Low Calorie Unrestricted Protein Diet Attenuates Renal Injury in Hypertensive Rats

Efrain Reisin, Silvia Azar, Bennett P. DeBoisblanc, Miguel A. Guzman, and Thomas Lohmann

In the present investigation we researched the effects of low calorie diet without protein restriction on the renal function and glomerular injury of uninephrectomized spontaneously hypertensive rats. We compared the findings with those that occurred in two different groups of uninephrectomized spontaneously hypertensive rats: one treated with oral hydralazine (10 mg/kg per day) and a second fed regular food. The low calorie diet and hydralazine treatment significantly reduced intra-arterial blood pressure in each group \( (p<0.05 \) and \( p<0.001, \) respectively). The control group showed at the end of the experiment a slight but not significantly different increase in the intra-arterial blood pressure. Low calorie diet was more effective in protecting the kidney function. Creatinine clearance after treatment was significantly higher in uninephrectomized spontaneously hypertensive rats on a low calorie diet than in either the hydralazine-treated or control groups \( (p<0.01). \) The 24-hour urinary protein excretion in the low calorie diet group was significantly lower than in the control group \( (p<0.05) \) and lower but not statistically different from the hydralazine-treated group. The mean glomerular injury index of the remaining kidney in the low calorie diet group was lower than in either the hydralazine-treated or control groups \( (p<0.05) \), and the mean mesangial expansion index in the low calorie diet group was significantly lower than in the control group \( (p<0.05). \) The favorable effect of low calorie diet on renal function was independent of protein restriction or sodium and potassium content. More studies are necessary to determine if these favorable effects on kidney function and glomerulosclerosis are induced by a renal hemodynamic phenomenon (decreased glomerular capillary pressure), by metabolic changes, or by a diminished kidney growth. (Hypertension 1993;11:971–974)

KEY WORDS • diet therapy • rats, inbred SHR • proteinuria • kidney glomerulus

Spontaneously hypertensive rats (SHR) develop glomerulosclerosis in the juxtamedullary nephrons at approximately 1 year of age. Unilateral nephrectomy accelerates the functional and glomerular damage to superficial as well as deep glomerulus. Some antihypertensive drug regimens, or a few dietetic maneuvers such as low protein diet, dietary carbohydrate restriction, or changes in the diet lipid composition attenuate renal damage. The effects of low calorie intake on the development of renal disease in normotensive rats and SHR are controversial. In the present investigation, we researched the effects of low calorie diet without protein restriction on the renal function and glomerular injury of unilaterally nephrectomized SHR (UNX-SHR). We also compared the findings with those that occurred in a group of UNX-SHR treated with oral hydralazine and in a control group of UNX-SHR given regular food to eat.

Methods

We used 2-month-old spontaneously hypertensive males of the Wistar Okamoto strain (Charles River Laboratories, Wilmington, Mass.). On arrival, they were housed singly in metabolic cages in a temperature-, humidity-, and light- (12-hour light/dark cycle) controlled room.

Diets

To obtain intakes that were low in calories but similar in protein, we increased the protein content of the experimental group’s diet by 34% and fed them 33% less food than the controls. Because sodium and potassium affect blood pressure and renal injury, those levels in the experimental diet were also increased by 36% and 35%, respectively, over those of the control diet. The experimental diet contained (in percentage): protein 31.4, fat 3.7, carbohydrate 38.5, potassium 3.1, sodium 1.1, phosphorus 0.6, calcium 0.8, and 13.15 kJ/g (3.13 kcal/g). The control diet (Rodent Laboratory Chow 5001, Purina, St. Louis, Mo.) contained (in percentage): protein 23.4, fat 4.5, carbohydrates 49, potassium 1.1, sodium 0.4, phosphorus 0.6, calcium 0.1, and 17.85 kJ/g (4.25 kcal/g). All rats received and consumed fixed food amounts during experimentation: 10 g for the low calorie group and 15 g for the normal calorie groups. All rats received tap water ad libitum.

Protocol

Two weeks after arrival, SHR left kidneys were removed (under light ether anesthesia), and the UNX-SHR were allowed to recover from surgery for 8 weeks. They were then randomly assigned to group 1, which

From the Departments of Medicine (E.R., B.P.D.) and Pathology (M.A.G.), Louisiana State University, New Orleans, University of Minnesota, Minneapolis (S.A.), and Ochsner Medical Foundation (T.L.), New Orleans.

Address for correspondence: Efrain Reisin, MD, Section of Nephrology, Department of Medicine, Louisiana State University, Room A 319, 1542 Tulane Avenue, New Orleans, LA 70112.
received the low calorie diet and untreated water; group 2, which received (to control for blood pressure effects) a normal calorie diet and hydralazine-treated drinking water (10 mg/kg body wt per day); or group 3, which received a normal calorie diet and untreated water. The diets were maintained until the rats were killed. Seven of the UNX-SHR completed all the experimental stages for groups 1 and 3 in group 2.

Body weight and 24-hour urinary protein excretion and creatinine clearance measurements were obtained before nephrectomy (2 weeks after arrival), 8 weeks after nephrectomy, and 4 weeks after dietary manipulation (end of experimentation). Arterial pressure and heart rate measurements were obtained 8 weeks after nephrectomy and 4 weeks after dietary treatment. End points at the time the rats were killed were: heart weight, left ventricle/heart weight index, right kidney weight, and renal morphology.

Procedures

Blood pressure determination and blood samples were obtained through a PE-50 cannula with a PE-10 tip inserted, under light ether anesthesia, into the right carotid artery or the lower abdominal aorta (at the time the rats were killed). The cannula was tunneled subcutaneously and externalized at the nape of the neck. Three hours after recovery, with the animals unrestrained in a plastic chamber (20x9x9 cm), the catheter tip was connected to a pressure transducer (model P23X2, Spectramed, Inc., Oxnard, Calif.) to record blood pressure and heart rate for 15 consecutive minutes on a polygraph (model 7E, Grass Instruments, Quincy, Mass.). All procedures were done according to the guidelines of the Institutional Animal Care Committee.

Tissue Processing, Analytical Methods, and Statistical Analysis

Tissue processing for light microscopy and the semiquantitative procedures used to assess glomerular injury score and the extension of mesangial matrix expansion are described in detail elsewhere. The PAS-stained sections were evaluated without knowing from which rat group they had been obtained. We defined glomerulosclerosis as the disappearance of cellular elements from the glomerular tuft, capillary lumen collapse, and folding of the glomerular basement membrane with entrappment of amorphous material. We considered that the mesangium was expanded if increased amounts of periodic acid-Schiff positive material was present in the mesangial region. All glomeruli/cross-section were assessed per specimen (a minimum of 100 glomeruli per animal from the cortex and juxtamedullary area). Lesion severity was graded from 0 to 4+ according to the percentage of glomerular involvement (0-100%). Glomerulosclerosis scores and mesangial expansion scores were done separately.

All serum and urine creatinines were analyzed on a Synchro CX3 machine (Beckman Corp., Brea, Calif.). Urine protein determinations were performed by sulfosalicylic acid precipitation. Results are expressed as mean±SEM, and all comparisons between groups were carried out using the generalized linear model procedure in SAS.

Results

Table 1 shows total body weight, left ventricle weight, left ventricle/total heart weight index, right remaining kidney weight, mean intra-arterial blood pressure, heart rate, creatinine clearance/100 g weight, at the different experimental stages in the three groups. The baseline and post-nephrectomy body weights are similar in all SHR groups. The weight of the left ventricle at the end of the experiment was significantly lower in the low calorie diet group than in the hydralazine-treated (232±10 vs 302±8 g; p<0.001) or control (232±10 versus 284±10 g; p<0.001) groups.

The weight of the left ventricle at the end of the experiment was significantly lower in the low calorie diet group than in the hydralazine-treated or control groups (p<0.05 and p<0.01, respectively), but the difference disappeared when the weights were indexed to the total heart weight.

The weight of the right (remaining) kidney was similar in all SHR groups. The low calorie diet and hydralazine treatment induced a significantly reduced intra-arterial blood pressure when compared with the control group: low calorie diet group, from 204±10 to 169±10 mm Hg (p<0.05); hydralazine-treated group, from 199±8 to 156±8 mm Hg (p<0.001); and control group, from 191±10 to 204±10 mm Hg (p=NS) before and after treatment, respectively.
Creatinine clearance at the end of the experiment was significantly higher in UNX-SHR on low calorie diets than in the hydralazine-treated and control groups ($p<0.01$).

The 24-hour urinary protein excretion at the end of the experiment was lower in UNX-SHR on low calorie diet than in the control group: $14.9\pm 6.7$ versus $36.5\pm 6.7$ g/24 hr per 100 g ($p<0.05$), respectively, and the hydralazine-treated group had an intermediate value of $20.3\pm 5.6$ g/24 hr per 100 g ($p=NS$).

The glomerular injury index and the mesangial matrix expansion in the remaining kidney were lower in the low calorie diet group than in the hydralazine-treated or control groups: $14\pm 2$ and $74\pm 16$ versus $20\pm 2$ ($p<0.05$) and $114\pm 16$ ($p=NS$) and versus $21\pm 2$ ($p<0.05$) and $131\pm 17$ ($p<0.05$), respectively.

**Discussion**

In the present study, the arterial pressure of the UNX-SHR is significantly reduced with both treatments: oral hydralazine and low calorie diet without sodium or protein restriction. Our findings, however, point toward other benefits induced by the nonpharmacological approach. Low calorie diet is also efficient in protecting the kidney function and glomerular sclerosis by mechanisms independent of protein restriction.

Our control group of UNX-SHR fed a regular diet reflects the abnormalities, previously described by other investigators, that are characterized by increased urinary protein excretion, mesangial expansion, and glomerulosclerosis.

Hydralazine has direct vasodilative properties that produce a relaxant effect on the vascular smooth muscle. Previous studies have shown that when hydralazine drastically decreases blood pressure, it induces a consistent increase in renal blood flow and decreases renal vascular resistance. Studies on the effect of hydralazine on glomerulosclerosis are lacking, but triple therapy with hydralazine, hydrochlorothiazide, and reserpine in UNX-SHR was effective in normalizing systemic pressure and in reducing glomerular capillary hydraulic pressure and the hemodynamic effect that ameliorates proteinuria and renal injury.

The same triple antihypertensive cocktail, but not the single use of the vasodilator hydralazine, causes regression of cardiac hypertrophy.

In our present study, treatment with hydralazine, despite the greatest decrease in systemic arterial pressure, allowed the UNX-SHR a pattern of deterioration in renal function and glomerular injury that was intermediate between the low calorie and control groups.

Earlier studies in different animal models have described the effect of various responses to a low calorie diet on renal function and glomerulosclerosis. Bras and Ross have shown the effects of life-long dietary regimens on glomerulosclerosis induced in rats by aging that appeared to be dependent on protein and carbohydrate intake. A low calorie (carbohydrate) intake, regardless of protein intake, reduced renal disease effects characterized by an increased glomerular intercapillary structure, basement membrane thickening, glomerular scarring, and tubular dilatation. Bras and Ross also demonstrated that low protein intake reduced renal disease index, but this effect depended on the intake of carbohydrates. Davis and coworkers have shown in male Wistar rats that calorie restriction decreased the age-associated decline in renal function and increased the survival percentage at 2 years of age. Low protein intake also improved renal function but decreased survival. Everitt and coworkers, using the same animal model, arrived at a different conclusion. They have shown that acute food restriction decreased protein excretion by 40% in 1 week with no further reduction in the second week, but a high protein calorie-restricted diet increased proteinuria excretion and glomerular injury. The beneficial effect of low calorie intake on the renal lesion in Everitt's experiment, however, was most probably affected by the well-known deleterious effect induced by high protein intake.

In our study, the UNX-SHR group fed low calorie diets were protein supplemented to the same levels of consumption as the other two groups. We also increased the sodium and potassium intake of the low calorie treated animals to abolish the effects of these two factors in the final results.

The favorable effect of the low calorie diet on renal function and glomerular injury demonstrated in this investigation may be explained by different theories. An earlier study has shown that a deficient energy intake may induce the use of more protein for gluconeogenesis and less for protein synthesis; consequently, a low calorie diet may have the same consequences as low protein intake. Others believe that alterations in cholesterol disposition between the various lipoprotein fractions may be responsible for the development of glomerulosclerosis. We have not measured lipoproteins in our study, but the hypocaloric effect may have affected the gluconeogenesis and the lipoprotein fractions.

The blood pressure decrease caused by low calorie intake in our study may also affect the renal function and glomerular lesion, as previously demonstrated with low protein diet, an approach that also decreased glomerular capillary pressure and plasma flow.

A recently published study performed in 5/6 nephrectomized Fisher rats demonstrated that 40% calorie restriction without protein restriction or sodium supplementation allowed glomerular hypertrophy. The same investigators observed a lessened increase of urinary protein excretion, glomerular filtration rate, kidney weight, and kidney insulin-like growth factor I content with an improved focal interstitial fibrosis and tubular atrophy in the diet-treated than in the sham-operated rats. The low sodium intake in the latter study may be a beneficial factor that diminished the compensatory kidney growth after 5/6 nephrectomy. We abolished the influence of the kidney growth factor in our investigation by adding sodium to the low calorie diet.

The slightly reduced phosphorus content in the low calorie diet that we offered to the UNX-SHR does not exert a protective effect on the remaining kidney because low phosphorus intake without protein dietary restriction fails to protect residual function in rats with severe and progressive renal failure.

In conclusion, the present study shows that both hydralazine and low calorie diet similarly reduce systemic arterial pressure, but low calorie intake is better in retarding the progression of proteinuria and glomerulosclerosis by mechanisms independent of protein re-
striction or sodium intake. More studies are necessary to determine if this favorable effect of a low calorie diet on kidney function and glomerulosclerosis is induced by a renal hemodynamic phenomenon (decreased glomerular capillary pressure), metabolic changes induced by decreased protein synthesis, or alterations in cholesterol disposition between the various lipoprotein fractions.

**References**

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