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Research Article

Effects of High Protein/Low Carbohydrate Rationed Formulated Diet on Glycemic Tolerance and Control, Body Weight and Organ Histomorphometry in Experimental Diabetic Rats

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Abstract

Metabolic response to carbohydrate and protein ingestion plays an important role in health and disease states. This experimentally-controlled designed nutritional study aimed to determine the effects of a high protein/low carbohydrate rationed formulated (HP/LC) diet on body weight, organs (kidneys, liver, heart, lungs, spleen and testes) histomorphometry, glycemic tolerance and control in experimental diabetic and healthy rats. Twenty-four male Wistar rats randomly categorized into four groups (n = 6, each): Healthy control group (HC), Healthy treated group (HT), Diabetic control group (DC) and Diabetic treated group (DT) were used for this study which lasted eight weeks. The animals were fed according to the experimental design with water *ad libitum*. Diabetes was inducted with freshly prepared alloxan monohydrate solution (150 mg/kg bwt, intraperitoneally). Body weights and fasting blood sugar concentrations were measured twice weekly while oral glucose tolerance test was conducted on the last day of the study after which, the organs were extracted for weight assessment and histomorphometric analysis. In this study, the high-protein/low-carbohydrate rationed formulated diet caused significant reduction in mean body weight gain both in treated diabetic (DT: 22.6%; P = .001) and healthy (HT: 5.8%; P = .007) rats compared with their respective controls which recorded significant (P < .05) increase in body weight gain (DC: 12.4%; HC: 11.2%). In DT and HT rats, glycemic tolerance and control improved significantly (DT > HT) while no visible lesions or distortion in organs histoarchitecture observed. In conclusion, high-protein/low-carbohydrate rationed formulated diet reduced body weight gain with improved glycemic tolerance and control without pathologic consequences on organs histoarchitecture in experimental diabetic and healthy rats.

Keywords: Body Weight; Experimental Rats; Formulated-diet; Glycemic Control; Histomorphometry

Introduction

Metabolic response to carbohydrate and protein ingestion plays an important role in health and disease states [1,2]. Uncontrolled weight gain poses increased risks of obesity and diabetes which are chronic non-infectious diseases with related pathogenesis. Diabetic individuals, who have their carbohydrate intake restricted, consume a greater proportion of fat, and such high fat intake has been linked with insulin resistance and poor glycemic control and profile [3]. The aim of diet therapy in diabetes is to achieve normoglycemia and maintain ideal body weight. In addition, dietary advice given in diabetes mellitus primarily aimed at averting symptoms of hyper- and hypoglycemia, eliminate or postpone second-

ary complications and normalize serum insulin concentrations, blood lipid abnormalities and blood pressure elevation. The rate of digestion and absorption of nutrient molecules can be a determinant factor for the metabolic control of some human chronic non-infectious diseases [4]. For this reason, there has been a growing interest in the biological utilization of dietary macromolecules by the human body on the large bowel physiology [5,6].

Dietary proteins of plant or animal origin are large macronutrient biomolecules consisting of one or more long chains of amino acid residues (polypeptides) essential for life support including catalysis of metabolic reactions, DNA replication, response to stimuli and transportation of molecules [7]. Inadequate or excess consumption of carbohydrates and proteins has detrimental effect on health with imposed risks on certain body organ physiology and histoarchitecture [1,8]. To abate or ameliorate such risks, a rationed formulated diet low in carbohydrates and high in protein with mix ratio (C25:P40) as shown in table 1 was designed to determine its effects on body weight, glycemic tolerance and control; organ weight and histomorphometry in diabetic and healthy rats. The rationale of this study is to develop a diet with dietary composition suitable for weight and glycemic control without consequential adverse effects on overall health in diabetic individuals.

Components	Ingredients	Test (HP/LC) Diet (% per 100g of feed)	Control diet (% per 100g of feed)
Carbohydrates	Maize	15	40
	Corn brown	5	10
	Wheat offal	5	5
Fat and oil	Palm kernel cake	14	10
	Groundnut cake	10	5
	Full fat soya	5	5
	Soya bean meal	5.5	5.5
Protein	Fish meal 72%	25	10
	Oyster shell	5	5
	Bone meal	10	4
Vitamins	Growth premix	0.25	0.25
Mineral salt	Salt	0.25	0.25
Additives	(Lysine, Methionine	0.1	0.1
	Total	100%	100%

Table 1: Percentage composition of control and test diets.

Materials and Methods

Experimental animals and diets

Twenty-four male Wistar rats weighing between 150 and 200g were purchased from a disease-free stock at the animal house of the Department of Physiology, Bowen University, Iwo, Osun State, Nigeria. They were fed initially with standard rat chow and water *ad libitum* for the two week acclimatization period in raised stainless steel cages with 6mm^2 mesh floor (to maintain some physical activity) kept in a well-ventilated animal house (at 23°C and a 12 h light and dark cycle). Replaceable numbered blotters papers were placed under each cage to catch the spilled diet that was measured to make up for the daily serving ration. The rats were weighed twice weekly to ensure that no rat outside the initial weight range was used. The entry point weight range was chosen to ensure that the rats used were mature enough to withstand the study protocol which lasted eight weeks. After acclimatization, the animals were randomly categorized into four groups according to the experimental design while their weight measurement continued. This study protocol using experimental animals was conducted in accordance with the National Institutes of Health guide for the care and use of laboratory animals [9] while the Animal Care and Use Review Committee of the institution approved the study.

Control and test diets composition

The composition of the diets used in this study, was based upon the standard diet formulas used to assess weight gain in rodents during commercial feeding studies. The diets were designed and prepared under nutritional guide with the assistance of an animal nutritionist. The mix ratio of protein and carbohydrates contents of the control (normal) and formulated (test) diets is shown in table 1.

Induction of diabetes

Rats in DC and DT groups after 15 hr overnight-fast, 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. By glucose oxidase method using a glucometer (ACCU-CHECK Active Roche, Mannheim Germany), diabetes was confirmed four days after induction by determining the fasting blood glucose (FBG) concentration using blood samples from the tail veins. Rats with FBG level > 150mg/dL were considered diabetic and used for this study since the level of serum glucose deemed to be normal in *Rattus norvegicus* ranges from 50-135mg/dL [10]. Diabetes was allowed to stabilize for 5 days before exposure to experimental diets.

Experimental design

This nutritional study was designed experimentally-controlled. The rats after two weeks acclimatization and induction of diabetes were randomly categorized into four groups of six rats each as follows:

- HC Group: Non-diabetic healthy rats fed with control diet
- HT Group: Non-diabetic healthy rats fed with test (HP/LC) diet
- DC Group: Diabetic rats fed with control diet
- DT Group: Diabetic rats fed with test (HP/LC) diet

Rats were monitored daily for food and water intake while their body weight and blood glucose levels were assessed bi-weekly.

Glycemic tolerance test

This was carried out on the last day of the eighth week of study. Animals in all groups were fasted 15 hours before the test with free access to water. Oral D-glucose load of 2gm kg^{-1} (dissolved in distilled water) was administered by an improvised cannula. Blood samples withdrawn from the tail vein of each animal were used to determine the fasting blood glucose (FBG) concentration at time 0 minute and subsequently at intervals of 30 minutes for 2 hours. The mean FBG concentrations obtained for each group were plotted against time to construct the glycemic tolerance curves.

Extraction and histomorphometric analysis of organs

After 8 weeks of test study, animals in all groups were given light anaesthesia using Ethyl ether in a glass dome after which, were dissected to extract the following organs: liver, heart, kidney, lungs, spleen and testes. Weights of the organs were measured and recorded as a percentage of final body weight together with the absolute values. The organs were grossly assessed for colour, texture, shape, size and visible lesions. Thereafter, tissues from the organs were processed histologically using standard laboratory histotechniques. 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).

Statistical analysis

Data were analyzed using appropriate statistical methods and programs of Microsoft Excel and SPSS version 22. Results (all mean values) are expressed as groups mean ± SEM. Comparisons between groups and the significant difference between the control and the treated groups were analyzed using Student t-test and one way analysis of variance (ANOVA) followed by Duncan's multiple range tests. A (9 x 3) repeated measures ANCOVA was performed on the weight gain data (using the total food intake as a covariable) to determine if there were any diet and time interactions. *P* values of < 0.05 were considered statistically significant.

Results

Effect of HP/LC diet on body weight gain and food intake

The initial and final mean body weights for each group and the total food intake are shown in table 2. At the onset of the experiment, no difference in the mean body weight exists between the control and the treated rats. However, at the end of the study, a significant reduction in the mean body weight (expressed in percentage) occurred in the treated rats (DT: 22.6%, P = .001; HT: 5.8%, P = .007) compared with their respective control (DC: 12.4%; HC: 11.2%). The observed difference reflects the remarkable weight-lowering impact of the HP/LC diet in diabetic rats. With respect to the total food intake for eight weeks, no significant change observed in treated rats compared with their respective control rats. However, repeated measures ANCOVA using the total food intake for each animal as a co-variable revealed that there was a significant effect of diet on weight while there was no interaction of diet and time over the eight weeks. A significant difference in food conversion ratio (food intake/weight gain) was observed between treated and control rats in both diabetic (P = .004) and non-diabetic rats (P = .012). The lower the ratio, the greater the weight-lowering effect of the test diet.

Effect of HP/LC diet on organ weight, gross morphometry and histoarchitecture

The gross assessment of all the extracted organs revealed normal morphological features in terms of shape, size, colour and texture with no visible lesions. The test diet had no effect on the mean weights of the extracted organs both in diabetic and healthy rats (Table 3). The tissues photomicrographs of the extracted organs in both treated healthy and diabetic rats also revealed normohistoarchitectures of the liver, kidney, spleen, lungs, testis and heart comparable with the control as shown in PLATES A - X. The above findings implied that this formulated diet has beneficial organoprotective effect in the experimental rats.

Parameter	Experimental Animal Groups			
	Diabetic		Healthy	
	DC	DT	HC	HT
Final mean body weight (g)	208.67 ± 6.71	140.33 ± 5.39	176.83 ± 2.62	147.83 ± 5.22
Initial mean body weight (g)	185.67 ± 7.18	181.17±3.36	159.00 ± 1.00	157.00 ± 4.07
Weight change (%)	12.39	-22.57**	11.20	-5.75*
Total food intake (g/8 weeks)	1210 ± 30	1226 ± 42	1243 ± 53	1260 ± 65
Food conversion ratio	52.61	-30.02	69.68	-130.00

Table 2: Effect of HP/LC diet on body weight gain (n = 6/group).

Values are expressed in mean ± SEM, *Significant (P < 0.05) when compared with healthy control.

**Significant (P < 0.05) when compared with diabetic control.

HC: Healthy Control; HT: Healthy Treated; DC: Diabetic Control; DT: Diabetic Treated.

Organs	Experimental Animal Groups/Mean Organ weights (g)			
	Diabetic		Diabetic	
	DC	DT	HC	HT
Heart	0.55 ± 0.05	0.50 ± 0.03	0.47 ± 0.02	0.62 ± 0.05
Liver	4.58 ± 0.40	4.67 ± 0.39	4.54 ± 0.47	5.00 ± 0.06
Spleen	0.51 ± 0.04	0.52 ± 0.05	0.50 ± 0.01	0.50 ± 0.06
Lungs	1.01 ± 0.05	1.05 ± 0.06	1.00 ± 0.04	1.06 ± 0.05
Testis	3.00 ± 0.29	2.83 ± 0.22	2.87 ± 0.23	3.05 ± 0.11
Kidney	0.96 ± 0.07	1.07 ± 0.07	0.91 ± 0.10	0.99 ±0.02

Table 3: Effect of HP/LC diet on mean organ weights (g).

Values are expressed in mean ± SEM (n = 6/group).

HC: Healthy Control; HT: Healthy Treated; DC: Diabetic Control; DT: Diabetic Treated.

Plate : PA–X showing tissue photomicrographs of the extracted organs from healthy and diabetic experimental rats.

HC: Healthy Control; HT: Healthy Treated; DC: Diabetic Control; DT: Diabetic Treated.

Effect of HP/LC diet on glycemic tolerance and control

The glycemic tolerance effect of the HP/LC diet was assessed by the incremental areas under the glycemic response curves as depicted in figure 1. The test diet significantly enhanced glycemic tolerance and status in treated (HT and DT) rats compared with their respective control (HC and DC). The mean blood glucose concentration at the end of the study was remarkably reduced in diabetic treated rats (185 mg/dL) compared with the diabetic control (225 mg/dL). This observation suggests the beneficial hypoglycemic effect of the formulated diet.

Figure 1: Effect of HP/LC Diet on Glycemic Tolerance (n = 6/group).

HC: Healthy Control; HT: Healthy Treated; DC: Diabetic Control; DT: Diabetic Treated.

Discussion

The effects of high-protein/low carbohydrate rationed formulated (HP/LC) diet on body and organ weight, glycemic tolerance/control and organ histomorphometry in male diabetic and healthy Wistar rats were determined in this experimentally-controlled designed nutritional study. Findings obtained revealed that HP/LC diet significantly reduced body weight gain and improved glycemic tolerance and control in experimental rats without pathological consequences on organ gross features and histoarchitecture. The above therefore, reflects the beneficial anthropometric, antidiabetic and organoprotective effects of the formulated diet in experimental rats.

Weight reduction in diabetes control is an essential target of interest in the dietary management of diabetes mellitus. This was demonstrated by the test diet in this study which significantly decreased the total body weight without a similar effect on organ weights. In contrast, the diabetic and healthy controls fed with standard diet recorded increased weight gain. This observed decrease in body weight agrees with the findings of our previous studies [1,8]. No significant change in the total food intake observed between the treated (HP/LC diet-fed) and the control rats. Repeated measures ANCOVA using the total food intake for each animal as a co-variable revealed that there was a significant effect of diet on weight while there was no interaction of diet and time over the 8 weeks. A significant difference in food conversion ratio (food intake/weight gain) was observed between treated and control rats. This was greatly influenced by the impact of the test diet on body weight. Previous studies on chronic consumption of high dietary protein without alteration in quantity and quality of carbohydrate component in the diet reported a link with functional and morphological changes in the body organ physiology and histoarchitecture. However, in this study, no such finding was observed which may result from the rationing of protein and carbohydrates contents of the diet as shown in table 1. Rationing protein and carbohydrates content of a dietary menu in normal and optimal proportion under nutritional guide would help in preventing unnecessary metabolic risks and complications in diabetic individuals.

The general goals of weight management are to reduce body weight, maintain a lower body weight over the long term and to prevent further weight gain. Weight gain as well as unfavorable changes in body composition has been reportedly linked with increased risk of medical disorders with decreased survival [11]. This general correlation is particularly useful for consensus data regarding diet-related disorders. Novelli., *et al.* [12] has shown that obesity is associated with adverse consequences of dyslipidemic profile and oxidative stress in the serum of rats. Increase in body weight is also associated with all-cause mortality [13,14]. Therefore, weight management should be the primary nutritional strategy in managing glycemic control in type 2 diabetes and other metabolic disorders [15].

Change in organ weight is a sensitive indicator for assessing general toxicity [16,17]. In theory, organ weight will be affected by the suppression of body weight as described by Michael., *et al.* [18]. In this study, no change in mean weight of kidneys, liver, heart, lungs, spleen and testes of treated rats observed. Gross features of the organs remain normal in shape, size, colour and texture with no visible lesions. These findings contrast the outcome effect of chronic consumption of diets high in protein and carbohydrates.

Glycemic tolerance improved in both healthy and diabetic treated rats in this study. However, the hypoglycemic impact of the diet was more marked in the diabetic rats which may be explained by the apparent high blood glucose level in diabetic condition. This improved glycemic tolerance may result probably from the delayed gastric emptying and decreased postprandial insulin spike. The lowering effect of HP/LC diet on blood glucose in experimental rats in this study is similar to the finding of other study [19] using human subjects which reported decrease in serum blood glucose, HbA_{1c} and insulin levels in untreated type 2 diabetic subjects following consumption of low carbohydrate/high protein diet. While a study [20] attributes the hypoglycemic effect due to low carbohydrates in the diet to the reduced store of glycogen and consequent decrease in glycogenolysis, other studies [21,22] indicate that gluconeogenesis remains constant irrespective of the amount of carbohydrate or gluconeogenic substrate in the diet.

The American Diabetes Association has recommended that diabetes treatment should include lifestyle changes, such as low fat, low carbohydrate and a reduced-calorie diet, to reduce cardiovascular risk factors and increase insulin sensitivity [23,24]. Therefore, lowering carbohydrates content in diets limits both the energy and glucose available to the body which results in increased use of fat oxidation and ultimate weight loss [25].

To develop an appropriate dietary plan menu suitable for weight and glycemic control, this study suggests that dietary menu rich in protein (especially phytoproteins) and low in carbohydrates in appropriate mix ratio should be encouraged. Such menu should be prepared using suitable processing and preparatory methods as studies have shown that processing and preparatory methods have effects on postprandial glycemic response and tolerance [26]. Therefore, it is hoped that in the nearest future, further studies involving the use of our various available local foods, prepared by different methods using appropriate mix ratio for proteins and carbohydrates be carried out to investigate their effects on weight and glycemic control and overall health. Meanwhile, it is advisable that individuals living with diabetes should be encouraged to consume diets low in carbohydrate and moderately rich in quality protein to achieve dietary control of their condition alongside other modalities of management.

Conclusions

This study revealed that a high-protein/low-carbohydrate rationed formulated diet significantly reduced body weight gain with improved glycemic tolerance and control in experimental male diabetic and healthy rats without consequential adverse effects on organ histoarchitecture and gross morphology. Therefore, the above findings suggest that consideration of dietary composition is vital in dietary management of weight and glycemic control. Meanwhile, longer-term studies of such a diet both in humans and experimental animals are suggested to rule out any possible adverse effect, if any.

Statement of Authorship

This work was carried out in collaboration between the authors. Author MMCA designed, supervised, performed the analysis and interpretation of data and wrote the manuscript of the study while author DOA assisted in the provision of essential materials and acquisition of data. Both authors read and approved the final manuscript for submission.

Conflict of Interest

No conflict of interest declared by the authors.

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