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The Effect of Level of Dietary Protein on Kidney Development and Function in Growing Rats

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ABSTRACT

This study was performed to investigate the effect of protein intake on kidney development and function in growing rats. Fourty-two male Spraque-Dawley rats of weighing 97.5± 1.9g were divided into 3 groups and given 5%, 15% or 50% casein diets for 6 weeks.

Body weight gain was higher in the 50% group. The kidney weight was selectively affected more by the level of dietary protein compared to the other organs.

DNA and RNA content were significantly higher in the 15% and 50% groups than in the 5% group but the differences disappeared when DNA and RNA were expressed per g of kidney weight. Protein and protein/g kidney content were increased with increasing level of protein in diet.

GFR/animal and GFR/100gB. W. were significantly higher in the 50% group compared to the 5% and 15% groups. There was no differences in PAH clearence and RBF. Osmolality was not affected by dietary protein level. BUN and urinary nitrogen excretion were increased with the increasing dietary protein level

Although urinary Ca excretion was not significantly difference among 3 grougs, the rats in the 5% group showed 30% less Ca excretion compared to the other groups.

Above results suggest that dietary protein level has a great effect on the kidney weight and GFR in growing rats. Especially the hyperfiltration inhanced by high protein diet may accelerate the kidney senescence.

KEY WORDS : dietary protein · GFR · RBF · osmolality · BUN · urinary Ca.

INTRODUCTION

The kidney is one of the most important organ to maintain body's internal homeostasis. It is, however, a vulnerable organ to be deteriorated in structure and function with advancing age[1~3]. Age-related reduced kidney function may lead to water, electrolyte, and acid-base imbalance, which is a contributing cause of the chronic diseases in late adulthood, such as renal and cardio-vascular diseases, hypertension, and diabetes mellitus[2].

Growth, development,and aging of kidney are under great infuluence of the diet, especialy protein level[4~6]. When animals are fed a high protein diet, increases in glomerular filtration rate(GFR) and in the kidney size are reported[7~9]. Also men

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with a normal mixed diet have a higher GFR than vegeterians with low protein intake[10].

At a normal level of Ca intake, increases in dietary protein are reflected in incremental increase in urinary Ca[11]. A decrease in the fractional resorption of Ca by the kidney tubule reported to be the most likely cause of the protein-induced hypercalciuria[12].

Although the excess of protein intake becomes an issue in western societies[4], sufficient protein intake is still recommended in Korea. However, some korean families from high socioeconomic class are reported to take more protein than RDA [13,14]. Due to the changes of dietary patterns and economic expansion in Korea, it is expected that protein consumption would be increasing further.

Therefore, we studied the effect of different levels of dietary protein on the development and function of kidney in growing rats.

MATERIALS AND METHODS

Fourty-two male rats of Spraque-Dawley strain weighing 97.5± 1.9g were devided into 3 groups and given either 5% (low protein), 15% (control) or 50% casein diets(high protein) for 6 weeks. Composition of the experimental diets are shown in Table 1. The contents of Ca and P in the diet were manipulated to be 0.6% and 0.4%, respectively. All rats were housed individually in wire bottomed cage and were allowed to eat and drink water ad. libitum.

The food intake and body weight were measured every week. Three days before the end of the experiment, all rats were placed in metabolic cage for the collection of 1-day(24 hours) urine specimen. After the experimental period, all rats were fasted for 12 hours. PAH(Para Amino Hipurate, 12.5mg/ml in 2% sodium sulfate) solution was injected subcutaneously in the lumbar region to 6 rats in each group. Fifty minutes after the injection, the rats were picked up over the funnel and the bladder drained by suprapubic pressure. Immediately following urine collection, blood was obtained by decapitation[15]. The rest of rats were also decapitated for blood collection and kidney, spleen, liver, and epididymal fat pad were quickly removed and weighed.

Osmolality of renal medulla and cortex were measured by osmometer(Fiske Associate, Catalog N. 110825) according to the Schmidt-Nielsen's method[16]. DNA and RNA contents in kidney were analyzed by Schmidt-Thanhouser's modification[17] of the diphenylamine reaction. Kidney protein was assayed by the method of Lowry[18], lipid by the method of Folch[19], blood urea nitrogen (BUN) by the method of Berthelot[20]. Urinary and plasma Ca were analyzed by Automic Absorption Spect(Perkin Elmer CO. 2380). Urinary PAH (U_{PAH}) and plasma PAH(P_{PAH}) which obtained from urine and plasma of rats injected PAH solution were analyzed by method of Goldring[15]. U_{PAH}, P_{PAH} and Hematocrit(Hct) were used to calculated PAH clearance(C_{PAH}) and renal blood flow(RBF), using following formulas.

$$C_{PAH}(ml/min) = \frac{U_{PAH} \times \text{daily urine volume}}{P_{PAH}}$$

$$RBF(ml/min) = \frac{C_{PAH}}{1 - Hct}$$

Urinary creatinine(Ucr) was measured by method of Folins and plasma creatinine(Pcr) by method of Folin & Wu[21]. Creatinine clearance(Ccr) was calculated as follows.

$$C_{Cr}(ml/min) = \frac{U_{Cr} \times \text{daily urine volume}}{P_{Cr}}$$

All values were presented as mean± S.E. Schffé test was used to test statistical significance.

Table 1. Composition of experimental diet.(g/kg diet)

	5% casein diet	15% casein diet	50% casein diet
Corn Starch	798.92	699.78	352.80
Casein	50.00	150.00	500.00
Corn Oil	100.00	100.00	100.00
Salt mixture[1)]	14.72	14.72	14.72
Vitamin mixture[2)]	10.00	10.00	10.00
$CaCO_3$	8.79	9.31	11.09
$Ca(H_2PO_4)_2$-H_2O	15.57	14.19	9.39
Choline Chloride	2.00	2.00	2.00

1) Salt mixture(mg/kg diet) : $MgCO_3$ 6900, $ZnCO_3$ 96, $FeSO_4$ $7H_2O$ 124, $CuSO_4$ · $5H_2O$ 20, $MnSO_4$ · H_2O 150, KI 1.3, NaCl 2300, Na_2CO_3 1600, K_2CO_3 3530 Na_2SeO_3 0.22

2) Vit mixture(mg/kg mix) : Thiamin · HCl 600, Riboflavin 600, Pyridoxine · HCl 700, Nicotinic acid 3000, D-calcium pantothenate 1600, Folic acid 200, Vitamin B_{12} 1, Colecalciferol 2.5 : Menadione 5.0, Retinyl palmitate : 400,000 IU Vit A activity, DL-tocopheryl acetate : 5,000 IU Vit E activity

Table 2. Food intake, protein intake, weight gain, and PER of rats fed on 5%, 15% and 50% casein diet for 6 weeks[1)]

Exp. Group	Food intake (g/6 weeks)	Protein intake (g/6 weeks)	Weight gain (g/6 weeks)	PER
5%	389.37± 24.60a	19.49± 1.23a	-3.28± 3.83a	-0.21± 0.22a
15%	624.90± 24.66b	92.69± 3.38b	159.07± 6.94b	1.61± 0.04b
50%	621.86± 34.65b	312.01± 17.25c	188.66± 13.68b	0.60± 0.02c

1) Mean± S.E. Value with different alphabet within the column were significantly different at $\alpha=0.05$ by Schffé test.

Table 3. Weights of liver, spleen, thymus, epididymal fat pad(EFP), and kidney[1)]

Exp. Group	Liver (g)	Spleen (g)	Thymus (g)	EFP (g)	Kidney (g)
5%	3.42± 0.28a	0.82± 0.02a	0.52± 0.02a	1.37± 0.05a	0.89± 0.01a
15%	8.00± 0.28a	1.36± 0.06b	0.80± 0.04b	3.19± 0.24b	1.85± 0.02b
50%	10.01± 0.51a	1.58± 0.08c	0.80± 0.05b	3.16± 1.36b	2.33± 0.66c

1) Mean± S.E. Value with different alphabet within the column were significantly different at ($\alpha=0.05$) by Schffé test. NS : Not significant at $\alpha=0.05$ by Schffé test.

RESULTS

Table 2 lists food intake, body weights, and protein efficiency ratio(PER). Food intake and body weight gains were significantly lower in 5% casein group than in 15% groups. PER of the 15% group was the highest followed by the 50% and 5% groups.

The weights of spleen, liver and epididymal fat pad were significantly lower in the 5% casein group compared to the other groups and no significant difference was seen between the 15% and 50% groups(Table 3). Kidney weight was increased significantly with increasing level of protein in the diet.

DNA and RNA contents of kidney were signifi-

Table 4. DNA, RNA, and protein contents of Kidney[1]

Exp. Group	DNA (mg)	DNA (mg/g)	RNA (mg)	RNA (mg/g)	RNA/DNA (mg/mg)	Protein (mg)	Protein (mg/g)	Pro/DNA (mg/mg)
5%	0.56± 0.01a	1.25± 0.04N.S	1.87± 0.01a	4.23± 0.25a	3.37± 0.11N.S	3.76± 0.35N.S	8.36± 0.51N.S	6.71± 0.43N.S
15%	1.43± 0.16b	1.57± 0.20	7.20± 0.07b	7.88± 0.30b	5.42± 0.59	6.77± 1.14	7.38± 1.36	5.53± 1.59
50%	1.90± 0.37b	1.61± 0.21	8.76± 1.47b	7.45± 0.53b	5.16± 0.88	13.16± 3.46	10.56± 2.57	6.21± 0.41

1) Mean± S.E. Value with different alphabet within the column were significantly different at $\alpha = /0.05$ by Schffé test. NS : Not significant at $\alpha = /0.05$ by Schffe test. Left kidney is used to measured of DNA, RNA and Protein contents.

cantly higher in the 15% and 50% groups compared to 5% group, however, DNA and RNA contents per g kidney weight did not differ(Table 4). Protein/whole kidney and protein /g kidney tended to be higher in high protein group, even though the differences were not significant.

There was no significant difference in renal osmolality among experiment groups(Table 5). GFR per animal(Table 5) was significantly higher in the 50% group and this difference was unchanged even when GFR was expressed per 100g body weight. PAH clearance and RBF were not affected by the dietary protein level(Table 5).

BUN and urinary N excretion was increased with increasing level of protein intake. Even though there was no significant difference in urinary Ca excretion, the rats in the 5% group showed 30% less Ca excretion compared to the other groups(Table 6).

DISCUSSION

The dietary protein level had a great effect on kidney weight in growing rats. The rats fed 5% casein diet for 6 weeks showed only half of the kidney weight of those fed 15% casein diet, whereas the kidneys of the rats in 50% group weighed 126% more than the 15% control group. When organ weights of 5% and 50% groups were compared to the 15% control group, liver weights were 42.8% and 112.6%, thymus 60.5% and 100 %, spleen 60.3% and 116.2%, respectively. Therefore, the kidney and the liver seem to be affected more by low protein diet than that of the spleen and the thymus. The kidney is also the most affected organ by extremely high protein diet, leading to hypertrophy. This result implies that protein nutrition plays a very important role in kidney development and function.

Selective stimulation of kidney growth by high

Table 5. Osmolality, GFR, and RBF[1)]

Exp. Group	Osmolality(mOsmole) Cortex	Medulla	GFR (ml/min)	GFR(ml/min/ 100 BW)	C_{PAH} (ml/min)	RBF ml/min)
5%	7.45± 5.81N.S	7.92± 1.12N.S	0.06± 0.03a	0.07± 0.03a	5,03± 1.28N.S	8.59± 2.18N.S
15%	6.15± 0.86	6.69± 0.56	0.24± 0.04a	0.09± 0.01a	3.56± 0.03	7.21± 0.69
50%	5.79± 0.29	6.47± 0.55	0.59± 0.08a	0.20± 0.02a	4.81± 0.43	8.77± 0.78

1) Mean± S.E. Value with different alphabet within the column were significantly different at $\alpha=0.05$ by Schffé test. NS ; Not significant at $\alpha=0.05$ by Schffé test.

Table 6. Blood urea nitrogen(BUN), urinary nitrogen, and urinary Ca contents[1)]

Exp. Group	BUN (mg/dl)	Urinary N (mg/day)	Urinary Ca (mg/day)
5%	5.66± 0.43a	4.73± 0.56a	0.49± 0.08NS
15%	9.75± 1.43b	12.27± 1.44a	0.71± 0.08
50%	12.58± 0.80b	36.84± 5.71b	0.70± 0.07

1) Mean± S.E. Value with different alphabet within the column were significantly different at $\alpha=0.05$ by Schffé test. NS ; Not significant at $\alpha=0.05$ by Schffé test.

protein intake has been indicated in studies from the 1930's[22)]. Millward[23)] reported that protein restriction reduced kidney weight more than brain weight.

In the 5% group DNA/g kidney was 21% less than that in the 15% control group, but protein content was higher by 13%, and consequently the protein/DNA ratio was higher than the other groups. These results may imply that low protein diet decreased DNA synthesis more than protein synthesis. Therefore, renal hyperplasia markedly reduced by protein restriction. Howarth[24)] observed the similar result in muscle of growing rats fed protein restricted diet.

GFR, measured by the clearance of creatinine, was increased with increasing dierary protein level. These results are similar to the already established studies. Pullman et al[25)] and Jakobson et al[26)] have found in rats that high protein feeding had an effect on increase in GFR and on the other hand GFR was decreases by feeding low protein diet. Many studies reported that RBF was also changed with the level of protein in the diet, parallel to the GFR change[27)]. In this study, however, there was no difference in RBF with 3 different casein levels in diet.

In accordance with many other reports[28)], BUN and urinary nitrogen were increased with increasing level of dietary protein. But no significant difference in BUN was found between the 15% and 50% groups. This result showed that the increasing amount of urea excretion accompanied by the increased GFR in relation to high protein diet had kept the level of BUN relatively constant.

Urinary Ca excretion tended to be higher in the 15% and 50% groups than in the 5% group. This result is good agreement with the many other reports shown hypercalciuria induced by high protein diet[29,30)]. Allen[30)] has found that high protein intake decreased Ca reabsorption in kidney tubule and resulted in hypercalciuria. On the other hand, Kim and Linkswiler[11] reported that increasing urinary Ca excretion by high protein intake was due to increase in GFR and decrease in Ca reabsorption. In the rats fed 5% casein diet, GFR and urinary Ca excretion were relatively low. However, urinary Ca excretion was nearly the same in the 15% and 50% groups neverthless there was a large difference in GFR between the two groups. Therefore, the increasing urinary Ca can not be explained by the increased GFR only, and some other factors besides of GFR have an effect on urinary Ca excretion. If high protein diet continues for life time,

the tendency of diet-induced hypercalciuria may exert an ill effect on bone health in later aged years.

In conclution, low protein diet decreased kidney weight and GFR, on the other hand high protein diet stimulated kidney growth and increased GFR in greater extent. It must be considered that high protein diet has two-sided effects ; it can stimulate kidney growth but gives overloading metabolites to be excreted which resulted in hyperfiltration. If glomerular hyperfiltration continues nephronic function may be rapidly deteriorated. Therefore, continued hyperfiltration caused by high protein intake from early in life may precipitate deterioration of kidney function with advancing age.

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식이내 단백질 수준이 성장기 흰 쥐의 신장 기능에 미치는 영향

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국문초록

본 연구에서는 식이단백질 수준이 성장기 흰쥐의 신장 발달과 기능에 미치는 영향을 조사하기 위해 평균체중이 97.5±1.9g인 숫컷 흰쥐를 5%, 15% 그리고 50% casein 식이로 6주간 사육하였다. 체중증가량은 단백질 수준이 높을수록 높았으며 특히 단백질수준이 신장무게에 미친 영향이 간, 비장, 흉선 등의 다른 장기에 비해 컸다. 신장내 총 DNA와 RNA 함량은 15%군과 50%군이 5%군에 비해 유의적으로 높았으나 단위 무게 당 DNA와 RNA 함량에서는 유의적인 차이가 없었다. 총 protein과 단위 g당 protein 함량은 식이내 단백질 수준이 높을수록 증가했다. 사구체여과율(GFR)은 식이단백질 수준이 높을수록 높았으나 신혈류(RBF)는 각 군 사이에 유의적인 차이를 보이지 않았다. 뇨질소배설량은 단백질 수준에 비례하여 유의적인 차이를 보였고 뇨칼슘배설량은 5%군이 낮은 경향을 보였다.

본 연구 결과로 성장기의 흰쥐에서 고단백식이는 신장의 무게증가를 촉진하며 사구체여과율을 증가시킴을 알 수 있다.