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Research Article

High Dietary Protein Intake Potentiates Metabolic and Renovascular Risks in Diabetic Rats

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Received: January 15, 2017; **Published:** February 01, 2017

Abstract

Diabetics who resolved in taking protein products due to restriction to consumptions of carbohydrates and fats have been reported to develop increased risk of renal problems and other associated metabolic disorders of public health importance. This experimentally-controlled designed nutritional study was carried out to determine the effect of high dietary protein intake on biochemical variables such as serum blood urea (BU) and total plasma protein (TPP) concentrations, renal histoarchitecture, total body and organ weight in alloxan-induced male diabetic rats. Thirty six male Wistar rats each weighing ≥ 200g were randomly categorized into four experimental groups (n = 9, each): Normal control (NC) fed with standard rat feed; Diabetic control (DC) fed with standard rat feed; Diabetic on high protein diet (DHP) and Normal on high protein diet (NHP). Diabetes was inducted with freshly prepared alloxan monohydrate solution (150 mg/dL, intraperitoneally). Rats were fed for a period of eight weeks according to the experimental design with water ad libitum while their weights were measured twice weekly and recorded. At the end of eight week, blood samples were taken from each rat to measure the TPP and BU concentrations while the animals were sacrificed to extract the kidney for gross analysis and tissue histology. Microsoft Excel and statistical program SPSS version 22 were used to analyze the data. P values < 0.05 were considered significant. High protein intake caused significant (p = 0.0004) reduction (4.13%) in weight gain in DHP rats compared with the DC rats (26.86%) and significant (P = 0.001) increase in TP and BU levels in DHP rats (TP – 11.42 g/dl; BU –97.30 mg/dL) compared with DC rats (TP – 7.48 g/dl; BU – 80. 25 mg/dL). 33.3% mortality in DHP rats was recorded before the end of study with autopsy revealing enlarged kidney with 20% increase in weight compared with DC rats. Renal photomicrographs of the high protein diet-fed rats showed mild (NHP rats) to moderate (DHP rats) cortical interstitial congestion, intratubular protein casts and renal capillaries enlargement. In conclusion, high dietary protein intake potentiates metabolic and renovascular risks in diabetic rats despite its beneficial impact on weight reduction.

Keywords: *Biochemical Variables; Body and Organ Weight; Diabetic Rats; High Protein Diet; Histomorphometry; Kidneys*

Introduction

The use of high-protein diets as alternative to the traditional calories-counting form of dieting has been fraught with controversies due to inconsistent findings and associated risks. Considerable debate has fraught the issues regarding protein intake requirements in normal and disease conditions. Such controversies exist either because research has not provided conclusive answers or those professionals are unaware of the research. People with diabetes mellitus need adequate and accurate information about protein on which to base their food decisions. Generally, proteins are polymers of amino acids, with each amino acid joined by peptide bond. The primary function of

dietary protein is to provide amino acids required for tissue growth and maintenance [1]. Inadequate or excess consumption of proteins is detrimental to health. Thus, optimal amount of proteins (plant and/or animal products) if not contraindicated, is expected to be consumed on daily basis. Diabetics who resolved in taking protein products due to restriction to consumptions of carbohydrates and fats have been reported to develop increased risk of renal problems and other associated metabolic disorders [2-4]. In the United States, approximately 16% of the average adult consumption of calories is from protein, and this has varied little over time [5]. While some studies [6-9] reported that no correlation exists between the amount of dietary proteins ingested and renal diseases when protein intake was in the range of usual dietary intake and rarely exceeded 20% of the total calories, other studies [10] in contrast, reported that an association exists between them when the amount of protein consumed exceeds 20% of the total calories especially in patients with hypertension and elevated HbA1c values. These observations made were significantly attributed to animal proteins than vegetable proteins. To help resolve this controversy and provide a more objective basis to guide dietary recommendations, this experimentally-controlled designed nutritional study was carried out to determine the effect of high dietary protein intake on biochemical variables such as serum blood urea (BU) and total plasma protein (TP) concentrations, renal histomorphometry, total body and organ weight in alloxan-induced male diabetic rats thus assessing the metabolic and renal impact of such diet.

Materials and Methods

Experimental Animals and Design

Thirty six adult male Wistar rats (*Rattus norvegicus*) weighing ≥ 200g were purchased from the disease-free stock of the department of Veterinary Physiology, University of Ibadan, Ibadan, Oyo State, Nigeria. They were fed initially with standard rat chow and water *ad libitum* for the 2 weeks acclimatization in raised stainless steel cages with 6 mm^2 mesh floor (to maintain same physical activity) kept in a well-ventilated animal house (at 23°C and a 12h 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. After acclimatization, the rats were randomly divided into four groups of 9 rats each: Normal control (NC) fed with standard rat feed; Diabetic control (DC) fed with standard rat feed; Diabetic on high protein diet (DHP) and Normal on high protein diet (NHP). Each group had a close entry value of mean body weight (Table 2) and coefficient of variation. All animal weights were measured twice weekly and recorded. This study using experimental animals was conducted in accordance with the internationally accepted principles for laboratory animal use and care [11] with the approval of the Animal Care and Use Review Committee of the Institution.

Test Diets, Composition and Feeding

The composition of the diets in this study was based upon the standard diet formulas used to assess weight gain in rodents during commercial feeding studies. The control (normal ration) and the test (high protein ration) diets were prepared from ingredients purchased from a commercial market in Ibadan metropolis, Oyo State, Nigeria according to the compositions (expressed in percentage) shown in Table 1. The animals were fed according to the experimental design for 8 weeks with water *ad libitum*. Body weight and total food intake of each group of rats were measured and recorded weekly while the food conversion ratio (food intake/weight gain) was calculated.

Ingredients	Normal ration (%)	High protein ration (%)
Maize	30	24
Corn bran	15.5	10
Wheat	15	15
Palm kernel cake	20	20
Groundnut cake	12	12
Soya bean meal	10.5	15.5
Fish meal 72%	3	14.5
Oyster shell	1	1

Bone meal	3	3
Salt	0.25	0.25
Growth premix	0.25	0.25
Total additives	100	100
Lysine	0.1	0.1
Methionine	0.1	0.1
Metabolizableenergy (kcal/kg)	2313.55	2330.25
Crude protein (%)	18.29	25.09

Table 1: *Composition of Control and Test Diets.*

Induction of Diabetes

After 15 hour overnight fast following acclimatization, rats in DC and DHP groups were injected by single intraperitoneal injection of 150 mg/kg body weight of freshly prepared 2% Alloxan monohydrate (Sigma chemicals, USA) dissolved in sterile 0.9% normal saline in a standard volumetric flask strapped with foil to prevent alloxan instability. Diabetes was confirmed 4-7 days later by use of glucometer (On Call Plus Blood Glucose Monitoring System, ACON Laboratories, Inc. San Diego, USA.) and compatible strips. Rats with Fasting Blood Glucose (FBG) level > 150 mg/dl were considered diabetic and used for this study since the level of serum glucose considered to be normal in *rattus norvegicus* ranges from 50 - 135 mg/dL [12]. Diabetes was allowed to stabilize for 5 days before exposure to experimental diets. Fasting blood glucose level of all rats in each experimental group was measured on weekly basis for the eight week study period.

Blood Collections and Biochemical Assays

The blood samples were collected from the tail veins of the rats 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. The sera obtained were used for the biochemical assay. Serum urea and total protein concentrations were determined by Colorimetric and Burette methods respectively while both values were read using spectrophotometer.

Kidney Extraction, Measurement and Histological Analysis

At the end of the study, animals in all groups were anesthetized using Ethyl Ether in a glass dome and then dissected to extract the kidneys which were rinsed and weighed. Tissues were histologically processed using standard laboratory histotechniques. Extracted kidneys were placed in 10% formalin solution for a day. 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 was analyzed using appropriate statistical methods and program of Microsoft Excel and SPSS v. 22. Results (all mean values) are expressed as group Mean ± SEM (Standard Error of Mean). Comparisons between groups and the significant difference between the control and the treated groups were analyzed using one way analysis of variance (ANOVA) followed by Duncan's multiple range tests. P values of < 0.05 were considered statistically significant.

Results

Effect of High Dietary Protein Intake on Body and Organ weights

Body Weight

The effect of high dietary protein intake on body and organ (kidney) weights is presented in Tables 2 and 3 respectively. Overall percentage weight gain after 8 weeks was significantly reduced (P = 0.001) in high protein diet-fed rats (DHP- 4.13%; NHP- 1.59%) compared with their respective controls (DC- 26.86%; NC- 32.91%) as suggested by standard ANOVA. High protein diets have similar effects on weight gain in both diabetic and non-diabetic rats but much more in diabetic rats. No significant difference observed in total food intake (P > 0.05) in High protein-fed rats compared with the control rats. Repeated measures ANCOVA using the total food intake for each animal as a covariable, revealed that there was a significant effect of diet on weight gain while there was no interaction of diet and time over the 8-week period. Mean weights at 8 weeks were significantly lower (p < 0.05) in DHP and NHP rats compared with their control rats while a significant difference (p < 0.05) was observed in the food conversion ratio (food intake/weight gain) between high protein-fed rats and the control rats.

Parameters	Experimental Animal Categories			
	Non-diabetic		Diabetic	
	NC	NHP	DC	DHP
Initial Weight (g)	200.51 ± 2.34	200.83 ± 2.34	200.55 ± 2.34	200.85 ± 2.34
Final Weight (g)	263.12 ± 2.48	197.66 ± 1.63	254.42 ± 183	192.56 ± 1.03
Weight change (%)	32.91	1.59[a]	26.86	4.13*

Table 2: *Effect of High Protein Diet Intake on Body Weight (n = 6).*
*Values are expressed in mean ± SEM, * Significant (p < 0.05) when compared with diabetic control- DC.*
aSignificant when compared with normal control- NC.

Organ (Kidney) Weight

Table 3 shows the effect of high protein intake on kidney weight during the eight week study period.

Parameter	Experimental Animal Categories			
	Non-diabetic		Diabetic	
	NC	NHP	DC	DHP
Kidney weight (g)	1.43 ± 0.01	1.50 ± 0.02[a]	1.46 ± 0.00	1.75 ± 0.02[b]

Table 3: *Effect of High Protein Diet Intake on Kidney Weight (n = 6).*
Values are expressed in mean ± SEM, b Significant (p < 0.05) when compared with DC, NC and NHP.
aInsignificant (p > 0.05) when compared with NC and DC.

High protein diet caused a significant (p < 0.05) increase (21.1 ± 2.0%) in the weight of the kidneys in DHP rats compared with the diabetic control rats whiles slight increase (4.9 ± 1.2%) observed in NHP rats kidneys was insignificant when compared with the normal control. The difference in values between NHP and DHP rats was significant (p < 0.05). Result of the autopsy of demised rats (33.3%) in DHP group also revealed kidney enlargement similar to those extracted at the end of study in the same group.

Effect of High Dietary Protein Intake on Biochemical Parameters

Serum Urea and Total Protein Concentrations

Effect of high dietary protein on serum urea and total protein concentrations is expressed in Table 4. A significant ($p < 0.05$) percentage increase in urea and total protein levels was observed in DHP and NHP rats compared with their respective control while the difference in urea and total protein concentrations between NHP and DHP rats was comparably significant ($p < 0.05$).

Parameters	Experimental Animal Categories			
	Non-diabetic		Diabetic	
	NC	NHP	DC	DHP
Serum Urea Concentrations (mg/dL)				
Initial week	39.42 ± 1.00	40.42 ±1.46	40.42 ± 0.56	40.02 ± 0.64
8th week	40.12± 1.25	45.05± 1.05	41.28± 1.26	57.38 ± 3.12
% Change	1.78	11.46*	2.13	18.39**
Total Protein Concentrations (g/dL)				
Initial week	6.56 ± 1.02	6.52 ± 0.11	6.64 ± 0.14	6.62 ± 0.24
8th week	6.84 ± 1.12	7.56 ± 2.13	7.21 ± 0.56	11.52 ± 1.22
% Change	4.88	15.95*	8.58	74.02**

Table 4: *Effect of High Protein Diet Intake on Serum Urea and Total Protein Concentrations. Values are expressed in mean ± SEM. ** Significant ($p < 0.05$) when compared with DC, NC and NHP. * Significant when compared with NC and DC.*

Effect of High Dietary Protein Intake on Renal Histoarchitecture

Under high power magnification (x 400) light microscopic examination, the photomicrographs (H and E stained) of the sectioned kidneys were closely examined. Photomicrographs of the NC and DC rats showed normohistoarchitecture of the kidneys while that of NHP and DHP rats displayed mild and moderate cortical interstitial congestion with tubular protein casts and peritubular vascular congestions respectively as shown in Figure 1 to 3 below.

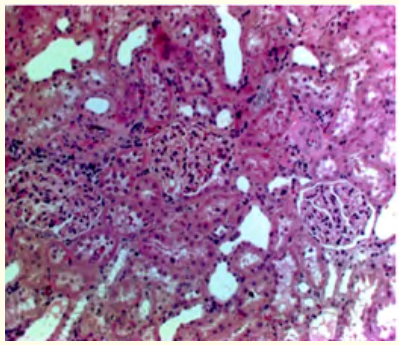

Figure 1: *Renal photomicrograph of NC and DC rats showing normal histoarchitecture.*

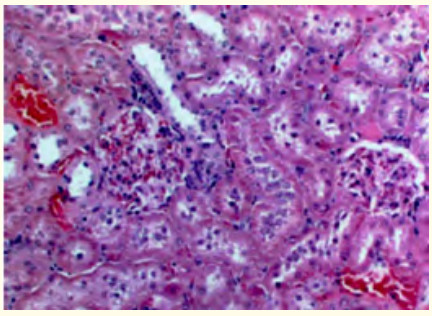

Figure 2: *Renal photomicrograph of NHP rats showing mild cortical interstitial and glomerular congestions with tubular protein casts and peritubular vascular congestion.*

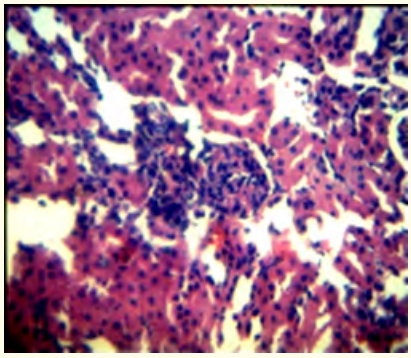

Figure 3: *Renal photomicrograph of DHP rats showing moderate cortical interstitial and glomerular congestions with tubular protein casts and peritubular vascular congestion.*

Discussion

This experimentally-controlled nutritional study evaluated the impact of high dietary protein intake on biochemical variables, renal histomorphometry, total body and organ weight in alloxan-induced male diabetic and non-diabetic rats thus, comparing results obtained between groups in order to assess the effect of high dietary protein in healthy and diseased states. The findings obtained revealed that high dietary protein intake significantly reduced weight gain, increased serum urea and total protein concentrations and caused histopathological changes in renal tissues with record of mortality in diabetic (DHP) rats. Results and findings in healthy rats were lower and milder with no record of mortality.

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 effect of high protein diet on body and kidney weight in healthy and diabetic rats was assessed. A significant reduction in mean body weight and an increase in renal size and weight were observed in DHP and NHP rats compared with their respective controls. Autopsy findings of the demised diabetic rats revealed similar changes in renal size and weight. The use of high dietary protein to reduce weight has long been employed [13]. However, non-persistent outcome resulting from such intake has cast doubt on the use of such diet in chronic reduction of body weight without adverse effect on overall health. Recently, diabetics who have restrictions to consumptions of carbohydrates and fats with resolution in increasing their protein intake, have been reported [14] to have increased risk of renal problems and other associated metabolic disorders of public health importance. Some studies [15-17] suggested

that diet rich in plant proteins has better metabolic and renal outcome than those rich in animal proteins. However, the amount of protein in diet may have specific effects. From broader dietary perspective, the choice of protein will inevitably influence other dietary components and may be a critical determinant for the health outcome. In this study, both plant and animal proteins were combined in proportion to formulate test diets used to assess the renal and metabolic impacts. The alteration in renal size and weight observed in this study calls for valid concern and caution as it may be since studies [18,19] have shown that 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 Michael [20] and observed in this study also. Recommendation of dietary protein therefore, should be considered with dietary guidelines that ensures optimal non-lethal ration for age, sex and race in healthy and diseased conditions. Most government and health-related agencies advised on 10 – 20% contribution of proteins to dietary energy. Exceeding this recommended value may promote negative health impact on individuals especially those with potential risk factors for renal and metabolic diseases.

Effect of high dietary protein on biochemical analytes was examined in this study which revealed asignificant increase in Blood Urea and Total Protein concentrations in normal and diabetic rats. The values obtained for diabetic rats were significantly higher than those of the normal rats. This observed increase may result from the increased metabolism of protein and associated nephron dysfunction of over 60% as reported by the study of Menezes., *et al*. [21] which stated that serum urea generally increases when there is loss of over 60% of nephron function. The dietary protein can modulate renal function when the amount consumed exceeds the recommended levels. The duration, type and amount of protein consumed are necessarily to be considered when determining its implication on health. Chronic consumption of high protein diet has been reported [22] to have renal impact due to hyperfiltration caused by increased glomerular pressure. Elevated urea level may result from increased amino acid oxidation which might impose strain on renal function thus resulting in nephropathy that may explain the mortality recorded in this study among the diabetic rats fed on high protein diet. Protein toxicity consequences resulting from chronic consumption of high protein diet constitutes health hazards and economic burden if not properly controlled. Therefore, diabetics who resolved in chronic consumption of protein products due to restriction to carbohydrates and fats should do so with moderations.

The photomicrographs of the kidney sections highlighted further the effect of high protein diet at tissue level. Pathological changes at tissue level may result from acute or chronic damage to organ from any etiological cause. Similarly, histological analysis of target organs monitoring for pathological changes enhances screening assessment of quality of product prior to recommendation for human consumption. The kidney sections under high power magnification (x 400) light microscope were closely examined. Photomicrographs of the NC and DC rats showed normohistoarchitecture of the kidneys while that of NHP and DHP rats displayed mild and moderate cortical interstitial congestion with tubular protein casts and peritubular vascular congestions respectively. These findings observed were similar to the results of other study [23] that observed cortico-vascular and glomerular changes in relation to high dietary protein. Meanwhile, the presence of mild histological changes observed in healthy rats on high protein diet in this study calls for caution. The arguments and controversies surrounding the belief that healthy individuals without preexisting risk for renal problems are free from negative renal impact of chronic high protein consumption should be revisited as the observed alteration in renal size and weight in normal rats in this study, in association with mild histoarchitectural changes and elevated urea concentrations signal an imminent protein toxicity which might with time, culminate in renal and other metabolic problems with associated increased risk of morbidity and mortality.

Conclusion

This eight week nutritional study demonstrated the health impact of high dietary protein in diabetic and healthy rats. It revealed that caution should be taken not to exceed dietary guidelines and recommendations in both healthy and diabetic individuals due to observed uremia, hyperproteinemia and altered renal histomorphometry resulting from protein toxicity. In conclusion, high dietary protein intake potentiates metabolic and renovascular risks with all-cause mortality in diabetic rats.

Conflict of Interest

No conflict of interest exists.

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