Food Restriction Beneficially Affects Renal Transport and Cortical Membrane Lipid Content in Rats\(^1,2\)

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ABSTRACT  
Food restriction (FR) exerts a variety of beneficial effects and may prolong life in both humans and animals. However, studies of its effects on the cortical brush border membrane (BBM) and basolateral membrane (BLM) lipid concentration, which may be pertinent to renal function, have not been reported in detail. We hypothesized that FR would decrease renal work and lower renal membrane lipid concentration. The changes in lipid concentration would be most dramatic in BBM because this membrane is the entry site for the recovery of filtered ions and nutrients. Young male Fischer 344 x Brown-Norway F1 rats consumed food ad libitum (AL) or were food-restricted (FR, 60% of AL consumption) for 6 wk. AL rats had higher fractional excretions of Na\(^+\), K\(^+\), and Cl\(^-\) than did the FR group (\(P < 0.001\)). Renal Na,K-ATPase activity in AL rats was 100% higher than in FR rats (\(P < 0.001\)), reflecting greater renal work. The work required for renal proton secretion was lower in FR than in the AL rats. In FR rats, all BBM phospholipid concentrations (phosphatidylserine, phosphatidylcholine, phosphatidylethanolamine, and sphingomyelin) were \(\sim 50\%\) lower than in the AL rats (\(P < 0.001\)). In the BLM, food restriction resulted only in lower phosphatidylcholine concentration, while the other phospholipids were unaffected. Plasma and renal membrane (BBM and BLM) cholesterol concentrations were significantly lower in FR than in AL rats. These results show that a nutritionally complete, but energy restricted, diet improves renal function. It also prevents renal membrane lipid deposition and decreases plasma cholesterol. Prolonged food restriction might attenuate the renal injury that occurs in obese humans as a consequence of insulin resistance and atherosclerosis.  


KEY WORDS:  
food restriction  
renal membrane lipids  
renal function  
F344 x BNF1 rats

Several recent studies have shown that rats fed an amount averaging 60% of that consumed by rats with free access to food increases mean life span by \(\sim 50\%\) (Frame et al. 1998, Keenan et al. 1998, Sprott 1997). Such food restriction (FR)\(^+\) was also reported to maintain tissue integrity, reducing the incidence of myocardial fibrosis, some endocrinopathies, carcinomas and environmental diseases (Cornwell et al. 1991, Frame et al. 1998, Keenan et al. 1998, Sprott 1997). Plasma glucose and insulin concentrations in food-restricted rats was 15 and 50% lower, respectively, than in those with free access to food (Masoro et al. 1992). Metabolic rate was only transiently altered by energy restriction (McCartar 1989).

Although the mechanism underlying these findings is not completely understood, short-term food restriction (40%, for 8 wk) has been shown to increase the capacity for enzymatic decomposition of hydroperoxides and to decrease oxidative stress in murine kidneys (Cadens et al. 1994).

Chronic food restriction in male Fischer 344 rats (40%, from 6 wk of age) suppressed the age-associated increase in malondialdehyde production and lipid hydroperoxide formation in liver mitochondrial and microsomal membranes (Laganiere and Yu 1987). Restricting intake also modified fatty acid composition in hepatocyte membranes such that linoleic acid content was increased and docosapentaenoic acid content was decreased (Laganiere and Yu 1987). A higher unsaturation/saturation index was indicative of the membrane’s resistance to peroxidation (Laganiere and Yu 1987). Food restriction also prevented the age-induced hyperparathyroidism and decreased the incidence of chronic glomerulonephritis that occur in rats (Kalu et al. 1984). Despite these observations, studies of the effects of food restriction on renal membrane lipid metabolism as it relates to renal transport have not been examined in detail. Such studies are relevant because alterations in renal tubular membrane lipids, per se, may hasten the progression of chronic renal failure (Mackenzie and Brenner 1998, Remuzzi et al. 1997).

In the present experiments we examined the effects of 6 wk of food restriction (60% of ad libitum [AL] intake) on selected...
aspects of renal function and on cortical brush border membrane (BBM) and basolateral (BLM) membrane lipid concentration in young male Fischer 344 x Brown-Norway F1 rats (F344 x BNF1). We compared the results we obtained to those of their age-matched littermates who consumed food AL. We postulated that if beneficial changes occurred, they would be associated with a decrease in renal work, measured by a fall in cortical membrane Na-K-ATPase activity and membrane transport. We further predicted that decreases in BBM and BLM phospholipid and cholesterol concentrations would occur. Such effects should render the renal membranes more resistant to oxidative injury.

MATERIALS AND METHODS

Animals. Male Fischer 344 x Brown-Norway F1 rats were obtained at 16 wk of age from the National Institute of Aging (NIA). At 14 wk of age, the NIA randomly separated the rats into two groups: one group was allowed free access to food (AL), while the other was subjected to food restriction (90% of the AL consumption). Over the subsequent 2 wk, the amount of food provided was gradually reduced so that by 16 wk of age, the FR group consumed 60% of that consumed by the AL rats. These rats (n = 10/group) were fed their respective diets throughout the experiment (an additional 6 wk). Both the decrease in energy and the amount of food given daily by us was prescribed by weight according to guidelines provided by the NIA (Spratt and Astad 1996). The nutrient NIH-31 diet (NIH Rat and Mouse/Adult 4F; Purina Mills, Richmond, IN) was used in this study.5 The FR rats were fed 60% (11.5–12.0 g/d, 20.95–21.86 kJ/d) of the amount given to the AL rats (19.16–20.0 g/d, 34.89–36.43 kJ/d). All rats were allowed free access to tap water and were housed in the animal facility with 12 h of daylight and 12 h of darkness. The protocol was approved by the Animal Care and Use Committee at our institution.

On the last day of the diet-treatment period, the rats were placed in metabolic cages (free access to water) for a 24-h urine collection as we have described previously (Sabatini et al. 1990). On the day they were killed, the rats were anesthetized, and blood samples were obtained from the aortas for measurement of pH, pCO2, and electrolytes. The kidneys were then removed for the biochemical studies described below. Urine and plasma electrolytes were measured for calculation of renal function by using the standard methods previously described (Sabatini et al. 1990). Plasma glucose and cholesterol concentrations were determined by CIBA-CORNING, Express Plus (Medfield, MA). Urine ammonium (after conversion to ammonia) was measured by an ion analyzer (Model 255, CIBA-CORNING) and an ammonia gas-sensing electrode (Model 95–12, Orion Research, Boston, MA). Titratable acid concentration was assessed by the amount of 0.1 mol/L NaOH used to titrate 1 mL of urine from the pH 7.4.

Renal cortical membrane preparations. Vesicles from brush border and basolateral membranes were prepared from rat renal cortex by previously described methods (Eiam-Ong and Sabatini 1999a and 1999b, Grassl and Aronson 1986). In brief, the kidneys were pooled. The cortical tissue was then added to the homogenizing medium, which is 200 mmol sucrose/L, 25 mmol potassium-glucosinate/L, 2 mmol disodium EDTA/L, and 10 mmol HEPES/L; the buffer was adjusted to pH 7.6 with tetra-methylammonium hydroxide (4°C). The cortex of each kidney was first separated from the medulla, and the tissue from the two kidneys was pooled. The cortical tissue was then added to the above solution (5 mL/g kidney wt.) and homogenized with a Teflon pestle-glass homogenizer. The homogenate was centrifuged at 40,525 × g (Beckman SW 28 rotor; Fullerton, CA) for 30 min (4°C). When making BLM, a preliminary centrifugation of the homogenate at 1,000 × g (Beckman SW 28) for 10 min was performed and the pellet discarded. The supernate was then centrifuged at 47,770 × g (Beckman SW 28) for 30 min. The resulting precipitate contained the purified BLM (Eiam-Ong and Sabatini 1999a and b, Hilden et al. 1989). Average enrichment in specific activity (final pellet/initial homogenate) of γ-glutamyltransferase, a brush border membrane marker, was 10.68 ± 0.45 (n = 10), a value not different from one reported by others (Glossmann and Neville 1971). The protein concentration of BBM ranged from 9 to 13 g/L (n = 10).

BLM. The lower, brown part of the 40,525 × g pellet was discarded, and the upper part was suspended in the homogenizing solution (Eiam-Ong and Sabatini 1999a and 1999b, Grassl and Aronson 1986). This mixture was centrifuged at 45,290 × g (Beckman SW 28) for 30 min. The upper part of the pellet containing the crude membrane was resuspended in an aliquot of the homogenizing medium. A solution containing 4 g Percoll and 19 g homogenizing medium was prepared to which 2 mL of the crude membrane suspension was added. This was followed by centrifugation at 32,811 × g (Beckman Ti 50.2 rotor) for 40 min to form a gradient (4°C). The distinct upper band in the upper half of the gradient was aspirated and pooled. These pooled fractions were centrifuged at 184,048 × g (Beckman Ti 50.2) for 60 min to remove the Percoll. The purified BLM was found above a hard Percoll pellet (Eiam-Ong and Sabatini 1999a and 1999b, Grassl and Aronson 1986). Average enrichment of Na-K-ATPase specific activity, a basolateral membrane marker, was 11.59 ± 0.89 (n = 10), a value not different from that reported by others (Grassl and Aronson 1986). The protein concentration of BLM ranged from 3 to 5 g/L (n = 10).

Enzyme measurements. Na-K-ATPase activity was measured in the BLM vesicles as the difference in activity found in the presence and absence of ouabain (Sabatini et al. 1990). γ-Glutamyltransferase activity in the BBM vesicles was measured by a standard method of the International Federation of Clinical Chemistry (IFCC) (CIBA-CORNING, Express Plus). Protein concentration was measured according to the Biuret method (CIBA-CORNING, Express Plus) after precipitation with 0.612 mol trichloroacetic acid/L and hydrolyzed in 0.5 mol NaOH/L.

Lipid measurements. Total lipids were extracted from both BBM and BLM based on a method (Davison and Wajda-Spohn 1961) that we modified (Eiam-Ong and Sabatini 1999a). The pellet fractions were extracted with chloroform-methanol (2:1, v/v) filtered to remove nonlipid substances, and the filtrates were washed by partitioning between the nonlipid phase (upper, aqueous) and the lipid-containing phase (lower, chloroform rich). The proportion of solvents was maintained at four parts chloroform-methanol (2:1, v/v) mixture to one part water. This procedure, which removes the nonlipid, water-soluble compounds from the chloroform-soluble lipids, was then divided into aliquots for the determination of cholesterol, total lipids, and the subsequent separation of the individual phospholipids.

To measure cholesterol, the sample was first evaporated under
nitrogen gas, and the residue was hydrolyzed with alcoholic KOH, followed by precipitation with digitonin (0.5 g digitonin in 50% aqueous ethanol). The precipitate was resuspended in glacial acetic acid, and the cholesterol concentration was determined colorimetrically after the addition of the Lieberman-Burchard reagent (20 parts ice-cold acetic anhydride: one part concentrated sulfuric acid) (Sperry and Webb 1950).

The individual phospholipids were separated by TLC (Cuzner and Davison 1967). The nitrogen-dried lipid residues, resuspended in 20 μL of chloroform methanol (2:1, v/v), were applied as narrow streaks to Silica Gel G plates (250 μm thick; Fisher, Houston, TX). The plates were developed in chloroform/methanol/ammonia (17:7:1, v/v/v). This system results in the qualitative separation of sphingomyelin (Spm), phosphatidylcholine (PC), phosphatidylethanolamine (PE), and phosphatidylserine (PS). After development, the chromatograms were allowed to dry and then were exposed to iodine vapor. The individual phospholipids were identified by using cochromatography of authentic standards (Sigma, St. Louis, MO). The phospholipids were scraped from the silica gel plates and ashed with 9.95 mol perchloric acid/L.

Phospholipid calculations were based on the assumption that, after converting lipid phosphorus to inorganic phosphate (Pi) by ashing, each phospholipid molecule yields 1 atom of Pi (Davison and Sperry 1950). The nitrogen-dried lipid residues, resuspended in 20 μL of chloroform methanol (2:1, v/v), were applied as narrow streaks to Silica Gel G plates (250 μm thick; Fisher, Houston, TX). The chromatograms were allowed to dry and then were exposed to iodine vapor. The individual phospholipids were identified by using cochromatography of authentic standards (Sigma, St. Louis, MO). The phospholipids were scraped from the silica gel plates and ashed with 9.95 mol perchloric acid/L digestion.

**Materials.** All chemicals and reagents were obtained from Sigma Chemical and were of highest quality.

**Statistical analyses.** All the data are expressed as means ± SEM. Statistical significance of differences (P-values < 0.05) was assessed using two-tailed, unpaired Student's t-test.

**RESULTS**

**Effects of food restriction on metabolic variables.** At the beginning of the experiment, the AL rats weighed 404 ± 7 g, and the FR rats weighed 294 ± 12 g, a 28% difference (P < 0.001). On the day of killing, 6 wk later, weight gain was 3 (P > 0.05) and 8% (P > 0.05) in the AL and FR groups, respectively (Table 1). Following 6 wk of FR, this group of rats maintained a body weight lower than that of the AL rats (P < 0.001). Plasma glucose, cholesterol and blood urea nitrogen were 12, 9 and 11% lower (P < 0.05), respectively, in FR rats than in AL rats (Table 1).

Food restriction did not affect plasma sodium, potassium, chloride, bicarbonate, phosphate, calcium, magnesium, creatinine or albumin (Table 1). Arterial pH was not altered by FR (data not shown).

**Effects of food restriction on urinary excretion of ions and solutes.** Food restricted rats had a 50% lower fractional excretion of sodium, potassium, chloride as well as urinary excretion of ammonium, titratable acid, and phosphate (P < 0.001) (Table 2). By contrast, urine HCO₃⁻ excretion did not differ between groups (3.77 ± 0.55 vs. 5.42 ± 0.87 mmol/L, FR vs. AL, respectively; P = 0.12). Daily urine volume in FR rats was 66% lower than that in the AL rats (P < 0.001), whereas, urine osmolality was unaffected, presumably reflecting the same ability to concentrate urine. Interestingly, the FR rats had a 12% lower creatinine clearance (P < 0.05) than did the AL rats (Table 2).

**Effects of food restriction on BLM Na,K-ATPase activity and renal membrane lipid concentration.** Na,K-ATPase activity in the basolateral membrane of FR rats was approximately one-half that of the AL group (P < 0.001). The enzyme activity was 14.44 ± 1.05 and 26.72 ± 1.85 μmol Pi/(mg protein × h) in FR and AL rats, respectively.

In the BBM (Fig. 1), 6 wk of FR significantly reduced the concentration of all phospholipids (phosphatidylserine, phosphatidylcholine, phosphatidylethanolamine and sphingomyelin) by ~50–60% compared to the AL group (P < 0.001). In the BLM (Fig. 2), FR lowered only phosphatidylcholine concentration by 50% (P < 0.01). Renal membrane cholesterol concentration was 50–60% lower in FR rats than in AL rats, both in the BBM (0.49 ± 0.02 vs. 1.22 ± 0.03 μmol/mg protein, FR vs. AL, respectively, P < 0.001) and in the BLM (0.30 ± 0.02 vs. 0.64 ± 0.03 μmol/mg protein, FR vs. AL, respectively, P < 0.05).

**DISCUSSION**

The ability of food restriction to increase longevity and retard aging has recently been of great interest to scientists (Frame et al. 1998, Keenan et al. 1998, Sprott 1997) because a delayed onset of several diseases was documented (Cornwell et al. 1991, Frame et al. 1998, Keenan et al. 1998, Sprott 1997). Such observations have profound implications for humans, however, the mechanisms underlying these effects are not completely understood. Our study was designed to examine the potential benefits of FR on the kidney, as regards aspects of renal function and cortical brush border and basolateral membrane lipid concentrations. Our results show that the changes that occurred with FR could be exploited to delay the onset of some renal diseases observed in overfed animals. These beneficial effects may also retard the progression to uremia (Mackenzie and Brenner 1998, Remuzzi et al. 1997).

In the present study, 6 wk of FR (60%) in young F344 x BNF1 rats significantly lowered renal membrane cholesterol (BBM & BLM) and phospholipid concentrations (BBM > BLM). FR rats were not malnourished because plasma

### TABLE 1

<table>
<thead>
<tr>
<th>Variables</th>
<th>AL</th>
<th>FR</th>
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<tbody>
<tr>
<td>Body weight</td>
<td>418.90 ± 14.11</td>
<td>318.40 ± 14.12**</td>
</tr>
<tr>
<td>Glucose</td>
<td>11.09 ± 0.32</td>
<td>9.72 ± 0.47</td>
</tr>
<tr>
<td>Cholesterol</td>
<td>1.01 ± 0.02</td>
<td>0.93 ± 0.03</td>
</tr>
<tr>
<td>Blood urea nitrogen</td>
<td>5.43 ± 0.25</td>
<td>4.84 ± 0.13</td>
</tr>
<tr>
<td>Na⁺</td>
<td>138.70 ± 1.17</td>
<td>138.06 ± 0.66</td>
</tr>
<tr>
<td>K⁺</td>
<td>4.54 ± 0.18</td>
<td>4.65 ± 0.12</td>
</tr>
<tr>
<td>Cl⁻</td>
<td>107.30 ± 1.36</td>
<td>106.40 ± 0.97</td>
</tr>
<tr>
<td>HCO₃⁻</td>
<td>24.20 ± 0.52</td>
<td>24.15 ± 0.65</td>
</tr>
<tr>
<td>PO₄⁻²</td>
<td>2.41 ± 0.17</td>
<td>2.51 ± 0.01</td>
</tr>
<tr>
<td>Ca²⁺</td>
<td>2.33 ± 0.03</td>
<td>2.08 ± 0.16</td>
</tr>
<tr>
<td>Mg²⁺</td>
<td>0.87 ± 0.06</td>
<td>0.83 ± 0.03</td>
</tr>
<tr>
<td>Creatinine</td>
<td>42.43 ± 0.88</td>
<td>42.43 ± 2.65</td>
</tr>
<tr>
<td>Albumin</td>
<td>31.30 ± 0.40</td>
<td>29.80 ± 0.60</td>
</tr>
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1 Values are mean ± SEM, n = 10. Asterisks indicate significantly different than the AL group (*P < 0.05, **P < 0.001), as analyzed using Student's t-test.
Manipulation of food intake has the positive effects of decreasing the incidence, severity and progression of some renal diseases (Gumprecht et al. 1993, Keenan et al. 1995, MacConi et al. 1997). While the restricting carbohydrate, fat, and minerals decreased the incidence, severity and progression of some renal diseases (Gumprecht et al. 1993, Keenan et al. 1995, MacConi et al. 1997). SCG/Kj mice exhibit clinical and histological evidence of acute crescentic glomerulonephritis, including proteinuria and hematuria, beginning as early as 3– 4 wk of age and die as 16-wk-old young adults with severe histological evidence of acute crescentic glomerulonephritis, including activation of autoreactive T cells (Urao et al. 1995). Food restriction (68%) in SCG/Kj mice (from 7 wk of age) delayed the onset of crescentic glomerulonephritis and extended the medium of the life span by 25% compared to overfed mice (Cherry et al. 1998). While the restricting carbohydrate, fat, and minerals (except for calcium and phosphorus) by 64% retarded growth in rats, it also prevented the development of end-stage renal pathology in the remnant kidney model of chronic renal failure (21 wk postablation) (Tapp et al. 1989). This occurred independent of the amount of protein in the diet. Twenty-one albumin concentration did not differ between groups. The changes found in this study were permanent, as most are also noted in 3-y-old FR rats (Eiam-Ong and Sabatini 1999a and b).

Two months of FR (50%) in MWF rats (an animal that develops spontaneous glomerular injury) significantly decreased urinary protein excretion compared to the overfed rats (18 ± 8 mg/d vs. 104 ± 32 mg/d, respectively) (MacConi et al. 1997). Glomerulosclerosis and tubulointerstitial changes were completely absent in the FR group (MacConi et al. 1997). The potential applicability of nutritional control for the treatment of autoimmune diseases in humans was suggested. Both fasting and a long-term, vegetarian diet were reported to be successful in treating rheumatoid arthritis in some patients (Kjeldsen-Kragh et al. 1991).

Mechanisms by which energy restriction may delay the loss of age-associated immune function include modulation of the fatty acyl composition of plasma membrane lipids or alterations in the concentration of phospholipids (Venkatraman and Fernandes 1992). Spleen cell membranes from FR rats (60%) showed higher linoleic acid (18:2) levels; significant decreases of arachidonic acid (20:4), docosatetraenoic acid (22:4) in phosphatidylcholine and phosphatidylethanolamine fractions were also noted (Venkatraman and Fernandes 1992). The same membranes had more binding sites for interleukin-2 (IL-2) and insulin and enhanced IL-2 production. Such modifications in membrane lipid may facilitate binding of IL-2 and insulin to their receptors, thus improving T cell function. Food restriction (60%, from 6 mo of age) in aged rats significantly increased levels of essential fatty acids and attenuated levels of long-chain polyunsaturated fatty acids in both phosphatidyli-
choline and phosphatidylethanolamine fractions from liver mitochondrial and microsomal membranes (Laganiere and Yu 1993). Despite changes in these fatty acyls, the concentration of the major phospholipids (i.e., phosphatidylcholine, phosphatidylethanolamine or phosphatidylinositol) in the membranes did not vary significantly with diet (Laganiere and Yu 1993). These results may be relevant to our data on renal cortical membranes in that at least phosphatidylethanolamine in BLM, the site of Na,K-ATPase, did not differ. In both membranes (BBM and BLM), however, phosphatidylcholine was lower in FR rats. We did not measure the effect of FR on renal mitochondrial or microsomal membranes, thus, we do not know whether it affects phospholipid concentration. No measure of fatty acyl composition was made in the renal tissue of FR rats. In addition, a lower fat intake by FR rats may explain the reduction in renal cortical membrane phospholipid deposition. If this were the sole reason, however, we would expect the phospholipids to be depressed equally in BBM and in BLM. This did not occur in our study. The lower amount of dietary fat consumed in FR rats could more easily explain the fall in BBM and BLM cholesterol concentration, but even here, the BBM seemed the more affected (i.e., it fell 75%, whereas BLM was ~50% lower).

Sphingomyelin is noted to be higher in atherosclerotic lesions than in normal arterial tissue (Schissel et al. 1996). Ceramide, a second messenger of sphingomyelin, is an important signal for renal injury and apoptotic response (Shayman 1998). There are no studies in renal membrane sphingomyelin or phosphatidylserine concentration in response to FR. Our study documented the first evidence that FR significantly decreases sphingomyelin and phosphatidylserine concentration in renal BBM. Such a fall in the deposition of sphingomyelin may reduce the propensity of the BBM to be injured. Further experiments are required to determine whether or not this is true.

Food restriction was shown to reduce the membrane rigidity that is induced with normal aging. It was noted in aged rats that FR (60%, from 16 wk of age) limited oxy-radical production in rat brain mitochondria (Gabbita et al. 1997). The fluidity of erythrocyte membranes derived from FR rats (60%) is higher (Levin et al. 1992), thus protecting the membranes against hemolysis (Pieri et al. 1996). These protective effects may be the consequence of decreased lipid peroxidation. The lower in membrane cholesterol concentration, a maneuver that also increases membrane fluidity, and as we noted in our studies, would be beneficial to prolonged and optimum cellular function in the renal tubules, both in BBM and BLM.

Basal Ca-ATPase activity in red blood cell membranes from aged rats was shown to be significantly reduced with FR (60%, from 16 wk of age), but responses to selected other stimuli were not changed, indicating that certain enzymes were operating more efficiently (Davis et al. 1991). Our data show that FR significantly lowered renal work that is required for proton secretion by 50%. This occurred along with a fall in BLM Na,K-ATPase activity and a reduction in the fractional excretion of Na⁺, K⁺, and Cl⁻. A reduction in Na⁺ and K⁺ intake (and possibly other electrolytes) in FR rats could explain the fall in the Na,K-ATPase enzyme. We believe the enzyme to be functioning more efficiently; however, it was not, more Na⁺ and K⁺, not less, would appear in the urine. Despite a fall in membrane Na,K-ATPase activity, both AL and FR rats showed identical levels of Na⁺ and K⁺ in plasma and muscle tissues (Eiam-Ong and Sabatini 1999a). We suggest that the lower energy expenditure by the kidney may reduce the accumulation of oxidatively damaged cell components. This would decrease renal membrane destruction, because of a favorable effect on phospholipid concentration. In the BBM, this adaptive change would affect the apical transporters, such as the proton-translocating -ATPase, the Na⁺/glucose and the Na⁺/PO₄⁻ cotransporters. In the BLM, the Na,K-ATPase would be affected. In the kidney, this enzyme provides virtually all of the energy for maintaining electrochemical gradients, secondary active transport and metabolism (Burckhardt and Gerger 1992). To document that the proximal tubule is more efficient, however, studies showing increased glucose or bicarbonate re-absorption would be required. It should be noted that urine HCO₃⁻ excretion did not differ between groups, despite the reduced Na,K-ATPase in FR rats.

Our study also showed that FR rats had lower plasma levels of glucose and cholesterol as well as 25% lower body weight. These data are consistent with evidence in animals and humans (Hansen and Bodkin 1993, Kemnitz et al. 1994, Lane et al. 1995, Ruhe et al. 1996, Wallford et al. 1992, Wang et al. 1997). The lower blood glucose and decreased body weight improve insulin sensitivity (Kemnitz et al. 1994, Lane et al. 1995, Ruhe et al. 1996, Wang et al. 1997), both of which prevent diabetes mellitus (Hansen and Bodkin 1993). We have reported that blood glucose concentration and body weight in the 3-y-old FR rats did not differ from the 4-mo-old FR rats (Eiam-Ong and Sabatini 1999a and 1999b).

Prolonged food restriction produces a wide array of effects on the cardiovascular system (Herlihy and Thomas 1992). FR (50%) lowered blood pressure in rats made hypertensive either by combined nephrectomy-deoxycorticosterone acetate treatment or by abdominal aortic coarctation (Swoap et al. 1995). Recently, Overton et al. (1997) showed that FR (40%, from 55 wk of age) decreased the development of hypertension in spontaneously hypertensive rats by reducing the activity of the sympathetic nervous system. Such a fall in the sympathetic nervous system activity may account for the lower creatinine clearance noted in the FR rats in our study. Such a reduction should result in a decrease in glomerular hyper perfusion, a factor known to cause glomerulosclerosis (Mackenzie and Brenner 1998). In the present study, our FR rats were not acetonemic because of a reduction in creatinine clearance. Moreover, FR significantly lowered blood urea nitrogen concentration, indicating that the rats were not catabolic. The low blood urea nitrogen also may explain the reduced proton excretion in the FR rats. In addition, a lower protein intake may decrease creatinine clearance and phosphate excretion in the FR rats. Collectively, these observations document some of the mechanisms for the beneficial effects of FR on the kidney. That FR causes lower renal membrane lipid deposition, glomerular filtration, renal energy expenditure and plasma cholesterol are all evidence that FR should delay either the onset or the progression of renal disease.

There are no studies of food restriction as related to the preservation of renal function in humans. The beneficial effects of protein restriction on the kidney were documented (Levey et al. 1996a and b, Pedrini et al. 1996). Clinical trials of energy restriction in renal disease patients need to be examined. The results may elucidate an additional noninvasive form of therapy to prevent the progressive nephropathy of aging.

In summary, the present study provides additional data as to the benefits of food restriction on the kidney. Six weeks of food restriction, without malnutrition, in F344 x BN.F1 rats improved renal function and prevented renal membrane lipid deposition. These findings might attenuate the incidence of renal disease related to the onset of diabetes mellitus and to the progression of glomerulosclerosis in humans. Clinical trials as to the efficacy of such an approach should be performed.
Studies should be performed to determine which component of the diet; which (i.e., carbohydrate, fat, or protein), if restricted, is beneficial (Kobayashi and Venkatachalam 1992, MacConi et al. 1997).

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LITERATURE CITED


