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Original Article

Rat model of food-induced non-obese-type 2 diabetes mellitus: comparative pathophysiology and histopathology

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Abstract: Based on the hypothesis that consistent hyperglycemia can result in insulin resistance, we explored the induction of non-insulin dependent diabetes mellitus (NIDDM) using diet of high glycemic/low fat index and compared the effects on the physiology and histology of the rats. The rats were divided into 3 groups. DM was induced in the first group by single intraperitoneal injection of 150mg/kg alloxan monohydrate and in the second group by feeding the rats with diet of high glycemic index/low fat for 8 weeks. The pathophysiology and histopathology of DM were studied. Hyperglycemia was recorded in the alloxan and food-induced groups respectively. Both groups were also positive for glycosuria, which confirmed the induction of DM. Concentrations of plasma potassium, calcium, protein and urea were higher ($p<0.05$) in the alloxan-induced than the food-induced diabetic rats, whereas food-induced rats recorded higher hematological indices than the alloxan-induced group. Coronary risk indices were higher in food-induced diabetic rats than the alloxan-induced, while activities of antioxidant enzymes were significantly higher ($p<0.05$) in alloxan-induced diabetic rats than the food-induced rats. Marked degenerations of the Islets of Langerhans was observed in pancreas of alloxan-induced diabetic rats, whereas, histological examination of the pancreas of food-induced and control rats revealed no visible lesion. Liver and kidney of all food and alloxan-induced diabetic rats showed marked degeneration of the hepatocytes and the glomeruli respectively. This study presents a rat model of type II diabetes mellitus using food of high glycemic/low fat index with its consequent ionoregulatory disruptions, acute anemia, hyperlipidemia, nephropathy and hepatopathy.

Keywords: Diabetes mellitus, alloxan-induced, food-induced, pathophysiology, histopathology

Introduction

Diabetes mellitus (DM) is a common metabolic disorder marked by elevated blood glucose concentration and excretion of glucose in urine [1, 2]. DM occurs either because of lack of insulin or the presence of factors that oppose the actions of insulin. The result of the insufficient action of insulin is an increase in blood glucose concentration higher than 160mg/dl which is above the normal value of 80-120mg/dl in humans [3]. Statistics have shown that about 10% of the world's population suffers from DM [4].

There are two major types of DM. Type 1 DM also known as Insulin Dependent Diabetes Mellitus (IDDM) is caused by massive loss of insulin secreting beta cells which could be as a result of viral or bacterial infection [5, 6]. Type 2 DM or Non-insulin Dependent Diabetes Mellitus (NIDDM) is caused by a combination of insulin resistance and altered insulin secretion, which disrupt the metabolism of glucose [6]. It is known that increasing age, obesity, diets rich in high glycemic index and physical inactivity are risk factors that enhance the chances of someone developing type 2 diabetes mellitus [7].

Majority of studies on IDDM in experimental animals are alloxan-induced diabetes mellitus

[8-11], while severeal studies [12-19] have presented rat models of NIDDM using high-fat/ low glycemic index diets.

However, studies [20, 21] demonstrated that consistent hyperglycemia causes insulin resistance in cultured human umbilical vein endothelial cells and skeletal muscle respectively. This study was therefore undertaken to explore the induction of NIDDM in Wistar strain rats by feeding them with diet of high glycemic/low fat index and to comparatively study the effects food- and alloxan-induced diabetes mellitus on the physiology and histology of the rats.

Materials and methods

The animals

Adult male albino rats of Wistar strain (Sprague Dawley) weighing between 150-180g were obtained from the animal house of the Department of Biological Sciences, University of Agriculture, Abeokuta. The rats were kept in rat cages at room temperature (27 ± 2^{0}C) and humidity (55 ± 5%) and a 12 hours cycle of light and dark. The rats were acclamatized for two weeks prior to commencement of the experiment. All experiments were performed in accordance with the National Institute of Health guidelines of care and use of laboratory animals [22].

Experimental set-up

Control: This group was made up of 20 rats. They were given standard animal pellet and water ad labitum.

Group AU: This group was made up of 20 rats. They were induced by single intraperitoneal injection of 150mg/kg of alloxan monohydrate dissolved in normal saline after an overnight fast. Surviving rats after 3 days with blood glucose concentration more than 200mg/dl of blood were considered as alloxan-induced diabetic rats and used as IDDM models for further study.

Group FU: This group was made up of 20 rats. They were induced by feeding the animals with food of high glycemic index for 8 weeks. White bread which has glycemic index value of 70 was fed to the rats, while granulated sugar with glycemic index value of more than 100 was dissolved in the drinking water at a concentration of 1g/ml. Surviving rats after 8 weeks with blood glucose concentration of 200mg/ml were considered as food-induced diabetic rats and used as NIDDM models for further study.

Periodic weighting of rats and blood collection

The rats were weighed weekly and recorded as mean weight per group. Also, percentage food consumption was recorded weekly. At the end of the experiment, the animals were anaesthetized with chloroform and blood was collected from each rat by cardiac puncture.

Blood chemical analysis

Blood glucose concentration was determined before and during the experiment by placing a drop of blood from the tail tip on strip of digital ACCU-CHEK advantage II glucose meter (Roche diagnostic, Germany). Glycosuria was determined by COMBI strips. Hydrogen ion concentration in whole blood was determined using an electric digital pH meter. Plasma total protein and urea concentrations were determined using the biuret and the Urease –Berthelot (enzymatic) colorimeter method respectively. Flame photometry method was used to evaluate the concentration of sodium and potassium in the plasma while colorimeter method was used to determine the concentration of calcium.

Lipid profile

Total cholesterol and triglyceride were determined by enzymatic (cholesterol oxidase) and (colorimeter) methods respectively. Low Density Lipoprotein (LDL) was estimated as the difference between total cholesterol and the content of the supernatant after precipitation of the LDL fraction, while the High Density Lipoprotein (HDL) was calculated from the data obtained.

Hematological studies

The PCV was determined by using the microhematocrit reader. The total hemoglobin concentration of the blood samples was estimated using the cyanomethemoglobin method. The white blood cell count (WBC), Red blood cell count (RBC), Mean cell hemoglobin (MCH), mean cell volume (MCV), and the mean cell hemoglobin concentration (MCHC) were also determined.

Table1. Glucose concentration of Food and Alloxan-induced diabetic rats.

Groups	Initial Glucose Concentration	Confirmatory Glucose level	Percentage Change
Control	81.0±3.3[a]	79.8±2.1[a]	-2.31
AU	86.5±2.4[b]	387.5±3.9[c]	+346.0
FU	87.7±2.6[c]	270.2±2.7[b]	+208.0

Values are mean± S.E; Values within a column having different superscripts are significantly different at p<0.05.

Enzyme assay

Catalase and peroxidase unit activities in the plasma were determined using the spectrophotometric method.

Histological studies

The pancreas, liver and kidney of each rat were collected and fixed in 10% formalin. The organs were processed routinely for histopathological evaluations.

Statistical analysis

Data obtained were expressed as mean ± SE. Significant difference between test and control groups was tested using student's t-test and correlation/regression of the SPSS computer software, version 16.0 at 95% confidence intervals (CI).

Results

Blood glucose concentration

Initial mean blood glucose concentrations of FU and AU groups are 86.85 and 87.75mg/dl (**Table 1**). Substantial increases in blood glucose concentrations were recorded in the FU and AU groups after feeding with high glycemic/low fat index diet for 8 weeks and after intraperitoneal injection of alloxan monohydrate respectively. The respective average blood glucose concentrations for the FU and AU groups were 270.27mg/dl and 387.35mg/dl. All induced rats also tested positive for glycosuria. Result showed significant difference (p<0.05) in the glucose concentrations of the diabetic groups compared to the control.

Body weight

There was marked reduction in mean body weight of the AU group after alloxan induction (**Figure 1**) with concomitant decrease in

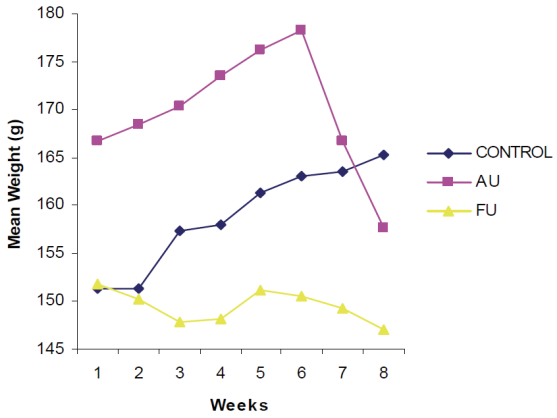

Figure 1. Mean weight of alloxan and food-induced rats. AU=Alloxan-induced group; FU=Food-induced group.

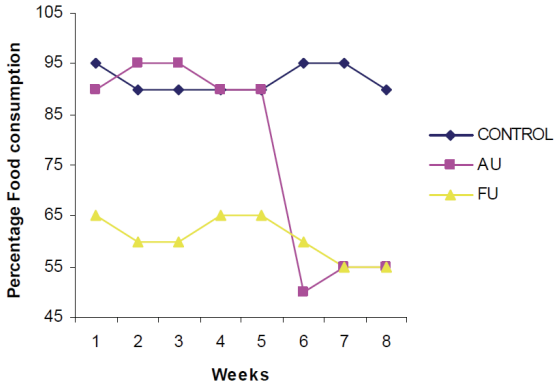

Figure 2. Percentage food consumption of alloxan and food-induced rats. AU=Alloxan-induced group; FU=Food-induced group.

percentage food consumption (**Figure 2**). Examination of the rats that died after alloxan injection showed pallor of the mucous membrane with dark coloration of the lips and corners of their eyes.

The body weight of food-induced rats showed irregular fluctuations throughout the period of feeding (**Figure 1**). For the first three weeks, a

Table 2. Blood Chemical Parameters of Food and Alloxan-induced diabetic rats.

Groups	Na^+ (mmol/L)	K^+ (mmol/L)	Ca^{2+} (mmol/L)	Total protein (g/L)	Urea (mg/dl)	Peroxidase Unit Activity (Unit/ml)	Catalase Activity (Unit/ml)
Control	98.4±3.2[a]	7.8±5.3[a]	2.4±1.2[a]	93.9±3.6[c]	65.4±2.6[c]	1.052±0.1[a]	0.631±0.4[a]
AU	107.6±2.4[b]	9.8±4.7[b]	3.7±1.7[b]	75.7±5.2[b]	48.8±2.6[b]	1.853±0.3[c]	1.516±0.1[c]
FU	109.8±3.9[c]	9.6±4.5[b]	2.5±1.3[a]	65.7±3.5[a]	31.2±3.6[a]	1.753±0.2[b]	1.409±0.1[b]

Values are mean± S.E; Values within a column having different superscripts are significantly different at $p<0.05$.

Table 3. Hematology of Food and Alloxan-induced diabetic rats.

Groups	PCV(%)	Hb(g/dl)	WBC($X10^6$)	RBC($x10^{12}$/l)	MCV($x10^{15}$/l)	MCH(pg)	MCHC(g/dl)
Control	45.4±2.4[c]	15.1±2.7[c]	16,000±6.2[a]	4.53±2.6[c]	9.93±2.6[c]	33.3±2.9[b]	0.34±0.2[ab]
AU	40.2±3.9[b]	13.4±3.2[b]	24,950±7.9[b]	4.35±2.6[b]	9.20±2.7[a]	30.8±2.6[ab]	0.33±0.8[a]
FU	31.8±5.2[a]	10.2±4.6[a]	27,250±7.3[c]	3.30±1.2[a]	9.39±2.5[b]	30.9±2.7[a]	0.33±0.5[a]

Values are mean ± SE; Values within a column having different superscripts are significantly different at $p<0.05$

steady decline in body weight was recorded, during which the rats consumed very little of the bread presented to them (**Figure 2**), while the entire sugar solution presented to them was consumed. During this period the animals appeared skinny, with marked areas of alopecia on their bodies. During the 4th and 5th weeks the animals recorded slight increase in their body weight, which did not reflect as improvement in the appearance of the animals. Steady decline in weight was also recorded during weeks 6, 7 and 8, which was also characterized by marked reduction in food consumption.

Blood chemistry

Ionoregulatory distruptions (hypernatremia, hyperkalemia ans hypercalemia) were observed in plasma of both AU and FU groups compared to the control rats (**Table 2**). Concentrations of K^+ and Ca^{2+} were however significantly higher ($p<0.05$) in alloxan- induced rats than in the food-induced rats. Concentrations of plasma urea and total protein were significantly lower ($p<0.05$) in FU than the AU group. Although both Catalase and Peroxidase activities were significantly higher ($p<0.05$) in FU and AU-induced diabetic groups than in the control, enzymes activities were however higher in AU diabetic rats than in the FU-induced group.

Hematology

Acute anemia was recorded in all diabetic rats as all haematological indices were lower in diabetic groups than the control (**Table 3**). Values of Packed Cell Volume (PCV), hemoglobin concentrations (Hb) and Red Blood Cell count (RBC) were however significantly higher ($p<0.05$) in the alloxan-induced diabetic rats than the food-induced group.

Plasma lipid

Concentraions of total cholesterol, triglyceride and Low Density Lipoprotein were higher ($p<0.05$) in FU group than the AU group (**Table 4**). The value of HDL was however significantly lower ($p<0.05$) in diabetic rats than in the control.

Histology of the pancreas, liver and kidney

Histology of the pancreas of the control and FU rats showed normal arrangement of the Islets of Langerhans of various sizes scattered throughout the exocrine tissue with no visible lession (**Figure 3A** and **B**). However, pancreas of alloxan -induced diabetic rats revealed marked degeneration of the Islet of Langerhans, with severe vacoulations of the exocrine tissue (**Figure 3C**).

Table 4. Plasma Lipid of Food and Alloxan-induced diabetic rats.

Group	Total cholesterol (mg/dl)	Triglyceride (mg/dl)	HDL (mg/dl)	LDL (mg/dl)	LDL/HDL	CRI
Control	84.6±4.4[a]	69.2±1.7[a]	30.0±2.3[b]	58.6±3.6[a]	1.95±1.4[a]	2.82±2.3[a]
AU	123.1±2.4[b]	94.5±2.3[b]	15.8±2.5[a]	95.4±4.4[c]	6.04±1.6[b]	7.79±1.5[b]
FU	130.8±3.2[c]	100.3±2.9[c]	15.3±3.5[a]	92.3±4.5[b]	6.03±2.4[b]	8.55±1.7[c]

Values are mean ± SE; Values within a column having different superscripts are significantly different at $p<0.05$

Liver of the control rats revealed normal arrangement of the hepatocytes within the liver parenchyma (**Figure 3D**). However, histological examination of liver of both food and alloxan-induced diabetic rats revealed marked degeneration with diffuse vacuolations of the hepatocytes (**Figure 3E** and **F**).

Histological examination of the kidney of control rats revealed no visible lesion as the renal corpuscles appeared normal in dense rounded structures, the glomeruli are surrounded by narrow Bowman's spaces (**Figure 3G**). Histological examination of kidney of both food and alloxan-induced diabetic rats revealed degeneration of the glomeruli with wider Bowman's spaces and diffuse vacuolation of the tissues (**Figure 3H** and **I**).

Discussion

DM is a disease characterized by marked increase in blood glucose and the presence of glucose in urine. The marked increase in blood glucose concentration observed in the rats after intraperitoneal injection of alloxan monohydrate confirmed the induction of insulin-dependent diabetes mellitus (IDDM). Earlier studies [2, 8, 9] also reported multiple increase in blood glucose concentration after a single intraperitoneal injection of alloxan monohydrate. This increase as reported by Bansal et al (1980) [10] is due to the toxic and destructive effect of alloxan monohydrate on the beta cells of the pancreas. This destruction is responsible for the inability of the pancreas to synthesize and secrete adequate amount of insulin necessary for the metabolism of carbohydrate.

The marked increase in blood glucose concentration and glycosuria observed in rats fed on white bread and concentrated sugar solution after 8 weeks confirmed the induction of non-insulin-dependent diabetes mellitus (NIDDM). The ability of white bread and sugar solution to induce diabetes mellitus may be because glucose derived from the high glycemic index diet produced persistently high level of insulin secretion from the pancreas, which ultimately resulted in post-receptor insensitivity to the released insulin [1]. This postulation is supported by the fact that histological examination of the pancreas of all food-induced rats revealed no degeneration of the Islets of Langerhans. This observation could offer explaination to the upsurge in the reported cases of DM in Africa particularly Nigeria where majority of our regular diets are rich in carbohydrate.

Mean body weights and percentage food consumption were observed to decrease drastically after a single intraperitoneal injection of alloxan monohydrate. Specific necrosis of the pancreatic islets in alloxan-induced rats affects the metabolism of glucose in the rats [11] and this could have been responsible for the decrease in weight observed in this study. Mortality recorded during this period was also high. Observation of eyes of rats that died after the injection revealed ocular opacity. The irregular change in body weight of rats fed with bread and sugar solution is a further testimony to the fact that excess glucose intake may result in metabolic disruption in the animals. This disruption may also be responsible for the inconsistency in weight change and food consumed by the rats as observation of the rats during this period showed that they looked pale, skinny and their hairs were turning yellow with many of them falling off.

Higher enzymes activity recorded in the diabetic rats is consistent with the findings of Klibber et al (1996) [11] that elevated extra and intra cellular glucose concentrations induced oxidative stress, which is as a result of increased production of oxygenated free radicals. The increased level of antioxidant enzyme could therefore constitute a physiological response to the glucose-

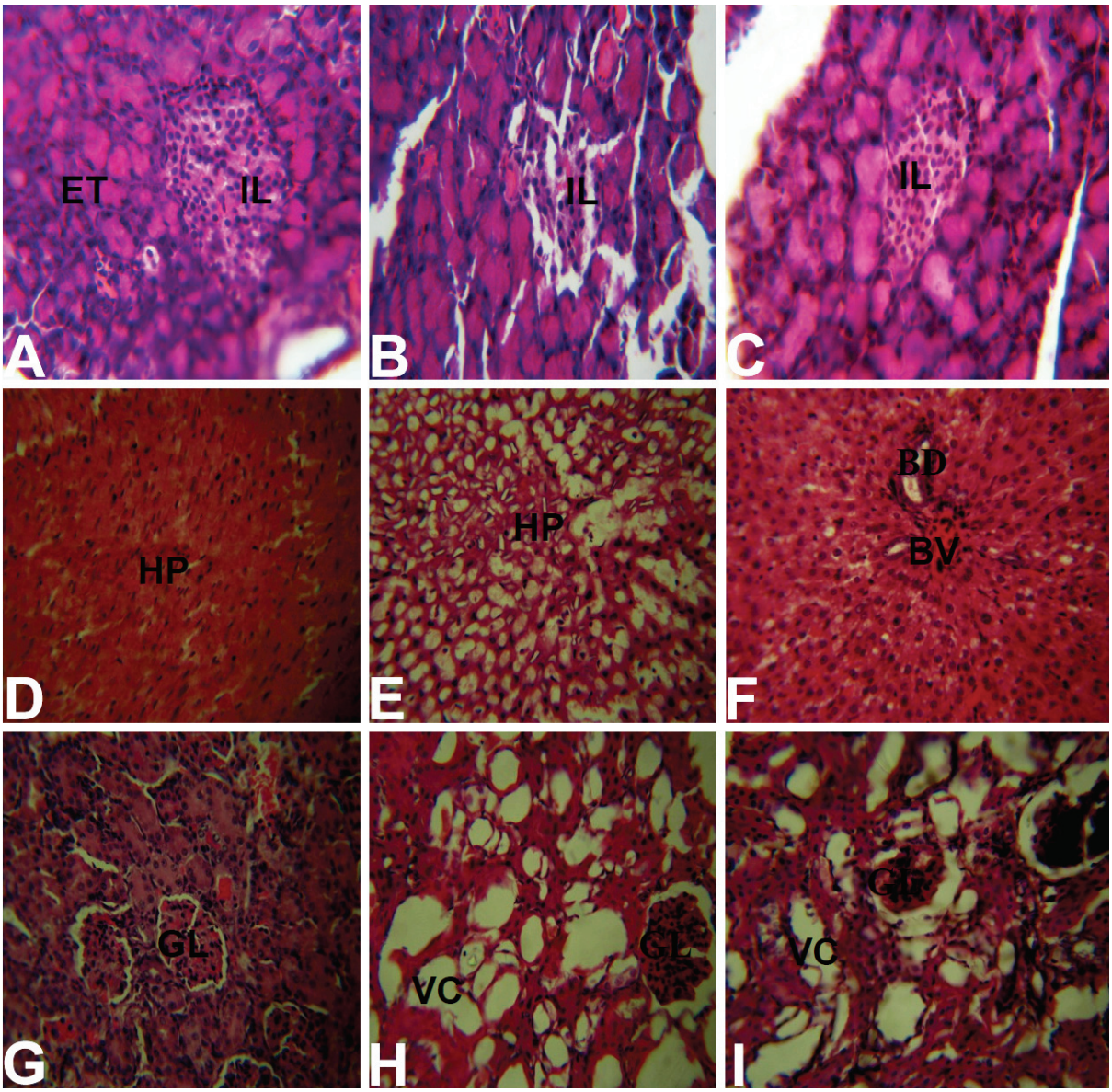

Figure 3. Histology of the pancreas, liver and kidney. **A.** Pancreas of control rat showing normal, appearance of the islet of Langerhans (IL) located in the exocrine tissue (ET). **B.** Pancreas of alloxan-induced diabetic rat, showing marked degeneration of the Islets of Langerhans (IL). **C.** Pancreas of food-induced diabetic rat showing normal appearance of the islets of Langerhans (IL). **D.** Liver of control rat show normal arrangement of the hepatocytes (HP) with no visible lesion. **E.** Liver of alloxan-induced rat show severe degeneration of the hepatocytes (HP) with numerous vacuolations. **F.** Liver of food-induced diabetic rat show hyperplasia of the bile ducts (BD) and congestion of the blood vessels (BV). **G**. kidney of normal rat show normal appearance of the glomeruli (GL). **H**. Kidney of alloxan-induced diabetic rat show marked degeneration of the glomeruli (GL) with glomerular atrophies and severe vacuolations (VC). **I**. Kidney of food-induced rats show marked degeneration of the focal areas of the glomeruli with severe vzcoulations. All panels were stained with H & E, magnification X 300.

induced oxidative stress in the diabetic rats. Anemia is a condition indicated by decreased haematological indices [23]. This study therefore demonstrated diabetes mellitus-induced anemia as all diabetic rats recorded lower haematological indexes than the control rats. Concentrations of total cholesterol, triglyceride, HDL, LDL, and CRI are indicators of cardiovascular disease [24, 25]. This study reported higher coronary risk indices in the FU

than AU rats. This therfore implied higher risk of cardiovascular diseases in food-induced diabetes mellitus.

The normal appearance of the pancreas of food-induced diabetic rat confirms the induction of NIDDM as a result of the insensitivity of receptors to insulin. The degeneration observed in the pancreas of alloxan-induced diabetic rats is due to the necrotic action of alloxan monohydrate on the β cells. Earlier work by Bansal (2002) [26] reported specific necrosis of the pancreatic islets after exposure of the islet to alloxan. This degeneration resulted in the inability of the pancreas to secrete adequate insulin for carbohydrate metabolism, which ultimately resulted in the onset of IDDM. Various degrees of degenerations observed in the liver hepatocytes and kidney glomeruli of the diabetic rats confirmed the pathological complications of food and alloxan-induced diabetes mellitus in the vital organs of the animal models.

This study demonstrated the induction of NIDDM by high glycemic/low fat diet in Wistar strain rats. The pathophysiological and histopathological effects observed affirmed the postulation that diet is a major contributing factor to the cause and pathology of diabetes mellitus.

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