Early Dietary Protein Restriction Slows Disease Progression and Lengthens Survival in Mice With Polycystic Kidney Disease¹

Koji Tomobe, Diana Philbrick, Harold M. Aukema, William F. Clark, Malcolm R. Ogborn, Anwar Parbtani, Hisahide Takahashi, and Bruce J. Holub²

K. Tomobe, D. Philbrick, H.M. Aukema, B.J. Holub, Department of Nutritional Sciences, University of Guelph, Guelph, Ontario, Canada
W.F. Clark, A. Parbtani, Department of Medicine, University of Western Ontario, London, Ontario, Canada
M.R. Ogborn, Section of Nephrology, Department of Pediatrics, University of Manitoba, Winnipeg, Manitoba, Canada
H. Takahashi, Laboratory Animal Center, School of Medicine, Fujita Health University, Toyoake, Japan
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ABSTRACT
The objective of these studies was to examine the effects of early dietary protein restriction on disease progression and survival in the DBA/2FG-pcy (pcy) mouse model of polycystic kidney disease. Male pcy mice of 70 days of age were fed either a normal protein (NP, 25% casein) or a low-protein (LP, 6% casein) diet for 105 days. At the end of the dietary treatment, kidney weight, kidney weight relative to body weight, and kidney water contents were almost 50% lower, and relative renal phospholipid and triglyceride contents were almost 50% higher, in mice fed the LP diet, indicating a marked reduction in the progression of cystic disease. Morphometric analyses also revealed a lower total and percent cyst area in kidneys derived from mice on the LP compared with the NP diet. There were no significant differences in final body weight, urine volume and osmolality, GFR, proteinuria, or plasma levels of protein and urea between these two groups. In a second study, it was found that all mice fed an NP diet from 70 days of age onward had died by 310 days of age, compared with a 42% survival rate in LP-fed mice at this age. Overall, the mean lifespan for pcy mice on the LP diet was 24% longer than that for those mice on the NP diet (310 ± 20 versus 251 ± 16 days; P < 0.01). These studies demonstrate that the introduction of a diet that has a reduced level of protein, yet one that provides adequate amounts of protein to prevent any signs of dietary deficiency, is effective in slowing down the progression of polycystic kidney disease in pcy mice. More important, in the long term, survival in pcy mice can be significantly improved by mice being fed the LP diet. Thus, early dietary protein restriction initiated in pcy mice before the manifestation of clinical symptoms of disease results in the attenuation of polycystic kidney disease progression.

Key Words: Polycystic kidney disease, dietary protein, survival

Autosomal dominant polycystic kidney disease (PKD) is one of the most common hereditary disorders, occurring in about 1 in 400 to 1 in 1,000 people in North America, with approximately 45% of those affected reaching ESRD by the age of 60 yr (reviewed in References 1 to 4). The considerable variation in the development of this disorder, even in people with the same genetic lesion, suggests that environmental factors strongly influence the progression of this renal disease. In addition, the observations that the development of autosomal dominant PKD is often asymmetrical (5) and that cystic kidneys often become smaller after dialysis or transplantation (6) also suggest that environmental factors play a key role in the pathogenesis of PKD.

Renal disease leading to renal failure usually follows a progressive and self-perpetuating course, in which, after a certain percentage of nephrons are lost, end-stage renal failure is inevitable. In order to delay the progression of renal disease, therefore, early intervention is important. A body of evidence already exists that suggests that dietary protein restriction may have a protective effect in established, late renal disease (reviewed in References 7 to 11). With respect to the efficacy of low-protein (LP) diets in PKD, specifically, in a study on patients with advanced PKD, Oldrizzi et al. (12) calculated that, compared with controls with PKD, the slope of reciprocal serum creatinine over an average of 3.5 yr for patients on LP diets was significantly lower than that for those on control diets. Grez et al. (13) studied a small number of PKD patients and found a markedly slower increase in serum creatinine in patients on LP diets supplemented with keto acids compared with controls. On the other hand, no evidence for a protective effect of protein restriction on late chronic renal insufficiency (including patients with PKD) was found in renal patients on LP diets for

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² Correspondence to Dr. B.J. Holub, Department of Nutritional Sciences, University of Guelph, Guelph, Ontario, Canada N1G 2W1.
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up to 2 yr (14). In contrast to these late intervention studies, no studies on the effects of early dietary intervention in human PKD have been undertaken.

We hypothesize that dietary protein restriction in the early stages of the disease would be effective in retarding the progression of PKD. In PKD, although only a relatively small number of PKD mice appear to develop cysts, cyst enlargement is considered to cause the destruction of the surrounding nephrons (15,16), resulting in hyperfiltration in the reduced number of remaining nephrons. In this way, PKD resembles renal ablation models of disease. Dietary protein may exacerbate hyperfiltration in PKD, as occurs in renal ablation models of disease (17–19).

To study the effects of dietary protein restriction on the early progression of PKD (20), we have used a murine model [DBA/2FG-pcy (pcy)] of autosomal dominant PKD (21,22). Morphometric analysis of kidneys derived from weanling pcy mice on normal protein (NP) and LP diets demonstrated that there was a significant beneficial effect of dietary protein restriction on the early progression of PKD in pcy mice. Kidney weight to body weight ratios were also lower and phospholipid to kidney ratios (in micromoles per gram) were higher in pcy mice on the LP diets compared with those on the NP diets. These parameters indicate early disease development in PKD (21–24) because kidney enlargement reflects early changes in PKD progression in humans (25,26) and in this mouse model (21) of the disease. Because there was a slight reduction in growth in these young animals, these measurements were useful for separating dietary effects on growth from those effects on the kidney specifically. This study was designed to further characterize the potential beneficial effects of early dietary protein restriction on the progression of PKD in mice that were slightly older (70 days of age) but that were still in the very early stages of the disease and exhibited no apparent clinical manifestations of disease. The effects of early dietary intervention on long-term survival were also assessed.

METHODS
Animals and Diets

The animal experimental protocol was in accordance with the Canadian Council for Animal Care guide and approved by the Animal Care Committee, University of Guelph. Male DBA/2FG-pcy (pcy) (21,22) mice were weaned at 30 days of age and housed individually in cages (23°C), humidity (60 to 65% R.H.), and light (12-h light-dark cycle)-controlled conditions. Laboratory chow and water were provided ad libitum. All experimental animals were started on semipurified diets when they were 70 days old, an age well before any clinical symptoms of renal cystic disease appear in these animals (21,22).

In Study I, 14 animals were fed isocaloric, semipurified NP or LP diets (Table 1) for 105 days. Radiolabeled inulin clearance studies were performed in conscious mice by a modification of the method of Jobin and Bonjour (27,28). Briefly, miniosmotic pumps (Alza Corporation, Palo Alto, CA) containing 25 μCi of [14C]inulin (Amersham Radiochemicals, Oakville, Ontario, Canada) were implanted in the neck region of the lightly anesthetized mice. After a 24-h equilibration period, the mice were put into metabolic cages (Med-Tech, Woodstock, Ontario, Canada) and fasted for 6 h before the urine collections were started. Urine was collected under minimal oil during the subsequent 18 h, during which time the mice were given wheat gluten cakes soaked with water sufficient for an 18-h period to prevent dehydration. At the end of the collection period, the animals were euthanized by CO2 overexposure and cardiac blood samples were collected in heparinized tubes. The [14C]inulin clearance was calculated from measurements of radioactivity present in aliquots of the plasma and urine samples as described previously (29). The remaining plasma and urine samples were kept at −80°C for routine clinical chemistry analyses.

Morphometry and Biochemical Analyses

At the time of euthanasia, the kidneys were rapidly removed, weighed, and frozen in liquid nitrogen for subsequent morphometric analyses (right kidney) and for tissue water, total phospholipid, and triglyceride contents (left kidney). Cyst area was determined from kidney sections as described previously (20).

Tissue water contents were measured as the difference between kidney wet weights and weights of the freeze-dried tissue. Lipids were extracted from the freeze-dried kidneys (29), and total phospholipid and triglyceride fractions were separated by thin-layer chromatography with heptane/isopropyl ether/acetic acid (60/40/3) as the mobile phase (30). Methyl ester derivatives of the fatty acids present in the total phospholipid and triglyceride fractions were analyzed by gas-liquid chromatography and used to quantify total phospholipid and triglyceride (31).

In Study II (survival study), 22 70-day-old pcy mice were fed the NP (N = 10) and LP (N = 12) diets, as described in Table 1, except that, in both diets, 20 g of cornstarch per 100 g of diet was replaced by 30 g of sucrose per 100 g of diet.

Statistics

All data are expressed as mean ± SE. The data in Study I were analyzed by t test. Data in Study II were analyzed by analysis of variance, with the model including effects for diet and litter (32). Differences were considered significant at P < 0.05.

### TABLE 1. Composition of experimental diets

<table>
<thead>
<tr>
<th>Ingredient</th>
<th>Dietary Group</th>
<th>NP (g/100 g diet)</th>
<th>LP (g/100 g diet)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Casein, Vitamin Free</td>
<td>25</td>
<td>6</td>
<td></td>
</tr>
<tr>
<td>Cornstarch</td>
<td>55.8</td>
<td>73.3</td>
<td></td>
</tr>
<tr>
<td>Com Oil</td>
<td>10</td>
<td>10</td>
<td></td>
</tr>
<tr>
<td>Mineral Mix</td>
<td>5.5</td>
<td>5.5</td>
<td></td>
</tr>
<tr>
<td>Choline Chloride</td>
<td>0.2</td>
<td>0.2</td>
<td></td>
</tr>
<tr>
<td>Vitamin-Methionine Mixb</td>
<td>1</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>Solka-floc (Fiber)</td>
<td>2.45</td>
<td>3.95</td>
<td></td>
</tr>
<tr>
<td>Antioxidant</td>
<td>0.05</td>
<td>0.05</td>
<td></td>
</tr>
</tbody>
</table>

* The diets were isocaloric and provided 418.2 cal/100 g.

† Provided 300 and 90 g of α-methionine per kilogram of mix for the NP and LP diets, respectively.
RESULTS

Study I

After 105 days on the diets, total kidney weights were lower by almost 50% in the mice on the LP diet compared with those on the NP diet (Table 2). These results are similar to those in our previous study, in which weanling mice were fed NP and LP diets for 90 days (20). In contrast to the previous work, there was no effect of dietary protein level on the final body weight of these mice because the animals in this study were started on the diets as adult mice (70 days of age), whereas in the previous study, weanling mice were used. On the basis of the measurements during Weeks 11 to 12 and 18 to 19, the daily intakes (per mouse) for food, energy, and protein for the NP and LP groups were 2.7 ± 0.2 and 3.5 ± 0.3 g (mean ± SE); 11.5 ± 0.8 and 14.8 ± 1.1 kcal, and 0.56 ± 0.04 and 0.17 ± 0.14 g, respectively. The fact that body weights, liver weights, and the liver weight to body weight ratios (Table 2) were not different between mice on the NP and LP diets shows that the dietary effects observed were specific to the kidney. In addition, the lower kidney weight to liver weight ratios in mice on the LP diet compared with the NP diet also demonstrated the specificity of the effect of the dietary treatment for the tissue affected by the disease.

Table phospholipid and triglyceride contents on a per-kidney basis were not significantly different in kidneys from mice on the different diets (Table 3). Relative to total weight, however, there were markedly higher levels of phospholipid and triglyceride in kidneys from mice on the LP diet compared with the NP diet. This can be explained in part by the fact that the total and relative amounts of water contained in the kidneys were lower in the mice on the LP diet (Table 4). The amount of water in the kidneys from mice on the LP diet was 46% lower compared with that in the kidneys from the mice on the NP diet (Table 4).

These data were also corroborated by the morphometric analyses, which also showed that the total section area (in square millimeters) of kidneys from mice on the LP diet was lower by 43%. The area (in square millimeters) represented by cysts was 61% lower in the kidney sections from mice on the LP diet compared with the NP diet, resulting in a 33% lower cyst area in kidney sections from mice on the LP diet (Table 4).

With respect to renal function measurements, there were no differences between the NP and LP groups for GFR, urine volume, osmolality, protein, or sodium levels. Urinary potassium levels in mice on the LP diet, however, were almost double those in mice on the NP diet (Table 5). There were no differences in plasma values for total protein or urea nitrogen between mice fed NP and LP diets (Table 5), demonstrating that protein in the LP diet was supplied at an adequate level for pcy animals at this age.

Study II

In the survival study, overall lifespan was significantly longer (by 24%) for the mice on the LP diet compared with that for those on the NP diet (310 ± 20 versus 251 ± 16 days; P < 0.01) (Figure 1). All mice on the NP diet had died by 310 days of age, compared with a 42% survival rate (5 of 12 mice still alive) in the mice fed the LP diet at this age (Figure 2).

DISCUSSION

Once a certain amount of renal damage occurs in kidney disease, the progression to renal failure continues, even if the original insult to the kidney has
TABLE 5. Plasma and urinary measurements from pcy mice fed NP and LP diets

<table>
<thead>
<tr>
<th>Measurement</th>
<th>Dietary Group</th>
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<th></th>
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</thead>
<tbody>
<tr>
<td></td>
<td>NP</td>
<td>LP</td>
<td></td>
</tr>
<tr>
<td>Urine Volume (mL/18 h)</td>
<td>5.3 ± 1.2a</td>
<td>3.6 ± 0.5</td>
<td></td>
</tr>
<tr>
<td>Osmolality (mosm/kg)</td>
<td>514 ± 42</td>
<td>536 ± 28</td>
<td></td>
</tr>
<tr>
<td>Protein (mg/dL)</td>
<td>34.8 ± 7.7</td>
<td>34.5 ± 8.9</td>
<td></td>
</tr>
<tr>
<td>Sodium (mmol/L)</td>
<td>17.2 ± 2.5</td>
<td>19.0 ± 2.3</td>
<td></td>
</tr>
<tr>
<td>Potassium (mmol/L)</td>
<td>12.7 ± 2.6</td>
<td>24.1 ± 3.2b</td>
<td></td>
</tr>
<tr>
<td>GFR (mL/min)</td>
<td>0.11 ± 0.07</td>
<td>0.10 ± 0.01</td>
<td></td>
</tr>
<tr>
<td>Plasma Total protein (g/L)</td>
<td>48.5 ± 4.3</td>
<td>51.6 ± 0.5</td>
<td></td>
</tr>
<tr>
<td>Urea (mmol/L)</td>
<td>23.0 ± 2.0</td>
<td>24.4 ± 2.7</td>
<td></td>
</tr>
</tbody>
</table>

a Means ± SE for seven animals/group.

b Significantly different from NP value (P < 0.05).

Figure 1. Lifespan of male pcy mice fed NP or LP diets from 70 days of age to death. Mice were provided either the NP (N = 10) or LP (N = 12) diets ad libitum (* P < 0.01).

Figure 2. Survival curve of male pcy mice fed NP or LP diets. Twenty-two mice of 70 days of age were provided either the NP (solid line) or the LP (dotted line) diets ad libitum. The number of animals surviving at the end of each month is shown as a percentage of total mice on each diet.

been removed. Therefore, any type of intervention in the early stages of the disease that delays this progression is highly desirable, particularly in PKD, for which there is no known treatment. We have previously demonstrated that the dietary protein restriction of weaning pcy mice was effective in attenuating the growth and enlargement of cystic kidneys (20). Beginning treatment at this early age, however, resulted in slightly compromised growth rates in the animals on the LP diets. The studies presented here (Table 4) demonstrated that it was possible to retard the growth and development of renal cysts in the pcy mouse model of PKD by initiating dietary protein restriction once the animals had reached adult weights (70 days of age) but had not yet manifested any clinical symptoms of renal disease. In these animals, body weights were not affected by the lower dietary protein levels and, together with normal serum protein values, ruled out the presence of protein deficiency in these animals. The kidney phospholipid to kidney weight (in micromoles per gram) ratio, which has been inversely correlated with the extent of cyst growth and cyst fluid accumulation (20), was higher in the LP animals (Table 3). Significantly, dietary protein reduction initiated at this early age resulted in a markedly lengthened lifespan in these animals (Figures 1 and 2).

Higher levels of dietary protein may adversely affect PKD by increasing renal hyperfiltration and perfusion. Because the number of functioning nephrons in PKD may already be reduced by cyst encroachment on surrounding renal tissue (15,16), the increased workload induced by an NP diet may shorten the functional lifespan of the remaining healthy nephrons (17,18). The reduction of functioning nephrons in PKD is analogous to the renal ablation experimental animal model, in which it has been shown that, although diets with higher levels of protein result in renal hypertrophy, the increased workload is associated with structural lesions, suggesting that this may be a maladaptive response in the long term. Although GFR values in the pcy mice used in this study appear to be lower than those in normal mice (unpublished observations), the reason for the lower values remains to be elucidated. In renal ablation models of disease, an LP diet reduced GFR, but in the long run it delays the deterioration of renal function (17,18). The same phenomenon could be occurring in the pcy mice on the LP diet and is suggested by the improved kidney parameters reported here, as well as the significantly longer survival rates in the pcy mice on the LP diet.

LP compared with NP diets may also exert their beneficial effects on PKD by decreasing renin activity (33), because abnormalities in the renin-angiotensin-aldosterone system have been documented in PKD (34-36). High-protein diets have been reported to increase plasma renin activity in studies of normal experimental rats and in studies of patients with renal disease (37-39). Another abnormality that may be exacerbated by NP diets in PKD is the increased activity
and/or reversed polarity of Na⁺K⁺ATPase (40,41), because high-protein diets have been shown to increase Na⁺K⁺ATPase activity in normal rats (42,43).

In contrast to the report by Tapp et al. (44), who found that food restriction and an accompanying growth retardation may contribute to the prevention in their remnant rat model, the lower disease progression and mortality in the pcy mice on the LP diet (6% casein), as observed here, could not be attributed to a lower food/caloric intake in this dietary group. The primary cause of death in the pcy mouse is uremia (21).

An interesting finding in this study was the higher urinary potassium concentrations in the mice on the LP diet compared with those in the mice on the NP diet. Because the renal excretion of potassium is impaired in ESRD, this finding may suggest that the renal secretion of potassium in mice on the LP diet is better preserved, leading to a higher output of urinary potassium. Whether or not pcy mice at this age are hyperkalemic, however, remains to be determined, because plasma potassium values were not determined.

In conclusion, early dietary protein reduction initiated in adult pcy mice before there were any clinical symptoms of renal disease resulted in reduced cyst growth and kidney size. In the long term, pcy mice on the LP diet lived significantly longer than their counterparts on the NP diet. If these findings can be reproduced in other models of PKD and are applicable to humans, the potential benefits of delaying renal failure by reducing protein intakes from double the nutritional requirement, as are generally consumed in the North American population, could be significant, both in terms of quality of life and economic costs.

ACKNOWLEDGMENTS

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