Defining an Efficient Model for Inducing Obesity and Metabolic Syndrome in Wistar Rats

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ABSTRACT

Obesity and metabolic syndrome are major health problems which often present a confounding therapeutic challenge. Gene-nutrient interactions are implicated in metabolic phenotypes like Metabolic Syndrome, and rats have been used extensively to study them. However, different models have been stated in literature. The aim of this study was to explore different models and determine the most efficient model(s) of achieving diet-induced metabolic syndrome in wistar rats. Wistar rats (n=208) weighing 100-120 g were used in this study. Rats were divided into gender pairs of 4 diets: standard rats' chow, standard rats' chow + 60% sucrose, high-fat (60% margarine) and high-fat (60% butter) and fed for nine (9) weeks. Body-mass Index (BMI), blood glucose, lipid profile, and insulin concentration were determined using standard methods. Male rats fed on HFD (butter)

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showed increases in all components of MS (Glucose=161.2±0.8 mg/dl, Insulin=800±69 ρmol/l, total cholesterol=96±3.2 mg/dl). Male rats fed on a high fat diet are the most efficient means of creating diet-induced obesity and metabolic syndrome in Wistar rats.

1. INTRODUCTION

Obesity has been described as "the sixth most important risk factor in terms of the number of deaths in the world" [1]. It is a disorder caused by excessive calorific intake [2] and characterized by increased lipid storage in increasing numbers of adipocytes, and general expansion of adipose tissue itself [3]. It modifies both endocrine and metabolic roles played by adipose tissue [3] and is also a major risk factor for metabolic disorders including metabolic syndrome, Type II diabetes mellitus, atherosclerosis, dyslipidemia, hepatosteatosis, hypertension etc [4]. On the whole, even though obesity and excessive adiposity are associated with metabolic and cardiovascular disorders, the particular location of body fat distribution has been shown to be a greater factor in predisposition to metabolic and cardiovascular health risk [5].

In humans, as well as in rodent models, the particular phenotype manifested is a function of interactions between individual genes and environmental influences, including epigenetic ones [6]. Since the constitution of the gene is normally unchanged, alterations in the local environment is the easiest means of manipulating or influencing the phenotype. These alterations are used in designing experimental phenotypes for use in laboratory study of animals, mimicking the phenotypes as observed in humans. Recent findings in nutritional science research have shown that diet is a potent environmental factor capable of influencing the phenotype of an animal [7]. Diet is a common influence in humans and is a means of inducing metabolic phenotypes which closely-resemble that in humans, without pharmacological or genetic manipulations. Rats are often utilised in animal models for studying metabolic conditions like obesity and metabolic syndrome [8.9], and both humans and rodents tend to gain weight with high-caloric intake [10]. However, it has been observed that under *ad libitum* feeding conditions, not all experimental rats will eventually manifest the obese metabolic phenotype [11].

In experimental studies, obesity is usually defined as any "significant increase in body weight or energy content" in comparison with control animals under the same conditions in the study [12]. This definition is dependent on the assumption that control animals used in the laboratory for research are lean and normal, thus many observations reveal variability in incidence, onset time and severity of many of the metabolic conditions and degenerative diseases. This study was carried out to determine the most efficient means of developing a high calorific diet which would induce obesity in Wistar rats, using the least possible number of animals and the shortest possible duration of feeding.

2. MATERIALS AND METHODS

2.1 Feed Composition

Animal feeds were mixed from individual constituents in particular compositions for each of the dietary groups. The mixes were then pelletised to ensure even distribution of components and consumption by animals. The control diet was derived from standard rats' feeds commercially-propounded and sold by Ladokun feeds®. Adequate nutritional requirements were ascertained and essential amino acids were added to the feeds to prevent under-nutrition. Feeds were subjected to bomb calorimetry to determine calorific content and analysed for appropriate macronutrient content by a nutritionist.

2.2 Animals

A total of 48 albino rats (24 males and 24 females) of the Wistar strain were used in this study. The animals were obtained from the Animal House of the College of Medicine, University of Ibadan, Nigeria. All studies were approved by the University of Ibadan Animal Ethics Committee. Handling and use of animals in this study were in accordance with the guiding principles for research involving animals as recommended by the declaration of Helsinki and the Guiding principles in the care and use of animals [13]. Animals were divided into pairs of 4 groups of 6 animals each and housed in well-

aerated experimental animal cages, maintained under standard lighting conditions. They were acclimatised for 7 days prior to commencement of the grouping. During this period, they were fed on standard rat chow (Ladokun feeds, Nigeria Limited®) and had access to clean drinking water.

2.2.1 Animal groupings

To achieve the overall aim, animals were divided into the following dietary groups, each with a male and a female component, and fed for nine (9) weeks:

- Group 1 (Control): Fed on normal rats' chow (26.5% protein, 40% carbohydrates, 29% fat, and 4.5% crude fibre)
- Group 2: Fed on normal rats' chow + 60% sucrose-in-water solution
- Group 3: Fed on a High Fat diet (HFD) (22% protein, 13.5% carbohydrates, 60% fat, and 4.5% crude fibre). Fat component was derived from margarine
- Group 4: Fed on a High Fat diet, however, fat component was derived from pure butter.

2.2.2 Measurement of anthropometric variables

Abdominal circumference (AC), Body length, and thoracic circumference (TC) were measured weekly using a tape measure. The abdominal circumference was measured immediately anterior to the hind-leg, body length was measured as nose-anus length, and thoracic circumference was measured immediately posterior to foreleg. These were used to determine the following:

- Body mass index (BMI) = body weight(g)/length^2 (cm^2).
- Lee index = cube root of body weight (g)/nose-to-anus length (cm) [14].

2.2.3 Determination of fasting blood glucose

Fasting blood glucose was assessed by the use of a glucometer based on the glucose oxidase method of determination. Prior to assessing fasting blood glucose, the animals were fasted overnight, but were allowed free access to water [15].

2.3 Lipid Profile

Total cholesterol, triglycerides and HDL-cholesterol were assessed in the serum of experimental animals. After the experimental period, animals were sacrificed and blood samples collected from them. The blood samples were centrifuged at 4°C for 20 min in a cold centrifuge and aliquots were then stored at -20°C until assayed.

2.3.1 Determination of total cholesterol

The levels of total cholesterol in the treatment groups were determined using Randox kits. A method of enzyme hydrolysis as described by [16], and [17] was used.

2.3.2 Determination of triacylglyceride concentration

This was measured by colorimetric method as described by [18] and [19].

2.3.3 Determination of HDL – cholesterol

This was determined in the serum by the method of [20]. Randox HDL – cholesterol precipitant kit was used.

i. The precipitant kit contains phosphotungstric acid (0.55 mmol/l) and magnesium chloride (25 mmol/l)
ii. Cholesterol content was determined using Randox kit reagent solution which is made up of 4 - aminoantipyrine (0.3 mmol/l), phenol (≥6 moll/l), peroxidase (≥ 0.5 u/ml), cholesterol esterase (≥ 0.15u/ml), pipe buffer (80 mmol/l, pH 6.8)

2.3.4 Plasma insulin determination

This ELISA is a solid phase two-site enzyme immunoassay as described by [21]. It is based on the direct sandwich technique in which two monoclonal antibodies are directed against separate antigenic determinants on the insulin molecule. During incubation insulin in the sample reacts with anti-insulin antibodies bound to the microtitration well and with peroxidase-conjugated anti-insulin antibodies. A washing step removes unbound enzyme labeled antibody. The bound conjugate is detected by reaction with 3,3,5,5-tetramethylbenzidine (TMB). The reaction is stopped by adding acid to give a colorimetric endpoint that is read spectrophotometrically at a wavelength of 450 nm using a microplate reader. ELISA kits were procured from Sigma-Aldrich®.

2.4 Statistical Analysis

Data obtained were expressed as mean ± standard error of mean (mean ± SEM). The

significance of the results was evaluated using analysis of variance (ANOVA) and the means were compared using Turkey-Kramer Multiple comparison Test. P<0.05 was regarded as statistically significant.

3. RESULTS

3.1 Effects of Different Diets on Anthropometric Variables

The results indicate that by the 9th week, Group 4 animals (fed on a high-fat diet derived from butter) showed significant increases in all anthropometric variables (Table 1). The males on that diet exhibited significant increases in body weight, thoracic and abdominal circumferences, BMI and Lee index (P<0.05) when compared with the control animals fed on the standard rats' chow. The females, however, exhibited an increase in abdominal circumference and Lee index only (Table 1). Group3 females (fed on a high-fat diet derived from margarine) only reflected an increase in Lee Index.

3.2 Effect of Different Diets on Fasting Blood Glucose in Wistar Rats

In individual comparison with the control, the results obtained show that fasting blood glucose was significantly elevated (P<0.05) in Group 2 animals (fed on a normal rats' chow + 60% sucrose-in-water solution). In Group 4 animals (fed on a high fat diet derived from butter), both

the male and female groups reflected a significant rise in fasting blood glucose (Table 2). However, this was a smaller increase when compared with that observed in Group 2 animals.

3.3 Effect of Different Diets on Triglyceride Level in Wistar Rats

Serum triglyceride concentration significantly (P<0.05) rose in Group 2 (fed on a normal rats' chow + 60% sucrose-in-water solution) male animals in comparison with control animals. Group 3 (fed on a high fat diet derived from margarine) and Group 4 (fed on a high fat diet derived from butter) male rats also exhibited significant increases in triglyceride level (Table 2). The greatest increase in triglyceride content was however observed in male animals fed on the high fat diet derived from butter.

3.4 Effect of Different Diets on High Density Lipoprotein (HDL-Cholesterol) Level in Wistar Rats

HDL-Cholesterol was significantly elevated (P<0.05) in Group 2 (fed on normal rats' chow + 60% sucrose-in-water solution) male rats in comparison with control animals. Group 4 (fed on a high fat diet derived from butter) male rats however had a decline in HDL-Cholesterol concentrations when they were compared with the control animals (Table 2).

Table 1. Anthropometric variables after 9th week

	Body weight (g)	Thoracic circumference (cm)	Abdominal circumference (cm)	Body-mass index	Lee index
Group 1 – males	168.3±8.7	10.500±0.300	12.583±0.210	0.38±0.01	0.261±0.001
Group 1 – females	119.0±7.2	9.250±0.158	12.000±0.151	0.31±0.01	0.253±0.001
Group 2 – males	140.3±8.3	9.929±0.213	11.857±0.237	0.34±0.01	0.257±0.002
Group 2 – females	120.7±2.5	9.000±0.109	11.786±0.124	0.33±0.01	0.257±0.001
Group 3 – males	132.1±5.9	9.111±0.105	11.444±0.230	0.35±0.01	0.261±0.002
Group 3 – females	134.4±2.2	9.000±0.141	11.800±0.141	0.37±0.01	0.270±0.002*
Group 4 – males	185.5±4.3*	13.000±0.100*	14.000±0.173*	0.46±0.02*	0.283±0.005*
Group 4 – females	137.2±4.9	10.100±0.071	11.900±0.071*	0.40±0.02	0.279±0.005*

Values are mean ± SEM for 6 animals per dietary group; P=0.05
*=significantly greater than control

3.5 Effect of Different Diets on Total Serum Cholesterol Level in Wistar Rats

In Group 2 (fed on normal rats' chow + 60% sucrose-in-water solution) male animals, total serum cholesterol was significantly elevated (P<0.05) in comparison with the control animals. The Group 4 (fed on a high fat diet derived from butter) male animals also showed a significant increase in total cholesterol concentration, however the increase observed in Group 2 rats was greater (Table 2).

3.6 Effect of Different Diets on Insulin Concentration in Wistar Rats

Insulin concentration significantly (P<0.05) rose in Group 2 (fed on a normal rats' chow + 60% sucrose-in-water solution) male animals in comparison with control animals. Group 3 (fed on a high fat diet derived from margarine) and Group 4 (fed on a high fat diet derived from butter) male rats also exhibited highly significant increases in their serum levels of insulin (Table 2). The greatest increase in insulin content was however observed in male animals fed on the high fat diet derived from butter.

4. DISCUSSION

Dietary origins have been proposed to be the main factor responsible for the different metabolic phenotypes observed in animals and humans. The most common and most-studied of these phenotypes is that resulting in the metabolic syndrome (MetS), and this has been studied severally using various rodent models. Monogenic models, such as the ob/ob mouse or the fa/fa (obese Zucker) rat do not replicate the disease in humans suitably, as the syndrome in humans is polygenic [22]. Also, pharmacologic and drug-induced manipulations which induce MetS in animals (e.g. the gold-thioglucose mouse model) are unphysiologic and therefore not true representations of the human disease [22].

Numerous dietary models with varying macronutrient compositions have been used to induce metabolic syndrome in animals. It has been observed that the disorders achieved by high-fat feeding resemble the human metabolic syndrome closely, and this may also extend to the cardiovascular complications [23,24]. In rats, different models have been utilised by researchers to this same end, however, the results reported have been contrasting. It is however generally accepted that high-fat diets can be used to generate a valid rodent model for the metabolic syndrome with insulin resistance and compromised β-cell function [25,26,27].

Many of these reports have described a sex-related response to feeding on these MetS diets. This has been adduced to sex differences in adipose tissue distribution. Females generally

Table 2. Serum concentrations of MetS markers at the end of the 9th week

	Fasting blood glucose (mg/dL)	Serum triglycerides (mmol/l)	HDL - cholesterol (mg/dl)	Total serum cholesterol (mg/dl)	Plasma insulin (ρmol/l)
Group 1 - males	99.8±0.4	1.3±0.2	33±0.8	84±4.9	512±78
Group 1 - females	99±0.5	2.0±0.4	28±0.3	70±4.5	522±31
Group 2 - males	184.7±0.7*	2.4±0.2*	39±1.4*	104±4.0*	767±68*
Group 2 - females	181.6±0.8*	1.9±0.4	30±1.8	72±5.6	548±74
Group 3 - males	115.8±0.4	2.1±0.3*	32±1.2	78.±3.5	756±87*
Group 3 - females	111.4±0.7	2.2±0.1	30±1.1	71±5.8	532±59
Group 4 - males	161.2±0.8*	2.9±0.2*	28±1.2*	96±3.2*	800±69*
Group 4 - females	163.8±0.6*	2.2±0.2	30±0.6	73±6.2	570±77

Values are mean ± SEM for 6 animals per dietary group; P=0.05
*=significantly greater than control

tend to have a higher adiposity throughout their lifespan in comparison to males [28,29] whereas males have more central adiposity which is a greater predisposing factor for MetS [30]. The findings of this study reflect this sexual dimorphism as it was observed that generally the male animals had a greater elevation in anthropometric variables in intra-group comparisons with corresponding female groups. In addition, in the high-fat diet (butter) group which exhibited all the components of Mets, the female animals only showed an elevation in fasting blood glucose and abdominal circumference. All evidences from research point to the different sex hormones released in the sexes as responsible for this dimorphism [31].

High-fat diets typically used in research usually contain about 32 to 60% of calories from fat [32,33]. The high fat diets used in this study contained 60% of calories from fat derived from either margarine or butter. The extent of the metabolic disorder induced by a chosen diet depends on the dietary regimen utilised and the specific rodent strain than on the species itself. For example, C57BL/6J mice develop obesity and insulin resistance similar to Wistar rats, while 129S6 [34] or A/J mice [35] do not.

The results from the preliminary study suggest that of our four chosen MetS diets; only the male animals fed on the high-fat diet (butter) gave rise to all of the accepted variables that constitute MetS. This group was characterised by obesity, hyperglycemia, hypertriglyceridemia, low HDL cholesterol, and hyperinsulinemia. This is in consonance with studies which report that diets based on saturated fatty acids induce the typical high-fat-diet phenotype, whereas diets containing polyunsaturated ω-3 fatty acids exert beneficial effects on body composition and insulin action [36]. The ability of a high-fat diet to induce obesity and MetS in animals has been adduced to energy density of the diet, rather than a purported increase in percentage of the dietary fat [37,38,39,40,41]. This corroborates the findings of this preliminary study as it was observed that although equal quantities of fat were fed to animals in both high fat diets (butter and margarine), only the HFD (butter) group showed significant effects in all MetS components. Butter is high in saturated fat content, while margarine contains trans-fatty acids. Trans-fatty acids are polyunsaturated fatty acids derived from partial hydrogenation of vegetable oils which are implicated in coronary heart disease and arteriosclerosis. Trans-fatty

acids reduce serum levels of HDL cholesterol and increase the levels of LDL cholesterol while serum cholesterol levels also increase with higher intake of saturated fats and cholesterol. Higher intake of trans-fats or saturated fats has been shown to be associated with increase drisk of MetS and coronary heart disease [42].

In male animals given the 60% sucrose-in-water solution, a significant increase in all MetS variables was also observed but there was no significant change in body weight and an elevation of HDL cholesterol, which negated a true MetS picture. It has been reported that an increased intake of refined carbohydrates, such as sucrose (composed of fructose and glucose), is associated with increased weight gain, hypertriglyceridemia, and insulin resistance (IR) in both humans and also in animal models [43,44]. In rodent models, diets containing high sucrose elevate triglyceride and glucose production in the liver, thus increasing availability of nutrients and ultimately leading to IR and hypertriglyceridemia [43,44]. The Wistar rat is an established model of sucrose-induced IR and hypertriglyceridemia [45,46,47]. It seems that the fructose component of sucrose is largely responsible for the hypertriglyceridemia and IR produced by high sucrose diets [48,49,50]. Chicco and co-workers observed that high sucrose diets do not appear to lead to excessive weight gain. This observation is corroborated by the findings in this study [51].

5. CONCLUSION

In deriving a most-efficient diet-induced model of MetS, a dietary formulation of pelletised high-fat diet with the fat component derived from pure butter is most ideal in producing results within the shortest possible time-frame. This diet gives rise to all the defining components of MetS, however, male rats are more likely to give the desired results due to their development of abdominal obesity.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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