

EFFECTS OF PROPORTIONAL HIGH-PROTEIN/LOW-CARBOHYDRATE FORMULATED DIET CONSUMPTION IN DIABETIC RATS: BENEFICIAL IMPACT ON GLYCEMIC AND WEIGHT CONTROL

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ABSTRACT

The metabolic response to nutrient ingestion and the rate of digestion and absorption of nutrient molecules in bowel physiology plays an important role in the metabolic control of some human chronic non-infectious diseases. This experimentally-controlled designed nutritional study which lasted eight weeks aimed to determine the effects of proportional high-protein/low-carbohydrate (HP/LC) formulated diet on glycemic tolerance, glycemic control, body weight, organ weight and organ morphometry in healthy and diabetic adult male Wistar rats. Twenty-four male Wistar rats purchased from a disease-free stock were randomly categorized into four groups ($n = 6$, each) after two weeks acclimatization period in raised stainless steel cages with 6 mm^2 mesh floor and replaceable numbered blotters papers placed under each cage in a well-ventilated animal house. Animal groups include: Healthy control group (HC), Healthy treated group (HT), Diabetic control group (DC) and Diabetic treated group (DT). The animals were fed according to the experimental design with water *ad libitum* for eight weeks. Diabetes was induced with freshly prepared alloxan monohydrate solution (150 mg/kg bw , 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 eighth-week study and subsequently followed by organs extraction after anesthesia for weight and gross assessment. Proportional high-protein/low-carbohydrate formulated diet caused significant reduction in mean body weight of treated diabetic (DT: 22.6%; $P = .001$) and healthy (HT: 5.8%; $P = .007$) rats while the control animals on control diet recorded significant ($P < .05$) increase in body weight gain (DC: 12.4%; HC: 11.2%). Glycemic tolerance and control improved significantly in diabetic treated rats over that of the healthy treated rats. Gross morphometry of the extracted organs (kidneys, liver, heart, lungs, spleen and testes) revealed sustained normal morphological features without any visible lesion. In conclusion, consumption of proportional high-protein/low-carbohydrate formulated diet enhanced body weight reduction and sustained normal organ morphological features with good glycemic tolerance and control in experimental rats, suggesting its dietary potentiality, safety and suitability to ameliorate obesity-related diabetes.

Key words: Body weight, diabetes, formulated-diet, glycemic control, obesity, Wistar rats, alloxan monohydrate



INTRODUCTION

Metabolic response to carbohydrate and protein ingestion plays an important role in health and disease states [1,2]. Diabetes and obesity 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 is primarily aimed at averting symptoms of hyper- and hypoglycemia, eliminate or postpone secondary 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. Previous studies [1,8] revealed that a diet rich in protein with unaltered carbohydrate amounts, imposed risks on certain body organ physiology and histoarchitecture. However, in this present study, we designed a formulated diet with high protein and low carbohydrate contents and determined its effects on glycemic tolerance/control, body weight, and organ weight/morphometry in healthy and diabetic rats with the rationale to assess its safety, potentiality and suitability for glycemic and weight control in obese-related diabetes.

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 6mm² 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 proportional composition of the control (normal) and test (HP/LC) diets for this study, as shown in Table 1, was designed and prepared under nutritional guide with the assistance of an animal nutritionist.

Induction of diabetes

After 15h overnight fast, rats in DC and DT groups 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 an experimentally-controlled design. The rats after 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 0 min and subsequently at intervals of 30 min for 2 h. The mean FBG concentrations obtained for each group were plotted against time to construct the glycemic tolerance curves.

Extraction and gross assessment 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 they were dissected to extract the following organs: liver, heart, kidney, lungs, spleen and testes. The organs were weighed and assessed for colour, texture, shape, size and visible lesions. Organs' weights were measured and recorded as a percentage of final body weight together with the absolute values.



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 \pm 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 AND DISCUSSION

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 was 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 and gross morphometry of organs

The effect of HP/LC diet on the mean weights of the extracted organs is presented in Table 3. No significant change was observed in mean organ weights of diabetic and non-diabetic healthy rats. This suggests the unlikely toxicity of the composed diet. Meanwhile, gross assessment revealed normal morphological features of the extracted organs in terms of shape, size, colour and texture with no visible lesions. These morphological features observed without alteration in organ weight (organo-protective effect) suggests the safety of this formulated diet.

Effect of HP/LC diet on glycemic tolerance

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 HP/LC diet significantly enhanced glycemic tolerance in treated (HT and DT) rats compared with their respective control (HC and DC). These antihyperglycemic and glycemic tolerant effects of the diet were remarkably reflected in the glycemic profiles of the DT and HT rats within the period of study.



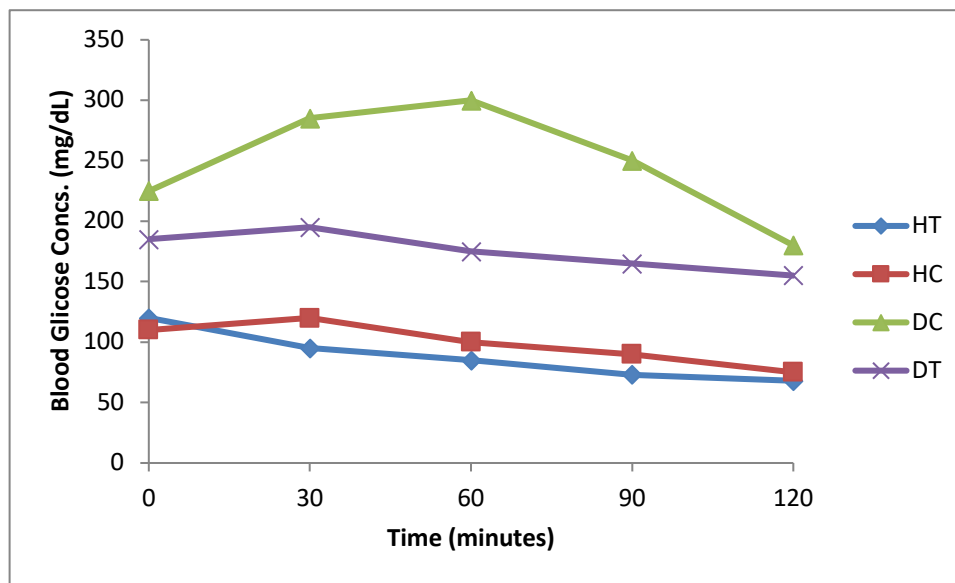


Figure 1: Effect of HP/LC Diet on Glycemic Tolerance (n = 6/group)

HC – Healthy Control; HT – Healthy Treated; DC – Diabetic Control;
DT – Diabetic Treated

The effects of proportional high-protein/low carbohydrate (HP/LC) diet consumption for eight weeks on body weight, glycemic tolerance, organ weights and organ morphometry in diabetic and non-diabetic healthy rats were determined in this experimentally-controlled designed nutritional study to assess its potentiality, safety and suitability for human consumption in dietary control of obesity-related diabetes. Findings obtained revealed that HP/LC diet significantly improved reduction in body weight, enhanced glycemic tolerance and control without alteration in organ weight and gross morphometry in experimental rats. This suggests its safety, potentiality and suitability in dietary control of obesity-related diabetes.

Weight reduction in diabetes control is an essential target of interest in the dietary management of obesity-related 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 was 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 proportionality of protein and carbohydrates mix ratio (in

terms of quality and quantity) in HP/LC diet used as shown in Table 1. This suggests that optimal desired weight reduction can be achieved through the rationing of protein and carbohydrate content mix ratio in diet under nutritional guide and monitoring.

The general goals of weight loss and management are to reduce body weight, maintain a lower body weight over the long term and to prevent further weight gain. The World Health Organization used body mass index (BMI) as the standard for recording obesity statistics since the early 1980s. However, in recent times, other anthropometric parameters including waist circumference have been included to assess body fat distribution. Weight gain as well as unfavorable changes in body composition have been reportedly linked with increased risk and decreased survival in some medical disorders [11]. This general correlation is particularly useful for consensus data regarding obesity or various other conditions. While obesity may be easily estimated from the BMI in rats, Novelli *et al.* [12] has shown that altered values were associated with a dyslipidemic profile and oxidative stress in the serum of rats, which may predict the adverse consequences of obesity.

A high protein/low carbohydrate diet caused no change in the length of rats in this study. Consequently, the observed reduction in weights of the treated rats in this study would automatically affect the BMI beneficially as obtained from the formula for determining this index in rodents [weight (g)/length square (cm²)], thus potentiating the beneficial anti-obesity effect of the HP/LC diet. Elevated BMI is associated with increased cardiometabolic risks. Obesity and overweight substantially increase the risk of morbidity from hypertension as a result of associated health conditions; dyslipidemia; type 2 diabetes; coronary heart disease; stroke; gallbladder disease; osteoarthritis; sleep apnea and respiratory problems; and endometrial, breast, prostate, and colon cancers. Higher body weights are also associated with increases in all-cause mortality [13,14]. Therefore, weight management should be the primary nutritional strategy in managing glycemic control in obesity-related type 2 diabetes [15].

The extracted kidneys, liver, heart, lungs, spleen and testes of the treated and control experimental rats were grossly examined and weighed after eight weeks of exposure to diets for comparison. No change was observed in the mean organ weights, while the gross assessment revealed normal shape, size, colour and texture with no visible lesions. 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]. However, in this study, with the composed HP/LC diet, no change in organ weight was observed, which contrast the aforementioned findings obtained in chronic consumption of high protein diet with disproportionate carbohydrate content.

This diet caused a significant decrease in mean FBG concentrations with improved glycemic tolerance in both healthy and diabetic treated rats in this study. However, the hypoglycemic effect was more marked in the diabetic than non-diabetic healthy rats, which may be explained by the apparent high blood glucose level in diabetic rats and better glucose handling in the healthy non-diabetic rats. This improved glycemic tolerance may result probably from the effect of the diet components mix ratio on bowel physiology, which 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 another study 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 [19]. While a study by Nilsson *et al.* [20] attributed 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] indicated 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 carbohydrate 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 dietary control of obesity-related diabetes, this study suggests that proteins especially phytoproteins obtained from locally available foodstuffs be mixed in proportion with carbohydrates found commonly in staple foods in the ratio of 20:30 using suitable processing methods [26] that favour low glycemic index and subsequently improve glycemic profile. Therefore, it is hoped that in the nearest future, further studies involving the use of various available local foods rich in quality proteins and carbohydrates and prepared by different methods using the above mix ratio be carried out to investigate their effects in ameliorating obesity-related diabetes. Meanwhile, it is advisable that individuals living with diabetes should be encouraged to consume diets low in carbohydrate and moderate in high quality protein to achieve dietary control of blood sugar levels among other management modalities.

CONCLUSION

This study revealed that consumption of proportional high-protein/low-carbohydrate diet improves glycemic control and body weight reduction without alteration in organ weight and morphological features. Therefore, the results suggest that the recommendation of proportionally formulated high-protein/low-carbohydrates diet in diabetic conditions could ameliorate the risk of obesity and uncontrolled hyperglycemia. 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 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



Table 1: Percentage composition of control and test diets

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

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

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



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

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

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

HC – Healthy Control; HT – Healthy Treated; DC – Diabetic Control

DT – Diabetic Treated

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