Original Article

Chronic High Fat Diet Induced Weight Gain, Hyperglycaemia and Cognitive Impairment in Albino Mice

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ABSTRACT

With the global rise in consumption of western diet, obesity is becoming a pandemic. High fat and high calorie diet (though more palatable) have been associated with a range of metabolic disorders. Oxidative stress and insulin resistance have a link to the dietary etiology of diabetes and obesity; on the other hand neuroinflammation and abnormal brain insulin signaling is said to cause cognitive decline when high fat diet (HFD) is consumed. In this study, mice were fed a home-made HFD with a total energy content of 5340kcal/kg. The overall energy contribution of saturated and unsaturated fat was about 70%. Animals were divided into Group I and II of 5 mice each. Group I (control group) were fed normal chow and water ad libidum. Group II (test group) were fed HFD and water ad libidum. HFD significantly ($p < 0.05$) increased the body weight of the test group (40 ± 7.7) as compared to control (30 ± 2.9). Blood glucose levels in the test group was also significantly higher (111.6 ± 5.2) compared to the control (77.6 ± 8.7). Morris water maze escape latency in the test group was not significantly different (68 ± 26.3) compared to the control (72 ± 20.9). Similarly, the time spent in target quadrant in the test group was not significantly different (20.8 ± 10.9) compared to the control (15.8 ± 5.6). In the Y-maze the control group had a significantly higher (29.6 ± 3.5) percentage alternations compared to the test group (21 ± 13.1). It is obvious that the chronic administration of HFD in mice altered the body's metabolic processes as evident in the significant weight gain, hyperglycemia and cognitive deficit. These can be attributed to the oxidative damage, insulin dysregulation and pro-inflammatory potentials of HFD.

Keywords: High Fat Diet, Morris Water Maze, Y-Maze, Hyperglycaemia, Dementia, Oxidative stress

INTRODUCTION

Diet is essential for the sustenance of life. It helps to maintain normal body physiology.¹ Modernization has inundated our society with an abundance of palatable foods that are consumed in high amounts, resulting in a rising prevalence of obesity and other metabolic disorders. In rodents, high fat diet increases hedonic eating which

results in obesity.^{2,3} The evolving connection between diabetes, obesity and dementia poses a serious public health challenge in today's society.^{4,5}

Research in recent times have shown the deleterious impact of high fat, high calorie diets on the various brain systems of animals and humans. Repeated intake of such diets has been linked to injury of the hippocampus, a brain structure responsible for promoting learning and memory.^{6,7} A key

component of cognition is memory and it is the earliest to depreciate, especially with ageing. Today, the prevalence of dementia (predominantly memory loss) is rising significantly.⁸ Various studies have suggested a strong link between diabetes mellitus and the most common dementia illness—Alzheimer's disease, via impaired insulin signaling. Insulin resistance is behind the major etiologies of dementia by inducing neuro-inflammation and oxidative stress.⁹ Another research also demonstrated that a 12 week high fat diet consumption in mice resulted in a significant increase in body weight, higher fasting blood glucose and insulin levels in plasma and lower glucose tolerance. It was found to elicit significant insulin receptor substrate (IRS-1) inactivation with accompanied increase in pro-inflammatory markers such as MAP kinase, p38 and JNK. They also noted a substantial decrease in long-term potentiation in the CA1 region of hippocampus indicative of impaired synaptic plasticity.¹⁰

The literature on the role of high fat diet in the etiology of many diseases (obesity, diabetes, dementia, cancers etc) are on the rise. The existing evidences so far are insufficient with some conflicting. Maze experimentations in the high fat diet model are also limited. This research seeks to study the effect of a long-term effect of high fat diet on weight gain and blood glucose and also memory assessment using multiple mazes.

MATERIALS AND METHODS

High Fat Diet (HFD) Composition

The HFD was constituted locally by an animal nutritionist of Faculty of Veterinary Medicine, Federal University of Agriculture, Makurdi. Benue State. Nigeria. The formula was designed using the method of Julia *et al*³ with slight modification. The diet was made from chow, tallow and soy oil at an inclusion rate of 60%, 25% and 15% respectively. The total caloric value of the diet was about 5340kcal/kg of which energy contribution of fat was about 70%. The fat component comprises of 60% saturated fat and 40% unsaturated fat

Animals

A total of 10 female albino mice weighing between 18-25g (5-6weeks) were obtained from the Animal House, College of Health Sciences, Benue State University, Makurdi. They were housed in polypropylene cages at the side laboratory of the Department of Physiology, College of Health Sciences and divided into two groups of 5 animals each. The control group was fed normal chow and water *ad libidum* for a period of 12 weeks. The second group was fed HFD and water *ad libidum* for a period of 12 weeks too.

Animal Weighing

A digital weighing scale was used to weigh the animals. Weighing was done weekly for the entire period of the experiment and results were recorded accordingly.

Blood Glucose Measurement

On the final week of the experiment, the animals were fasted overnight and their blood glucose were measured using the glucose test strip which is based on the glucose oxidase method. The animals' tails were wiped with spirit-soaked cotton wool. A small snip was made with a scissors at the tail tip and a drop of blood was applied to the end tip of the test strip in the glucometer (On Call Plus II® Blood Glucose Test Strip by ACON laboratories, San Diego USA; Product number: 1150730003). Readings were taken thereafter.

Maze

Morris water maze (MWM)

MWM as described by Morris¹¹ with slight modification was used to assess learning and memory in the mice. Instead of a plastic water tank, we used an inflatable plastic pool measuring 1.47m in diameter. The pool was manufactured by Intex® development company limited, Hong Kong and it was purchased at a local store in Makurdi town. The pool was divided into four quadrants and filled with water at room temperature to a depth of 14cm to allow the animal swim freely. A sealed cylindrical plastic container was submerged 1cm below the surface of the water to serve as the hidden escape platform. To eliminate bias, the water was colored with powdered milk serving as a camouflage. The

MWM experiment lasted for 4 consecutive days.⁸ The first 3 days were the acquisition trial days while the 4th day was the probe trial day. On each day of the acquisition trial the animals were trained to locate the hidden platform fixed in a particular quadrant throughout the experiment. They were gently placed in each of the four quadrants sequentially and allowed a period of 120 seconds to locate the hidden platform. If they fail to locate it the animal will then be gently assisted to the platform and allowed another period of 30 seconds to build cohesive memory and adequate visual representation of the pool. With progression in the days of acquisition trial, time to find the escape platform were recorded and animals that locate the platform faster were noted as having better learning and memory. On the day of the probe trial the hidden platform was removed and each animal was given just one chance of swimming for a period of 120 seconds. The time spent in the target quadrant was recorded. Prolonged time spent in the quadrant that hitherto housed the platform was measured as an index of good memory recall.

Y-Maze

Y maze is a tool used to assess working and spatial memory in mice. It is based on the innate preference of an animal for novelty. A mouse with intact working memory and functional prefrontal cortex will remember the arms previously visited and show a tendency to enter a previously un-entered arm. An intact spatial reference memory and functional hippocampus can also be tested by the Y-maze^{8,12,13} The maze was constructed from wooden material with the three identical arms at an angle of 120° to each other. On commencement of the experiment one of the arms was closed with a wooden shutter and the mouse placed in the centre of the maze to explore the two open arms for a period of 5 minutes. The animal was then given a rest interval of 30 minutes before the second trial; this time with all arms opened, the animal explores all three arms for another period of 5 minutes. In between trials the maze was wiped with 70% ethyl alcohol to obliterate all olfactory cues. The maximum spontaneous alternations (i.e. total number of arms entered minus 2) and the actual alternations

(i.e. either ABC, ACB, BAC, BCA, CAB OR CBA) were measured. Percentage alternations was then calculated as:

$$\text{percentage alternations} = \frac{\text{actual alternations}}{\text{maximum alternations}} \times 100$$

The percentage alternation is directly proportional to memory index.

Statistical Analysis

Data obtained from the study were expressed as mean $\pm$ SEM. The differences between groups were analyzed by the independent samples t-test using the SPSS statistical tool version 20. Values of $p < 0.05$ were considered significant.

RESULTS

Body weight

The chart shows a statistically significant gain in body weight in the HFD group compared to the control group. * indicates statistical significance ($p < 0.05$)

Figure 1. The difference in body weights before and after the experiment

Blood glucose

The chart shows a statistically significant rise in blood glucose level in the HFD group compared to the control group. *indicates statistical significance ($p < 0.05$)

Figure 2. Blood glucose levels after overnight fast

Figure 3. Acquisition trial time to locate hidden platform in MWM

Escape latency in MWM

The chart shows no statistically significant difference in escape latency at $p < 0.05$

Time spent in target quadrant in MWM

The chart shows no statistically significant difference in time spent in target quadrant at $p < 0.05$

Figure 4. Probe trial time in target quadrant in MWM experiment

Percentage spontaneous alternations

The chart shows statistically significant reduction in percentage alternations in the HFD group compared to the control group. *indicates statistical significance ($p < 0.05$)

Figure 5. Percentage spontaneous alternations in Y-maze experiment

DISCUSSION

The research proved that chronic administration of high fat diet in mice impacted significantly on their body weight, blood glucose and brain function. There was a marked weight difference in the high fat diet-fed mice compared to the control at the end of 12 weeks. This may be due to the fact that high fat diet drives hedonic hunger thus increasing food consumption rate, positive energy balance and weight gain. Also, the reward circuit systems of the brain are activated, this include ventral tegmental area, nucleus accumbens, amygdala and prefrontal cortex³. This finding was supported by that of David and John;¹⁴ that high fat diet induces weight gain by inhibiting hypothalamic neurogenesis and disrupting the orexigenic and anorexigenic balance. Findings by Rossi *et al*¹⁵ also showed that high fat diet impairs the lateral hypothalamic brake

region on feeding in the brain of mice. We also found the blood glucose level to be significantly higher in all high fat diet-fed mice compared to control. This result which is also supported by some literatures^{16,10,17}. Zhingang *et al*¹⁰ revealed that mice that consumed high fat diet for a period of 12 weeks had a significantly higher fasting blood glucose and insulin levels. It was found to elicit significant insulin receptor substrate (IRS-1) inactivation plus elaboration of inflammatory markers. A similar study by Maria and Bo¹⁸ demonstrated that a compromise in insulin response and islet dysfunction were responsible for sustained hyperglycemia in high fat-fed mice. In this study also we found that in the Y-maze experiment the animals fed on high fat diet performed poorly demonstrating an impaired learning and visuo-spatial memory. However, there was no significant finding in the MWM experiment. This finding is corroborated by John *et al*¹⁹. They found that prolonged consumption of high fat diet by mice did not affect their performance in the MWM even though it did in other neurobehavioral models. A number of studies have demonstrated that chronic high fat diet consumption results in impaired spatial memory in mice tested on the Y-maze.^{20,21,22,23} Younghua *et al*²⁰ attributed the cause of impaired performance in the Y-maze to hippocampal oxidative stress as evidenced by decrease in brain catalase activity, glutathione peroxidase activity, total antioxidative capacity plus an increase in malondialdehyde levels.

CONCLUSION

This study has shown that high fat consumed for a long time causes an unhealthy weight gain which may be due to impairment in the anorexigenic neurons of the hypothalamus. Also, sustained hyperglycemia was seen in these animals, which have been linked to impaired insulin sensitivity. Cognitive impairment was also seen as evidenced by poor performance on the Y-maze which is possibly due to neuroinflammatory and oxidative damage to the brain particularly the hippocampus. Further studies will be required to elucidate more molecular mechanisms for such findings.

Author Contributions

All authors participated in the laboratory work, manuscript drafting and proof reading

Conflict of Interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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