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LABORATORY INVESTIGATION

Effects of dietary protein restriction and oil type on the early progression of murine polycystic kidney disease

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Effects of dietary protein restriction and oil type on the early progression of murine polycystic kidney disease. A paucity of research data exists on the potential for early dietary modification to directly retard cystic growth and proliferation in polycystic kidney disease (PKD). We have therefore examined the relative effects of dietary protein levels and oil type on the progression of disease in a murine model of PKD. In the first study, weanling DBA/2FG-*pcy* (*pcy*) mice were fed either a normal (NP), 25%, or low (LP), 6%, casein diet with 10% of either sunflower seed oil (SO) (containing n-6 fatty acids), or fish oil (FO) (containing n-3 fatty acids), in a 2 × 2 design. At the end of the dietary treatment, kidney weight relative to body weight was higher in mice on the NP diets. In addition, kidney phospholipid to kidney weight (μmol/g) was lower in *pcy* mice on NP diets, indicating that the increased kidney size was largely due to increased cyst development. Replacement of dietary SO with FO resulted in alterations in renal phospholipid fatty acid compositions: 18:2 n-6, 20:4 n-6, and 22:5 n-6 were lower, and 20:5 n-3, 22:5 n-3, and 22:6 n-3 were higher in FO-fed animals. No effect of dietary lipid type on disease progression was noted, however. In a second study, morphometric analysis revealed an 11% lower percentage cyst area and a 46% lower total cyst area (mm^2) in kidney sections derived from mice on LP diets compared to NP diets. These results indicate that early dietary protein restriction in PKD prior to clinical manifestation of symptoms of the disease may have a significant impact on the pathogenesis of PKD.

There is a growing body of evidence which suggests that dietary protein restriction may have a protective effect in established, late renal disease [reviewed in 1–4]. Several large prospective trials testing this hypothesis in patients with established chronic renal failure are presently in progress. With respect to the efficacy of low protein (LP) diets in polycystic kidney disease (PKD) specifically, Oldrizzi et al [5] studied patients with advanced PKD on LP diets for an average of three and one-half years. They calculated that compared to controls with PKD, the slope of reciprocal serum creatinine over time for the patients on LP diets was significantly lower than for those on control diets. Gretz, Korb and Strauch [6] 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 to controls. Recently, Locatelli et al [7] studied renal patients on LP diets for up to two years and found no evidence for a protective effect of protein restriction on late chronic renal insufficiency, including patients with PKD. In PKD, cyst enlargement is considered to cause destruction of surrounding nephrons [8, 9], resulting in hyperfiltration in the remaining nephrons. Dietary protein may accelerate disease progression by increasing the hyperfiltration of remaining healthy nephrons, as occurs in renal ablation models of disease [10, 11, 12].

Diets enriched in fish oil (FO) containing n-3 fatty acids in human and animal forms of renal disease have also been shown to be beneficial in some, but not all, renal disorders [reviewed in 13–15]. Recently, cytokines and eicosanoids [derived from tissue arachidonic acid (20:4 n-6)] have been implicated in cystic kidney disease [16]. The formations and actions of these have been found in other studies to be affected by dietary FO containing n-3 fatty acids [13, 15, 17, 18].

No research data exist on the potential for early manipulation of dietary protein level to modify cyst growth and proliferation in PKD, particularly before the presentation of clinical symptoms or abnormal measures of renal functioning. In the present study, we have used a murine model of autosomal dominant PKD [19, 20] to study the effects of dietary manipulation [protein level and oil type (n-6 vs. n-3 fatty acids)] on the early progression of PKD. We have measured kidney weight to body weight ratios; this has been used to indicate cyst development in PKD [20–23] since kidney enlargement reflects early changes in PKD progression in humans [24, 25] and in this mouse model [23] of the disease. For example, despite considerable renal enlargement in *pcy* mice at 18 weeks of age, blood urea nitrogen is not elevated in these mice. Progressive renal insufficiency, however, develops in *pcy* mice after 18 weeks of age [23]. In addition, we have measured renal phospholipid composition (level and fatty acid profiles), as well as phospholipid to kidney ratios (μmol/g), which appear to be inversely related to the progression of PKD. To further study the effects of protein restriction on cyst growth, morphometric analysis of kidneys derived from mice on NP and LP diets were also performed. The results presented herein provide supportive evidence for a significant beneficial effect of dietary protein restriction on the early progression of PKD, regardless of oil type.

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Table 1. Composition of experimental diets

Diet ingredient	% Of diet by weight			
	NP-SO	NP-FO	LP-SO	LP-FO
Casein, vitamin-free	25	25	6	6
Cornstarch	25	25	44	44
Sucrose	30	30	30	30
Sunflower seed oil	10	1	10	1
MaxEPA oil	—	9	—	9
Mineral mix	5.5	5.5	5.5	5.5
Choline choride	0.2	0.2	0.2	0.2
Inositol	0.1	0.1	0.1	0.1
DL-methionine	0.3	0.3	0.09	0.09
Vitamins	0.03	0.03	0.03	0.03
Solka-floc(fiber)	3.87	3.87	4.08	4.08

Methods

Animals and diets

The experimental animal used was the DBA/2FG-*pcy* (*pcy*) mouse, a model of autosomal dominant PKD [19, 20]. Twenty-eight male *pcy* mice were weaned at 30 days of age and randomly divided into four groups; one mouse died of unknown causes during the course of the experiment. Mice were housed individually in a temperature (23°C), humidity (60 to 65% R.H.), and light (12 hours light:12 hours dark) controlled environment. Water and diet were provided *ad libitum*.

Study 1. Mice were fed semi-purified diets containing either a normal (NP, 25% casein) or low (LP, 6% casein) level of protein, and either sunflower seed oil (SO) rich in the n-6 fatty acid, linoleic acid (18:2 n-6), or a FO concentrate (MaxEPA, R.P. Scherer, Canada) rich in n-3 fatty acids (eicosapentaenoic acid, 20:5 n-3, plus docosahexaenoic acid, 22:6 n-3), in a 2 × 2 design. The FO enriched diet contained 1% SO to supply adequate amounts of the essential fatty, 18:2 n-6. Ethoxyquin was added as an anti-oxidant at a level of 0.05% [26]. The composition of these diets is given in Table 1. All mice were weighed at the beginning and end of the study, and feed consumption was monitored for a seven day span during the study. Blood samples taken from the orbital sinus plexus after eight weeks on study were pooled for serum albumin analysis. When the mice were 120 days of age, they were sacrificed after CO_2 anesthesia.

Study 2. For morphometric studies, weanling mice (3 per group) were fed LP and NP diets exactly as above except that corn oil (rich in 18:2 n-6) was used as the lipid source for both groups such that these diets differed only in protein level. Mice were weighed at the beginning and end of the study, and sacrificed at 120 days of age.

Biochemical analysis

In study 1, the kidneys and livers were immediately removed at sacrifice, frozen in liquid nitrogen, and stored at −80°C until further analysis. Kidney lipids were extracted [27] and total phospholipid fractions were purified by thin-layer chromatography using heptane/isopropyl ether/acetic acid (60/40/3, by volume) as the mobile phase [28]. Fatty acid methyl ester derivatives were formed from the isolated phospholipid fractions and analyzed by gas-liquid chromatography to determine their fatty acid content as previously described [29].

Morphometric analysis

Kidneys from mice in study 2 were fixed in 10% formalin. Prior to embedding in paraffin, the kidneys were bisected in the coronal plane through the hilum of the kidney. Five micron sections were stained with hematoxylin and eosin using standard techniques. Morphometric analysis was performed on a Nikon Labplot microscope equipped with a Cohu high resolution black and white video camera with a computer interface via a Visionplus board. Image analysis was performed using the densitometry and fluorometry module (IM4100) of the Image-measure program (Phoenix Biotechnology Inc., Federal Way, Washington, USA). At a object to screen magnification of 142×, tubular luminal area was measured fluorometrically over the entire kidney excluding the papilla. The screen was divided into a grid of 12 sections and fluorometric measurements taken of each section. Measurements were repeated in non overlapping sections of the kidney until the entire organ had been covered. Measurements were performed in duplicate from two alternate five micron sections taken from near the centre of the kidney. A minimum of 400 measurements were taken from each kidney.

Statistics

All data are expressed as the mean ± standard error. The data from the study 1 were analyzed by analysis of variance followed by the protected least significant differences test when interactions were present. The model included effects for protein level, oil type, and litter. Student's *t*-test was used for the study 2, using individual measurements as the base unit of study. Differences were considered significant at $P \leq 0.05$, interactions at $P \leq 0.10$ [30].

Results

Study 1

The average initial body weight for mice in all groups was 16.5 ± 0.3 g (mean ± SE, $N = 27$), with no significant differences between the four groups. At the end of the study, the overall body weights of mice on the NP diets were higher than for those on the LP diets (Table 2). FO-feeding also resulted in higher body weights compared to SO-feeding. The mice on the LP diets consumed 3.3 ± 0.2 g of diet per day, as compared to 2.2 ± 0.1 g/day for the mice on the NP diets ($P < 0.001$). There was no difference in food consumption between mice fed SO versus FO (2.7 ± 0.2 vs. 2.8 ± 0.2 g/day). The lower final body weight and increased feed consumption by the mice on the LP diets indicated that these mice may have been marginally protein deficient. Serum albumin levels, however, were in the normal range for mice on all diets (2.9 ± 0.0 and 2.8 ± 0.1 g/dl, for mice on NP and LP diets, respectively).

When kidney and liver weights were expressed in absolute values or as a proportion of body weight, both were elevated in mice on the NP diets, but oil type had no effect on these parameters (Table 2). Kidneys were enlarged to a much greater extent than livers in the mice on the NP diets as evidenced by their significantly higher kidney weight to liver weight ratios.

Total renal phospholipid and cholesterol were both elevated in mice on the NP diets; renal phospholipid was also higher in the FO-fed mice (Table 3). When renal phospholipid and cholesterol were expressed per gram of kidney, however, only dietary protein level had an effect on these two parameters. The total phospholipid to kidney weight ratio (μmol/g) was 48%

Table 2. Effect of dietary protein level and oil type on body and organ weights and ratios of *pcy* mice

	NP-SO	NP-FO	LP-SO	LP-FO	Effects[a]
Body wt. *g*	24.8 ± 0.8	28.2 ± 0.7	22.4 ± 0.8	22.6 ± 0.8	a,c
Kidney wt. *g* (total)	1.69 ± 0.17	2.32 ± 0.28	0.83 ± 0.04	0.84 ± 0.08	a
Kidney wt./body wt. *g/100 g*	6.83 ± 0.79	8.17 ± 0.85	3.74 ± 0.25	3.68 ± 0.27	a
Liver wt. *g*	1.29 ± 0.06	1.42 ± 0.05	1.01 ± 0.7	0.87 ± 0.6	a
Liver wt./body wt. *g/100 g*	5.17 ± 0.12	5.05 ± 0.18	4.47 ± 0.19	3.86 ± 0.22	a
Kidney wt./liver wt. *g/g*	1.34 ± 0.19	1.63 ± 0.19	0.86 ± 0.10	0.99 ± 0.12	b

Each value represents mean ± SE for 6 to 7 animals per group.
[a] $P < 0.001$, [b] $P < 0.01$, main effects of protein level
[c] $P < 0.05$, main effects of oil type

Table 3. Effect of dietary protein level and oil type on phospholipid and cholesterol in *pcy* mouse kidneys (both) on different diets

	NP-SO	NP-FO	LP-SO	LP-FO	Effects[a]
Phospholipid *μmol*	12.08 ± 1.12	15.02 ± 1.02	8.30 ± 0.66	8.98 ± 0.52	a,b
Cholesterol *μmol*	2.64 ± 0.12	2.90 ± 0.20	1.67 ± 0.10	1.66 ± 0.10	a
Phospholipid/kidney wt. *μmol/g*	7.34 ± 0.71	6.91 ± 0.76	10.14 ± 0.96	11.00 ± 0.70	a
Cholesterol/kidney wt. *μmol/g*	1.61 ± 0.09	1.31 ± 0.10	2.04 ± 0.12	2.02 ± 0.08	a
Cholesterol/phospholipid *molar ratio*	0.22 ± 0.03	0.20 ± 0.03	0.21 ± 0.02	0.19 ± 0.01	NS

[a] $P < 0.001$, main effects of protein level; [b] $P < 0.05$, main effects of oil type; NS, not significant.

Table 4. Effect of dietary protein level and oil type on renal phospholipid fatty acid composition in *pcy* mouse kidneys

Fatty acid	NP-SO	NP-FO	LP-SO	LP-FO	Effects[a]
16:0	21.0 ± 0.4	23.2 ± 0.3	20.5 ± 0.2	23.0 ± 0.4	d
16:1	0.2 ± 0.0	1.0 ± 0.1	0.3 ± 0.1	1.7 ± 0.2	c,d
18:0	17.9 ± 0.3	17.8 ± 0.2	19.1 ± 0.4	19.2 ± 0.3	a
18:1	7.3 ± 0.1	7.6 ± 0.4	7.3 ± 0.2	7.3 ± 0.3	NS
18:2(n-6)	10.8 ± 0.1	4.6 ± 0.2	12.0 ± 0.3	4.9 ± 0.2	b,d
20:3(n-6)	0.6 ± 0.1	0.5 ± 0.0	0.6 ± 0.0	0.5 ± 0.0	e
20:4(n-6)	21.2 ± 0.6	9.3 ± 0.2	21.5 ± 0.6	9.6 ± 0.2	d
20:5(n-3)	tr	7.2 ± 0.3[f]	tr	8.6 ± 0.2[f,g]	int
22:0	1.4 ± 0.0	1.2 ± 0.1	1.5 ± 0.0	1.3 ± 0.1	d
22:4(n-6)	1.6 ± 0.0	0.1 ± 0.0	1.5 ± 0.0	0.1 ± 0.0	c,d
22:5(n-6)	9.0 ± 0.8	0.1 ± 0.0[f]	6.3 ± 0.8[g]	0.2 ± 0.0[c]	int
22:5(n-3)	0.2 ± 0.0	1.6 ± 0.0	0.2 ± 0.0	1.7 ± 0.0	d
22:6(n-3)	3.5 ± 0.2	19.3 ± 1.1[f]	4.2 ± 0.3	15.3 ± 0.8[f,g]	int
24:0	1.8 ± 0.0	2.2 ± 0.1[f]	1.9 ± 0.0	2.2 ± 0.1[f]	int
24:1	0.8 ± 0.0	0.7 ± 0.0	0.7 ± 0.1	0.6 ± 0.0	c

Values represent mol percent of total fatty acids; tr, trace. Other minor fatty acids have been omitted from the table. Abbreviations are: NS, not significant; int, interaction between protein level and oil type.
[a] $P < 0.001$, [b] $P < 0.01$, [c] $P < 0.05$, main effects of protein level; [d] $P < 0.001$, [e] $P < 0.05$, main effects of oil type
[f] Oil effect within that level of protein, $P < 0.05$ (NP-FO vs. NP-SO, or LP-FO vs. LP-SO)
[g] Protein effect within that oil type, $P < 0.05$ (NP-FO vs. LP-FO, or NP-SO vs. LP-SO)

higher (overall) on the LP versus NP diets. The renal cholesterol to phospholipid ratio was not altered by the dietary treatments.

In general agreement with previous studies of this model [31], replacement of dietary SO with FO resulted in marked changes in renal phospholipid fatty acid composition (Table 4). FO-feeding resulted in significantly lower levels of n-6 fatty acids (including 18:2 n-6, 20:4 n-6, and docosapentaenoic acid, 22:5 n-6) and higher amounts of n-3 fatty acids (including 20:5 n-3 and 22:6 n-3) in renal phospholipid.

Study 2

In agreement with study 1, kidneys from mice on the LP diets were markedly less enlarged than kidneys from mice on NP diets. Kidney weights were 0.85 ± 0.03 g versus 1.82 ± 0.08 g (mean ± SE, $P < 0.001$), and kidney weights as a percentage of body weight were 3.74 ± 0.24 versus 7.41 ± 0.57 ($P < 0.005$), for mice on LP and NP diets, respectively. Morphometric analysis of sections obtained from these kidneys (Figs. 1 and 2) revealed that the percent cyst area was lower in kidneys obtained from mice on LP versus NP diets (47.1 ± 0.1 % vs. 53.2 ± 0.2 %, respectively, $P < 0.001$). The total cyst area per section was 46% lower in mice on LP diets compared to mice on NP diets (27.2 ± 1.2 mm^2 vs. 50.5 ± 2.9 mm^2, $P < 0.005$).

Discussion

In previous studies (unpublished observations), we compared normal control DBA/2J mice with diseased *pcy* mice on chow

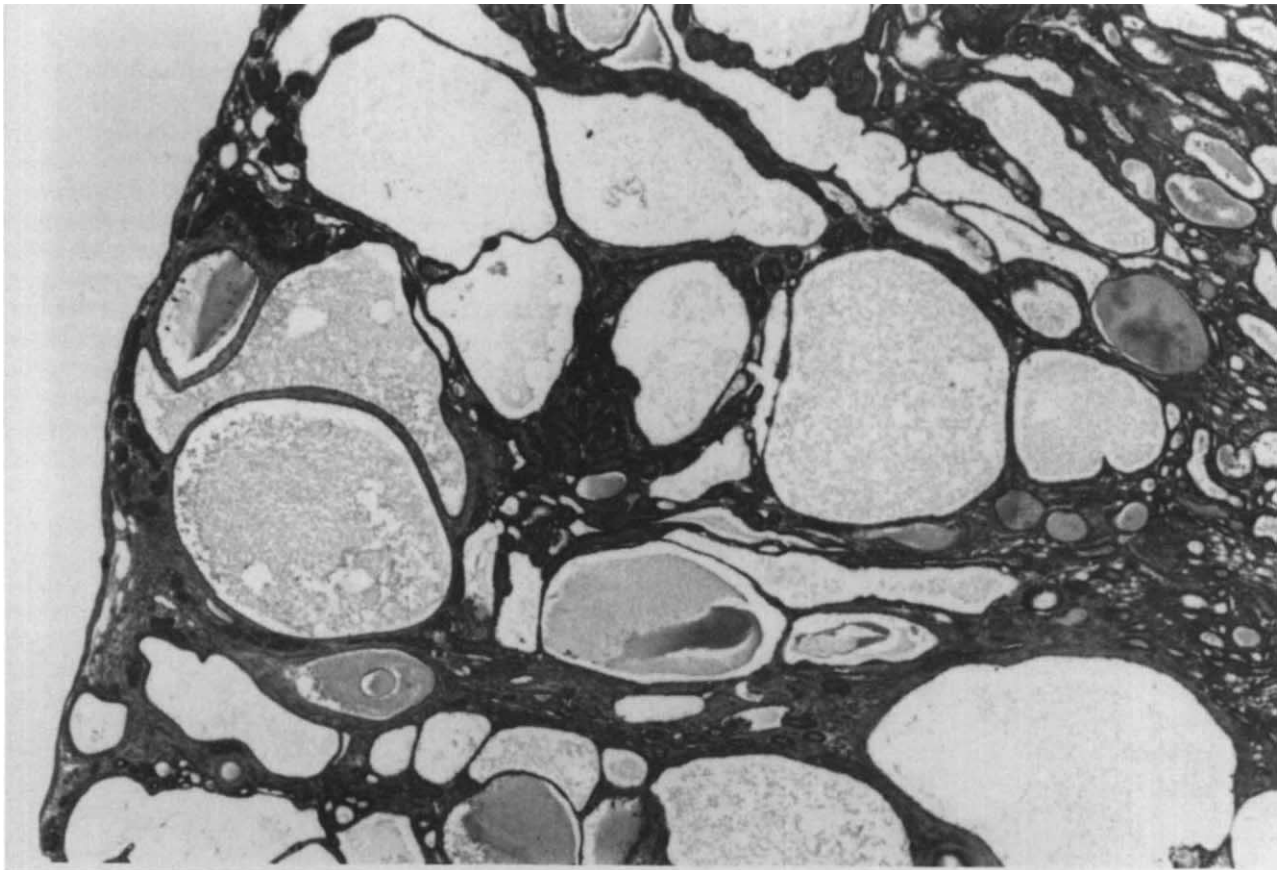

Fig. 1. *Light micrograph of kidney section obtained from* pcy *mouse on NP diet (magnification, ×45).*

diets containing 17% protein. Normal and diseased mice of 120 days of age had kidney weight to body weight ratios of 1.9 ± 0.1 and 5.3 ± 0.4, respectively, while kidney weight to liver weight ratios were 0.4 ± 0.0 and 1.3 ± 0.1, respectively. Another parameter which appears to reflect (inversely) the extent of cyst growth and cyst fluid accumulation is the kidney phospholipid to kidney weight (μmol/g) ratio. In normal versus diseased chow-fed mice of 120 days of age, kidney phospholipid to kidney weight ratios were 43.6 ± 3.3 versus 10.6 ± 1.7 μmol/g, respectively. This ratio varies inversely with cystic disease progression; as cysts enlarge, a greater proportion of the kidney is taken up by cyst fluid, thus decreasing the phospholipid (cellular membrane component) to kidney weight ratio. In the present study, we used these parameters to evaluate the progression of cyst growth in *pcy* mice, since clinical measures of renal function (serum blood urea nitrogen and creatinine) do not reveal any abnormalities at this early stage of the disease, both in this model [23] and in humans [24, 25]. Data from the morphometric analysis (showing an 11% lower percent cyst area and 46% lower total cyst area in kidney sections from mice on LP diets compared to NP diets) confirms the validity of these parameters in *pcy* mice.

Total kidney weights and kidney weights as a percentage of body weight were lower in the *pcy* mice on the LP diets. The level of dietary protein, however, also had a similar, but lesser effect on liver weights and liver weights expressed as a percentage of body weight, despite the absence of cysts in the livers. This higher liver weight (and presumably part of the increased kidney weight) on the NP diets is probably due to the fact that dietary protein has a hypertrophic effect on liver and kidney apart from disease [32]. To account for this effect, in study 1 we measured kidney weight in relation to liver weight, thus using liver weight as an internal standard. The higher kidney weight to liver weight ratios on the NP diets confirms that, in addition to increasing normal tissue growth, NP diets (compared to LP diets) also accelerate cystic growth and disease progression. Kidneys from the mice on LP diets also had significantly higher phospholipid to kidney weight ratios, confirming that these kidneys contained a smaller proportion of cysts and cyst fluid relative to normal tissue (associated with membrane phospholipid). Consistent with cholesterol being a cellular membrane component, a higher kidney cholesterol to kidney weight ratio was also found in *pcy* mice on the LP diets.

Compared to LP diets, NP diets may adversely affect PKD by increasing renal hyperfiltration and perfusion. Since the number of functioning nephrons in PKD may already be reduced by cyst encroachment on surrounding renal tissue [8, 9], the increased workload induced by NP diets may shorten the functional lifespan of the remaining healthy nephrons [10, 11]. 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

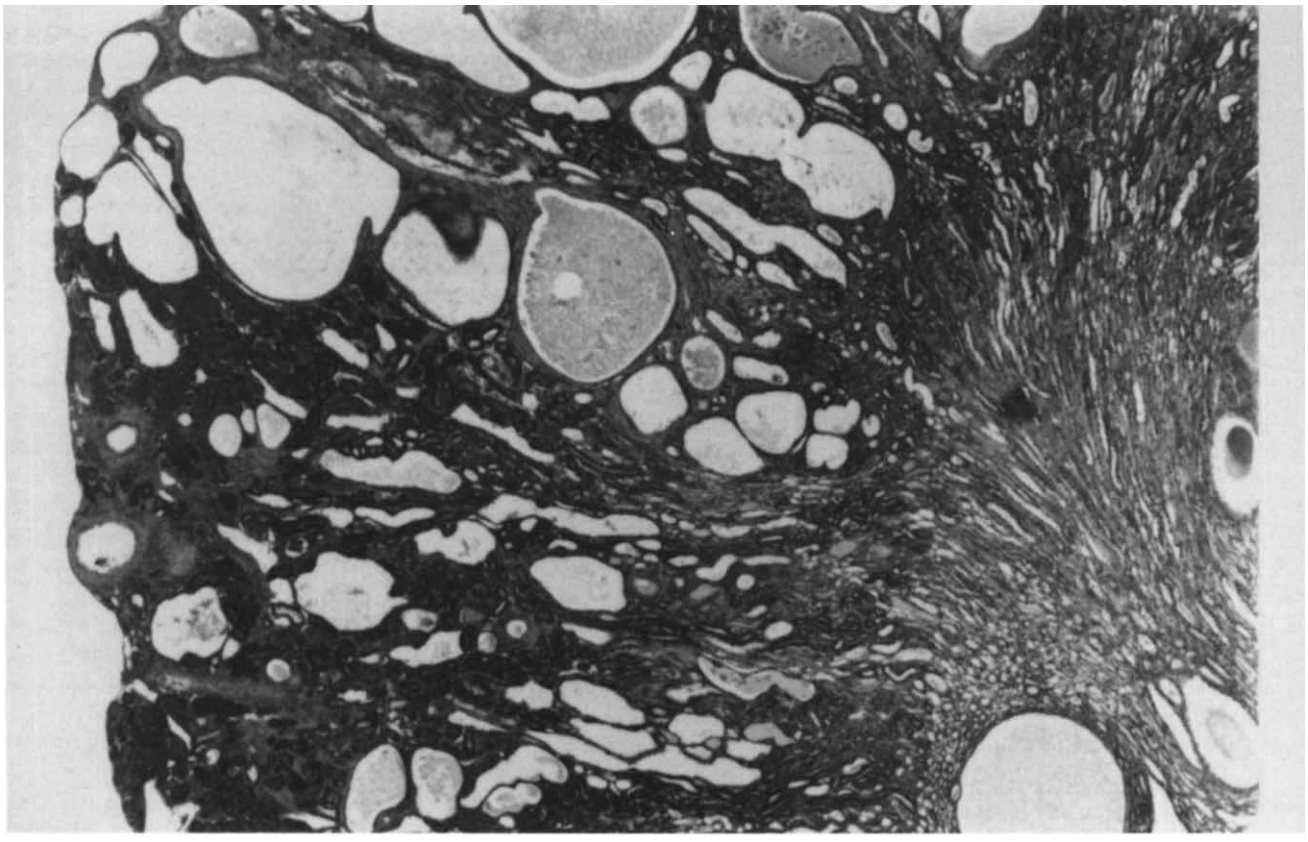

Fig. 2. *Light micrograph of kidney section obtained from* pcy *mouse on LP diet (magnification, ×45).*

hypertrophy, the increased workload is associated with structural lesions, suggesting that this may be a maladaptive response in the long term. NP diets may also exert their deleterious effects on PKD by increasing renin production, since abnormalities in the renin-angiotensin-aldosterone system have been documented in PKD [33–35]. High protein diets have been reported to increase plasma renin activity in studies of normal experimental rats and in studies of patients with renal diseases [36–38]. Another abnormality which may be exacerbated by NP diets in PKD is the increased activity and/or reversed polarity of Na^+K^+-ATPase [39, 40], since high protein diets have been shown to increase Na^+K^+-ATPase activity in normal rats [41, 42].

We found no protective effect of dietary FO on the early progression of PKD in study 1. An earlier study with *pcy* mice in which dietary treatment was initiated a month later and for a shorter time period indicated that FO diets may have some moderate beneficial effects [31]. Replacing dietary SO with FO from weaning to death, however, did not improve survival in *pcy* mice [43]. These studies suggest that if there is any beneficial effect of dietary FO in *pcy* mice, it may be limited to a small time period in the course of the disease. With respect to the fatty acid alterations in renal phospholipid, of note was the dramatic replacement of the 22-carbon n-6 fatty acids, adrenic acid (22:4 n-6) and docosapentaenoic acid (22:5 n-6), and the reduction by more than 50% of 18:2 n-6 and 20:4 n-6, by the n-3 fatty acids, 20:5 n-3, docosapentaenoic acid (22:5 n-3), and 22:6 n-3, with dietary FO relative to SO. The role of eicosanoids in the development of PKD in *pcy* mice appears not to be important since protein restriction altered disease progression, but did not affect the level of available eicosanoid precursor as 20:4 n-6, while oil type did affect 20:4 n-6 levels, but not the early progression of PKD. Increased levels of PGE_2 have been reported in renal cyst fluid derived from patients with PKD [16], but the relative formation of eicosanoids (thromboxanes, prostaglandins, leukotrienes) remains to be studied in this disease.

In conclusion, early dietary protein restriction appears to retard cyst development and disease progression in this murine model of PKD. It is possible to markedly alter renal phospholipid fatty acid compositions by replacing dietary SO with FO, but these alterations did not affect the early progression of cystic disease in *pcy* mice. These results indicate that very early dietary protein restriction in PKD prior to clinical manifestation of the disease may have a significant impact on the pathogenesis of PKD.

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