Enhanced Platelet Aggregation, Hyperinsulinemia and Low Testosterone Level in Monosodium Glutamate Obese Rats

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Abstract: The aim of this study was to assess the effect of obesity induced by high fat diet and monosodium glutamate (MSG) on platelet aggregation, hematologic parameters, plasma insulin and testosterone in adult male rats. Male rats were distributed into 3 groups: Control group, receiving control diet. High fat diet (HFD) -induced obesity group, receiving high fat diet. Monosodium glutamate (MSG) - treated group, receiving control diet and MSG in a dose of 150 mg/Kg b.w. daily by gavage. The HFD and MSG- obese rats showed increased values of final body weight (BW), body mass index (BMI) and Lee index as well as the percentage gain of these parameters compared with their matching controls. Erythrocyte count, hemoglobin content, the total and differential leukocyte count, the platelet count and the platelet indices (mean platelet volume, and platelet distribution width) were not significantly changed in the different studied groups compared to their matched control group. However, ADP-induced platelet aggregation was significantly increased in the MSG-obese rats as compared to either control rats or HFD-obese rats. Radioimmunoassay measurement revealed significant increase in plasma insulin level in MSG-obese group compared to their matched control group. In contrast, plasma testosterone level was significantly decreased in MSG-obese group compared to their matched control and HFD-obese groups. A significant positive correlation was displayed between BMI and ADP-induced platelet aggregation in the studied rats. Moreover, a positive correlation was observed between plasma insulin level and ADP-induced platelet aggregation in MSG-obese rats. Ingestion of MSG in adult male rats produced platelet hyperaggregation, hyperinsulinemia, hypoandrogenemia and hence could be responsible for the initiation of atherothrombosis. These findings raise a concern about the safety of MSG use.

Key words: Obesity, platelet aggregation, monosodium glutamate, hyperinsulinemia, hypoandrogenemia

INTRODUCTION

Obesity is a complex multifactor disease characterized by excessive accumulation of adipose tissue that may impair health. The prevalence of obesity is rapidly reaching epidemic properties in developed nations (Flegal et al., 2002; Freedman et al., 2002). Insulin induced a dose- dependent decrease of platelet aggregation to ADP in healthy subjects (Trovati et al., 1995). However in insulin- resistant subjects, a reduced antiaggregatory effect of insulin was observed (Russo et al., 2007). Monosodium glutamate, the sodium salt of glutamic acid is a food additive used as a flavoring agent for enhancing taste, may be associated with increased risk of overweight in humans (He et al., 2008). Among obesity induction models, administration of monosodium glutamate has been used as a tool to study obesity (Nakagawa et al., 2000).

Platelets play a central role in normal thrombosis and hemostasis, as well as contributing greatly to diseases such as stroke and myocardial infarction. Despite the presence of glutamate in the platelet granules; the role of glutamate in haemostasis is still unknown. Activated platelets release glutamate, platelets express α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid receptor (AMPA) subunits, and glutamate increases agonist-induced platelet activation (Morrell et al., 2008).

A small number of studies have been devoted to the possible effect of obesity especially following

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treatment with monosodium glutamate on platelet function. Accordingly, this study was carried out to investigate the possible effect of MSG-induced obesity on platelet function.

MATERIALS AND METHODS

This study was carried out on 40 male Wistar rats of average weight. Rats were purchased from the Egyptian Organization for Biological products and vaccines, El -Agouza (VACSERA) (Cairo). The rats were left for 10 days as a period of acclimatization during which all rats were fed a standard control diet prepared in our laboratory. Meals were introduced daily at 11 A.M. The weights of food remaining in the food bin in each cage were recorded. The body weight of each rat was recorded every 10 days before food presentation. Rats were randomly distributed into 3 groups:

a) Control group, receiving control diet (n=16).

b) High fat diet (HFD) - induced obesity group, receiving high fat diet (n=12).

c) Monosodium glutamate (MSG) - treated group, receiving control diet and MSG in a dose of 150 mg/Kg b.w. (Graham et al., 2000) by gavage, (n=12).

Body mass index (BMI) was assessed every 10 days till reaching BMI significantly higher than the control rats (Novelli et al., 2007). Obesity was further confirmed by assessment of Lee index, and this was considered as the end of the experimental period.

Induction of experimental obesity by high fat diet was introduced by feeding rats with high fat diet. It was prepared by increasing the fat content of diet to 11-12%. Diets were designed in our laboratory; the composition of control diet is: (protein, 12- 13%; carbohydrates, 50- 51%; fats, 4- 5% and calories, (total calories by 100gm = 283.4 Kcal). The composition of high fat diet is: (protein, 19%; carbohydrates, 45- 46%; fats, 11- 12% and calories, (total calories by 100gm = 335 Kcal).

Anthropometric parameters: The body weight [BW, (g)] and body length (naso-anal length) [length, (cm)] were measured and used to determine the following parameters: Body mass index [BMI; body weight (g)/length^2(cm^2)]; Lee index [cube root of body weight (g)/ length (cm)] (Nascimento et al., 2008).

Experimental Procedures:

At the end of the experimental period, overnight fasted rats were weighed and anaesthetized with intraperitoneal injection of pentobarbitone sodium (Abbott laboratories), in a dose of 40mg/kg body weight. A midline incision was made, and the abdominal aorta was exposed and cannulated. Three blood samples were collected. One ml of blood was drawn into a tube containing EDTA, and kept at room temperature for assessment of a complete blood picture within 1 hour from blood collection.

Three ml blood were collected into chilled plastic tube containing sodium citrate 3.8% (9 volumes of blood to 1 volume of citrate), and gently mixed. The citrated blood was used for preparation of platelet rich plasma (PRP) and platelet poor plasma (PPP), for testing platelet aggregation. A third blood sample was collected in a tube containing 1 drop of heparin (5000 I.U. /ml, Nile CO.) and used for hormonal assessment. Complete blood picture was performed by the use of Sysmex kx-21N counter, Japan.

Platelet aggregation was tested by the turbidimetric technique; according to the method of Mustard et al. (1964). PRP was prepared and used for platelet aggregation as described by Youssef, (2002) using Chrono-Log Automatic Aggregometer (model 540, Chrono-Log Corporation, Harverton, USA), coupled with computer and printer. The aggregating agent used was ADP at a final concentration of 10 μM/L.

Hormonal Assay:

Testosterone and Insulin radioimmunoassay: Plasma testosterone and insulin levels were measured with rat-specific radioimmunoassay kits (Coat-A-Count; Diagnostic Products Corp. (DPC), Los Angeles, CA). Assays were performed according to the manufacturer’s instructions.

Statistical Analysis:

Data were expressed as means ± SEM. Statistical significance of data was determined using a one-way analysis of variance (ANOVA) with post-hoc test, significance calculated by least significant difference (LSD) multiple range-test to find inter-group significance. The correlations among variables were determined by linear regression analysis. The level of significance was accepted as P < 0.05.

Results:

Data of the present study are summarized in tables (1-2) and illustrated in figures (1-3).
Fig. 1: Tracing of ADP-induced platelet aggregation (%) in control rats (A), High-fat diet-obese rats (B) and monosodium glutamate-obese rats (C).

Fig. 2: Graph showing correlation of BMI (gm/cm²) and ADP-induced platelet aggregation in the studied rats. Control rats, □ High fat diet-obese rats □ and monosodium glutamate-obese rats •.

**Anthropometric Parameters:**
Initial BW, BMI and Lee indices showed insignificant difference among all studied groups. Final BW, BMI and Lee indices as well as the percentage gain of these parameters were increased significantly in rats of the HFD and MSG-treated groups compared to their matching controls.

**Plasma Glucose Level:**
Showed nonsignificant difference among all studied groups.

**Testosterone Radioimmunoassay:**
Plasma testosterone was significantly decreased in MSG-treated group compared to their matched control group as well as compared to HFD-obese group.
Fig. 3: Graph showing correlation of plasma insulin (μlU/ml) and ADP-induced platelet aggregation (%) in the monosodium glutamate-obese rat group.

Table 1: Initial Body weight (BW), Final BW, BW gain%, Final body mass index (BMI), BMI gain%, Lee index, Lee index gain %, plasma glucose, insulin, and testosterone in the studied groups.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Control</th>
<th>HFD-induced obesity</th>
<th>MSG-treated</th>
</tr>
</thead>
<tbody>
<tr>
<td>Initial BW (gm)</td>
<td>132.3±5</td>
<td>140.5±4.5</td>
<td>143.9±6.2</td>
</tr>
<tr>
<td>Final BW (gm)</td>
<td>248.3±9.8</td>
<td>353±8.8</td>
<td>348.1±10</td>
</tr>
<tr>
<td>BW gain %</td>
<td>90.7±9.2</td>
<td>153.3±8.1</td>
<td>146.6±11.7</td>
</tr>
<tr>
<td>Final BMI</td>
<td>0.53±0.01</td>
<td>0.66±0.008</td>
<td>0.65±0.01</td>
</tr>
<tr>
<td>BMI gain%</td>
<td>34±4.5</td>
<td>58.8±4.3</td>
<td>63.7±6.1</td>
</tr>
<tr>
<td>Lee index</td>
<td>0.29±0.002</td>
<td>0.305±0.001</td>
<td>0.305±0.002</td>
</tr>
<tr>
<td>Lee index gain %</td>
<td>3.9±1.1</td>
<td>7.9±1.3</td>
<td>10±1.6</td>
</tr>
<tr>
<td>Pl. glucose (mg%)</td>
<td>80.9±4</td>
<td>88.3±9.2</td>
<td>82.5±3.7</td>
</tr>
<tr>
<td>Pl. insulin (μlU/ml)</td>
<td>18.8±2.1</td>
<td>27.9±4.4</td>
<td>32.5±4.5</td>
</tr>
<tr>
<td>Pl. testosterone (ng/dl)</td>
<td>89.8±12.6</td>
<td>88.4±15.9</td>
<td>44.8±6.5</td>
</tr>
</tbody>
</table>

In parenthesis is the number of observations.

(a) Significance calculated by least significant difference (LSD), P<0.05 from control group.
(b) Significance calculated by least significant difference (LSD), P<0.05 between HFD-obese and MSG-obese groups.

Table 2: Total and differential leucocytic counts, RBCs count, hemoglobin content (Hb), platelet count, ADP- induced platelet aggregation, mean platelet volume (MPV), and platelet distribution width (PDW) in the studied groups.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Control</th>
<th>HFD-induced obesity</th>
<th>MSG-treated</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total W.B.Cs (x10^3/ μl)</td>
<td>3.8±0.48</td>
<td>2.5±0.21</td>
<td>3.3 ± 0.2</td>
</tr>
<tr>
<td>Neutrophils%</td>
<td>6.3±0.9</td>
<td>9.6±1.9</td>
<td>5.7±1.2</td>
</tr>
<tr>
<td>Lymphocytes%</td>
<td>78.1±3.3</td>
<td>77±5.5</td>
<td>72.7±3.8</td>
</tr>
<tr>
<td>RBCs count (x10^12/μl)</td>
<td>7.3±0.12</td>
<td>7±0.14</td>
<td>7.2±0.12</td>
</tr>
</tbody>
</table>
Table 2: Continue.

<table>
<thead>
<tr>
<th></th>
<th>Group 1 (16)</th>
<th>Group 2 (12)</th>
<th>Group 3 (12)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hb content (gm %)</td>
<td>12.8±0.2</td>
<td>12.8±0.3</td>
<td>13±0.1</td>
</tr>
<tr>
<td>Platelet count (x10^3/μl)</td>
<td>902.4±33.1</td>
<td>909.2±38.4</td>
<td>932.7±31.8</td>
</tr>
<tr>
<td>Platelet aggregation (%)</td>
<td>50.3±2.4</td>
<td>51.2±2.2</td>
<td>60.7±2.9ab</td>
</tr>
<tr>
<td>MPV(fl)</td>
<td>5.4±0.1</td>
<td>5.3±0.1</td>
<td>5.4±0.1</td>
</tr>
<tr>
<td>PDW(fl)</td>
<td>6.2±0.17</td>
<td>6.1±0.14</td>
<td>6.2±0.14</td>
</tr>
</tbody>
</table>

In parenthesis is the number of observations (a) Significance calculated by least significant difference (LSD), P<0.05 from control group (b) Significance calculated by least significant difference (LSD), P<0.05 between HFD-obese and MSG-obese groups

**Insulin Radioimmunoassay:**

Plasma insulin was significantly increased in MSG-treated group compared to their matched control group.

**Erythrocyte and Leucocyte Parameters:**

Non-significant changes were observed in erythrocyte count and hemoglobin content, the total and differential leucocytic count in the different studied groups, compared to their matched control group.

**Platelet Parameters:**

All the studied groups showed non-significant changes in either the platelet count or any of the platelet indices (mean platelet volume, MPV, and platelet distribution width, PDW) as compared to their matched control groups. Enhanced ADP-induced platelet aggregation was encountered in the MSG-treated group as compared to either control group or HFD-obese group (figure 1).

**Correlation Studies:**

A significant positive correlation was displayed between BMI and ADP-induced platelet aggregation in the studied rats. Also, a positive correlation was observed between plasma insulin level and ADP-induced platelet aggregation in MSG-obese rats.

**Discussion:**

Induction of obesity by high fat diet and monosodium glutamate in the present study resulted in increase in BW, BMI, Lee index, and their respective percentage value compared with their matching controls. However, ADP-induced platelet aggregation, increased plasma insulin level and decreased plasma testosterone level were only found in MSG-obese rats.

Researchers reported that MSG-obesity is an early-onset obesity (Matysková et al., 2008) resulting from MSG-induced lesions in arcuate nucleus. The relationship between MSG-induced damage of the arcuate and the metabolic changes that produce obesity in MSG-treated mice was attributed to the lack of leptin receptors in arcuate nucleus (Dawson et al., 1997), causing leptin resistance related to overweight/obesity. Studies have indicated an important role for leptin in regulating food intake and energy balance (He et al., 2008).

Hyperinsulinemia in MSG-obese rats observed in this study, is in agreement with Marno et al. (1994); Matysková et al. (2008) and Sartin et al. (1985) who demonstrated hyperinsulinemia in rats, mice and golden hamsters neonatally treated with monosodium glutamate. Hyperinsulinaemia is an early marker of insulin resistance (Kapoor et al., 2005). Dolnikoff et al. (1988) reported that MSG-obese rats have high plasma levels of corticosterone, and that this hormone could play an important role in this metabolic alteration (Guillaume-Gentil et al., 1993). Also, the increased insulin level could be the result of the reported decrease of glucose transporters (GLUT4) in insulin-sensitive tissues of MSG-obese mice (Machado et al., 1993). Other factors, such as high parasympathetic activity, have been suggested to be involved in the cause of hyperinsulinemia in fasting MSG-obese rats (Balbo et al., 2002).

This study revealed a significant reduction of plasma testosterone in MSG-treated rats. Low testosterone level is associated with a high BMI (Phillips, 1993). The increase of BMI was not the contributive factor for the decrease of testosterone level in our study as the HFD-obese rats showed a comparable testosterone level as the control rats. The cause of decreased testosterone could be due to decreases plasma LH although it was not measured in this study. Plasma LH, FSH, testosterone levels were reported to be decreased in prepubertal neonatally treated MSG rats. Moreover, these animals revealed a significant reduction in testis weight and the number of Sertoli and Leydig cells per testes (França et al., 2006).
Pitteloud et al. (2005) suggested that insulin resistance is associated with a decrease in Leydig cell testosterone secretion in men. This relationship may, in fact, be bidirectional. Insulin signaling in the brain plays an important role in regulating reproductive function (Bruning et al., 2000). Insulin promotes GnRH secretion in a hypothalamic GnRH neuronal cell line (Bureclin et al., 2003) and stimulates gonadotropin secretion from pituitary cell cultures (Adashi et al., 1981), and testosterone secretion from cultured Leydig cells (Bebakar et al., 1990; Lin et al., 1986). In animal studies, lowering plasma insulin levels decreases pituitary LH content and plasma LH levels (Benitez and Diaz, 1985). In obese men, acute hyperinsulinemia causes a modest increase in testosterone levels (Pasquali et al., 1997), whereas lowering insulin levels with diazoxide reduces serum testosterone levels (Pasquali et al., 1997). This stimulatory effect of insulin on the hypothalamic-pituitary-gonadal (HPG) axis appears to contradict the inverse relationship between testosterone and insulin levels noted in epidemiological studies (Pasquali et al., 1991). However, this apparent paradox could be explained by the decreased sensitivity of the HPG axis to insulin action in insulin-resistant states.

The association between hypogonadism and insulin levels in men has also been reported (Kapoor et al., 2005). Pitteloud et al. (2005) demonstrated a positive correlation between serum testosterone levels and insulin sensitivity in men. Hypogonadism may cause insulin resistance via dysregulation of fatty acid metabolism, and low testosterone levels may represent an additional environmental factor contributing to decreased expression of genes involved in oxidative metabolism (Pitteloud et al., 2005). In male rats, acute castration induces significant insulin resistance (Holmang A, Bjorntor, 1992). In men, low testosterone levels predispose to central obesity (Tsai et al., 2000) and predict the development of both the metabolic syndrome (Laaksonen et al., 2004) and diabetes mellitus (DM- type 2) (Svarberg et al., 2004). The impact of testosterone administration on insulin sensitivity in men is still unclear, with some (Marin et al., 1993), but not all (Liu et al., 2003), studies showing an improvement.

In the current study, the absence of significant change in the total or differential leucocytic count or any of the erythrocytes parameters may indicate that MSG in the applied dose in the current study was not toxic for the hemopoietic cells. In the present study, the significant positive correlation between BMI and ADP-induced platelet aggregation demonstrated in the groups studied, may be ascribed to hyperinsulinemia, which leads to enhanced platelet aggregation. The increase of insulin level in MSG-obese rats and the significant increased ADP-induced platelet aggregation only in this group of obesity ascertain this assumption. Moreover, a significant positive correlation between ADP-induced platelet aggregation and plasma insulin level was demonstrated in the MSG-obese group.

Insulin is generally thought to reduce platelet responses to the agonists ADP, collagen, thrombin, arachidonate, and platelet-activating factor (Trovati et al., 1988). However, Vinik et al. (2001) studied the effects of insulin on platelets of obese insulin-resistant patients compared with lean healthy control subjects. Insulin impaired the inhibition of platelet aggregation in response to proaggregatory agents in obese subjects compared with lean subjects.

Ishida et al. (1996) suggested that insulin inhibits platelet function by both prostaglandin E1-dependent and-independent mechanisms. Ferrannini et al. (1999) reported that insulin inhibits lipolysis and platelet aggregation. In the presence of insulin resistance, hyper-aggregation and anti-fibrinolysis may create a prothrombotic milieu. Preliminary evidence indicates that hyperinsulinemia per se may be pro-oxidant both in vitro and in vivo. In the kidney, insulin spares sodium and uric acid from excretion; in chronic hyperinsulinemic states, these effects may contribute to hyperuricemia. ADP-induced platelet aggregation was enhanced in hyperuricemic rats (Mohamed et al., 2007).

The low testosterone level encountered in the MSG-obese rats could be the second cause of enhanced platelet aggregation in the present study. Animal experiments showed that androgens at physiological doses inhibit oxidative-stress-induced platelet aggregation via its receptor, which is associated with the reduction of thromboxane A2 release from platelets (Li et al., 2007).

In conclusion, the aforementioned observations suggested that ingestion of MSG at dose levels of 150 mg/kg body weight in adult male rats produced hyperinsulinemia, hypoandrogenemia and platelet hyperaggregation and hence could be responsible for the initiation of atherothrombosis. These findings raise a concern about the safety of MSG use.

REFERENCES


