ORIGINAL ARTICLE

COMPARISON OF DIET INDUCED METABOLIC SYNDROME WITH ARTIFICIALLY INDUCED METABOLIC SYNDROME IN A RAT MODEL

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Background: Metabolic syndrome is an emerging health problem. The diets rich in fats and refined carbohydrates and monosodium glutamate are considered major risk factors for this emerging epidemic. The aim of this study was to determine whether high fat high carbohydrate (HFHC) diet, Monosodium glutamate (MSG) diet or their combination is faster/more potent inducer of MS. **Methods:** Twenty male Sprague Dawley rats were randomly divided into four groups. Group 1 was given normal rat chow, group 2 was given HFHC diet, group 3 was given MSG in diet and group 4 was given both HFHC and MSG in their diet for 20 weeks. Body weight, blood pressure, lipid profile and glycaemic indices were determined at the end of study. **Results:** After 20 weeks of study, HFHC and MSG groups showed full blown Metabolic syndrome (MS) with development of obesity, hypertension, hyperglycaemia and dyslipidemia. However, group 4 which was given combination diet did not develop features of MS. **Conclusion:** Both HFHC and MSG containing diets when given alone are potent inducers of MS in rat model rather than their combination.

Keywords: Metabolic syndrome, high fat high carbohydrate diet, monosodium glutamate, obesity, hypertension, dyslipidemia, insulin resistance

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INTRODUCTION

Non-communicable diseases (NCD) have become a major cause of morbidity and mortality in today's world in both developed and developing countries. Among these NCDs, Metabolic Syndrome (MS) has emerged as a global epidemic.[1] Metabolic syndrome or syndrome X is a cluster of medical abnormalities characterized by presence of three or more of the following pathologies: hypertension, hyperglycaemia, dyslipidemia, obesity and insulin resistance.[2] Other co-morbidities with MS include pro-thrombotic and pro-inflammatory states, non-alcoholic steatohepatitis, reproductive disorders and certain types of cancer.[3] Patients with MS are at two fold increased risk of developing cardiovascular disease (CVD) and five fold increased risk of type 2 diabetes mellitus compared to normal people.[4]

The prevalence of MS is rapidly increasing worldwide primarily due to sedentary lifestyles and unhealthy eating habits.[5] Other contributing factors and mechanisms include insulin resistance, adipose tissue dysfunction, chronic inflammation, oxidative stress, circadian disruption, microbiota, genetic factors etc.[6] Strategies aimed at primary prevention are required to ameliorate its further rise as well as reduction of associated morbidity and mortality.[3]

Studies have revealed that consumption of diet and beverages rich in fats and sugars is clearly responsible for increased incidence of MS. Saturated fats and sugars when taken together, become greater risk factor for development of MS than fats or sugars given alone.[7,8] High fat high carbohydrate (HFHC) diet provides excess energy than needed in the body which is then stored in adipose tissue resulting in its expansion and inducing obesity. Increased adiposity is associated with generation of oxidative stress (OS) and local inflammatory changes. OS leads to impaired glucose tolerance and high insulin secretion from pancreas. Increased reactive oxygen species and generation of Angiotensinogen by adipose tissue are responsible for high blood pressure. HFHC diet also induces *de novo* hepatic lipogenesis.[9]

Several epidemiological studies reveal the association of dietary consumption of the chemical Monosodium glutamate (MSG), also called *Ajino moto*, with metabolic disorders such as obesity, hypertension and metabolic syndrome. Its consumption is increasing worldwide as a flavour enhancer, in parallel with the epidemic of metabolic syndrome.[10] It is a sodium salt of the naturally occurring amino acid L-glutamate which elicits a unique taste called 'umami'.[11] It increases the sapidity of food and produces a flavour which cannot be provided by other food additives.[12] It is present in most processed foods, mostly hidden on ingredient labels and listed under other names. Chronic MSG intake may cause glutamate induced damage to arcuate nucleus, disrupting energy regulation axis. It leads to damage to leptin signalling through hypothalamus, causing leptin resistance, overweight and obesity.[13] Chronic administration of MSG induces OS in experimental animals and changes metabolic and endocrine indicators. It induces hypertension due to excessive OS and renal sodium and water retention.[11] It increases hepatic

gluconeogenesis resulting in hyperglycaemia which then causes increased insulin secretion. It also induces insulin resistance via induction of oxidative stress.[14]

Being a rapidly emerging epidemic like disease, researchers are probing more into detailed mechanism of metabolic syndrome and strategies for its prevention. Animal models have a key role in understanding the disease pathophysiology. The aim of this study was to determine whether HFHC diet, MSG diet, or their combination is more potent inducer of MS.

MATERIAL AND METHODS

This was an experimental study. The protocols were approved by the Ethics Committee of PGMI, Lahore, and were conducted in accordance with the guidelines for animal care. The sample size was calculated at 90% power of study and 5% level of confidence. The calculated sample size was five in each group.[15] Twenty male Sprague Dawley rats aged 4–5 weeks, weighing 50–70 g were kept in a controlled temperature of 23±2 °C under natural light and dark cycle, and were fed with standard Rat chow and water *ad libitum* for one week.

After one week of Acclimatization, rats were randomly divided into 4 groups of 5 each with almost equal body weights and were placed on following diets for 20 weeks:

Rats in Group-1 were fed with normal rat chow composed of 48% carbohydrates, 21% protein, 3% fat, 5% fibre, 13% moisture, 8% ash and traces of calcium and phosphorus. Group-2 rats were fed with rat chow in which MSG was mixed in an amount of 5 g MSG/Kg body weight of rats and dose was adjusted per 100 gm diet.[16] Rats in Group-3 were given rat chow in which HFHC diet was mixed. High fat diet consisted of 15% beef tallow (15 gm beef tallow in 100 gm rat chow), 1% cholesterol (1 gm cholesterol in 100 gm rat chow) and 0.5% sodium deoxycholate (0.5 gm in 100 gm rat chow).[17] High fructose diet consisted of 15% fructose (15 gm fructose in 100 ml water) in drinking water.[2] Group-4 rats were fed with rat chow in which both HFHC diet and MSG were mixed.

Body weights of animals were measured weekly using a digital lab weighing scale. Blood pressure was measured at the end of the study on a non-invasive blood pressure measuring system using a pulse transducer connected to PowerLab®. Measurements were considered valid only when 3 consecutive readings did not differ by more than 10 mmHg. After 12 hour overnight fasting, blood samples were taken from cardiac puncture using a disposable syringe with 26 gauge needle under chloroform anaesthesia. After 30 minutes, serum was separated by centrifuge at 3,000 rpm, and stored at -20 °C in serum cups.

Total cholesterol (TC), triglycerides (TGs) and high density lipoproteins (HDL) were measured by Colorimetric method with spectrophotometer. Blood Glucose was measured by Oxidase method with spectrophotometer. Serum Insulin was measured with ELISA with rat insulin kit (Bioassay Technology Laboratory, China), using Sandwich ELISA technique. Serum LDL was calculated with formula: LDL cholesterol=TC-HDL-(TG/5). HOMA-IR was calculated with formula:

HOMA-IR=Fasting Insulin×Fasting Plasma Glucose/405

Data was analysed using SPSS-22. Normality of data was checked by Kolmogorov-Smirnov test. Normally distributed results were presented as Mean±SD. One way ANOVA was applied for comparison among study Groups. Post Hoc Tuckey test was applied to analyse the differences of means among groups, and $p \leq 0.05$ was taken as statistically significant.

RESULTS

At the end of study, intergroup comparison by ANOVA showed significant difference among groups. Results of Post Hoc Tuckey test showed that rats in MSG and HFHC group exhibited a significant weight gain as compared to normal control (NC) group. However, body weight of HFHC+MSG group was significantly less than other groups.

One-way ANOVA showed significant differences of blood pressure among groups. Post Hoc Tuckey test showed that both HFHC and MSG groups showed a significant increase when compared to NC although hypertension was developed more in HFHC than MSG group. There was no significant difference of HFHC+MSG group with NC Group. (Table-1).

Lipid profile changes were variable in different groups at the end of the study, Serum Triglycerides (TG) changes being significant in HFHC group, Serum Cholesterol (TC) changes significant in combination group while Serum HDL was significantly decreased in MSG rats. (Figure-1).

Fasting Hyperglycaemia was clearly developed in MSG and HFHC groups only showing significantly higher values when compared to NC. MSG administration induced higher serum insulin levels both alone as well as in combination diet although results were statistically non-significant while HFHC diet did not induce any remarkable change in insulin level. Results of HOMA-IR indicate that MSG induces higher insulin resistance. (Table-2).

Table-1: Effects of different diets on body weight and BP of rats (n=5 in each)

Groups	Body Weight (gm)	Blood Pressure (mmHg)
NC	262±33.98	99.80±6.61
MSG	335±31.81 *(p=0.006)	124.80±6.18 *(p=0.000)
HFHC	335.60±29.1 *(p=0.005)	133.60±6.84 *(p=0.000)
HFHC+MSG	191.40±18.88 *(p=0.007)	109.80±3.11

*Post Hoc Tuckey test

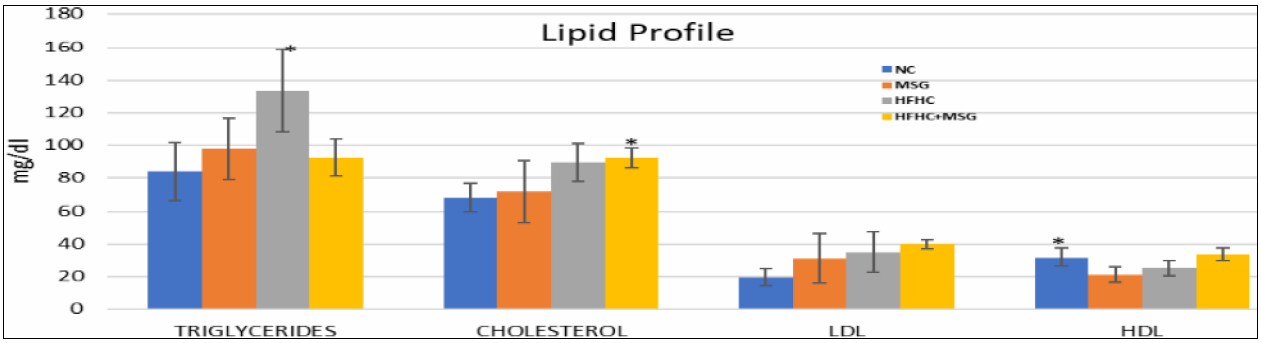

Figure-1: Effects of different diets on Lipid profile of rats

*p<0.05 as compared to NC (Analysed by Post Hoc Tuckey test)

Table-2: Effects of different diets on Glycaemic indices of rats

Groups	Fasting Glucose (mg/dl)	Serum Insulin (mIU/L)	HOMA-IR
NC	84.20±8.58	1.34±1.11	0.27±.23
MSG	157.60±27.75 *(p=0.000)	3.04±2.80	1.17±1.16
HFHC	152.80±8.46 *(p=0.000)	1.78±0.27	0.67±.14
HFHC+MSG	81.80±8.16	3.08±0.31	0.61±.07

*Post Hoc Tuckey test

DISCUSSION

Metabolic syndrome (MS) is a multifactorial condition with alarming rate of prevalence nowadays. To evaluate the pathophysiology of MS in humans, it is crucial to establish appropriate experimental animal models which mimic the disease state in humans.[18] Among rodents, rats and mice are the most common animal models for investigating MS and in rats, Sprague Dawley rats and Wister rats are most commonly used rodents.[19]

Several previous studies have shown the successful induction of MS in animal models with HFHC diet and also with MSG.[7,20] Literature review reveals different experiences in success of induction, duration of induction and parameters affected by MS.[18] we have conducted this study to evaluate whether diet, chemical or a combination of both is potent and faster inducer of MS in rat model.

There was significant development of obesity, hypertension, fasting hyperglycaemia and hyper-triglyceridemia in rats fed HFHC diet as compared to NC rats. Our results confirm the findings of several other researchers.[17,21,22] However, the levels of serum insulin and HOMA-IR although raised from NC rats were statistically not significant which could be due to shorter duration of study. Pathophysiological changes by HFHC diet include excess energy in body resulting in high serum free fatty acid concentration, hypertrophy of adipose tissue, increased oxidative stress and insulin resistance among various other mechanisms.[21,22]

We observed that MS was also successfully induced in MSG rats with development of significant obesity, hypertension, hyperglycaemia, and decreased HDL levels as compared to NC rats. The values of serum insulin and HOMA-IR although higher than NC and HFHC groups but were statistically non-significant which may be due to our rat model in which adult rats were recruited for study, shorter duration of study or insufficient dose of MSG. Our results also coincide with previous human as well as animal studies.[10,13,23–25] Increased oxidative stress, changes in fat and carbohydrate metabolism, sodium and water retention by kidney might be possible mechanisms responsible for development of MS by monosodium glutamate.[11] Another research shows that MSG is linked with obesity, type II diabetes, and the metabolic syndrome as its intake in healthy Chinese adults correlates with the resulting increase in body mass index regardless of energy intake.[23]

On the contrary, rats of combination group (HFHC+MSG) showed reduction in weight gain when compared with NC rats, which might be due to the decreased food intake (noted by leftover food pellets after 24 hrs) and early satiety. Our findings coincide with findings of Onaolapo *et al*.[26] who noted that obesity, hyperglycaemia and dyslipidemia did not develop with this combination diet. These findings are contrary to the findings of some other researchers.[27] This may be due to antagonistic effects of both diets when given in combination as demonstrated by Su *et al*[28] in their research on growing pigs. They observed antagonistic effects of both diets on most fatty acid receptors in gut wall resulting in reduced activity of most fatty acid receptors and resultant altered lipid metabolism. The effects of this combination diet may also be due to less food consumption and early satiety, reduction in lipid peroxidation and possible interaction between the diets altering the expression of lipid metabolizing genes.[26]

CONCLUSION

HFHC diet and diets containing MSG when given individually, are effective inducers of MS in rat model and may be considered for induction of MS in experimental animals. Combination model failed to induce features of MS.

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NZ: Literature search, Data collection
AHS: Data analysis
KI: Data Collection, Editing of manuscript
RMY: Technical/Lab work, Data collection
MS: Concept, Design of work, Proof reading

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