Effects of fibre-enriched diets on tissue lipid profiles of MSG obese rats

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\textbf{A B S T R A C T}
In order to investigate the influence of some fibre-enriched diets on tissue lipids in an animal model of obesity induced by the administration of monosodium glutamate (MSG), obese rats were fed diets containing 30\% of Acha, Cassava, Maize and Plantain for five weeks and weight gain, feed intake and leee index were recorded. The lipid profiles of plasma, erythrocytes, kidney, heart and liver as well as hepatic 3-hydroxy-3-methylglutaryl-CoA (HMG-CoA) reductase activity were measured. The diets significantly ($p < 0.05$) reduced weight gain and leee index in the obese rats. Obesity-induced increase in plasma and erythrocytes lipid levels was significantly ($p < 0.05$) reduced by these diets. MSG-induced obesity also resulted in a significant increase ($p < 0.05$) in hepatic cholesterol level which was reduced by the diets. MSG-obesity was characterised by a significant ($p < 0.05$) increase in cholesterol, triacylglycerol and phospholipids in kidney and this was reversed by the diets except Maize which did not reverse the increased cholesterol level. Only Acha reversed the obesity-induced increase in heart cholesterol and phospholipids. The increased activity of hepatic HMG-CoA reductase associated with obesity was also significantly ($p < 0.05$) reduced by the diets. In conclusion, dyslipidemia associated with MSG-induced obesity could be attenuated by consumption of fibre-enriched diets.

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\section{1. Introduction}
Increased food consumption in excess of energy requirements, coupled with excessive body fat present a growing human health problem (Mozes et al., 2004). Obesity, which is defined as body fat excess, usually develops slowly as a result of long-term alterations in energy balance. In general, when food intake exceeds energy expenditure, the retained energy is deposited as fat (Dolnikoff et al., 2001). Obesity is a highly prevalent disorder associated with decreased life expectancy and increased morbidity because of its combination with a variety of other disorders including hyperglycemia, hyperlipidemia, hypertension and consequently cardiovascular diseases carrying significant economic cost (Gunven et al., 1999). These disorders are often associated with both qualitative and quantitative changes in lipid composition of several tissues in the body.

Dietary habits are considered one of the factors contributing to obesity; hence, a scrupulous dietary intervention is indicated in its management (De Filippo et al., 2010). This dietary intervention should include diets that regulate food intake and energy balance.

African diets are known to be high-fibre and low-fat (De Filippo et al., 2010; Thian et al., 2006). While the rural African population live agrarian life style, urban population is becoming westernized (De Filippo et al., 2010). In Nigeria, for example, the rural populations consume more of carbohydrate and fibre-rich food like Cassava, Acha, Plantain and Maize more than western-type processed food and beverages (Olamakaiye et al., 2010). Thian et al. (2006) reported that in sub-saharan Africa, obesity is becoming a problem with 10–30\% of men and 15–45\% of women in West Africa being either overweight or obese.

In general, it is believed that humans are more suited to resist famine than overabundance of food (called the “thrift gene hypothesis”) and; hence, it has been argued that the easy and related inexpensive availability of energy-dense food is responsible for the current obesity epidemic (Das, 2010). The energy balance is very tightly controlled by hypothalamic factors. Hence, the gut-brain axis and the cross-talk between gut hormones and hypothalamic factors are important in the regulation of food intake, energy balance, and development of obesity (Das, 2010). This knowledge has been under utilised in developing experimental murine model of obesity by intraperitoneal administration of neonatal rats with monosodium glutamate (MSG). This study was therefore aimed at studying the effects of some fibre-enriched diets on MSG – obesity – induced perturbations in lipid metabolism.
2. Materials and methods

2.1. Animals and diets

Male neonatal albino rats were used for the experiment and obesity was induced as described by Nakagawa et al. (2000) with slight modification. Briefly, the neonates were treated with MSG (4 mg/kg body weight) intraperitoneally five times (2, 4, 6, 8 and 10 days after birth). Physiological saline was administered in a similar fashion to the control animals. The rats were weaned on the 21st day and raised normally thereafter, and studied at the age of 10 weeks. At the tenth week, Lee indices of the animals were calculated as \( \frac{W - W_0}{W_0} \times 100 \). 25 rats with Lee index of 0.3 or more were considered obese. A total of 30 rats (25 obese surviving rats, 5 normal rats (not treated with MSG)) were used and they were divided into 6 groups of 5 rats each and randomly assigned to the experimental diets (Table 1). The 6 groups of rats were designated as follows:

- **Group 1. Normal-C**: Rats fed normal diet.
- **Group 2. MSG-C**: Obese rats fed normal diet.
- **Group 3. MSG-Acha**: Obese rats fed Acha.
- **Group 4. MSG-Cassava**: Obese rats fed Cassava flakes.
- **Group 5. MSG-Maize**: Obese rats fed Maize.
- **Group 6. MSG-Plantain**: Obese rats fed unripe Plantain flour.

The rats were fed for five weeks on the experimental diets. The composition of the experimental diet (Table 1) was based on the AIN-93 semisynthetic diet and were prepared to contain 30% of the fibre enriched diets. The animals were given food and distilled water ad libitum during the experimental period. Food consumption was measured daily while weight gain was measured weekly. The Ethical Committee for Conduction of Animal Studies at the Department of Biochemistry, University of Agriculture, Abeokuta, Ogun State approved the experimental protocol and all animals were cared for in accordance with the principles and guidelines of the committee.

2.2. Tissue collection

At the end of the experimental period, blood was collected from the animals into heparinised tubes by cardiac puncture under light ether anesthesia after an overnight fast. The plasma was separated from the erythrocytes by centrifuging the whole blood at 5000 rpm for 10 min. Erythrocytes were washed three times with methanol mixture (2:1, v/v) as described by Folch et al. (1957). Phospholipid concentrations in aliquots of the chloroform–methanol extracts of each of the plasma and erythrocyte total cholesterol and triglycerides were determined using an indirect method described by Rao and Ramakrishnan (1975). In this method, the concentrations of HMG-CoA and mevalonate are determined in the liver homogenate and the ratio of HMG-CoA/mevalonate is taken as an index of the activity of HMG-CoA reductase. An increase in the ratio indicates decrease whereas a decrease in the ratio indicates increased HMG-CoA reductase activity.

2.3. Biochemical analyses

2.3.1. Plasma lipid profiles

Plasma concentrations of total cholesterol and triglycerides were determined with commercial kits (Cromatest linear chemicals, Montgat Spain). HDL cholesterol and triglycerides were determined in plasma with same commercial kits for total cholesterol while Coronary Risk Index (CRI) was estimated as the ratio of plasma total cholesterol (HDL + VLDL) to cholesterol while Coronary Risk Index (CRI) was estimated as the ratio of plasma total cholesterol to HDL cholesterol as described by Ademiyiwa et al. (2008).

2.4. Statistical analysis

Calculations were made using the software “SPSS 13.0 for Windows” and data were expressed as Mean ± SEM of five readings. Analysis of Variance (ANOVA) was carried out to test for the level of homogeneity at \( p < 0.05 \) among the groups using Duncan’s Multiple Range Test (DMRT) to separate the heterogeneous groups.

### Table 1

<table>
<thead>
<tr>
<th>Composition of diet in g/100 g</th>
<th>Fish Meal</th>
<th>Acha</th>
<th>Cassava</th>
<th>Maize</th>
<th>Plantain</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>20</td>
<td>20</td>
<td>20</td>
<td>20</td>
<td>20</td>
</tr>
<tr>
<td>Groundnut oil</td>
<td>5</td>
<td>5</td>
<td>5</td>
<td>5</td>
<td>5</td>
</tr>
<tr>
<td>Mineral mix</td>
<td>3.5</td>
<td>3.5</td>
<td>3.5</td>
<td>3.5</td>
<td>3.5</td>
</tr>
<tr>
<td>Vitamin mix</td>
<td>1</td>
<td>1</td>
<td>1</td>
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<td>1</td>
</tr>
<tr>
<td>Cellulose</td>
<td>5</td>
<td>5</td>
<td>5</td>
<td>5</td>
<td>5</td>
</tr>
<tr>
<td>Maize starch</td>
<td>50</td>
<td>25</td>
<td>25</td>
<td>25</td>
<td>25</td>
</tr>
<tr>
<td>Sucrose</td>
<td>15</td>
<td>10</td>
<td>10</td>
<td>10</td>
<td>10</td>
</tr>
<tr>
<td>Choline bitartrate</td>
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<td>0.2</td>
<td>0.2</td>
<td>0.2</td>
<td>0.2</td>
</tr>
<tr>
<td>DL-Methionine</td>
<td>0.3</td>
<td>0.3</td>
<td>0.3</td>
<td>0.3</td>
<td>0.3</td>
</tr>
<tr>
<td>Acha</td>
<td>30</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cassava</td>
<td></td>
<td>30</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Maize</td>
<td></td>
<td></td>
<td>30</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Unripe Plantain flour</td>
<td></td>
<td></td>
<td></td>
<td>30</td>
<td></td>
</tr>
</tbody>
</table>

| * Mineral mix and vitamin mix contains the following in g/100 g: Calcium phosphate 49.50, sodium powder 11.80, potassium sulphate 5.20, sodium chloride 7.40, magnesium oxide 2.40, potassium citrate 22.40, ferric citrate 0.60, manganese carbonate 0.35, cupric carbonate 0.03, zinc carbonate 0.16, chromium potassium sulfate 0.055, potassium iodate 0.001, sodium selenate 0.001, choline chloride 0.50, thiamine HCl 0.06, riboflavin 0.03, niacin 0.30, calcium pantothenate 0.16, biotin 0.01, vit D3 0.025, vit B12 0.10, vit E acetate 1.00, pyridoxine 0.07, folic acid 0.02, vit A acetate 0.08.

3. Results

The MSG treated rats gained more weight and consumed less feed than the control rats. There was no significant \( p < 0.05 \) difference in the Lee indices among all the MSG treated groups before treatment with the diets. However, the diets brought about a significant \( p < 0.05 \) decrease in the Lee indices of the animals. Acha and unripe Plantain flour containing diets brought the lee index to levels similar that of the normal rats (Table 2). There was no significant \( p < 0.05 \) difference in heart weight among all the groups. Kidney weight of the MSG-treated rats and that on Cassava containing diet were also significantly \( p < 0.05 \) lower than the normal rats. Liver weight of the MSG treated rats were significantly \( p < 0.05 \) lower than that of the normal rats and the other groups (Table 2).

MSG intake induced dyslipidemia in both plasma and lipoproteins and this was characterised by increased cholesterol, triacylglycerol and phospholipids. Intake of the diets attenuated the MSG-induced dyslipidemia (excluding total and HDL-phospholipids where Acha further significantly \( p < 0.05 \) increased their concentrations). Unripe Plantain flour containing diet had the most...
Effects of diets on blood lipid profiles.

Table 3

<table>
<thead>
<tr>
<th>Group</th>
<th>Normal-C</th>
<th>MSG-C</th>
<th>MSG-Acha</th>
<th>MSG-Cassava</th>
<th>MSG-Maize</th>
<th>MSG-Plantain</th>
</tr>
</thead>
<tbody>
<tr>
<td>Erythrocyte cholesterol</td>
<td>47.82 ± 1.40a</td>
<td>85.80 ± 1.03a</td>
<td>65.11 ± 1.04a</td>
<td>66.59 ± 1.04a</td>
<td>70.11 ± 1.04d</td>
<td>73.33 ± 1.04c</td>
</tr>
<tr>
<td>Erythrocyte phospholipids</td>
<td>157.40 ± 1.04a</td>
<td>184.47 ± 2.09a</td>
<td>155.27 ± 3.07b</td>
<td>139.02 ± 3.07a</td>
<td>145.76 ± 3.07c</td>
<td>131.69 ± 3.07a</td>
</tr>
<tr>
<td>Plasma total cholesterol</td>
<td>81.17 ± 2.09a</td>
<td>112.42 ± 3.29b</td>
<td>92.06 ± 1.99a</td>
<td>90.51 ± 1.99c</td>
<td>98.11 ± 1.99c</td>
<td>83.71 ± 2.09b</td>
</tr>
<tr>
<td>Plasma total triacylglycerol</td>
<td>57.69 ± 1.99a</td>
<td>156.43 ± 1.36d</td>
<td>87.3 ± 2.11c</td>
<td>80.95 ± 2.11d</td>
<td>71.92 ± 2.11d</td>
<td>67.39 ± 1.99b</td>
</tr>
<tr>
<td>Plasma total phospholipids</td>
<td>108.59 ± 3.20a</td>
<td>131.74 ± 3.46a</td>
<td>166.69 ± 3.16b</td>
<td>102.96 ± 3.16c</td>
<td>124.39 ± 3.16d</td>
<td>80.73 ± 2.09a</td>
</tr>
<tr>
<td>HDL cholesterol</td>
<td>36.9 ± 1.85a</td>
<td>17.63 ± 1.75c</td>
<td>30.08 ± 1.11a</td>
<td>37.09 ± 1.11a</td>
<td>49.83 ± 1.11a</td>
<td>25.70 ± 0.34d</td>
</tr>
<tr>
<td>HDL triacylglycerol</td>
<td>23.45 ± 1.04a</td>
<td>32.07 ± 1.86d</td>
<td>33.64 ± 1.33c</td>
<td>35.49 ± 1.06c</td>
<td>36.22 ± 0.61d</td>
<td>23.70 ± 1.44c</td>
</tr>
<tr>
<td>HDL phospholipids</td>
<td>9.91 ± 0.10a</td>
<td>37.4 ± 1.09a</td>
<td>60.21 ± 0.29b</td>
<td>16.32 ± 1.30c</td>
<td>21.11 ± 0.44d</td>
<td>10.41 ± 0.08b</td>
</tr>
<tr>
<td>LDL cholesterol</td>
<td>32.71 ± 1.74a</td>
<td>63.50 ± 2.46c</td>
<td>44.52 ± 2.78a</td>
<td>37.22 ± 2.76a</td>
<td>33.89 ± 2.30a</td>
<td>44.53 ± 2.55b</td>
</tr>
<tr>
<td>LDL triacylglycerol</td>
<td>11.54 ± 0.27a</td>
<td>28.31 ± 0.72a</td>
<td>17.46 ± 0.54a</td>
<td>16.20 ± 0.25c</td>
<td>14.38 ± 0.32d</td>
<td>13.47 ± 0.20b</td>
</tr>
</tbody>
</table>

Values expressed as mg/dL and each value represents the mean ± S.E.M. n = 5. Values within the same row with different alphabets are significantly (p < 0.05) different from each other.

Cholesterol, triacylglycerol and phospholipid levels in the heart of the MSG-treated rats were significantly (p < 0.05) higher than that of the normal rats. While other diets resulted in a significant increase in heart cholesterol and phospholipid, Acha containing diets consumption gave a significant decrease in the heart cholesterol while Acha and unripe Plantain flour reduced heart phospholipid. The MSG treated rats had a triacylglycerol level significantly (p < 0.05) lower than the normal rats. Consumption of Acha and Cassava containing diets gave triacylglycerol levels similar to that of the diets resulted in a significant (p < 0.05) decrease in their concentrations. However MSG treatment brought about a significant (p < 0.05) decrease in the level of phospholipid in the kidney when compared to the normal control. This reduction was significantly (p < 0.05) reversed by Maize-containing diets while other diets led to a further significant (p < 0.05) drop in the concentration of phospholipid in the kidney (Table 4).

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of the normal rats while Maize containing diets gave levels significantly \((p < 0.05)\) higher than the normal rats (Table 4).

Hepatic cholesterol levels in the MSG-treated rats were significantly \((p < 0.05)\) higher than that of the normal rats. Consumption of the diets however resulted in a significant \((p < 0.05)\) decrease in the cholesterol concentrations. Acha had the most pronounced effect in lowering hepatic cholesterol while Maize had the least effect. MSG treatment brought about a significant decrease in hepatic phospholipids. Consumption of unripe Plantain flour brought about significant \((p < 0.05)\) decrease while Acha and Cassava significantly \((p < 0.05)\) increased phospholipid levels. Hepatic TG level in MSG-treated rats was similar to that of the normal rats, however Acha and Cassava significantly \((p < 0.05)\) increased the concentration of TG while the concentration of TG in the other diets was similar to that of the normal and MSG-treated rats (Table 4).

The MSG treated rats had a coronary risk index value of about 2.9 times higher than the normal rats \((p < 0.05)\). Rats fed with Cassava and Maize containing diets brought the ratio to levels similar to that of the normal rats. Treatment with MSG led to about 4-fold increase in the ratio of atherogenic risk index when compared with the normal control. Intake of the diets reduced the ratio, except the animals fed Acha and unripe Plantain flour, to levels similar to that of the normal animals. (Fig. 1) HMG-CoA/Mevalonate ratio is inversely proportional to the activity of HMG CoA reductase in the liver of the animals. The HMG CoA reductase activities of MSG-treated rats were significantly higher than others while Acha had the lowest HMG CoA reductase activity. The activity of the enzyme of the obese rats fed the diets was between 1.1 and 4.5 times lower when compared with the obese rats not fed the diets. (Fig. 2)

4. Discussion

The findings of this study indicated that 10 week-old rats neonatally treated with MSG exhibited obesity with increased Lee in-
et al., 2000; Nagata et al., 2006). Dyslipidemia was expressed as a general agreement with data from other studies (Nakagawa). Erythrocyte lipid profile presented in this work is in association with dyslipidemia in the rat tissues. The response of plasma levels of corticosterone (Guimarães et al., 2002) as well as increased lipogenesis and reduced lipolysis in adipose tissues (Dolnikoff et al., 2001) occurring despite their normophagia or even hypophagia (Zhang et al., 1994; Martinkova et al., 2000).

When the hypothalamic ventromedial nucleus and arcuate nucleus are destroyed in rats by treatment with MSG in the neonatal stage, there is development of obesity as the rats grew (Nakagawa et al., 2000). Novelli et al. (2007) reported that adult rats (age 60 days) model of obesity experience a cessation of growth and development with a concomitant accumulation of fat in the abdominal region, leading to an increase in weight without a corresponding increase in the body length. This may lead to chronic exposure of tissues to elevated concentrations of lipids which may contribute to tissue dysfunction and obesity-related disease such as diabetes, hypertension, cardiovascular disease and metabolic syndrome (Muolio and Newgard, 2006).

The present investigation shows that unripe Plantain flour consumption by the obese rats led to weight loss while Cassava and Maize had reduced weight gain as compared with the MSG-obese rats fed on control diets. Unripe Plantain has been reported to be useful in managing diabetes and obesity due to its low glycemic index which is the consequence of its small concentration of free sugars and rapidly digestible starch (Ramdath et al., 2004). The potential mechanisms of weight reduction by these diets may include antihyperglycemic effects, inhibition of lipid droplet accumulation in fat cells without affecting adipose conversion, inhibition of fatty acid synthesis, or inhibition of adipocyte differentiation (Kim et al., 2003). In particular, high-fibre diet feeding is accompanied by greater satiety and less hunger than low-fibre weight-reducing diets. Hence, an explanation for the reduced weight gain in rats on fibre-enriched diets is that nutrient absorption is limited and its caloric utilisation is different (Mazur et al., 1990).

As also observed in this study, MSG-induced obesity was associated with dyslipidemia in the rat tissues. The response of plasma and erythrocyte lipid profile presented in this work is in general agreement with data from other studies (Nakagawa et al., 2000; Nagata et al., 2006). Dyslipidemia was expressed as an increase in plasma total cholesterol, plasma triacylglycerol, RBC cholesterol and RBC triacylglycerol with a decrease in HDL cholesterol. Also total cholesterol: HDL cholesterol and LDL cholesterol:HDL cholesterol ratios were increased. In man at least, these ratios are considered to increase the risk of cardiovascular dysfunction, especially when total cholesterol level is elevated (Dabai et al., 1996). It was therefore noteworthy that the diets used in this study were able to improve plasma and RBC dyslipidemia and reduce atherogenic and coronary heart disease risk index in MSG-obese rats. This observation is consistent with the findings of Adamson and Mbajorgu (1985) who reported that the serum lipid levels were reduced in rabbits fed Cassava and Unripe Plantain flour-based diets. They attributed their observations to the type and amount of dietary fibre in the diets they used.

Several mechanisms have been suggested to be responsible for the influence of dietary fibre on blood lipids. One is ascribed to the water soluble fibres (Kritchevsky, 1988; Madar and Odes, 1990), which when present in gastrointestinal tract increases viscosity and interferes with micelle formation and lipid absorption (Gallagher et al., 1993; Superko et al., 1988). In addition, certain fibre varieties bind or adsorb bile acids and neutral sterols, enhancing their removal from enterohepatic circulation leading to lower cholesterol in the various pools. If the absorption of lipids in the small intestine was decreased, it would be expected that the amount of cholesterol, triacylglycerol and perhaps phospholipids in the pools would also decrease but all pools of lipid are not affected in the same way and often times, the effect of different fibres is to shift the balance of lipid among the various compartments and pools (Adamson and Mbaajorgu, 1985; Mehta and Kaur, 1992).

Short-chain fatty acids (SCFA)-acetate, propionate and butyrate are produced in large quantities from the fermentation of dietary fibres in the colon. Studies have indicated that there might be a connection between SCFA and lipid metabolism. Wolever et al. (1991), using rectal infusions of acetate alone, propionate alone, or a combination of acetate and propionate, showed that propionate inhibited the utilisation of acetate for cholesterol synthesis. It is possible that the blood levels of these SCFAs in circulation and the amount in the organs examined in this study were responsible for the variation in the amount of lipids present in them.

The fibre-enriched diets used in this study also resulted in a reduction of hepatic HMG-CoA reductase activity of the rats. SCFAs produced by bacterial fermentation of dietary fibre, in particular propionate, have been suggested to down regulate the activity of this enzyme (Chen et al., 1984) leading to the hypolipidemic effect.

Fig. 2. HMG CoA/Mevalonate ratio of the animals exposed to different experimental diets. Bars represent the means ± SEM (n = 5). Bars with different alphabets are significantly (p < 0.05) different from each other.
of dietary fibres. It has been established that the intake of fermentable fibres increases SCFA levels in the hepatic portal blood (Hara et al., 1998). This study therefore established that all of the fibre-enriched diets were able to improve dyslipidemia in MSG-induced obesity in rats. The unripe Plantain flour diet was the most effective in bringing about body mass reduction in the obese rats. Also, the study provided an unequivocal demonstration of the lipid-lowering effects of the fibre-enriched diets used in this study in dyslipidemic MSG-induced obese rats. These effects were associated with modifications of plasma lipoprotein distribution, RBC lipid levels and HMG-CoA reductase activity.

Conflict of interest

The authors declare that there is no conflict of interest.

References