associated with increased oxidative stress in animals was reported (Diniz *et al.*, 2005; Egbuonu *et al.*, 2009). The serum MDA concentration decreased dose dependently in the rats exposed to a combination of ARG and MSG, indicating enhanced antioxidant capacity. Although, MSG-induced increase in MDA concentration was reported in rats (Egbuonu *et al.*, 2009), ARG attenuated oxidative stress in animal models (Dasgupta *et al.*, 2006). Thus, the decreased MDA concentration noted in the ARG plus MSG-treated rats may suggest the overriding antioxidant potential of ARG over MSG. This result seems to support the decreased T-Chol concentration recorded in this study, since Kumar *et al.* (2006) associated increased cholesterol in the blood with increase in oxidative stress.

Increased TAG levels are risk factors related to arteriosclerosis that, by thickening the walls of larger blood vessels, results in heart attack (Ochei and Kolhatkar, 2008). Results of the present study revealed that T-Chol and TAG concentrations of ARG plus MSG-fed rats decreased, indicating improved lipid

metabolism, probably due to decreased lipogenesis and enhanced lipolysis (Afifi and Abbas, 2011). In apparent support of this result, administration of L-arginine (2%) in drinking water decreased T-Chol and TAG concentrations but in combination with L-ornithine (2%) (Al-Omair, 2009), whereas, L-arginine (3%) decreased T-Chol and TAG in normal and hypercholesterolemic rats (Harisa, 2011). The present results on T-Chol and TAG concentrations appear to be supported by the increased water intake noted in this study that could enhance fatty acid oxidation via enhanced TAG hydrolysis resulting in the decreased serum TAG concentration of the rats. MSG-induced increase in T-Chol concentration (Afifi and Abbas, 2011; Egbuonu *et al*., 2010a; Egbuonu *et al*., 2010b; Egbuonu and Osakwe, 2011) and TAG concentration (Afifi and Abbas, 2011; Diniz *et al*., 2005; Egbuonu *et al*., 2010b; Egbuonu and Osakwe, 2011) were reported in rats which suggests that ARG may antagonize the adverse influence of MSG on lipid metabolism. However, in a similar study, ARG plus MSG increased T-Chol concentration, but on comparison with control rats dosed 1 mL kg^{-1} body weight of distilled water (Egbuonu *et al*., 2010a).

CONCLUSION

In conclusion, the results suggest that exposure to ARG plus MSG, such as their concomitant use in diets and drugs, may significantly improve feed efficiency, lipid metabolism and antioxidant capacity in the male rats. The nutritional implication of the results is noteworthy, warranting further and probably better controlled, investigations to collaborate these preliminary findings.

This study has a number of limitations. For instance, the small sample size used in order to justify the importance of the study in relation with the number of animals may limit the present study. Furthermore, the effect of exposure to ARG or MSG alone ought to be studied for comparison with that of concomitant exposure to ARG and MSG. Therefore, careful interpretation of the data is required to forestall invalid extrapolation. Nevertheless, the findings of this study will serve as a reference point for comprehensive studies, possibly addressing the noted limitations, on the effect of concomitant ingestion of ARG and MSG in animals.

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