Research Article

Effects of Monosodium Glutamate on Some Metabolic Parameters in Adult Male Albino Rats

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Abstract:

Aim of work: Monosodium glutamate (MSG) is a globally consumed food additive that has no use-limit and recently reported to be health hazardous. This study aimed to study the effects of chronic oral MSG intake on obesity development, the changes in some carbohydrate and lipid metabolic parameters, and the possible role of nitric oxide (NO). **Materials and Methods:** Twenty four adult male albino rats were classified into ٤ equal homogenous groups according to two weeks' oral treatment as follows: ١- **Control** group,(saline vehicle) ٢- **MSG** treated group; **(**٤g/kg/day**)**, ٣- **N^G-Nitro-L-Arginine Methyl Ester (L-NAME)** treated group**; (**٢٠ mg/kg/day**)**; to block nitric oxide synthase (NOS), ٤- **L-NAME+ MSG** treated group; combined as in groups ٢ and ٣. Blood samples were then collected after decapitation for biochemical analysis, gastrocolic omentum (GCOF) excised and weighed, and liver and gastrocnemius muscle taken for glycogen assay. **Results: MSG** produced a significantly higher body weight, food intake, Lee index and GCOF, muscle and liver glycogen while L-NAME significantly lowered them except muscle glycogen and when combined no significant change from control was found except a lowered GCOF. MSG produced a metabolic syndrome picture of hyperglycemia, hyperinsulinemia, increased insulin resistance, and dyslipidemic atherogenic lipid profile with significantly higher peroxides (malondialdehyde; MDA). L-NAME produced similar effects to MSG but more significantly higher, and the combination was more significantly worse. Finally MSG produced no significant change in serum glutamate, nitric oxide and ghrelin, while L-NAME significantly lowered them whether alone or in combination with MSG. **In conclusion:** chronic MSG intake was found to be obesigenic, diabetogenic and increased cardiac risk factors due to disturbed carbohydrate and lipid metabolism. A peripheral mechanism stimulating the gut-brain axis *via* neuroendocrine mechanism involving NO is the most probable, but, NO is not involved in its systemic metabolic disturbances. The results could alarm for the health hazards of chronic MSG consumption in humans.

Key words: Monosodium glutamate, N^G-Nitro-L-Arginine Methyl Ester, Obesity, Ghrelin, Nitric oxide.

Introduction

Monosodium glutamate (MSG) is the sodium salt of glutamic acid; the most common amino acid found in nature and also produced in the body with a diversity of metabolic effects. In addition, it is a widely used flavor enhancing food additive added to packaged foods as a preservative, and to stimulate the "umami" delicious taste sensation of protein that is considered the fifth taste sensation in addition to salt, sour, sweet and bitter senses[١].

Taste receptors are dimers of $T_١$ and/or $T_٣$ receptors. $T_١$ receptors are of three types; $T_١R_١$, $T_١R_٣$, and $T_١R_٣$. $T_١R_١/T_١R_٣$ is the umami taste receptor, and it can only bind and is stimulated with L-glutamate. It is a G-protein coupled receptor. In addition, other glutamate receptors are either metabotropic (G-protein coupled receptors), or ionic channel receptors including $Ca^{٢+}$-channel receptors. Together with chemo-sensor cells, they are widely distributed in the mucosal cells of the gastrointestinal tract (GIT) and not restricted to the oropharynx. They are coupled to enteroendocrine cells and afferent vagal terminals by paracrine signaling. This diversity of receptors and their broad distribution throughout the GIT makes glutamate an important cell signaling

molecule transferring gut-brain information either through the vagus nerve or via humoral mechanism involving the release of GIT hormones. Thus, it is not only important as a taste modulator affecting food intake, but also for the digestion, absorption, metabolism and energy homeostasis [٢,٣].

L-glutamate plays an important role in body metabolism. It acts as an interface between carbohydrate and amino acid metabolism; being a key substrate for transamination reactions and the product; alpha ketoglutarate is an intermediate in citric acid cycle. It modulates the functions of pancreatic islet cells and it is a constituent of glutathione; an important endogenous anti-oxidant scavenger. In addition, it is an important central and peripheral neurotransmitter [٤,٥].

Some studies suggested that the effects of glutamate on neuroendocrine cells or vagal nerve afferents of the gut are mediated via other signaling molecules as ٥-hydroxytryptamine (٥-HT) through ٥-$HT_٣$ receptors and/or nitric oxide (NO)[١]. On the other hand, controversial results were obtained about the effects of glutamate on food intake, body weight gain and obesity development. While Boutry et al.,[٦] showed that acute or chronic glutamate supplementation had no effect on food intake or obesity development, Contini et al.,,[٧] described a glutamate model of obesity in rats.

This work was designed to study the effects of chronic oral monosodium glutamate intake on: ١) obesity development, ٢) the changes in some carbohydrate and lipid metabolic parameters, and ٣) the possible role of NO.

Material and methods:

I- Animals

Twenty four adult male albino rats (Sprague dawley strain) were used. Their weight ranged between ١٥٠ and ١٦٠ grams at the beginning of this study. Rats were housed in groups of six in stainless steel mesh bottomed cages providing adequate space and light at room temperature with natural light\dark cycles for one week for acclimatization to lab conditions. Rats were fed a standard diet of commercial rat chow and tap water ad libitum until the time of the experiment. During the acclimatization period, daily food intake was measured to know the mean daily food intake per rat. All the procedures followed with the rats were in accordance with our institutional guidelines. The protocol was ethically approved by The Laboratory Animals Maintenance and Usage Committee of Faculty of Medicine, Minia University.

Rats were then grouped into four equal and homogenous groups according to treatment protocol as follows:

١- **Control group (C):** in which each rat was given ٢ ml oral saline (vehicle) daily by gavage for ٢ weeks.

٢– **Monosodium glutamate (MSG) treated group**; in which each rat was given MSG, ٤g/kg/day dissolved in saline (Sigma, USA) by gavage for two weeks. This is the most widely used dose in studying MSG hazards in rats[٨].

٣- **N^G-Nitro-L-Arginine Methyl Ester (L-NAME) treated group;** in which rats were given ٢٠ mg/kg/day of L-NAME (Sigma, USA) dissolved in saline by gavage for two weeks [٩].

٤- **L-NAME+MSG treated group;** in which each rat was given L-NAME followed after one hour with MSG by gavage in the same doses as in groups ٢ and ٣ daily for two weeks.

During the two weeks' treatment period, rats were allowed free access to the standard rat chow and water ad libitum. The composition of standard rat chow (g/kg diet) was; Fat ٥٪, carbohydrates ٦٥٪, proteins ٢٠.٣٪, fiber ٥٪, salt mixture ٣.٧٪, and vitamin mixture ١٪ and provided ٣.٠ kcal/g of diet.

Rats were followed throughout the two weeks' experimental study by:

- Body weight; was determined at the start, end of the first and second weeks
- Food intake was daily determined and the mean at the start, end of first and second weeks was determined.
- Lee index: Lee index is used to determine obesity in rats using body weight and naso-anal length. It was

measured at the beginning of the study, at the end of the first and at the end of the second week. Lee index was calculated for each rat according to the following formula:

Cube root of body weight (g) X ١٠ / nasoanal length (mm)

Rats with Lee index ≥ ٠.٣ were considered obese [١٠].

At the end of the treatment period, rats were sacrificed after an overnight fast by decapitation and blood samples were collected from the jugular vein, allowed to clot at room temperature, and then centrifuged at ٣٠٠٠ rpm for ١٥ min. in a cooling centrifuge (Hettich centrifuge). The serum layer was then withdrawn into identified eppendorf tubes and stored at -٢٠ °C till the time of assay.

II- The weight of gastrocolic omentum:

Rats were opened via ventral abdominal incision. Peritoneal omental adipose tissue was removed by lifting the intestines and cutting the intermediate fat free, starting at the distal end close to the appendix, the whole gastrocolic omentum was weighed [١١].

III- Biochemical analysis:

Serum samples were analyzed for:

١. Lipid profile: Total cholesterol (TC), triglycerides (TGs), low density lipoprotein (LDL-c), high density lipoprotein (HDL-c) were determined using enzymatic colorimetric methods (Bio-Diagnostic kits, Egypt).
The atherogenic index was calculated according to Ikewuchi et al., [١٢]:
Atherogenic index of plasma= log [Triglyceride/HDLcholesterol]

٢. Glucose concentration was determined by enzymatic (glucose oxidase) colorimetric methods, using kits purchased from Bio-diagnostic, EGYPT using spectrophotometer (Spectronic ٢٠٠٠, BAUSCH & LOMB).

٣. Insulin concentration; was assayed using enzyme-linked immunosorbent assay (ELISA) kit (United Biotech Ink; UBI, MAGIWELTM).

٤. Homeostasis Model Assessment of Insulin Resistance (HOMA-IR)[١٣]: was calculated according to the following formula:
HOMA-IR= Serum fasting glucose (mg/dl) X Serum fasting insulin (μU/ml) / ٤٠٥

٥. Serum glutamate was determined by a colorimetric glutamate assay kit (BioAssay Systems' EnzyChrom™ glutamate assay kit, USA), depending on glutamate dehydrogenase catalyzed oxidation of glutamate, in which the formed NADH reduces a formazan (MTT) Reagent. Its absorbance was read at ٥٦٥ nm using spectrophotometer.

٦. Serum nitric oxide was determined by a colorimetric nitrite assay kit (Bio-diagnostic, Egypt), depending on the color intensity that develops following reaction of Griess reagent with nitrite. Its absorbance was read at ٥٢٠ nm using spectrophotometer.

٧. Serum malondialdehyde (MDA) was determined by the thiobarbituric acid method described by Ohkawa et al., [١٤].

٨. Ghrelin concentration was determined by enzyme-linked immunosorbent assay (CUSABIO, CHINA) using ELISA apparatus (SLT-SPECTRA, Salzpurg).

All methods followed the instruction manual of the manufacturer.

IV- Determination of hepatic and muscle glycogen:

This was done according to the method described by Kemp and Adrienne [١٥] and adopted by Ramu et al.,[١٦]. In brief, pieces of muscle or liver tissues were weighed, homogenized in ٥ ml TCA reagent and boiled in a boiling water bath for ١٥ minutes to extract glycogen, then, centrifuged and ١ ml of the supernatant containing glycogen was boiled again with ٣ml of concentrated sulfuric acid in a water bath for ٦.٥ minutes then cooled. The intensity of the pink color which is proportionate to glycogen concentration was read spectrophotometrically at ٥٢٠ nm.

Glycogen concentration was deduced from a standard curve prepared at the same time.

Statistical Analysis

Statistical analysis was performed using Graph pad Prism ٥ software and significant difference between groups was done by one-way ANOVA followed by Tukey-Kramar post hoc test for multiple comparisons with a value of P ≤٠.٠٥ considered statistically significant

Results

The results clearly demonstrated that MSG pre-treatment significantly increased body weight, food intake and Lee index as compared to the control group from the start up to the end of the study. However, L-NAME pre-treatment reversed the effects of MSG treatment on the body weight, Lee index and food intake reaching levels insignificant from control group in L-NAME+MSG treated group. The levels were significantly lowered than the control group in L-NAME treated group (table ١). On the other hand, the gastrocolic omentum weight showed the highest mean value in the MSG treated group, while the lowest mean value was found in the L-NAME treated groups (figure ١).

As shown in table ٢, MSG treated rats showed atherogenic serum lipid profile. There were significant higher values in the TC, TGs, LDL-c with insignificant change in HDL-c level but with a significant higher value in the atherogenic index. L-NAME significantly increased TG and LDL-c than MSG, and when combined L-NAME did not have antagonizing effect on the MSG disturbed lipid profile, but aggravated it producing a significantly higher TC, TGs and atherogenic index as compared with either L-NAME or MSG treated groups alone.

The changes in glucose homeostasis are shown in table ٣. MSG treatment produced significantly higher fasting serum glucose, insulin and HOMA-IR levels as compared to the control group. In addition, L-NAME treatment alone showed significantly higher hyperglycemic effects than MSG treated group, and when combined, L-NAME did not antagonize MSG disturbed glucose homeostasis, but aggravated it. The hepatic and muscle glycogen content were significantly high in MSG treated groups (with or without L-NAME) and low in L-NAME treated group among all experimental groups. So, L-NAME did not block the MSG glycogenic effect although it reduced both liver and muscle glycogen content by itself.

In the present study, MSG treated group showed significantly higher MDA level as compared with the control group. On the other hand, L-NAME administration either alone or with MSG significantly increased MDA level as compared with MSG treated group with insignificant difference between them (figure ٢).

As regard NO level, MSG treated group showed insignificant difference as compared with the control group, while L-NAME administration either alone or with MSG significantly lowered NO level as compared with control and MSG treated groups (figure ٣).

As shown in table ٥, serum glutamate and ghrelin were significantly lower in L-NAME and L-NAME+MSG treated groups as compared to control group, while MSG treatment failed to produce any significant change when compared with the control group.

Table (١): changes in body weight (g), Lee index and food intake (g/day) in different groups

Parameters	Groups			
	Control	MSG	L-NAME	L-NAME +MSG
Body weight (g)				
- **Initial**	١٥٢.٥±٣.٢	١٥٠.٩ ±٢.٣	١٥٣.٨ ± ٢.٤	١٥٠.٣ ± ١.١
- **One week**	١٦٩.٣± ١.٥	١٩٥.٨± ٥.٣[a]	١٧٥.٨± ٣.٩ [b]	١٦٤.٣± ٤.٤[b]
- **Final**	١٧٨.٥ ± ٢.٢	٢١٨.٥ ± ٤.٤[a]	١٦٤.٥ ± ٣.٣[ab]	١٧٤.٥±٢.١[bc]
Lee index				
- **Initial**	٠.٢٨٤±٠.٠٠٢	٠.٢٨١±٠.٠٠٤	٠.٢٨٨±٠.٠٠٣	٠.٢٨٨±٠.٠٠٣
- **One week**	٠.٢٨٥±٠.٠٠٣	٠.٣١±٠.٠٠٤[a]	٠.٢٨٥±٠.٠٠٣[b]	٠.٢٨٣±٠.٠٠٥[b]
- **Final**	٠.٢٨٣±٠.٠٠٢	٠.٣٣±٠.٠٠٣[a]	٠.٢٦٦±٠.٠٠٤[ab]	٠.٢٧٧±٠.٠٠٢[bc]
Food intake				
- **Initial**	١٥±١.٦	١٦±٢.٠	١٥.٤±٢.١	١٥.٢±١.٩
- **One week**	٢٢.٥±١.٦	٣١.٤±١.٢[a]	١٦.٤±٠.٩[ab]	٢٣.٦±١.٩[bc]
- **Final**	٢٥.٩±٠.٨	٣٣.٤±٢.٠[a]	١٣.٣±٠.٨[ab]	٢٦.١±٠.٩[bc]

Data are expressed as mean ± S.E.M. of ٦ rats in each group. a: Significant from control group, b: Significant from MSG treated group, c: Significant from L-NAME treated group respectively, P < ٠.٠٥. MSG: Monosodium glutamate, L-NAME: N^G-Nitro-L-Arginine Methyl Ester.

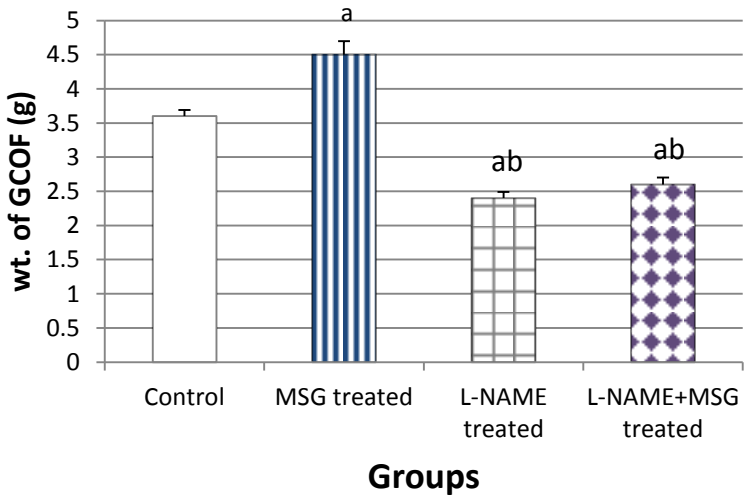

Figure ١: The weight of the gastrocolic omentum (GCOF) in different groups. Data are expressed as mean ± S.E.M. of ٦ rats in each group. a: Significant from control group, b: Significant from MSG treated group, c: Significant from L-NAME treated group respectively, MSG: Monosodium glutamate, L-NAME: N^G-Nitro-L-Arginine Methyl Ester.

Table (٢): serum lipid profile and atherogenic index (Aix) in the different studied groups

Parameters	Groups			
	Control	MSG	L-NAME	L-NAME +MSG
TC(mg/dl)	٩٨.٩٨ ± ٢.٧	١١١.٥ ± ٢.٩	١٤٨.٦ ± ١.٦	١٥٩.٤ ± ١.٣ [abc]
TGs (mg/dl)	١٠٠ ± ٣.٢	١٣٤ ± ١.٥ [a]	١٤٧.٨ ± ٢.٦ [ab]	١٥٩.٦ ± ١.٤ [abc]
HDL-c (mg/dl)	٣٧.٦ ± ١.٦	٤٠.٥ ± ٠.٧	٤١.٧±١.٩	٤٢.٦±١.٧
LDL-c (mg/dl)	٣٩.٩ ± ٠.٦	٥١.٢ ± ٢.١ [a]	٦١.٢ ± ٢.٩ [ab]	٥٨.٧ ± ٢.٥ [ab]
Aix	٠.٤١±٠.٠٢	٠.٥٢±٠.٠١ [a]	٠.٥٤±٠.٠١ [a]	٠.٥٨±٠.٠١ [abc]

Data are expressed as mean ± S.E.M. of ٦ rats in each group. a: Significant from control group, b: Significant from MSG treated group, c: Significant from L-NAME treated group respectively, MSG: Monosodium glutamate, L-NAME: N^G-Nitro-L-Arginine Methyl Ester.

Table (٣): Serum glucose, insulin, HOMA-IR, liver and muscle glycogen concentrations in the different studied groups

Parameters	Groups			
	Control	MSG	L-NAME	L-NAME +MSG
Serum glucose (mg/dl)	٩٨.٨ ± ١.٨	١٧٩ ± ٢.١ [a]	٢٠٣ ± ٣ [ab]	٢٠٥.٣ ± ٣.٧ [ab]
Serum insulin (µU/ml)	٧.٥ ± ٠.١	١٢.١ ± ٠.٥ [a]	١٤.٨ ± ٠.٤ [ab]	١٤.٤ ± ٠.٣ [ab]
HOMA-IR	١.٦٦ ± ٠.١٣	٤.٨٣± ٠.٢٥ [a]	٦.٦٨ ± ٠.٢٩ [ab]	٦.٥٦ ± ٠.٢٤ [ab]
Liver glycogen (mg/g tissue)	١٩.٩٨ ± ٠.٥	٣٤.٢٥ ± ٠.٦ [a]	١٤.٤ ± ٠.٥ [ab]	٣٢.١ ± ١.٢ [ac]
Muscle glycogen (mg/g tissue)	٢.٧ ± ٠.٠٣	٨.٨± ٠.٠٩ [a]	٣.٧ ± ٠.٠٥ [b]	١٠ ± ٠.٠٧[ac]

Data are expressed as mean ± S.E.M. of ٦ rats in each group. a: Significant from control group, b: Significant from MSG treated group, c: Significant from L-NAME treated group respectively, MSG: Monosodium glutamate, L-NAME: N^G-Nitro-L-Arginine Methyl Ester. HOMA -IR: Homeostasis Model Assessment of Insulin Resistance.

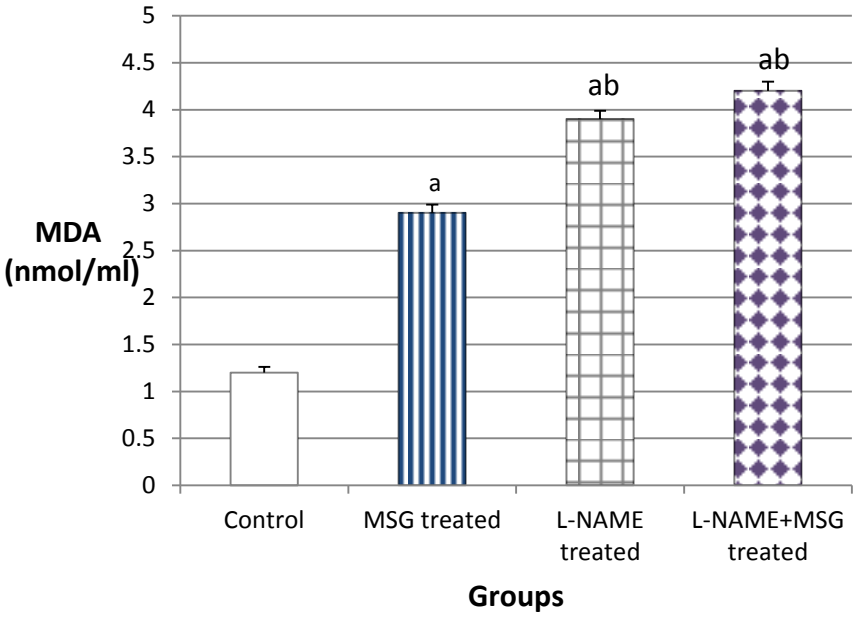

Figure ٢: Serum MDA concentration in the different studied groups. Data are expressed as mean ± S.E.M. of ٦ rats in each group. a: Significant from control group, b: Significant from MSG treated group, c: Significant from L-NAME treated group respectively, Monosodium glutamate, L-NAME: N^G-Nitro-L-Arginine Methyl Ester, MDA: malondialdehyde.

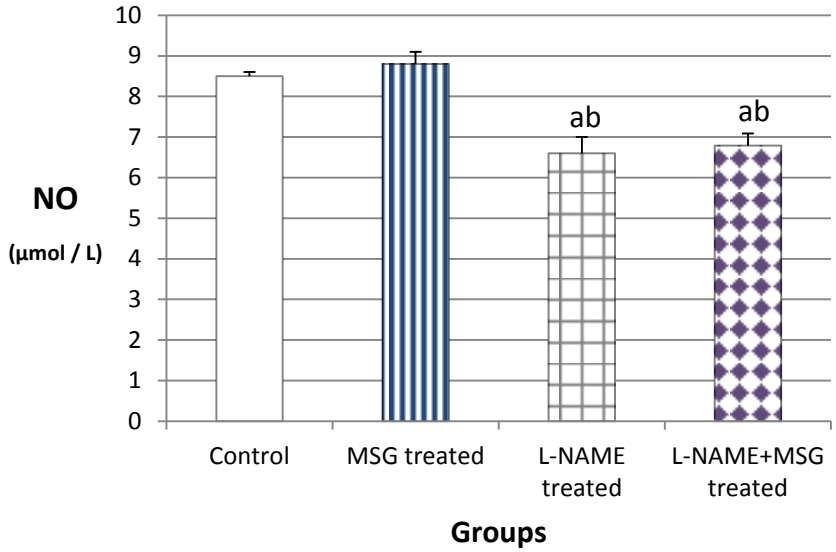

Figure ٣: Serum NO concentration in the different studied groups. Data are expressed as mean ± S.E.M. of ٦ rats in each group. a: Significant from control group, b: Significant from MSG treated group, c: Significant from L-NAME treated group respectively, Monosodium glutamate, L-NAME: N^G-Nitro-L-Arginine Methyl Ester, NO: Nitric oxide.

Table (٥): Serum Glutamate (mg/dl) and ghrelin (ng/ml) concentrations in the different studied groups.

Parameters	Groups			
	Control	MSG	L-NAME	L-NAME+MSG
Serum glutamate	١٤.٨ ± ٠.٣	١٤.٢ ± ٠.٣	١١.٠٣ ± ٠.٣ ab	١٠.٧ ± ٠.٥ ab
Serum ghrelin	٦.٦ ± ٠.٢	٦.٢ ± ٠.٠٩	٤.٧ ± ٠.٠٦ ab	٤.٦ ± ٠.٢ ab

Data are expressed as mean ± S.E.M. of ٦ rats in each group. a: Significant from control group, b: Significant from MSG treated group, c: Significant from L-NAME treated group respectively, P < ٠.٠٥. MSG: Monosodium glutamate, L-NAME: N^G-Nitro-L-Arginine Methyl Ester.

Discussion

MSG is a freely used food additive both as a preservative and an enhancer of food palatability based on the stimulant effect of glutamate to "umami taste receptors" under physiological conditions. Being categorized as "Generally considered as safe"; GRSA-list of foods by the Food and Drug Administration (FDA) of the United States, it had no specific daily intake limit, although globally, much controversy has been generated concerning its safety [١٧].

In the present work, chronic daily MSG intake significantly increased food intake, body weight, and Lee index with disturbed carbohydrate and lipid meta-bolism towards a metabolic syndrome picture with a significant hyperglycemia, hyperinsu-linemia, increased insulin resistance (increased HOMA-IR) and atherogenic lipid profile. These metabolic changes can be explained by broad distribution of taste receptors in the gut and their neural and hormonal gut-brain signals to regulate metabolic homeostasis[١٨]. Glutamate is the only amino acid which can stimulate afferent fibers of the gastric branch of vagus in the stomach[٢,٣]. In the present work, glutamate could have increased appetite through the orexigenic effect of vagus via the metabotropic glutamate receptors according to Torii[١]. He also suggested NO as the mediator. Blocking NO synthesis with L-NAME in this study completely prevented this MSG effect, as food intake, body weight and Lee index were reduced and became insignificantly different from the control group in the combined group.

The role of ghrelin in the MSG-induced increase in food intake was investigated in this work. Ghrelin; the orexigenic gut hormone secreted from the oxyntic gland cells of the gastric mucosa was not significantly increased in fasting serum of MSG treated rats of the present work. Maximal serum ghrelin levels occur during fasting [١٩] as measured in this study. As far as we know, no previous research was reported about variation in serum ghrelin in MSG-treated mice or rats; however, Vancleef et al., [٢٠] have recently found a stimulant effect of MSG on its release from a ghrelinoma cell line in ex vivo study. Our findings exclude a direct central effect of ghrelin on the hypo-thalamus, to mediate the MSG-stimulated food intake, yet an indirect peripheral role through vagal afferent stimulation could not be ignored and is compatible with the findings of Berthoud[٢١], and Torii et al.,[٥] that ghrelin's appetite-stimulating effect was abolished in rats with sub-diaphragmatic vagotomy and in rats with capsaicin-induced vagal deafferentation. Furthermore, ghrelin receptors were found on vagal afferents. Clinically, ghrelin does not stimulate food intake in vagotomized patients.

Similar to ghrelin, serum glutamate or NO was not found to be significantly increased by MSG in this work. Therefore, we can exclude a direct effect of ingested MSG on the brain, nor the systemic involvement of NO as a mediator for its action. Many mechanisms tightly control serum glutamate levels and could explain the present finding. Ingested glutamate is extensively absorbed and metabolized in the intestine by a first pass effect. Alpha ketoglutaric is formed consequent to transamination of glutamate via glutamic-oxaloacetic (GOT) and glutamic-puruvic (GPT) transaminases, and is rapidly incorporated to kreb's cycle generating ATP in the gut; that is why it is considered an oxidative fuel [٢٢]. Furthermore, increased plasma transaminases,[٢٣] in addition to the liver, gut and muscle have been found to clear blood stream of injected glutamate within ١٠ minutes according to Leibowitz et al.,[٢٤]. They added that glutamate could also be converted to important biochemical molecules; glutamine, glutathione, proline and arginine and could not cross the BBB under normal condition.

In experimental animals, contro-versial results concerning the effect of MSG on body weight were reported. Contini et al., [٧] agreed with our obesigenic effect of MSG, while Boutry et al.,[٦] and Tordoff et al., [٢٥] found no change. On the contrary, suppression of body weight gain, fat deposition, and plasma leptin levels were found by Kondoh and Torii[٢٦], and ascribed these effects to increased energy expen-diture inspite of increased food intake. Also, chronic MSG intake was reported to damage the gastric mucosa[٢٧], ileum [٢٨], and liver [٢٩] associated with mal-digestion and mal-absorption. In humans, contro-versial data suggest that MSG consumption either increases[٣٠] or has no effect on the risk of becoming overweight[٣١]. Thus, these controversial results show that the effect of MSG depends on the dose, duration, species and degree of toxicity on GIT mucosal integrity and function.

Increased food intake and the positive energy balance resulting from MSG administration in the present work was obesigenic as marked by the significant increase in GCOF and a Lee index > ٠.٣. Increased leptin release from visceral fat adipocytes following saturation of the fat depot opposes insulin and explains the developed picture of metabolic syndrome characterized by insulin resistance as evidenced by increased HOMA-IR together with hyperglycemia according to Abel et al., [٣٢] Furthermore, hyperinsulinemia could also be due to increased vagovagal reflex activity to pancreatic islets by the stimulating effect of MSG, in addition to increased release of incretin hormones from the GIT enteroendocrine cells as Glucose-dependent insulinotropic peptide (GIP) and glucagon like peptide ١ (GLP-١). These peptides have trophic secretory effects on β-cells of the pancreas[٤,١]. Finally, MSG could alter the expression of muscarinic receptor subtypes in the pancreas with increased ratio of insulinotropic to insulin-ostatic subtype receptor expression $(M_١+M_٣)/(M_٢+M_٤)$ in pancreatic islets according to Miranda et al.,[٣٣] They also

reported that atropine treatment could reduce insulin secretion in MSG-treated rats.

Liver and muscle glycogen were significantly higher in MSG treated rats of the present work. This finding is logic in the presence of high food intake, fasting hyperglycemia and hyperinsulinemia and is compatible with Bevzo[٣٤]. MSG; being a gluconeogenic amino acid, metabolized mainly in the muscle and liver [٣٤] could flood the Kreb's cycle and spare glucose as high protein diet do. Stepien et al.,[٣٥] found that this diet promotes lipid oxidation; an effect mediated by altering the transcription of the corresponding enzymes. The MSG-induced higher liver glycogen in the present work, could be a switch off mechanism attempting to feedback inhibit the hypothalamus by stimulating afferent hepatic vagal fibers according to the "hepatostatic hypothesis"[٣٦]. However, it may have not reached its threshold due to short duration or the peripheral MSG stimulation is stronger.

Dyslipidemia with the development of atherogenic lipid profile signifies increased leptin release from adipocytes after saturation of the fat depot that opposes insulin, hence increased hormone-sensitive lipase activity[٣٧]. These results are consistent with Egbuonu and Osakwe[٣٧] and Singh et al.,[٣٨] and prove the cardiac health hazards of MSG. The significantly higher MDA due to MSG treatment is an indicator of oxidative stress and could be due to increased α-ketoglutarate from glutamate, flooding the Kreb's cycle and generating free radicals via α-ketoglutarate dehydro-genase or reduced antioxidant enzyme activity [٣٩].

L-NAME treated rats in the present study showed reduced serum NO with consequent reduced food intake, body weight, hyper-glycemia with insulin resistance, lowered liver and muscle glycogen, dyslipidemia with atherogenic lipid profile, and increased MDA as a marker of oxidative stress. NO, is a cell signaling molecule in nearly every tissue of the body, formed from the amino acid L-arginine by the three isoforms of nitric oxide synthases (NOS); neuronal; (nNOS), endothelial (eNOS) and inducible (iNOS) to regulate different metabolic functions. In the hypothalamus, it stimulates the appestat to increase food intake[٤٠]. It increases glucose uptake and oxidation in all tissues by increasing blood flow, stimulating glucose transporter; $GLUT_4$, and stimulating mitochondrial biogenesis and respiratory chain complexes. In addition, it increases insulin release from beta cells and its sensitivity, promotes glycogenesis, and decreases gluconeogenesis. It is also a powerful free radical scavenger[٤١]. That is why blocking NO synthesis by L-NAME produced the above findings.

When L-NAME was given with MSG, some metabolic changes induced by MSG were completely reversed to insignificant levels from control. These included: food intake, body weight, Lee index and GCOF. This is explained by; either the MSG peripheral appetite stimulant effect is NO dependent, hence blocked by L-NAME or the anorexic effect of reduced NO in the hypothalamus could nullify the peripheral stimulant effect of MSG. In support of the peripheral effect, serum ghrelin; the GIT hormone was significantly lowered in the presence of L-NAME.

The other metabolic functions concerning glucose, and lipid homeostasis were aggravated in the direction of hyper-glycemia and dyslipidemia. This indicated that NO is not a mediator for MSG on these processes, otherwise L-NAME would have blocked and not aggravated them. Although L-NAME alone lowered liver and muscle glycogen, yet it failed to lower it in the presence of MSG. The mechanisms controlling liver and muscle glycogen may be different; since liver, but not muscle glycogen can contribute to blood glucose and so, need further research.

Conclusion

Chronic MSG intake is hazardous; obesigenic, diabetogenic and increases cardiac risk factors due to disturbed carbohydrate and lipid metabolism. A peripheral effect through stimulating neuronal and/or enteroendocrine gut-brain axis is the most probable. The insignificant

changes in serum glutamate and ghrelin exclude a direct effect on the appestat center through the blood brain barrier. NO could be a mediator of the peripheral MSG stimulated appetite mechanism, but not its systemic metabolic disturbances.

References:

١. Torii K. Brain activation by the umami taste substance monosodium-L-glutamate via gustatory and visceral signaling pathways and its physiological significance due to homeostasis after a meal. J Oral Biosci. ٢٠١٢; ٥٤(٣):١٤٤-١٥٠.

٢. San Gabriel A, Nakamura E, Uneyama H, Torii K. Taste, visceral information and exocrine reflexes with glutamate through umami receptors. J Med Invest. ٢٠٠٩; ٥٦: ٢٠٩S-٢١٣S.

٣. San Gabriel A. Taste receptors in the gastrointestinal system. Flavour, ٢٠١٥; ٤:١٤.

٤. Kondoh T, Mallick H, Torii K. Activation of the gut-brain axis by dietary glutamate and significance in energy homeostasis. Am J Clin Nutr. ٢٠٠٩; ٩٠(٣): ٨٣٢S-٨٣٧S.

٥. Torii K, Uneyama H, Nakamura E. Physiological role of dietary glutamate signaling via gut-brain axis due to efficient digestion and absorption. J Gastroenterol. ٢٠١٣; ٤٨(٤): ٤٤٢-٤٥١.

٦. Boutry C, Bos C, Matsumoto H, Even P, Marnich D, Tome D, Blachier F. Effects of monosodium glutamate supplementation on glutamine metabolism. Front Biosci. ٢٠١١; ٣: ٢٧٩-٢٩٠.

٧. Contini M, Riera, N, Mahieu S. Kidney and liver functions and stress oxidative markers of monosodium glutamate-induced obese rats. Food and Public Health. ٢٠١٢; ٢(٥):١٦٨-١٧١.

٨. Okwudiri O, Sylvanus A, Peace I. Monosodium glutamate induces oxidative stress and affects glucose metabolism in the kidney of rats. Int J Biochem Res Rev. ٢٠١٢; ٢(١): ١-١١.

٩. Mohamed R, Sabry M, Shawky H, Tawadrous A, Gharib D. Interaction between Renin Angiotensin System and Apelin/APJ System in Hypertensive Rats. Der Pharma Chemica. ٢٠١٦; ٨(٤):٣٢-٤٩.

١٠. Campos G, Volpato I, Calderon M, Rudge R, Damasceno D. Effect of obesity on rat reproduction and on development of their adult offspring. K.E. Braz J Med Biol Res. ٢٠٠٨; ٤١: ١٢٢-١٢٥.

١١. Caesar R, Drevon C. Pancreatic contamination of mesenteric adipose tissue samples can be avoided by adjusted dissection procedures. J Lipid Res. ٢٠٠٨; ٤٩: ١٢-٥٥.

١٢. Ikewuchi C, Ikewuchi C, Ifeanacho M. Attenuation of salt-loading induced cardiomegaly and dyslipidemia in wistar rats by aqueous leaf extract of Chromolaena odorata. Pharmacol Pharmacy. ٢٠١٤; ٥, ١٦٠-١٧٠.

١٣. Ma F, Qiao L, Yue H, Xie S, Zhou X, Jiang M, Zhang W, Qi J, Wang L, Xu K. Homeostasis model assessment-insulin resistance (HOMA-IR); a key role for assessing the ovulation function in polycystic ovary syndrome (PCOS) patients with insulin resistance. Endocr J. ٢٠٠٨; ٥٥(٥):٩٤٣-٩٤٥.

١٤. Ohkawa H, Ohishi N and Yagi K. Assay of lipid peroxides in animal tissues by thiobarbituric acid reaction. Anal Biochem.١٩٧٩; ٩٥: ٣٥١-٣٥٨.

١٥. Kemp A, Adrienne J. A colorimetric micro-method for the determination of glycogen in tissues. Biochem J. ٢٠٠٨; ٥٦(٤):٦٤٦-٦٤٨.

١٦. Ramu R, Shirahatti P, Swamy N, Zameer F, Dhananjay B, Prasad M. Assessment of in vivo anti-diabetic properties of Umbelliferone and Lupeol constituents of Banana (Musa sp. var. Nanjangud Rasa Bale) flower in hyperglycaemic rodent model. PLoS One. ٢٠١٦; ١١(٣): e٠١٥١١٣٥.

١٧. Eweka A, Om'Iniabohs F. Histological studies of the effects of monosodium glutamate on the ovaries of adult wistar rats. Ann Med Health Sci Res. ٢٠١١; ١ (١): ٣٧-٤٣.

١٨. Julio-Pieper M, Flor P, Dinan T, Cryan, J. Exciting times beyond the brain: metabotropic glutamate receptors in peripheral and non-neural tissues. Pharmacol. Rev. ٢٠١١; ٦٣(١): ٣٥-٥٨Burrin D, Stoll B. Metabolic fate

and function of dietary glutamate in the gut. Am J Clin Nutr. ٢٠٠٩; ٩٠(٣): ٨٥٠S–٨٥٦S.

١٩. Yin X, Li Y, Xu G, An W, Zhang W. Ghrelin fluctuation, what determines its production? Acta Biochim. Biophys Sin. ٢٠٠٩; ٤١ (٣): ١٨٨–١٩٧.

٢٠. Vancleef L, Van Den Broeck T, Thijs T, Steensels S, Briand L, Tack J, Depoortere I. Chemosensory signalling pathways involved in sensing of amino acids by the ghrelin cell. Sci Rep. ٢٠١٥; ٥:١٥٧٢٥.

٢١. Berthoud H. The vagus nerve, food intake and obesity. Regul Pept. ٢٠٠٨; ١٤٩(١-٣):١٥–٢٥.

٢٢. Burrin D, Stoll B. Metabolic fate and function of dietary glutamate in the gut. Am J Clin Nutr. ٢٠٠٩; ٩٠(٣): ٨٥٠S–٨٥٦S.

٢٣. Egbuonu A, Ezeanyika L, Ejikeme P, Obidoa O. Histomorphologic alterations in the liver of male wistar rats treated with L-arginine, glutamate and monosodium glutamate. Res J Environ Toxicol. ٢٠١٠; ٤(٤): ٢٠٥-٢١٣.

٢٤. Leibowitz A, Boyko M, Shapira Y, zlotnik A. Blood glutamate scavenging: Insight into neuroprotection. Int J Mol Sci. ٢٠١٢; ١٣, ١٠٠٤١-١٠٠٦٦.

٢٥. Tordoff M, Aleman T, Murphy M. No effects of monosodium glutamate consumption on the body weight or composition of adult rats and mice. Physiol Behav. ٢٠١٢; ١٠٧(٣):٣٣٨-٣٤٥.

٢٦. Kondoh T, Torii K. MSG intake suppresses weight gain, fat deposition, and plasma leptin levels in male Sprague-Dawley rats. Physiol Behav. ٢٠٠٨; ٩٥: ١٣٥-١٤٤.

٢٧. Falalieieva T, Kukhars'kyĭ V, Berehova T. Effect of long-term monosodium glutamate administration on structure and functional state of the stomach and body weight in rats. Fiziol Zh. ٢٠١٠; ٥٦(٤): ١٠٢-١١٠.

٢٨. Eweka A, Om'Iniabohs F. Histological studies of the effects of monosodium glutamate on the small intestine of adult wistar rats. Electron J Biomed. ٢٠٠٧; ٢: ١٤-١٨.

٢٩. Eweka A, Igbigbi P, Ucheya R. Histochemical studies of the effects of monosodium glutamate on the liver of adult wistar rats. Ann Med Health Sci Res. ٢٠١١; ١(١): ٢١-٢٩.

٣٠. He K, Du S, Xun P, Sharma S, Wang H, Zhai F, Popkin, B. Consumption of monosodium glutamate in relation to incidence of overweight in Chinese adults. China Health and Nutrition Survey (CHNS). Am J Clin Nutr. ٢٠١١; ٩٣: ١٣٢٨-١٣٣٦.

٣١. Shi Z, Luscombe-Marsh N, Wittert G, Yuan B, Dai Y, Pan X, Taylor, A. Monosodium glutamate is not associated with obesity or a greater prevalence of weight gain over ٥ years: findings from the Jiangsu Nutrition Study of Chinese adults. Br J Nutr. ٢٠١٠; ١٠٤(٣):٤٥٧-٤٦٣.

٣٢. Abel E, O'Shea K, Ramasamy R. Insulin resistance: metabolic mechanisms and consequences in the heart. Arterioscler Thromb Vasc Biol. ٢٠١٢; ٣٢: ٢٠٦٨-٢٠٧٦

٣٣. Miranda R, Agostinho A, Trevenzolib I, Barella, L, Franco C, Trombini A, Malta A, Gravena C, Torrezan R, Mathias P, de Oliveira J. Insulin oversecretion in MSG-obese rats is related to alterations in cholinergic muscarinic receptor subtypes in pancreatic islets. Cell Physiol Biochem. ٢٠١٤; ٣٣(٤):١٠٧٥-١٠٨٦.

٣٤. Bevzo V. Effect of prolonged administration of sodium glutamate in some carbohydrate metabolism. Inter Scientific J. ٢٠١٦; ٣(٦): ١٨-٢٦.

٣٥. Stepien M, Gaudichon C, Fromentin G, Even P, Tome D, Azzout-Marniche D. Increasing protein at the expense of carbohydrate in the diet down-regulates glucose utilization as glucose sparing effect in rats. PLOS One. ٢٠١١; ٦(٢): e١٤٦٦٤.

٣٦. López-Soldado I, Zafra D, Duran J, Adrover A, Calbó J, Joan uinovart J. Liver glycogen reduces food intake and attenuates obesity in a high-fat diet–fed mouse model. Diabetes. ٢٠١٥; ٦٤:٧٩٦–٨٠٧.

٣٧. Egbuonu A, Osakwe O. Effects of high monosodium glutamate on some serum markers of lipid status in male Wistar rats.J Med Med Sci.٢٠١١;٢(١): ٦٥٣-٦٥٦.

٣٨. Singh K, Sharma J, Kaur A, Pushpa Ahluwalia P. Alteration upon oral

ingestion of monosodium glutamate in various lipid and lipoprotein fractions in serum of adult male rat. J Life Sci. ٢٠١١; ٣(١): ١٧-٢١.

٣٩. Sharma A. Monosodium glutamate-induced oxidative kidney damage and possible mechanisms: a mini-review. J Biomed Sci. ٢٠١٥; ٢٢ (٩٣): ٢-٦.

٤٠. Malfatti C, da Silva L, Pereira R, Michel R, Snak A, dos Santos F. Acute hypothalamic administration of L-arginine increases feed intake in rats. Rev Nutr. ٢٠١٥; ٢٨(١):٥٥-٦٣.

٤١. Dellamea B, Leitão C, Friedman R, Canani L. Nitric oxide system and diabetic nephropathy. Diabetol Metab Syndr. ٢٠١٤; ٦(١): ١٧.