Tropical Journal of Nephrology Vol.2 No2 December, 2007 99-105

The Effects of Low and Normal Protein Soyabean Based Diets on Chronic Renal Insufficiency in Rats

Fadupin GT[1], Keshiro OO[2] and Arije A[3]

[1-2] *Department of Human Nutrition, College of Medicine, University of Ibadan, Nigeria and*
[3]*Department of Medicine, University College Hospital, University of Ibadan, Nigeria*

ABSTRACT

The main objective of this study is to determine the effects of two different levels of soyabean-based protein intake on chronic renal insufficiency in Sprague-Dawley strain albino rats. An experiment was conducted comparing the effects of the consumption, for six months, of soyabeans at 21 % protein (normal protein, or NP) and 14% protein (low protein, or LP) on the food intake, body weight, urinary output, proteinuria, blood urea and serum creatinine levels of 12 nephrectomized rats (NR) and 12 normal rats used as control (CT). Statistical comparison of the results shows a reduction in food intake as well as less body-weight gain in the NR compared to the control (P=0.001). Also, the NR had significant reduction in 24-hour urinary output, elevated proteinuria, blood urea and serum creatinine than the control. The food intake of the nephrectomized rats on the normal protein diet (NR–NP) as well as their gain in body weight were significantly lower than the nephrectomized rats on the low-protein diet (P=0.001). Blood urea and serum creatinine were also higher significantly in the NR-NP compared with the corresponding values in the NR-LP ($P < 0.001$). The study shows that controlled dietary restrictions using low-protein soyabeans diet can be effective in ameliorating the effects of renal insufficiency without inducing malnutrition, as a low soyabean protein diet has maintained better food intake, body weight, blood urea and serum creatinine than normal soyabean protein diet in the nephrectomized rats.

Keywords: *Low soyabean based protein diet, renal failure, rats.*

INTRODUCTION

One of the disorders in which diet can be applied as a therapy is chronic renal failure (CRF)[I]. This is a kidney malfunction caused by diseases that affect the parenchyma over several months or years, and generally progressively worsens until there is irreversible decline in glomerular filtration rate (GFR). Once a critical level of renal functional deterioration is reached and CRF is established, it usually progresses inevitably to end-stage renal failure (ESRF), even if the initiating event or condition is resolved or eradicated [2].

Chronic renal failure is a disease that affects the young and the old globally. Clinical evidences and studies in animals reveal that proper dietary treatment involving restriction of protein is an integral part of the management of CRF. Proper dietary management provides a temporary stabilization or occasionally exhibits improvement in renal function for months and, in some cases, years [3-6].

Recently, both animal and human trials have shown that, not only protein, restriction, but also modifications in the type of protein consumed, has favourable effects on renal health, because not all proteins are equal in their biological value. Also, researchers to date still argue whether low-protein diets will induce malnutrition in renal insufficiency [1, 4, 5]. Documented evidence of Anderson and Wardle reports [3, 7] indicate that soybean protein may specifically benefit renal patient [5]. However, animal and human researches targeting the effects of Soya beans on renal insufficiency are still limited. [3, 8] Thus, there is a need to further evaluate the effectiveness of Soya bean in CRF.

Corresponding author: **Dr Grace T. Fadupin**

Department of Human Nutrition, College of Medicine, University of Ibadan, Ibadan, Nigeria

Certain experimental manipulations and long-term study are difficult or rather impossible in human subjects. Hence, animal experiments are often carried out for results that are projectable to humans in clinical situations. Such experiments might produce similar biochemical scenario in humans as created in laboratory animals. As a preliminary step to ascertain the beneficial dietary levels of Soya bean-based protein diet on chronic renal insufficiency, an animal experimentation was carried out. In the study, Sprague-Dawley Strain albino rats were used to determine which of the two levels (14% or 21%) of soya bean-based protein diet would be beneficial to food intake, body weight gain, urinary output, proteinuria, blood urea and serum creatinine of nephretomized rats used as equivalents of animals with CRF.

MATERIALS AND METHODS

Animals and experimental design

Twenty four albino rats, raised on standard chow and weighing between 170 and192g, were randomly distributed into four groups I, II, III and IV. Each group consisted of six albino rats with a mean weight of approximately 183.5 ± 2.13g.

Twelve of the albino rats (Groups I and III), after being anaesthetized, underwent 2/3 nephrectomy performed by veterinary surgeons. The right kidney was removed by excision, followed by ablation and cauterization of one-third (at the upper pole) of the left kidney. Rats in groups II and IV were not subjected to surgery and therefore had the two kidneys intact, served as control. All the nephrectomized rats received ampicillin injection immediately after surgery as a prophylaxis against infection.

Diet design

On the day of surgery, the rats for nephrectomy, (groups I and III) had nothing to eat or drink, while the control groups (II and IV) were given tap water only. After surgery, all the rats were fed on only maize starch, '*ogi*' and tap water for the next six days. This procedure was adopted to stabilize the nephrectomized rats from post-operations shock, and to allow their residual kidneys to rest.

On the seventh day, all the animals were housed in individual metabolic bottom-screened cages according to their groups (I, II, III, and IV). The animals continued to feed on maize starch and tap water until the eighth day after operation.

On the eighth day, the animals were assigned to two dietary regimens namely low-protein (LP) and normal-protein (NP) which contained 14g proteins and 21 g protein in 100g soyabean diets respectively. Group I (nephrectomized) and II (control) had 14% soyabean protein diet (LP diet) while groups III (nephrectomized) and IV (control) had 21 % soyabean protein diet (NP diet). The 14% protein (LP) diet provided the minimal protein requirement while 21 % protein (NP) diet is a normal-protein diet for normal rats [8]. The protein was provided by hulled, cooked and sun-dried soybean flour (a plant protein of high biological value) which Anderson, *et al* [3] suggested may specifically benefit renal patients. The other calories were supplied by maize starch, '*ogi*' and sucrose and as shown in table I. The diets, in powdered form, were isocaloric and the protein provided 14g and 21g protein in 100g of the diets respectively. Vitamins, minerals and fiber content were identical in the diets, and their compositions conform to the diet proposed by the Committee on Laboratory Diet, Institute of Laboratory Animal Resources, Assembly of Life Science, [9]. The daily food intake of the animals was determined by filling their feeding cup, but not up to the brim, to prevent or minimize spillage. Any leftover was deducted from the original quantity. All the animals were allowed to eat and drink water ad libitum throughout the six months of the experiment.

Three weeks after operation, the initial assessments of food intake, body weight, tail length, urinary excretion and urinary protein of each group of the experimental rats were determined as the mean values for each group of animals. The weights and tail lengths of the rats were measured to an accuracy of 0.1g and 0.1mm respectively after anaesthetizing them lightly to make them relax and sleep. At the end of the sixth month, the initial assessments were repeated and blood sample was obtained by ocular artery cannulation to evaluate their serum creatinine, blood urea, serum phosphate, and serum albumin using the SLIM- Auto Analyzer. The packed cell volume of the rats was also determined.

STATISTICAL ANALYSIS

The SPSS package version 10.0 was used to summarise the data as, means, and standard deviation. The data was also analysed by 2 ways analysis of variance to assess the main effects of LP vs. NP on

the food intake, anthropometric indices and urinary assessment of the rats.

For biochemical indices a pair-wise comparison, using the student t-test was used to identify which of the diets had a statistically significant effect more than the others.

RESULTS

Food intake and anthropometric variables

The initial mean daily food intakes, body weight and tail length of the groups of rats (NR-LP and CT-LP) as shown in table 2, were similar. Six months after the experiment began, the food intake of the NR-LP (18.3± 0.32g/day) was significantly less compared to the control (CT-LP) rats whose food intake had increased to 20.1±0.41g per day (P>0.05).

rats also varied with the different level of protein intake as the final food intake of the NR-NP was significantly less than that of the NR-LP (P<0.001). However, the actual daily protein intake of the NR-NP was still 0.8g more than the protein intake of the NR-LP.

Also, after six months the mean body-weight gain of the NR-LP and NR-NP diet was significantly lower than that of the controls (P<0.05), while the mean body weight gain (85.4 ± 3.33g) of the NR-LP was significantly more than that (76.9±4.34g) of the NR-NP (P<0.05).

It was also observed at the end of the experiment that the nephrectomized rats had shorter tail length gain than the controls (P<0.05). Shorter

TABLE 1: Composition of the diet

INGREDIENT	14% Protein *(14 g p/100g food)*	21% Protein *(21 g p/100g food)*
Soya beans (g)	41.5	62.3
Corn flour *'ogi'* (g)	43.0	22.2
Sucrose (g)	5.0	5.0
Cellulose (g)	5.0	5.0
Vitamin mix (g)	0.5	0.5
Mineral mix (g)	5.0	5.0

Conform with the diet proposed by the Committee on Laboratory Diet: Institute of Laboratory Animal Resources, Assembly of Life Science. National Research Council Report; Common Wealth Bureau of Nutrition (1979).

Similarly, among the rats on the normal-protein soyabean diet, the final food intake of the NR-NP (16.2±2.13g/day) was significantly less than that of the CT-NP which ate 20.4±0.31g/day (P<0.05). The nephrectomized rats took less food than the controls and less than their initial food intake. The extent of reduction in food intake among the nephrectomized tail length was also observed among the NR-NP than among the NR-LP group (P<0.05). The low-Protein diet maintained better food intake, body-weight and tail-length gain among the nephrectomized rats than the normal-protein diet.

TABLE 2: Changes in food intake and anthropometric indices of the experimental rats placed on the different protein diets

Food Intake and Antropometric *Variables*	Types of Diet NR-LP *(n = 6)*	CT-LP *(n = 6)*	NR-NP *(n = 6)*	CT NP *(n = 6)*
1.Food intake (g)/24 hours				
(a) Initial	19.7 ± 0.21	19.9 ± 0.32	19.8 ± 0.92	19.6 ± 0.4
(b) Final	18.3 ± 0.32	20.1 ± 0.41	16.2 ± 2.13	20.4 ± 0.31
Mean difference	-1.4 ± 0.09	0.4 ± 0.14	-3.6 ± 0.57	0.78 ± 0.14
2. Body weight (g)				
(a) Initial	183.8 ± 4.03	183.3 ± 3.94	184.6 ± 3.71	183.2 ± 4.32
(b) Final	269.2 ±10.11	290.3 ± 5.10	261.5 ± 13.45	299.2 ± 15.91
Mean difference	85.4 ± 3.33	107.08 ± 0.54	76.9 ± 4.34	116.00 ± 4.87
3. Tail length (mm)				
(a) Initial	147.6 ± 0.82	148.0 ± 2.33	149.5 ± 6.32	148.2 ± 3.83
(b) Final	198.7 ± 5.71	203.5 ± 3.31	179.5 ± 41.11	209.6 ± 15.81
Mean difference	51.10 ± 2.17	55.4 ± 0.92	50.03 ± 5.89	61.40 ± 5.53

n = Number of Rats;
NR – LP = Nephrectomized rats on low-protein diet
CT – LP = Control rats on low-protein diet
NR - NP = Nephrectomized rats on normal protein diet
CT – NP = Control rats on normal protein diet.
** Values are based on 95% confidence interval.*

TABLE 3: Twenty-four (24) hour urinary parameters of the experimental rats

VARIABLES	NR-LP *(n = 6)*	CT-LP *(n = 6)*	NR-NP *(n = 6)*	CT-NP *(n = 6)*
24-Hour Urinary Excretion (ml)				
(a) Initial	7.2 ± 0.21	7.2 ± 0.33	7.3 ± 0.23	7.3 ± 4.07
(b) Final	5.1 ± 0.43	7.1 ± 0.41	3.9 ± 0.32	8.04 ± 0.25
Mean difference	-2.1 ± 0.23	0.52 ± 0.13	-3.40 ± 0.13	0.67 ± 0.16
24-hour Urinary Protein Excretion (mg/dl)				
(a) Initial	6.3 ± 0.13	6.3 ± 0.14	6.8 ± 0.3	7.0 ± 0.92
(b) Final	18.5 ± 0.62	7.4 ± 0.71	40.7 ± 4.33	7.9 ± 2.16
Mean difference	12.16 ± 0.23	1.13 ± 0.28	34.35 ± 1.67	2.88 ± 0.75

n = Number of Rats
NR – LP = Nephrectomized rat on low-protein diet
CT – LP = Control rats on low-protein diet
NR - NP = Nephrectomized rats on normal protein diet
CT – NP = Control rats on normal protein diet.

** Values are based on 95% confidence interval.*

Urinary excretion of the rats

It is shown in table 3, that the initial 24-hour urine excretion and proteinuria by the four groups of rats was similar. At the end of the six months study, however, the daily urine excretion reduced and proteinuria increased in the nephrectomized rats. These symptoms were more severe in the NR-NP.

As shown in table 4, significantly higher serum creatinine and blood urea levels were observed in the nephrectomized rats on either of the two diets compared to the controls (P<0.05). Serum creatinine and blood urea were also significantly elevated in the NR-NP, than in the NR-LP diet (P<0.05). Normal-protein soyabean diet and reduction in renal mass were associated with significantly higher serum creatinine and blood urea in the experimental rats. This indicates that the mean rate of decline in renal function was greater in the nephrectomized rats that were placed on the normal-protein diet.

However, the PCV of the NR-NP group (28.8±3.92%) was significantly lower than that of the NR-LP (33.8±0.41%) (P <0.05). Only one of the nephrectomized rats put on normal-protein died during the period of study (fifth month).

DISCUSSION

Studies in Nigeria on the dietary management of chronic renal insufficiency, using experimental animals are limited. However, such studies elsewhere in the world have in recent years produced encouraging results, not only on the beneficial effects of protein restriction, but also in the modification of types of protein prescribed for renal health [10-13].

Like previous works (6, 8, 11, 12), this study used subtotal nephrectomy (2/3 nephrectomy) to induce chronic renal insufficiency in albino rats. The study also determined the chronic effects of two protein levels, using soyabean (a vegetable protein

TABLE 4: The final biochemical indices of the animal

Indices	NR-LP (n=6)	CT-LP (n=6)	NR-NP (n=6)	CT-MP (n=6)
Serum Creatinine (mg/dl)	2.0 ± 0.31	0.8 ± 0.11	3.3 ± 0.42	0.8 ± 0.14
Blood Urea (mg/dl)	47.1 ± 0.53	22.2 ± 0.32	87.3 ± 0.31	23.7 ± 0.72
Serum Phosphate	6.6 ± 0.10	5.3 ± 0.61	8.4 ± 0.60	6.8 ± 0.7
Serum Albumin (g/dl)	3.6 ± 0.12	4.0 ± 0.31	3.0 ± 0.43	4.7 ± 0.81
Packed Cell Volume (%)	33.8 ± 0.41	34.2 ± 0.53	28.8 ± 3.92	37.8 ± 0.52

n = Number of Rats
NR – LP = Nephrectomized rat on low-protein diet
CT – LP = Control rats on low-protein diet
NR - NP = Nephrectomized rats on normal protein diet
CT – NP = Control rats on normal protein diet.
** Values are based on 95% confidence interval.*

Serum phosphate concentration was significantly higher in NR-NP compared with the serum phosphate value in the NR-LP (P<0.05). Similarly albumin concentration was greater in the control than in the nephrectomized rats (P<0.05). While the serum albumin of NR-LP was significantly higher than the NR-NP (P <0.05).

No significant difference was observed in the PCV of the NR-LP and CT-LP (P>0.05).

of high biological value) based diet, in the experimental albino rats with reduced and normal functioning renal mass.

In previous studies such as [6, 8, 11, 12] refined foods, such as casein, were used to determine the effects of the intake of very low protein (7%), low-protein (14%), or high protein (27%), in chronic renal insufficiency in rats with reduced and normal renal mass. In the present animal experiment,

however, hulled, cooked and sun-dried soy bean based protein which has been reported to offer health advantage [3] was used to compare the effects of low-protein (14%) and normal-protein (21%) diets on the food intake, anthropometric and some biochemical indices of both nephrectomized and normal experimental rats.

During the fifth month of the study, only one of the nephrectomized rats put on the normal soybean protein diet died. No other animal died throughout the six months of the study. This mortality rate is low compared with the 25% mortality rate reported by Hostetter *et al* (12) in their four months study on similar nephrectomized rats, where the effects of very high 27% of protein) and very low protein 7% protein diet using casein as the test proteins were compared. In general, at the end of the six months feeding trial of this study, all the nephrectomized rats on both diets had marked reduction in food intake compared to their initial food intake. The nephrectomized rats' food intake, body weight and tail-length gain were significantly less, compared to the normal rats on the same level of diet ($P<0.05$). This shows that renal insufficiency has some adverse effects on food intake, bodyweight and increase in tail length (growth) of the rats. Also, compared to the nephrectomized rats on the low-protein diet, the nephrectomized rats on the normal-protein diet had a significant reduction in food intake, and also in body-weight gain ($P<0.05$). This observation shows the beneficial effects of low-protein diet even when soyabean, a vegetable protein based diet of high biological value is taken [3]. This study shows that a chronic reduction in functioning renal mass induces anorexia, weight loss and growth retardation, but these effects are more severe when rich, rather than low protein is fed in chronic renal insufficiency. Generally, normal protein diet, as against low-protein diet, is known to improve food intake and maintain normal body weight. However, the reverse obtains in renal insufficiency, even when protein of high biological value is fed. [1, 3, 7, 14].

Also, the nephrectomized rats after six months, (whatever their diets) were observed to have a significant reduction in urine output, elevated proteinuria, blood urea appearance, serum creatinine and phosphate concentrations compared with their initial values, and also when compared with the values of the controls ($P<0.05$). This shows the progressive nature of CRF whatever the diet [1, 6]. However, compared with the nephrectomized rats put on low-protein diet, the nephrectomized rats put on normal-protein diet had significantly lower 24-hour urine excretion, higher proteinuria, blood urea appearance, serum creatinine and phosphate concentrations and also had lower serum albumin and PCV value ($P<0.05$). Increase in proteinuria is an indicator of greater prevalence of sclerotic glomeruli, causing progressive damage to the remnant functioning renal mass, while oliguria, increases in serum creatinine and blood urea appearance are indicators of severity of chronic renal insufficiency [6, 14]. It then follows that higher-protein rather than lower-protein diet even from soybean, will aggravate the uremic toxicity of chronic renal insufficiency. Unlike the observations made on the nephrectomized rats, the final 24-hour urine excretion, urinary protein, serum creatinine, serum urea or albumin were not significantly different in the normal rats put on the low or the normal-protein diets. However, serum phosphate and PCV were significantly higher in the control on the normal-protein compared with the control on the low-protein diet. The nephrectomized rats on the low-protein diet also had better PCV level than their counterpart on normal-protein diet ($P<0.05$).

These findings document the better nutritional status of the nephrectomized rats put on low-protein diet and the adverse effect of higher level of protein diet in renal insufficiency [1, 11].

Thus, the findings of this study establish the progressive nature of chronic renal insufficiency and its adverse effects, such as anorexia, weight loss, elevated proteinuria, blood urea appearance and higher serum creatinine and serum phosphate concentration. These negative responses were more severe when higher protein diet rather than appropriate low-protein diet was fed. The observations of this study also document the beneficial effects of low-protein rather than higher protein intake in chronic renal insufficiency in experimental albino rats.

Thus, low-protein as against normal soyabean-based-protein diet is recommended as the low-protein soyabean-based diet resulted in better nutritional status and less uremic toxicity in the experimental rats with renal insufficiency. Further research is required to test the beneficial effect of low-protein soyabean-based diet on a longer term in animal and humans.

ACKNOWLEDGEMENTS

The authors would like to acknowledge the support from the Senate Grant Funds of the University of Ibadan. They are also grateful to the Veterinary

Surgeons and Pathologists of the Faculty of Veterinary Medicine, the Laboratory staff of the Department of Human Nutrition, University of Ibadan and also to the Staff of Chemical Pathology of the University College Hospital, Ibadan, for their technical assistance in the study.

REFERENCES

1. Giovannetti S. Dietary treatment of chronic renal failure: Why is it not used more frequently? Nephron 1985; 40: 1-12.
2. Barsotti G. Morrelli E, Cupisti A, Meola M, Danib and Giovannetti S . A low nitrogen, low-phosphorus vegan diet for patients with chronic' renal failure. Nephron 1996; 74(2): 390-394.
3. Anderson J.W, Blake J.E, Turner J and Smith B.M. Effects of soy protein on renal function and proteinuria inn patients with type 2 diabetes. Am. J. Clin. Nutri. 1998; 68 (Suppl): 1475 - 13535.
4. Locatelli F, Albert D and Graziani G . Progressive randomized multi Centre trial of protein restriction on the progression of chronic renal insufficiency. Lancet 1991; 337:1299-1304.
5. Fouque D, Laville M and Broissel J Controlled protein diets in chronic renal insufficiency. British Med. Journal 1992; 304: 216-220
6. Yanagisawa H, Kurishara N, Klahr S, Morrissey J and Wada O. Dietary protein restriction in isolated glomeruli from rats with bilateral ureterial obstruction. Kidney International 1994; 46: 245-251
7. Wardle E.N.: Soy protein diet therapy in renal diet. J. Renal Nutr. 1995; 5: 234-238
8. Kleinknecht C, Salusky I, Broyer M and Gublern M. Effects of various protein diets on growth renal function and survival of uremic rats. Kidney International 1979; 15: 534-541.
9. Committee on Laboratory Animal Diets: Institute of Laboratory Animal Resources Assembly of Life Sciences. National Research Council Report: Common Wealth Bureau of Nutrition. Nutrition Abstract and Reviews, B: Livestock feeds and feeding 1979; 49: 413-419.
10. El-Nahas A.M, Master-Thomas S and Brady S.A. Selective effect of low-protein diets in chronic renal disease. Br. Med. J. 1984; 189:1337-1341.
11. Kenner CH, Evan AP, Blomgren P, Arnoff G.P and Luft FC. Effect of protein intake on renal function and structure in partially nephrectomized rats. Kidney Int. 1985; 27: 739-50.
12. Hostetter TH, Meyer TW, Rennke HG and Brenner BM. Chronic effects of dietary protein in the rat with intact and reduced renal mass. Kidney Int. 1986; 30: 500- 517.
13. Bankir L and kriz W . Adaptation of the kidney to protein intake and to urine concentration activity. Similar consequences in health and chronic renal failure. Kidney International 1995; 47: 7-24.
14. Brenner BM, Meyer TW and Hostetter TH. Dietary protein intake and the progressive nature of kidney disease: the role of aerodynamically mediated injury in the Pathogenesis of progressive glomerular sclerosis in aging renal ablation and intrinsic renal disease. New England Journal of Medicine. 1982; 307: 652-659.
15. Alvestrand A, Butch H, Gutierrez A and Bergstrom J. Progression of chronic renal failure in man is influenced by frequency and quality of clinical follow up. Kidney International 1985; 27: 240-247.
16. Zadik Z, Frishberg Y, Drukker A, Blachar Y, Lotan D, Levi S and Reifen R. Excessive dietary protein and sub optimal calorie intake have a negative effect on the diseases before and during growth hormone therapy. Metabolism clinical and experimental 1998; 47 (3): 264-268.
17. Klahr S. and Purkerson ML . Effects of dietary protein on renal function and on the progression of renal disease. Am. J. Clin. Nutr. 1988; 47:146-152.