

Original Research Article

Comparative Effects of Low- and High-Glycemic Index Diets on Biochemical Variables and Organ Histology in Alloxanized Diabetic Rats

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ABSTRACT

This experimentally-controlled designed study compared the effects of low- and high-glycemic index diets on biochemical variables and organ histology in alloxan-induced diabetic rats (DR).

Effects of low-GI (fried yam) and high-GI (roasted) diets on organ and body weights, pancreas histology, glycemic tolerance (GT) and lipid profile (LP) were determined and compared in the adult alloxan-induced (150mg/kg intraperitoneally) diabetic rats of 3 groups (8 rats each) fed with standard rat feed (control), fried and roasted yam diets respectively for six weeks. Lipid profile and glycemic tolerance were analysed and determined using a dry-chemical automatic analyzer and oral D-glucose load of 2 gm kg⁻¹ dissolved in distilled water respectively. Organs were extracted and weighed while pancreas histoarchitecture examined after 6 weeks of feeding. Data were analyzed using ANOVA and Student's t test while values of $P < 0.05$ were considered significant.

Postprandial glycemic response to low-GI (GI = 36%) diet showed improved GT (IAUC = 3082.5mg/dl.min) over that of high-GI (GI = 93%) diet (IAUC = 8332.0mg/dl.min). A significant increase ($P < 0.05$) in mean body weight of rats was observed in all groups after six weeks of feeding with the highest increase (24.8%) observed in DR on high-GI diet (initial weight = 251.0±1.6g; final weight = 312.3±5.9g) and the lowest increase (9.2%) in DR on low-GI diet (initial weight = 250.7±1.1g; final weight = 273.2±1.7g). No significant change was observed in mean organ weights. Pancreas histology of DR on high-GI diet showed degeneration with degranulation and vacuolation of the islet β cells while regeneration of some β cells was observed in DR fed with low-GI diet. Triacylglycerides (52.2±1.0mg/dL) and cholesterol (60.5±1.5mg/dL) increased in DR fed with fried yam (low-GI) compared with those fed on roasted yam (high-GI).

The low-GI diet used in this study showed improved glycemic tolerance and histoarchitecture of pancreatic islets over that of high-GI diet. Although both diets are prepared from same source, difference in their processing methods reflects their observed impact on the biochemical variables. Fried yam as one of severally known low-GI diets may be incorporated in diabetic menu. However, long-term consumption of fatty foods should be avoided.

Key Words: Low- and high-glycemic index diets; glycemic control; lipid profile; pancreas; diabetic rats

1. INTRODUCTION

Human diet contains many types of carbohydrates, each of which contributes to different physiologic responses. Diets rich in rapidly digested carbohydrates (High-GI diets) have been suggested to have detrimental effect on health due to high level of postprandial blood glucose and insulin responses associated with them (1) while slowly digested carbohydrates (Low-GI diets) may protect against chronic diseases (2). Dietary carbohydrates have an essential physiological role in the body. The rate of digestion and absorption of carbohydrates can be a determinant factor for the metabolic control of some human chronic non-infectious diseases (3). For this reason, there has been a growing interest in the biological

utilization of carbohydrates by human body, especially referring to starch and dietary fiber and their effects on the glycemic response and index and on the large bowel physiology (4,5). Currently, the importance of glycemic index studies is linked to the possible therapeutical and physiological effects of diets with low GI on healthy, obese, diabetic and hyperlipidemic subjects. The GI has also been related to colon diseases and physical activity (6).

Glycemic index values have been published for a wide range of foods and have been used in several studies to design low-glycemic load diets for diabetic subjects. Research on GI indicates that even when foods contain the same amount of carbohydrate (i.e., carbohydrate exchanges), there are up to fivefold differences in glycemic impact (7). Current dietary recommendations emphasize the quantity rather than the quality of carbohydrate, despite the fact that carbohydrate source and nature profoundly influence postprandial glycemia (8). In addition, several prospective observational studies have found that the overall GI and glycemic load of the diet, but not total carbohydrate content, are independently related to the risk of developing type 2 diabetes (9,10), cardiovascular disease (11), and some cancers (12,13). However, not all studies are in agreement, and further research is needed (14). Although logic suggests that low-GI diets should improve glycemic control, the findings of randomized controlled trials have been mixed; some studies have shown statistically significant improvements (15,16) whereas other studies have not (17,18). As a result, the issue of the GI has been fraught with controversy and has polarized the opinions of leading experts (19,20).. Currently there is no universal approach to the optimal dietary treatment for diabetes due to controversy about how useful the glycemic index (GI) is in diabetic meal planning.

The aim of diabetes management is to normalize blood glucose levels, since improved blood glucose control through diets is associated with reduction in development and progression of complications, improved quality of life, medications minimization, and increase life expectancy. While the American Diabetes Association acknowledges that use of low-GI foods may reduce postprandial hyperglycemia but asserts that there is not sufficient evidence of long-term benefit to recommend their use as a primary strategy for the dietary control of diabetes (21), the European Association for the Study of Diabetes in contrast, recommends the use of low-GI foods in dietary control of diabetes mellitus (22). To help resolve this controversy and provide a more objective basis to guide dietary recommendations, this study was conducted to compare the effects of high- and low-GI diets on biochemical variables (glycemic tolerance (GT), lipid profile (LP) and organ weights) and organ histology (pancreas) in alloxan-induced diabetic rats thus assessing their impact on glycemic profile and overall diabetic control.

2. MATERIALS AND METHODS

2.1 EXPERIMENTAL ANIMALS AND DIETS

Twenty four (24) male albino Wistar rats weighing 250g suitable for the experimental study were purchased from the disease-free stock of the animal house of the department of Veterinary Physiology, University of Ibadan, Ibadan, Oyo state, Nigeria. The rats were kept in polypropylene plastic cages and

maintained at normal and standard laboratory conditions of temperature ($28 \pm 2^{\circ}\text{C}$) and relative humidity ($46 \pm 6\%$) with 12-hour light-dark cycle and adequate ventilation to acclimatize to their environment. The rats were weighed twice weekly to ensure that no rat outside the initial weight range of 250g-300g was used. The rats were initially fed with commercially available standard rat feed (Ladokun feeds Nig. Ltd.) purchased from a commercial branch depot and water *ad libitum* during the period of acclimatization and thereafter were grouped into 3 groups (8 rats each) and fed with fried yam (low-GI diet), roasted yam (high-GI diet) and standard rat feed (control) respectively for a period of 6 weeks. The weights of the rats were recorded for the six weeks period of the study prior to laboratory investigations.

This study using experimental animals was conducted in accordance with the internationally accepted principles for laboratory animals (23).

2.2 INDUCTION OF DIABETES

After 15 hour overnight fast, rats in all groups A, B and C were injected intraperitoneally with freshly prepared alloxan monohydrate (Sigma chemicals, USA) dissolved in sterile normal saline at a dose of 150 mg/kg body weight. Diabetes was confirmed 4-7 days later by use of glucometer (On Call Plus Blood Glucose Monitoring System, ACON Laboratories, Inc. San Diego, USA.). Rats with Fasting Blood Glucose (FBG) level $> 150\text{mg/dl}$ were considered *diabetic* and used for this study (24).

2.3 TEST DIETS

Two test diets (roasted and fried yams) processed from white yam (*Dioscorea rotundata*) were used for this comparative experimental study. To ensure their suitability for the study as low- and high-GI diets based on GI international classification of foods, their glycemic indices using modified Wolever method (25) were determined using 10 volunteered diabetic subjects following their written consent and ethical approval of the experimental protocol by U.I/U.C.H Institutional Review Committee of the Institute for Advanced Medical Research and Training (IMRAT) with the assigned number UI/EC/07/0092. The determined GI of roasted yam is 93% (high-GI: $>70\%$) while that of fried yam is 36% (low-GI: $<55\%$). Their proximate nutrient compositions were determined using standard methods of food analysis (26,27) Proximate compositions of the diets and their corresponding GI are shown in Table 1 below.

Table 1: Glycemic index and proximate nutrient composition of low-GI (fried yam) and high-GI (roasted yam) diets

Test meals	Food energy (kcal)	Moisture (%)	Protein (g)	Fat (g)	Carbohydrate (g)	Fiber (g)	Ash (g)	Glycemic index (%) GI= $\frac{1}{2}(\text{IAUC}/\text{IAUCS}) \times 100\%$
Fried Yam	383.81 $\pm$ 0.11	54.33 $\pm$ 0.01	8.10 $\pm$ 0.01	2.20 $\pm$ 0.01	79.07 $\pm$ 0.00	2.67 $\pm$ 0.01	1.57 $\pm$ 0.00	36 $\pm$ 2.71
Roasted Yam	379.52 $\pm$ 0.02	51.39 $\pm$ 0.00	9.41 $\pm$ 0.01	0.59 $\pm$ 0.01	80.40 $\pm$ 0.02	3.27 $\pm$ 0.01	0.51 $\pm$ 0.01	93 $\pm$ 4.04

Values of proximate nutrient composition are means $\pm$ SEM of three determinants while GI values are mean $\pm$ SD; GI =glycemic index; IAUC=incremental area under curve (test diet); IAUCS= incremental area under curve (standard diet)

2.4 EXPERIMENTAL DESIGN

The animals after 2 weeks acclimatization period and induction were randomly divided into 3 broad categories of eight (8) rats each:

GROUP A: Diabetic rats fed on high-GI (Roasted yam) diet – DRG group.

GROUP B: Diabetic rats fed on low-GI (Fried yam) diet – DFG group.

GROUP C: Diabetic rats fed on normal standard rat diet (Control Diabetic) – DCG group.

2.5 BIOCHEMICAL ASSAYS

2.5.1 Oral Glucose Tolerance Test (OGTT)

The OGTT was conducted at the end of the 6 weeks of study. Animals in all groups after 15 hour overnight fast with free access to water were treated with an oral D-glucose load of 2 gm kg^{-1} (dissolved in distilled water) administered by means of cannula. Blood samples were withdrawn from the cordal (tail) vein of each animal (tail snipping) to determine the fasting blood sugar concentration at time 0 minute (before ingestion of glucose) and subsequently at intervals of 30, 60, 90, 120 and 150 minutes after oral glucose administration.

2.5.2 Lipid profile test (LPT)

The lipid profile was conducted at the beginning and then 6 weeks later at the end of the study for comparison. Blood samples from the Posterior Vena Cava vein were collected and transferred into the k_3 EDTA (Ethylene Diamine Tetraacetic Acid) sample bottles. Samples were centrifuged at 3000 revolutions to obtain the plasma fractions which was kept in a refrigerator (at -70°C) until used and the sera obtained were used for the biochemical assay of the lipid profile. Plasma concentration of total cholesterol (TC), high density lipoprotein (HDL) and Triacylglycerol (TAG) were measured by the enzymatic colorimetric method after centrifugation using a dry-chemical automatic analyzer AU-5200 OLYMPUS (Randox Laboratories, San Francisco, USA). LDL level was determined by the Friedewald formula (28) as follows:

$$\begin{aligned}\text{VLDL (mg/dL)} &= \text{TAG}/5 \\ \text{LDL (mg/dL)} &= \text{TC} - \text{VLDL} - \text{HDL}\end{aligned}$$

2.5.3 EXTRACTION OF ORGANS AND ORGAN WEIGHTS

After 6 weeks of test study, animals in all groups were given light anaesthesia using Ethyl Ether in a glass dome and then dissected to extract some organs. Organ weights (liver, heart, kidney, lungs, spleen and testes) were measured and recorded as a percentage of final body weight together with the absolute values while the pancreatic tissues were histologically examined.

2.5.4 Histology of Pancreas

Histological examination was based on an earlier protocol (29). Slices of the pancreatic tissue were fixed in 10% formalin solution for 24 h. All samples were then dehydrated in graded ethanol series, cleared in

toluene and embedded in paraffin wax; 5-6 μm sections were routinely stained with Harris hematoxylin and eosins stains (Sigma-Aldrich) and were assessed under light microscope (Nikon Eclipse E400).

2.6 STATISTICAL ANALYSIS

Data was analyzed using appropriate statistical methods and program of Microsoft Excel and SPSS v. 17. GI values are expressed in mean $\pm$ SD while other results are expressed as group mean $\pm$ SEM. Comparisons between groups and the significant difference between the control and the treated groups were analyzed using one way analysis of variance (ANOVA). Values of $P < 0.05$ were considered significant.

3. RESULTS

3.1 BODY AND ORGAN WEIGHTS

As shown in Table 2 below, the mean body weights of the rats were almost the same (~ 250 g) in all groups at the start of the study. At the time of sacrifice, the mean body weight increased significantly ($P < 0.05$) in all groups with highest increase (24.8%) in diabetic rats group (DRG group) fed with high-GI diets ($312.33 \pm 5.85\text{g}$) and lowest increase (9.2%) in diabetic rat group (DFG group) fed with low-GI diets ($273.17 \pm 1.72\text{g}$). Both low- and high-GI diets had significant impacts on body weight but much more with high-GI diets. No significant change was observed in the mean weights of organs (liver, heart, kidney, lung, spleen and testes).

3.2 GLYCEMIC TOLERANCE TEST

Postprandial glycemic response to low-GI diet showed improved glycemic tolerance over that of high-GI diet with incremental area of low-GI diet = 3082.5mg/dl.min while that of high-GI diet = 8332.0mg/dl.min . High-GI diet displayed quicker and higher glycemic responses to oral glucose challenge as compared to the Low-GI diet which displayed slower and decreased glycemic responses to oral glucose challenge. Control diet showed relative normoglycemic response curves. Figure 2 below shows the mean glycemic tolerance curves to test and control diets.

3.3 LIPID PROFILE

Table 3 below shows the comparative effects of the test diets on the lipid profile of experimental diabetic rats. After 6 weeks of diet, levels of the constituting parameters of the lipid profile (except HDL) increased in all groups but much more significant in fried yam (low-GI) fed group than roasted yam (high-GI) fed group.

**Table 2. Effects of low- and high-GI diets on mean body and organ weights of experimental rats.
(n = 8)**

Experimental Animals				188
Body Weight (g)	Control/Standard (DCG)	High-GI Diet (DRG)	Low-GI Diet (DFG)	
Initial	251.00±1.61 ^a	254.67±2.46 ^a	250.67±1.12 ^a	
Final	297.67±6.23 ^a	312.33±5.85 ^b	273.17±1.72 ^b	
Organ weights				
SPLEEN	0.78±0.00 ^a	0.73±0.00 ^a	0.75±0.02 ^a	
KIDNEYS	1.46±0.00 ^a	1.39±0.00 ^a	1.42±0.02 ^a	
LUNGS	1.18±0.00 ^a	1.17±0.00 ^b	1.13±0.06 ^b	
HEART	0.49±0.00 ^a	0.58±0.02 ^a	0.53±0.02 ^a	
LIVER	5.56±0.00 ^a	5.52±0.04 ^a	5.54±0.04 ^a	
TESTES	2.12±0.01 ^a	2.44±0.06 ^a	2.32±0.10 ^a	

Means with the different letter (superscripts) within the same row are significantly different at P value < 0.05. DFG = Diabetic Group fed with fried yam (Low-GI); DCG = Diabetic Group fed with normal standard rat feed; DRG = Diabetic Group fed on roasted yam (High-GI).

Table 3: Effects of low- and high-GI diets on lipid profile of experimental diabetic rats (n= 8)

Time (weeks)	Experimental groups		
	Low-GI Diet (DFG)	Control Diet (DCG)	High-GI Diet (DRG)
Total cholesterol (TC) mg/dl			
0	42.00±1.47 ^a	41.20±1.05 ^a	41.23±1.25 ^a
6	60.50± 1.50 ^b	47.22±6.85 ^a	45.40 ±2.60 ^a
Triacylglycerol (TG) mg/dl			
0	21.05±1.30 ^a	20.45±2.00 ^a	20.05±2.30 ^a
6	52.24±1.00 ^b	28.34±2.90 ^a	29.54±2.70 ^a
High density lipoprotein cholesterol (HDL- C) mg/dl			
0	8.85 ±1.85 ^a	8.75 ±1.05 ^a	8.95 ±1.75 ^a
6	8.35±1.50 ^a	10.46±2.34 ^b	11.00±2.80 ^b
Low density lipoprotein cholesterol (LDL- C) mg/dl			
0	28.94±0.94 ^a	28.36±0.80 ^a	28.27±0.96 ^a
6	41.70± 0.80 ^b	31.09±3.93 ^a	28.49±0.74 ^a

Means with the same letter in same row are not significantly different. P value < 0 .05 is significant.
DFG = Low-GI fed group; DCG = control diet fed group; DRG = High-GI fed group

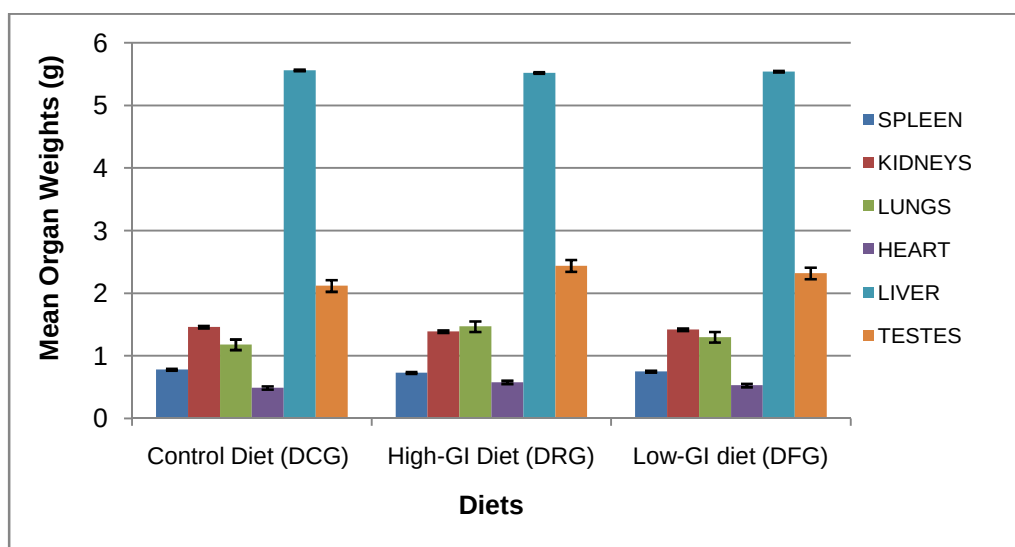


Fig. 1: Effect of test diets on the mean organ weights of experimental rats after 6 weeks.

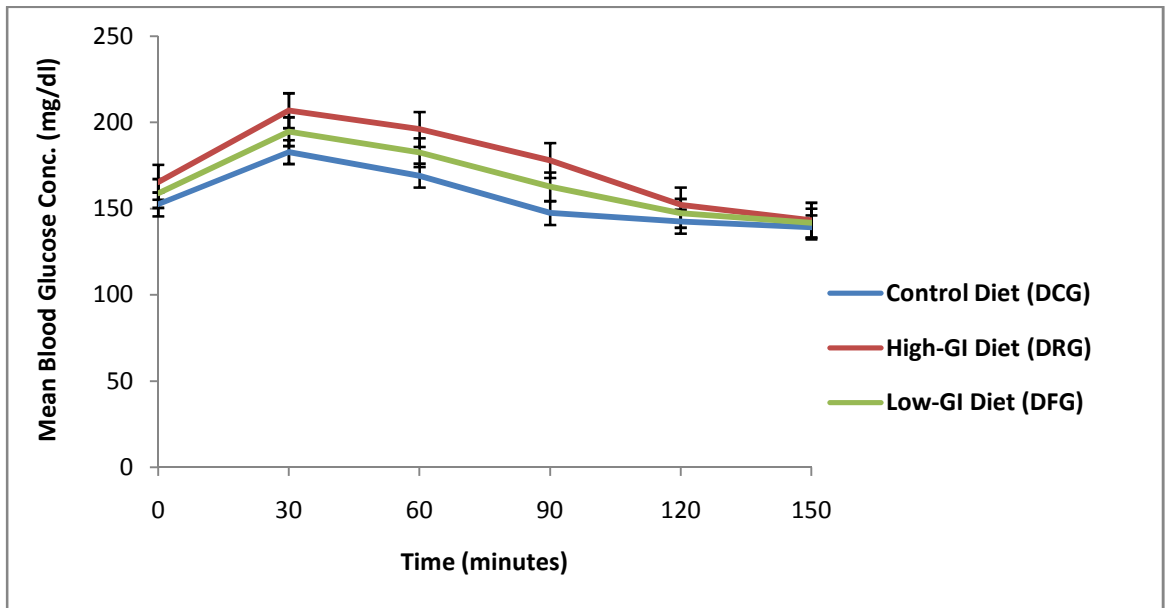


Fig. 2: Mean glycemic tolerance curves to test and control diets

3.4 HISTOLOGICAL ANALYSIS

The photomicrographs of the pancreas (figures 3–5) under high power magnification light microscopic examination were closely examined in experimental rat groups. Pancreatic islet cells of experimental rats in all groups exhibited degenerated islets with degranulation and vacuolization of β -cells. However, the histoarchitecture of the rats fed with low-GI diet showed some visible regeneration of the β cells which was not observed in the rats fed with high-GI diet.

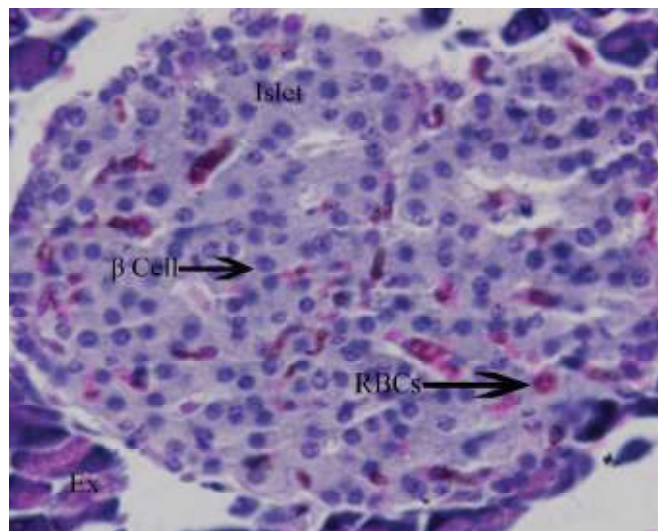


Fig. 3: Normal Photomicrograph of the pancreas from a non-diabetic rat demonstrating normal histoarchitecture. Blood capillaries are surrounded by centroacinar cells containing serous acini (hematoxylin and eosin; original magnification x400). EX: Exocrine pancreas, β cell: beta cells, RBCs: red blood cells.

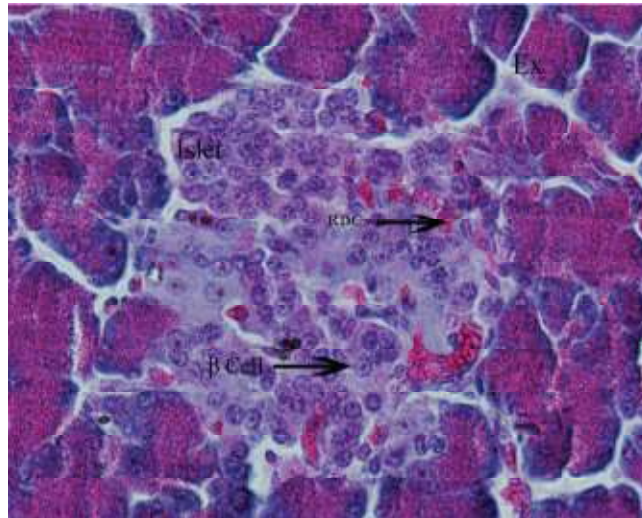


Fig. 4: Photomicrograph of the pancreas from diabetic rat on high-GI diets showing the exocrine region and islets of Langerhans with damaged β cells due to necrosis (degranulation and degeneration) and a decreased number of β cells. (hematoxylin and eosin; original magnification x400) EX: Exocrine pancreas, β cell: beta cells, RBCs: red blood cells.

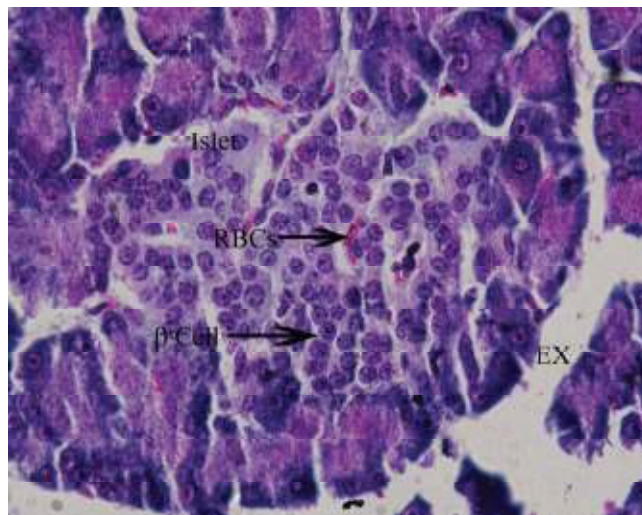


Fig. 5: Photomicrograph of the pancreas from a diabetic rat on low-GI diet (DFG group) showing degenerated serous acini, with also visible regeneration of β cells (hematoxylin and eosin; original magnification x400). EX: Exocrine pancreas, β cell: beta cells, RBCs: red blood cells

4. DISCUSSION

Recently, focus on GI of foods has attracted attention by some nutrition researchers to enable the development of data based nutritional table with GI values of foods (30) that would help in providing information on dietary guidelines and recommendations by the dieticians in the dietary management of diabetics. However, the use of GI in the dietary management of chronic metabolic disorders and food related disorders such as diabetes mellitus, obesity and dyslipidaemia has generated lots of controversy in the world population with regards to use of diets with low glycemic index (GI). As a result, contrasting recommendations have been proffered around the world. Currently there is no universal approach to the optimal dietary treatment for diabetes due to controversy about how useful the glycaemic index (GI) is in diabetic meal planning.

At the commencement of this study, the mean body weights of the rats (250 ± 5.5 g) were almost the same in all groups but at the time of sacrifice, i.e 6 weeks after, mean body weight was significantly increased in all groups with highest increase (24.8%) observed in diabetic rats on High-GI diet (312.33 ± 5.85 g) and lowest increase (9.2%) observed in diabetic rats on Low-GI diet (273.17 ± 1.72 g). Both low- and high-GI diets intake had significant effects ($p < 0.05$) on body weight however, the high-GI diets had more effect on body weights compared to low-GI diets. Some of the rats in the high-GI diet fed group and few in the low-GI diet fed group were observed to be obese (>300 g). This observed increase in the body weight may result in addition to the GI from the amount of food consumed per day by the individual experimental rats. Based on the preliminary study, average food per day served to each group of rats was about 500g ($n=8$). However, rats in high-GI diet group consumed relatively more than those in low-GI group which may be as a result of the different palatability and appetite stimulating of the experimental diets. This observation agrees with the findings of few studies which suggested that total dietary fat intake and overconsumption of high-GI foods are linked to an increased risk of obesity (31) and diabetes (32). Thus dietary precaution should be taken when recommending dietary menu to the diabetic.

No significant change in the mean weights of organs in all the experimental groups was observed. This finding agrees with the report of other study (33). Organ weight measurement is important to access general toxicity because any change in organ weight is a sensitive indicator of toxicity. In theory, organ weight will be affected by the suppression of body weight as described by Marshall (34). In this study, the Low- and the High-GI diets used did not affect the organs' mean weights of the experimental rats.

High-GI diet fed rats displayed quicker and higher GRs to oral glucose challenge while Low-GI diet fed diabetic rats showed decreased and delayed (slower) GRs to oral glucose challenge. This observation agrees with the findings of other studies which reported significant decreased glycemic responses to diets that are high in fiber and oil in comparison to more traditional grain-based feeds (35) and also for grain-based feeds that are top-dressed with oil (36). Reduction in GR to low-GI diet feeding is partly due to the lower starch and sugar content and also likely to be influenced by the presence of oil in a

feed. Fat delays the peak but not the total glucose response for the more fat or acid a carbohydrate food contains, (or, the more fat or acid in the stomach, during digestion) the slower the carbohydrate food is converted to glucose and absorbed into the bloodstream. The presence of fat and/or acid retards the emptying of the stomach (37,38). The above reason explains the reduction in postprandial glycemia and good glycemic tolerance observed with the fried yam (low-GI) in this study. The glycemic response curves in the experimental rats peaked at 30 minutes of oral glucose administration in all groups.

The main rationale for providing a high carbohydrate intake has been the possibility of decreasing dietary fat and cholesterol intake, since diabetics who have their carbohydrate intake restricted consume greater proportion of fat. Such high fat intake has been associated with raised blood lipids and an increased risk of cardiovascular diseases (39). In this study, levels of the constituting parameters of the lipid profile (except for HDL) increased in all groups but much more significant in fried yam fed groups than roasted yam fed groups. HDL level decreased much more in rats fed with fried yam compared with those fed on roasted yam and control groups. However, very high carbohydrate diet has been observed to result in a rise in fasting triglycerides in hyperlipidemic patients, in diabetics and in normal subjects (40). Such carbohydrate-induced lipidemia has been linked to the high insulin levels stimulated by the high carbohydrate diet (41). The low-GI diets (fried yam) used in this study promotes good glycemic response but due to high fat content, it poses some risk of elevated lipid profile as shown in this study. Thus, consumption of excess fatty foods should be avoided while trying to achieve good glycemic control.

Experimental and control diabetic rats photomicrograph exhibited degenerated islets with degranulation and vacuolization of β -cells. However, visible regeneration of some islet β cells was observed in the histoarchitecture of the rats fed with low-GI diet. This finding agrees with the result of Vessby (42) which reported the implication of certain metabolic and diet-related disorders in the disruption of the architecture of the pancreas and the improvement of the tissue with low-GI diets. This observation raises some hope of improving the course of diabetes with good dietary recommendations using low-GI diets

5. CONCLUSION

Low-GI diets poses little risk for obesity and raises hope of improving the course of diabetes with good dietary recommendations. However, excessive consumption of fat in the course of achieving good glycemic profile and control should be avoided especially in hypertensive diabetics because of the long-term cardiovascular risks posed on blood lipid profile. low-glycemic index diets with low fat should be encouraged. Dietary advice regarding the content and type of dietary carbohydrate in the diabetic diet has to be individualized although low-GI foods have proved favorably in the dietary management and control of type 2 diabetes mellitus as shown and supported by this study and other different research findings.

COMPETING INTEREST

No competing interests exist.

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IMPLICATION FOR HEALTH POLICY/PRACTICE/RESEARCH/MEDICAL EDUCATION

This manuscript provides evidence based experimental results for the better understanding of the role of diets in the management and control of diabetes. It also provides basis for dietary recommendation of low glycemic index diets for good glycemic control and profile in diabetics.

AUTHOR'S CONTRIBUTION

The proposal was designed, implemented and manuscript prepared by the corresponding author.

FINANCIAL DISCLOSURE

No financial interest.

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