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Full Length Research Article

The Effects of Controlled Intake of Selected Protein Foods on Nephrectomized Rats

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ABSTRACT

Dietary management of patients with chronic renal failure (CRF) has remained a very tasking one, especially as it affects the source and quantity of protein in diets. This study was carried out to determine the effects of low-protein diets from common protein foods on 36 ⅔-nephrectomized albino rats (NR). NR were placed on isocaloric 14% protein diets incorporating cooked beef (A), smoked catfish (B), cooked beef and smoked catfish (C), cooked beef, smoked catfish and sun-dried, hulled, red cowpeas (D), cooked egg white (E), and sun-dried hulled, red cowpeas (F) for a period of six months during which food intake, anthropometric parameters, blood urea and serum creatinine levels were measured. Food intake decreased significantly ($p<0.05$) in the NR placed on all experimental diets, with F>A>C=D>B>E. The same trend was obtained for body weight gains. All the NR had proteinuria and reduction in 24–hour urine output; these were greatest in NR placed on diet F and lowest in those on diets E and B. Blood urea and serum creatinine levels of NR on diet E were significantly lower ($p<0.05$) than the corresponding levels for NR on diets B>C>A>D>F. In conclusion, this study has shown that cooked egg white and smoked catfish had more beneficial effects on food intake, anthropometric parameters, blood urea and creatinine levels in NR. It is recommended that low-protein diets containing egg white and fish be offered to patients suffering from early to moderately severe CRF as conservative therapy to ameliorate its symptoms in patients.

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Key words: food, protein, nephrotomy, rats, intake

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INTRODUCTION

The role of diet in the management of most diseases, be it in the acute or chronic states, has been recognized over the years. Comprehensive nutritional therapy based on appropriate needs and sound clinical judgment has thus been widely utilized in facilitating the success of medical and surgical treatment, recovery from illness or injury, and in the maintenance of good health (Williams, 1994).

Studies in animals and clinical evidence have revealed that proper dietary treatment involving restriction of protein and phosphorus is an integral part of the management of chronic renal failure (CRF). Restriction of protein and phosphorus alleviates the symptoms of uraemia and markedly slows down the progress of renal disease. Diet therapy also ameliorates uraemic toxicity over a long period of time. It provides a temporary stabilization and occasionally an improvement, in renal function for months or even years (Giodiano, 1982, Giovannetti, 1985; Zandik *et al*., 1998). It has been shown that a low-protein, low-phosphorus diet protects remnant glomeruli from further sclerotic changes and delays end stage renal disease (ESRD) (Zandik *et al*., 1998). Thus, an appropriate diet is critically important in the management of patients with CRF, because it offers several advantages and benefits (Giovannetti and Maggmore, 1964; Giovannetti, 1985; Barsotti *et al*., 1996).

Several studies have helped to advance the knowledge of the pathophysiologic mechanism of nephron destruction. Much of this information is based on the results of studies in experimental animals. It has been known for a number of years that if a critical percentage of renal mass tissue is removed in a variety of species, proteinuria and progressive deterioration of renal function will develop, resulting in renal failure and death of the animals (Chanutin and Ferris, 1932; Morrison, 1962; Kleinknecht *et al*., 1979). To induce renal insufficiency for the purposes of studying the effect of selected sources of protein foods on body weight, tail length, renal function and the structure of the kidneys, albino rats were used because all these manipulations and long-term study are impossible in man. This study was carried out with the aim of evaluating the effects of a variety of selected sources of low protein foods on the food intake, anthropometric and biochemical parameters of nephrectomized albino rats

MATERIALS AND METHODS

Nephrectomy of experimental rats

Thirty-eight albino rats raised on standard chow (i.e. rat diet) and weighing between 168 to 195g were anaesthetized and underwent ⅔-nephrectomy performed by veterinary surgeons. This was carried out by the excision of the right kidney, followed by ablation and cauterization of ⅓ of the left kidney at the upper pole. All the nephrectomized rats received ampicillin injection immediately after surgery to mitigate bacterial contamination of surgical sites. Only two (5%) deaths were recorded among the rats after surgery.

Diet Design

From the day of surgery, the rats were fed on only corn starch and tap water *ad libitum* for the next 6 days. This procedure was adopted to stabilize the nephrectomized rats from post-operation shock and to allow their residual kidney to rest. On the 7th day post-nephrectomy, the rats were randomly distributed into six groups (Groups A, B, C, D, E and F) of 6 nephrectomized rats each with an average weight of 182.8±3.21g. The rats were housed in individual metabolic bottom-screened cages according to their groups.

On the 8th day post-operation, groups A, B, C, D, E and F were assigned to low protein experimental diets (Table 1). The protein in the diets was provided from oven-dried cooked beef (diet A), smoked cat fish (diet B), equal quantities of protein from cooked beef and smoked catfish (50/50 by protein) (diet C), equal quantities of protein from cooked beef, smoked catfish and hulled, cooked, sundried red cowpeas (diet D), cooked egg white (diet E), and hulled, cooked, sundried red cowpea (diet F), respectively. The diets differed exclusively only in their protein sources. The diets were isocaloric and the protein provided 14% of the caloric content of each of the diets, which are the minimal requirements for maximal growth in growing rats (Kleinknecht *et al*., 1979). Other components of the diets were corn starch, corn oil, vitamin mix, mineral mix and fiber (Table 1). The composition of the diet used in feeding the animals also conformed to the one proposed by the Committee of Laboratory Animal Resources (1979). All the animals were allowed to eat and drink *ad libitum* throughout the six months that the experiment lasted.

Data Collection: Three weeks post-operation, the initial measurements of the food intake, body weight, tail lengths and 24-hours urinary excretion of the rats were undertaken. The mean food intake and urinary excretion of each group of nephrectomized rats were determined (Hostetter *et al*., 1986), while levels of protein in their urine were determined by the sulforsalicilic acid method (Davidson and Henry, 1969). The weights and tail lengths of the rats were also measured at monthly intervals. At the end of the 6-week feeding trial, 2ml of blood was obtained from each rat by ocular artery cannulation into heparinized tubes. Plasma levels of phosphate, calcium, sodium, potassium, chlorides, bicarbonate, blood urea, plasma creatinine, plasma proteins (albumin and globulin), packed cell volume (PCV), erythrocyte (RBC) counts and haemoglobin IHb) concentration of the nephrectomized rats were determined as described by Ogunsanmi *et al*. (2002).

Statistical analysis: Data collected were expressed as range, mean ± standard deviation (SD) and percentages as necessary. Comparisons of the levels of food intake, anthropometric and biochemical indices of the rats in the various groups were subjected to two-way analysis of variance and Duncan's multiple range test (Duncan, 1959; SAS, 1987).

RESULTS

Food intake and anthropometric parameters of the nephrectomized rats

Table 2 shows the initial and final food intake, body weights and tail lengths of the nephrectomized rats categorized according to the different diet groups. At the beginning of the experiment, daily food intake was similar in all the groups of rats ($p>0.05$). However, at the end of six months, all the rats in each group consumed less but varied amounts of food than at the commencement of the experiment ($p<0.05$). The mean difference in the initial and final food consumption between the groups of the nephrectomized rats on the individual diets was significant ($p<0.05$). The least final food consumption was observed in the group of rats on F diet, while the greatest food consumption was observed in rats on E diet, followed by those on diet B (Table 2). Also, increases in mean body weight gain and tail length were observed in all the different groups of rats, but the pattern of body weight and tail length gains among the rat groups varied. The least mean body weight gain and tail length increase (81.5±4.27g and 48.9±2.36mm, respectively) were observed in the rats on the F diet. The greatest mean body weight and tail-length gains (91.5±1.4g and 56.6±0.52mm, respectively) were observed in the rats on the E diet, followed by the rats on the B diet (86.8 ± 3.19g) and 52.5± 0.56mm, respectively). A comparison of the effect of the different diets showed a significant increase ($p<0.05$) in body weight gain of rats placed on the E diet than the those placed on the other diets. The rats on diet E also had longer but insignificant ($p>0.05$) gain in mean tail length than all the other rats placed on the other diets.

Urine excretion by nephrectomized rats

The initial 24-hour urine excretion of all the groups of rats was similar (Table 3). This ranged from 7.2±0.37ml to 7.4±0.11ml/day. However, at the end of the experiment, varied reduction in the 24-hour urinary output: -2.1±0.12ml, -1.6±0.07ml, -1.9±0.14ml, -2.2±0.20ml, -1.1±0.22ml and -3.0±0.16ml were observed in the nephrectomized rats on the A, B, C, D, E and F diets, respectively. The reduction in the 24-hour urinary excretion was greatest in the rats placed on the F diet (-3.00±00.16ml/day) and lowest in rats on the E and B diets (-1.15±0.22 and -1.62±0.07ml/day), respectively.

Table 1: Composition of the low-protein (14% CP) diets of the experimental rats

Composition (g/100g)	Diet A	Diet B	Diet C	Diet D	Diet E	Diet F
Protein	13.6	4.8	9.2	11.2	15.2	3.0
Corn Starch	70.9	79.7	75.3	73.3	69.3	81.5
Corn oil	5.0	5.0	5.0	5.0	5.0	5.0
Cellulose	5.0	5.0	5.0	5.0	5.0	5.0
Vitamin mix	1.0	1.0	1.0	1.0	1.0	1.0
Mineral mix	4.5	4.5	4.5	4.5	4.5	4.5
Total (g)	100.0	100.0	100.0	100.0	100.0	100.0

Table 2: Food intake and anthropometric measurement changes in nephrectomized rats fed different sources of low-protein diets

	Diet Groups					
Variables	**A***	**B**	**C**	**D**	**E**	**F**
Food Intake (g/24 hr)						
(a) Initial	19.4±0.3	20.1±0.2	19.9±0.4	19.9±0.4	20.2±0.1	20.4±0.1
(b) Final	17.3±0.3	18.7±0.5	18.1±0.1	18.0±0.2	19.2±0.2	17.5±0.1
Mean difference	-2.1±0.1	-1.4±0.2	-1.8±0.3	-1.8±0.1	-1.1±0.1	-2.8±1.7
Body Weight (g)						
(a) Initial	182.9±4.1	183.4±4.0	182.8±3.8	183.2±4.1	183.8±3.3	182.9±4.2
(b) Final	268.5±10.3	270.2±13.4	268.6±13.4	268.7±9.9	275.3±5.7	264.4±4..3
Mean difference	82.3±1.1	86.8±3.2	85.8±4.0	85.5±3.2	91.5±1.5	81.5±4.3
Tail Length (mm)						
(a) Initial	147.2±0.8	146.4±0.7	147.2±0.9	146.9±0.7	147.6±1.0	147.0±0.6
(b) Final	197.9±4.8	196.9±5.8	197.6±4.8	198.6±4.6	204.1±1.0	195.5±6.3
Mean difference	50.7±1.8	52.5±0.6	51.4±2,0	51.2±0.0	56.6±0.5	48.9±2.4

***number of rats in each group = 6**

***Abbreviations**: A = Cooked beef, B = Smoked cat fish, C = Cooked beef + smoked cat fish; D = Cooked beef + smoke cat fish + Hulled cooked sundried red cowpeas; E = Cooked egg white, F= Hulled sun dried red cowpea*

Table 3: 24-hour urinary excretion and urinary protein of nephrectomized rats fed different low-protein diets

	Diet Groups					
Variables	**A***	**B**	**C**	**D**	**E**	**F**
24-hours urinary excretion (ml)						
(a) Initial	7.3±0.3	7.4±0.1	7.3±0.3	7.2±0.4	7.3±0.1	7.4±0.1
(b) Final	5.2±0.3	5.8±0.2	5.5±0.2	5.0±0.3	6.2±0.1	4.3±0.3
Mean difference	-2.1±0.1	-1.6±0.1	-1.9±0.1	-1.2±0.2	-1.1±0.2	-3.0±0.2
24-hour urinary protein (mg/dl)						
(a) Initial	6.5±0.3	6.6±0.5	6.5±0.3	6.5±0.3	6.3±0.0	6.3±1.1
(b) Final	19.9±0.2	13.9±1.9	15.9±2.3	20.3±1.8	10.1±0.7	20.6±9.0
Mean difference	13.4±0.1	7.3±0.7	9.5±0.9	13.8±0.6	3.8±0.3	14.4±5.6

***number of rats in each group = 6**

***Abbreviations**:*

A = Cooked beef, B = Smoked cat fish, C = Cooked beef + smoked cat fish; D = Cooked beef + smoke cat fish + Hulled cooked sundried red cowpeas; E = Cooked egg white, F= Hulled sun dried red cowpea

Table 4:
Biochemical parameters of the nephrectomized rats fed with selected food sources of low-protein diets

Parameters	Diet Groups					
	A*	**B**	**C**	**D**	**E**	**F**
Plasma creatinine	2.9+0.12	2.2+0.07	2.7+0.14	2.8+0.15	2.0+0.04	3.20+0.09
(mg/dl)	50.2+0.60	44.1+0.30	47.5+0.47	50.7+0.90	34.1+0.16	66.2+0.75
Blood urea (mg/dl)	3.6+0.06	3.9+0.04	3.7+0.07	3.4+0.04	4.9+0.23	3.0+0.14
Serum albumin (g/dl)	5.3+0.05	5.4+0.04	5.3+0.06	5.2+0.04	6.2+0.47	4.5+0.36
Total Protein (g/dl)	33.7+0.52	34.5+0.84	34.3+0.52	33.3+0.52	37.7+0.52	31.8+0.50
PCV (%)	6.8+0.08	5.4+0.07	6.1+0.08	6.8+0.15	5.0+0.14	8.3+0.32
Phosphate (mg/dl)	5.3+0.07	5.3+0.14	5.2+0.10	5.4+0.27	4.6+0.27	5.5+0.29
Calcium (mg/dl)	174.6+3.94	174.2+1.40	174.0+3.30	146.2+63.68	137.5+60.12	141.6+1.47
Sodium (g/dl)	3.3+0.21	3.2+0.15	3.3+0.19	3.18+0.15	3.3+0.25	5.1+0.34
Potassium (mΣq/dl)	126.3+0.79	127.7+1.00	126.7+1.65	127.2+0.93	127.4+1.03	120.6+0.59
Chloride (mΣq/dl)	23.3+0.38	23.3+3.69	23.9+0.72	23.0+0.72	23.9+3.56	24.3+0.97
Bicarbonate (mEq/dl)						

number of rats in each group = 6
***Abbreviations**:*
A = Cooked beef, B = Smoked cat fish, C = Cooked beef + smoked cat fish; D = Cooked beef + smoke cat fish + Hulled cooked sundried red cowpeas; E = Cooked egg white, F= Hulled sun dried red cowpea

The mean reduction in the 24-hour urinary excretion of the rats on the F diet was significantly greater ($p<0.05$) than values observed for other groups of rats.

The group of rats on the F diet had the highest degree of proteinuria, followed by those on the D diet, while those on the E diet had the lowest proteinuria. The group of rats on the A, D and F diets had the highest proteinuria, which were significantly greater ($p<0.05$) than the values in the other groups of rats on the other sources of protein diets.

Haematology and plasma biochemical parameters of the nephrectomized rats

Hematological parameters (PCV, RBC counts and Hb concentration), mean plasma creatinine, blood urea, albumin and total plasma protein levels of the nephrectomized rats, according to their diets, are presented in Table 4. There were no significant differences in the haematological parameters of the rats irrespective of the diets groups. The group of rats on E diet had the lowest serum creatinine (2.0±0.04mg/dl), while group F had the highest value (3.2±0.09mg/dl) which was significantly higher ($p<0.05$) than the serum creatinine of the rats on the E and the B diets. Similarly, blood urea was markedly elevated in the rats on the F diet, while rats on the E diet had the lowest blood urea level. Plasma albumin concentration of the rats on the E diet (4.9±0.23g/dl) was significantly higher ($p<0.05$) than the levels of 3.6±0.06g, 3.9±0.04g, 3.7±0.07g, 3.4±0.04g and 3.0±0.1.4g/dl for rats fed A, B, C, D, and F diets, respectively. Furthermore, the rats which were placed on the E diet had higher ($p<0.05$) plasma albumin and total protein concentrations than those on the other diets.

The plasma phosphate concentration of the group of rats on the B and E diets, 5.0±0.14mg/dl and 5.7±0.07mg/dl, respectively were significantly lower ($p<0.05$) than those rats on the A, C, D and F diets which had serum phosphorus concentrations of 6.8±0.08mg, 6.1±0.08mg, 6.8±0.15mg and 8.3±0.32mg/dl, respectively. The rats placed on diet F had the highest plasma phosphate concentration. There was no significant variation ($p>0.05$) in plasma calcium levels of the rats given A, B, C, D and F diets, while rats on the E diet had the lowest ($p<0.05$) plasma calcium level of 4.6±0.27mg/dl. Furthermore, the plasma sodium concentration of the groups of rats on A, B and C diets were similar, ranging from 174.0±3.30mg/dl in the ones on C diet to 174.6±3.94mg/dl on the rats on the A diet. Also, the plasma potassium and bicarbonate concentrations of the rats on the F diet were significantly higher than those of the other groups of rats (<0.05), while these (K, and $HC0_3$) concentrations were similar in the other groups of rats ($p<0.05$).

DISCUSSIONS

It is generally well known that not all protein food sources are of equal value to the body and that both the quantity and quality of dietary proteins are important in the development and progression of CRF. In order to ensure adequate supply of essential amino acids, Giovannetti (1985) and Wilkins (1996) recommended up to 60-75% of the protein needs from protein of high biological value for patients with CRF or other renal diseases which should be in the form of egg, meat, fish and poultry. However, Newburg and Curtis (1928) observed that rats fed diets with dry liver as a source of protein developed renal lesion faster than when an equivalent amount of casein protein was fed to a similar group of rats; whereas a diet of beef containing equivalent amount of protein to liver or casein was neither as damaging as the liver alone diet or as agreeable as the casein diet. According to Levey *et al*. (1998), protein quality, the lipid-lowering and antioxidant effects of protein foods may contribute to the progression of chronic renal failure.

In this study all the low-protein diets provoked similar though not identical, severity of symptoms in the nephrectomized rats. Varied levels of reduction in food intake, mean body weight gain and tail length increase were noted in the nephrectomized rats groups compared to their initial values, whatever the diets. The nephrectomized rats that were placed on the egg white diet, followed by the rats that were placed on the fish diet alone ate more food and had better body weight and tail length gains than the rats fed the other diets. Reduction in food intake, low body weight and tail length gains were more apparent in the rats fed the cowpea diet alone, followed in descending order by the rats on the mixture of beef, fish and cowpea diet; beef diet; mixture of beef plus fish diet; and fish diet respectively than observed in rats groups that were placed on the egg white diet alone.

Restricted protein diet was incriminated as a possible cause of anorexia and chronic malnutrition which are commonly observed in rats with substantial loss of renal mass (Chantler *et al*., 1974, Diaz *et al*., 1975). The observations made in the course of this study suggest that the severity of anorexia as well as the amount of body weight and tail length gain were influenced by the different food protein sources and their mixtures fed the rats with renal insufficiency. However, despite a reduction in food intake, all the nephrectomized rats still had body weight and tail length increase. It could then be suggested that low protein diet, most especially of animal origin, no matter the source, when appropriately utilized, will enhance body weight and possibly growth, even in children. However, egg white diet and fish protein diet ameliorated anorexia and increased body weight and tail length of the nephrectomized rats better than the other diets.

The degree of decrease in urine output, increased proteinuria, blood urea and creatinine elevation have been suggested as indicators of renal deterioration (Lalich and Allen, 1971). All the groups of nephrectomized rats used in this study, whatever their protein food source, developed a decrease in their daily urine output, had increases in proteinuria, serum creatinine and blood urea values. These values were significantly greater in the nephrectomized rats put on cowpea diet alone, compared to the values of other nephrectomized rats fed with the other diets ($p<0.05$). The nephrectomized rats fed with the egg white diet followed by the fish diet had better daily urinary output, lower proteinuria, serum creatinine and blood urea appearance than the rats placed on the other diets. The diets containing the food protein mixtures were intermediate in these biochemical responses. It seems clear from these findings that rats with substantial reduction in functioning renal mass, even when placed on low-protein diet will ultimately present renal responses such as oliguria, proteinuria, serum creatinine and blood urea elevation, which indicate progressive deterioration in renal function. However, the severity of these renal responses could be influenced by the protein food source or their mixtures in the diet ingested. For example egg albumin diet and fish protein diet induced better renal function in the nephrectomized rats than beef or cowpea diet, or their mixtures.

The nephrectomized rats on egg albumin diet ate more food and excreted less protein than rats which were placed on beef, fish, or cowpea, or their mixtures. They also had significantly higher levels of serum albumin, total plasma protein and packed cell volume ($p<0.05$). Thus it is clear from this study that as substantial functioning renal mass is lost, the progression of renal damage is certain, but the rate of deterioration could be attenuated when a low-protein diet is adopted. Also, the degree of ureamic symptom amelioration could be related to the specific food protein in the diet.

Lower levels of serum phosphorus and other electrolytes were observed in the nephrectomized rats fed the egg white diet and fish diet than in those placed on the other diets. These findings further suggest the efficacy of low-protein diet from egg albumin and fish protein. It also suggests that the choice of protein foods and the proportion in which they are mixed could be a major factor in the rate at which the symptoms of chronic renal failure are ameliorated with low-protein diets.

Conclusions and Recommendations

The low-protein diets made from cooked beef alone, smoked catfish alone, mixtures of equal quantities of cooked beef plus smoked-catfish with hulled cooked sundried red cowpeas, and cooked egg white alone respectively, all provoked varied levels of reduction in food intake, mean body weight and tail length gain, oliguria, proteinuria, and both blood urea and serum creatinine appearance in albino rats with renal insufficiency. The nephrectomized rats that were placed on the egg white diet ate more food and had better anthropometric parameters than the other nephrectomized rats which were placed on the other diets. Anorexia and the least anthropometric parameters were more apparent in the nephrectomized rats group on the cooked hulled sundried red cowpea low-protein diet. Hence, egg white and fish protein should be recommended more in the diet of patients with chronic renal insufficiency, since the severity of anorexia, the anthropometric and biochemical parameters of the nephrectomized rats were found to be influenced by types and biological quality of proteins.

The egg white diet alone or the catfish diet alone had better ameliorating effect on these indices. Egg white and fish protein sources induced better nutritional status and renal function responses than the other sources of protein fed to the nephrectomized rats.

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