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Dietary Soy Protein Effects on Inherited Polycystic Kidney Disease Are Influenced by Gender and Protein Level

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Abstract. The effects of dietary soy protein compared to casein were examined in male and female CD1-*pcy*/*pcy* (*pcy*) mice with polycystic kidney disease. Animals 10 wk of age were fed purified diets containing either soy protein isolate or casein given at a level of 17.4 or 6% protein. After 13 wk on the diets, body weights and serum concentrations of albumin and protein indicated that protein nutrition was adequate on all diets. Overall, animals fed soy protein *versus* casein had 28% lower ($P = 0.0037$) relative kidney weights (g/100 g body wt), 37% lower ($P = 0.0089$) cyst scores (% cyst area × relative kidney weight), and 25% less ($P = 0.0144$) kidney water (g). Dietary protein reduction resulted in 30% lower ($P = 0.0010$) relative kidney weights, 25% lower ($P = 0.0327$) cyst scores, and 35% less ($P = 0.0001$) kidney water. Analysis of interactions between main effects revealed that the effects of soy protein on kidney size were significant only in females, and that effects of soy protein on cyst score were significant only in animals on the low protein diets. In addition, differences in kidney weights and cyst score due to protein reduction were significant in animals fed soy protein, but not in those fed casein as the protein source. These results show that both dietary protein source and level significantly affect polycystic kidney disease in *pcy* animals, with the effects of dietary soy protein being most pronounced in female animals fed the low protein diets and the effects of protein reduction being most pronounced in animals fed soy protein-based diets.

Autosomal dominant polycystic kidney disease (ADPKD) occurs in 1 in 400 to 1 in 1000 people in North America, making it one of the most common genetic diseases and the most frequently inherited nephropathy. The genetic locus responsible for approximately 85% of cases is found on chromosome 16 (PKD1) and for most other cases on chromosome 4 (PKD2). A third locus also has been reported (reviewed in references (1–3)). The considerable variation in the development of this disorder, however, even in related individuals with the same genetic lesion, suggests that modifier genes and/or environmental factors also influence the progression of this disease.

The progression of cystic renal disease in many animal models of PKD can be altered by adding specific non-nutritive compounds to the diet or by manipulating the constituents already present in the diet. Compounds added to the diet that ameliorate PKD in animal models include enalapril, losartan, methylprednisolone, and HCO_3^- (4–6), whereas NH_4Cl and NaCl (4,6,7) worsen the disease. Dietary restriction of K^+ accelerates PKD, while restriction of NaCl or protein delays its progression (4,7–10). Of these dietary modifications, reduction in protein content is significant because it is a dietary manipulation that can reduce the development of renal cysts and prolong survival without negatively affecting body growth (8,9). Studies in animal models of PKD indicate that reduced dietary protein intake early in the disease process significantly retards the progression of disease (7–10), suggesting that reduction of protein intake in patients with nonazotemic PKD may be a very important mode of treatment for this disorder. With the advent of the potential for genetic screening for PKD, positive diagnosis can be made long before any apparent deterioration in renal function occurs; this would allow time for appropriate diet therapy to have an impact on disease progression.

Another potential therapeutic approach to PKD involves altering the type of dietary protein. Two recent reports indicate that dietary soy protein reduces renal cyst growth in animal models of PKD (11,12). Plant protein derived from soybean may have unique effects on this renal disease, as soy-based diets have been shown to have beneficial effects on the kidney in humans (13–15) and in some animal models of renal diseases (16,17). Soy protein contains phytoestrogens; these may influence the steroid hormone balance, which appears to play a role in some models of PKD (18–20).

Previous studies on dietary protein intake effects in animal models of PKD have used male animals only. Although some dietary treatments affect disease progression similarly in males and females, others have invoked gender-specific responses (7,21). In humans with PKD, there appears to be a gender-related difference in the progression of the disease, with females succumbing to renal failure approximately 5 yr later than males (22–25), although this finding is not universal (26).

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Disease progression in the Han:SPRD-*cy* rat model of PKD also is influenced by gender and steroid hormones (19,20), suggesting that hormonal influences play a role in the progression of this disease.

The studies described herein tested the hypothesis that altering dietary protein level and protein source has different effects on disease progression in male and female animals. We examined the effect of dietary soy protein in conjunction with the effect of protein restriction (to a low, yet growth-maintaining level) early in the disease process in male and female CD1-*pcy*/*pcy* (*pcy*) mice, an animal model of PKD. These studies indicate that gender and dietary protein source are important factors in the ability of reduced dietary protein intake to retard the progression of PKD.

Materials and Methods

Animals

The animal experimental protocol was in accordance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals and was approved by the University Animal Care and Use Committee. Breeding pairs of the *pcy* mouse model of PKD (27,28) were obtained from V. H. Gattone II, University of Kansas Medical Center. Male and female *pcy* mice were weaned at 4 wk of age and housed in temperature- (22 to 24°C), humidity- (50 to 60% relative humidity), and light- (12-h light/dark cycle) controlled conditions.

Diets and Experimental Protocol

At weaning, all experimental animals were provided the (control) AIN-93G rodent diet (29) and were maintained on this diet until 10 wk of age. Food and water were consumed *ad libitum*. All diet ingredients were purchased from Dyets, Inc. (Bethlehem, PA). At 10 wk of age, a time point well before any clinical symptoms of renal cystic disease appear in this model of PKD (26), a total of 48 animals were placed on one of four isocaloric diets. These diets contained either 17.4 g protein per 100 g diet (normal protein, NP) or 6 g protein per 100 g diet (low protein, LP); protein was derived from either casein or soy protein isolate. Six male and six female animals were placed on each diet, resulting in a 2 (protein levels) $\times$2 (protein sources) $\times$2 (genders) study design. The diet with the NP level in which casein was the protein source is the (control) AIN-93G rodent diet; details of this diet have been published by the American Society of Nutritional Sciences Committee on Rodent Diet Composition (29). The other diets were identical except that casein was replaced with an equivalent amount of protein from heat-treated soy protein isolate in the soy-based diets, and protein was replaced by an equivalent amount of carbohydrate in the LP diets to maintain equal energy density of the diets. Notably, phosphorus levels were similar in both protein sources, resulting in similar levels of phosphorus in casein- and soy-based diets (calculated to be 3504 and 3492 mg/kg in the casein- and soy-based NP diets, respectively). Animals were fed the experimental diets for 13 wk and weighed biweekly. Food and water intake were determined during weeks 6 and 10 of this feeding period. During each of these weeks, food intake was determined for three successive 48-h periods. Food cups containing fresh food each time were weighed at the beginning and end of each 48-h period. Food intake was calculated by determining the difference in food cup weights, adjusting for any spillage that occurred. Water intake was calculated during these weeks by measuring the difference in water bottle weights at the beginning and end of the weekly change of water. Animals were killed at the end of the feeding period, serum was collected, and kidneys and livers were removed, weighed, and immediately frozen in liquid nitrogen.

Laboratory Analyses

The left kidney was lyophilized to determine water content. The right kidney was fixed in alcoholic Bouin's reagent and embedded in paraffin blocks. Sections (4 μm) were mounted on slides and stained with periodic acid-Schiff for visualization. Morphometric analyses of randomly selected sections were performed using an Olympus BX60 microscope equipped with a Dage MTI CCD72 camera with a computer interface. A trained, blinded observer performed the image analysis using Microcomputer Imaging Device software (Imaging Research, Inc., St. Catharine's, Ontario, Canada). At an object-to-screen magnification of $\times$470, cyst area was determined from non-overlapping fields until the entire kidney section was covered. To give an estimate of cyst volume, cyst area was multiplied by right kidney weight divided by body weight; this was referred to as cyst score. Total serum protein was determined by the method of Bradford (30), and serum albumin was measured using Sigma kit 631 reagents (Sigma, St. Louis, MO). Serum urea nitrogen (SUN) and creatinine concentrations were determined using reagents from Sigma kits 640 and 555, respectively.

Statistical Analyses

Data were analyzed by ANOVA of a $2 \times 2 \times 2$ design. Normality of the data was tested using the Shapiro–Wilk's W test. If necessary, data were normalized by logarithmic transformation. Data are reported as untransformed means $\pm$ SEM. Differences and interactions were considered significant at $P < 0.05$. Where interactions were significant, contrasts were used to determine differences between simple effects.

Results

Protein Source Effects

The dietary source of protein had a significant effect on the size and fluid content of the total kidney and the proportion of the kidney that was occupied by cysts. Overall means for protein level, protein source, and gender effects are shown in Tables 1 and 2, and individual group means are shown in graphs of relative kidney weight (Figure 1), cyst score (Figure 2), kidney water content (Figure 3), and SUN (Figure 4). In all animals fed diets containing soy protein isolate as the protein source, kidney weights were 28% lower than in all animals consuming diets containing casein as the protein source. Kidney weights expressed relative to body weights also were 28% lower in animals fed diets containing soy protein compared with casein. The main effect of protein source for both absolute and relative kidney weight was highly significant. However, because there was a significant interaction between protein source and gender effects, simple effects were analyzed. Figure 1 shows that the effect of soy protein on kidney size was significant only in female animals.

In addition to having less enlarged kidneys, the area of the kidney occupied by cysts was 19% lower in animals fed soy protein compared with casein. Examples of kidney sections for each treatment are shown in Figure 5. Cyst score also was calculated by multiplying cyst area by the right kidney weight relative to body weight to give an estimate of cyst volume. Overall, cyst score was 37% lower in animals fed diets con-

Table 1. Overall effects of dietary protein level, dietary protein source, and gender in *pcy* mice

Parameter	Protein Level			Protein Source			Gender			SEM
	Normal	Low	*P* Value	Casein	Soy	*P* Value	Male	Female	*P* Value	
Body weight (g)	37.0	35.5	0.2898	36.9	35.6	0.2907	38.5	34.0	0.0009	0.88
Kidney weight[a] (g)	1.44	0.98	0.0002	1.41	1.01	0.0009	1.41	1.01	<0.0001	0.10
Kidney weight/body weight[a] (g/100 g)	3.94	2.75	0.0010	3.89	2.81	0.0037	3.68	3.02	0.0019	0.20
Cyst area (% of section)	28.3	26.0	0.3380	30.0	24.3	0.0239	27.9	26.3	0.5107	1.7
Cyst score[b,c]	0.59	0.44	0.0327	0.63	0.40	0.0089	0.60	0.44	0.0154	0.07
Kidney water (left only) (g)	0.543	0.351	0.0001	0.510	0.384	0.0144	0.523	0.372	<0.0001	0.041
Food intake[d] (g/d)	3.45	3.90	0.0096	3.57	3.78	0.1383	3.81	3.55	0.0831	0.09
Water intake[d] (g/d)	4.41	3.20	0.0046	3.65	3.96	0.3529	3.71	3.90	0.5611	0.22

[a] Interaction between gender and protein source, $P < 0.05$.
[b] Cyst score is calculated as % cyst area × right kidney weight/body weight.
[c] Interaction between protein level and protein source, $P < 0.05$.
[d] Average of weeks 6 and 10 of study.

Table 2. Overall effects of dietary protein level, dietary protein source, and gender on serum measures in *pcy* mice

Parameter	Protein Level			Protein Source			Gender			SEM
	Normal	Low	*P* Value	Casein	Soy	*P* Value	Male	Female	*P* Value	
Serum creatinine (mg/dl)	0.38	0.40	0.3170	0.41	0.37	0.2028	0.41	0.37	0.0859	0.02
Serum urea nitrogen (mg/dl)	30.7	22.9	<0.0001	29.5	24.1	0.0002	27.1	26.5	0.3855	1.0
Serum protein ($\mu g/\mu l$)	51.1	49.4	0.3699	49.5	51.0	0.4586	49.5	51.0	0.4313	1.4
Serum albumin (g/dl)	3.17	3.16	0.8175	3.07	3.26	0.2884	3.01	3.32	0.1484	0.13

taining soy protein compared with casein. However, there was a significant interaction between protein level and protein source. Simple effect analysis of this parameter revealed that the effect of soy protein was significant only in animals on the LP diets (Figure 2). Kidney water content was 25% lower in animals on the soy protein diets, suggesting less accumulation of cyst fluid. When expressed relative to total body weight (mg/100 g), the kidney water content also was significantly lower (by 24%) in soy protein-fed animals (data not shown). Although there were no significant interactions among any of the main effects for kidney water, data on the individual group means (Figure 3) had similar patterns to individual group means for relative kidney weight (Figure 1) and cyst score (Figure 2), indicating that soy protein effects were greatest in females and in animals on the LP diets.

Serum creatinine values were not different in animals fed these two protein sources, indicative of the fact that this measure of renal function is not altered in *pcy* animals at this age (our unpublished observations). SUN values were 18% lower in all animals fed the soy protein compared with animals fed the casein-based diets. At the end of the dietary treatment, body weights, and serum protein and albumin concentrations were similar in animals fed casein or soy protein diets, demonstrating that the quality of the soy protein was adequate to maintain growth and provide adequate protein nutriture. Food and water intakes also were similar in animals fed both sources of protein, indicating that the level of protein intake was similar in casein- and soy-fed animals.

Protein Level Effects

Dietary protein reduction significantly affected renal size, cyst score, and fluid content in *pcy* animals (Table 1). After 13 wk on the diets, kidneys were 32% smaller in animals on the LP diets compared to those on the NP diets. Kidney weights expressed relative to body weight were 30% lower in animals on the LP diets. With respect to cyst score estimates, kidneys from animals on the LP diets had a 25% lower cyst score than kidneys from animals on the NP diets. Simple effect analysis, due to interactions between main effects (see above), revealed that protein level effects on kidney size and cyst score were significant in the soy-fed animals, but not in those fed casein (Figures 1 and 2). The amount of kidney water also was lower in animals on the LP diets, indicating less accumulation of cyst fluid.

SUN concentrations were 25% lower in animals on the LP diets compared to those on the NP diets. Although food intake was higher in the animals on the LP compared with NP diets, the total amount of protein consumed was still 61% lower than the quantity consumed by the animals on the NP diet (23.4 g/d compared with 60.0 g/d). Despite the lower protein intake, at the end of the dietary treatment, body weights and serum protein and albumin concentrations were similar in animals on

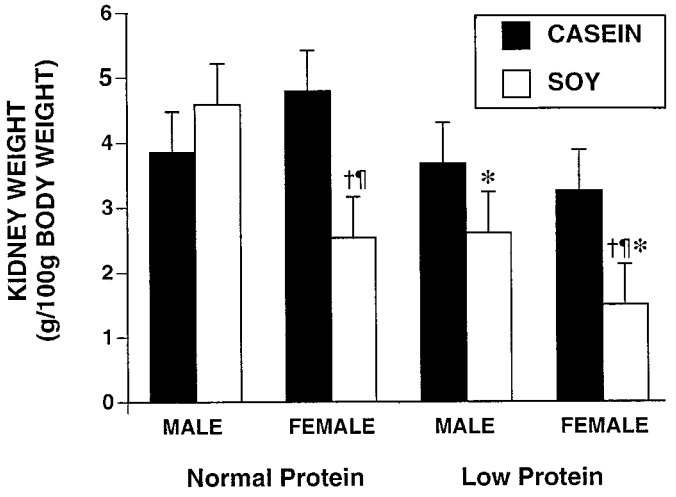

Figure 1. Effects of protein source, protein level, and gender on kidney size (g/100 g body wt) in *pcy* mice. There was a significant interaction between gender and protein source effects. $^{\dagger}P < 0.05$, protein source effect: significantly different from casein-fed females given the same level of protein. $^{*}P < 0.05$, protein level effect: significantly different from soy protein-fed animals of the same gender. $^{\P}P < 0.05$, gender effect: significantly different from soy-fed males given the same level of protein.

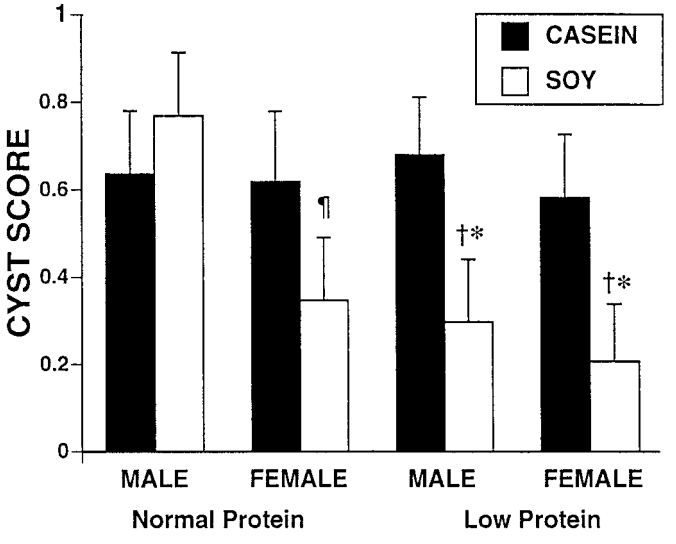

Figure 2. Effects of protein source, protein level, and gender on cyst score (% cyst area × right kidney weight/body weight) in *pcy* mice. There was a significant interaction between gender and protein source effects. $^{\dagger}P < 0.05$, protein source effect: significantly different from casein-fed animals of the same gender given the same level of protein. $^{*}P < 0.05$ for males, $P = 0.07$ for females, protein level effect: significantly different from soy protein-fed animals of the same gender. $^{\P}P < 0.05$, gender effect: significantly different from soy-fed males given the same level of protein.

the NP and LP diets, indicating that the animals on all diets obtained adequate dietary protein. Serum creatinine values were also similar in animals on the NP compared with the LP diets. Water intake was 27% lower in the animals on the LP diets.

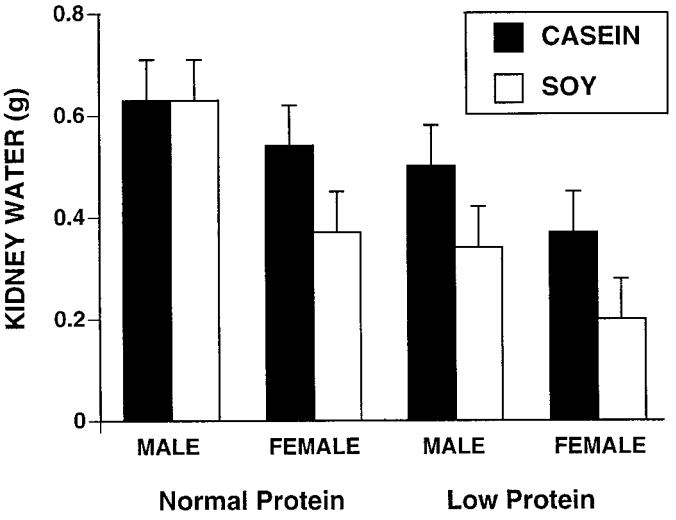

Figure 3. Effects of protein source, protein level, and gender on kidney water content (g) in *pcy* mice. Main effects of protein source, protein level, and gender are significant. For significance levels, see Table 1.

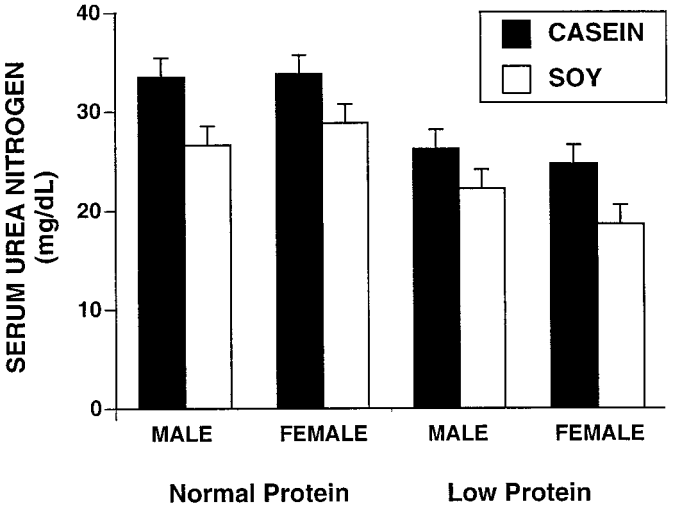

Figure 4. Effects of protein source, protein level, and gender on serum urea nitrogen concentrations (mg/dl) in *pcy* mice. Main effects of protein source and protein level are significant. For significance levels, see Table 2.

Gender Effects

Kidney size, cyst score, and water content also were affected by gender in the *pcy* mice in this study (Table 1). However, there were interactions in kidney size and cyst score analyses (as indicated above), and simple effect analyses revealed that the gender differences in kidney size and cyst score were present only in soy protein-fed animals (Figures 1 and 2). Kidney water content was 35% lower in female compared with male animals. At the end of the dietary treatment, SUN, serum creatinine, serum protein, and serum albumin concentrations were similar in male and female animals. Food and water intakes also were similar.

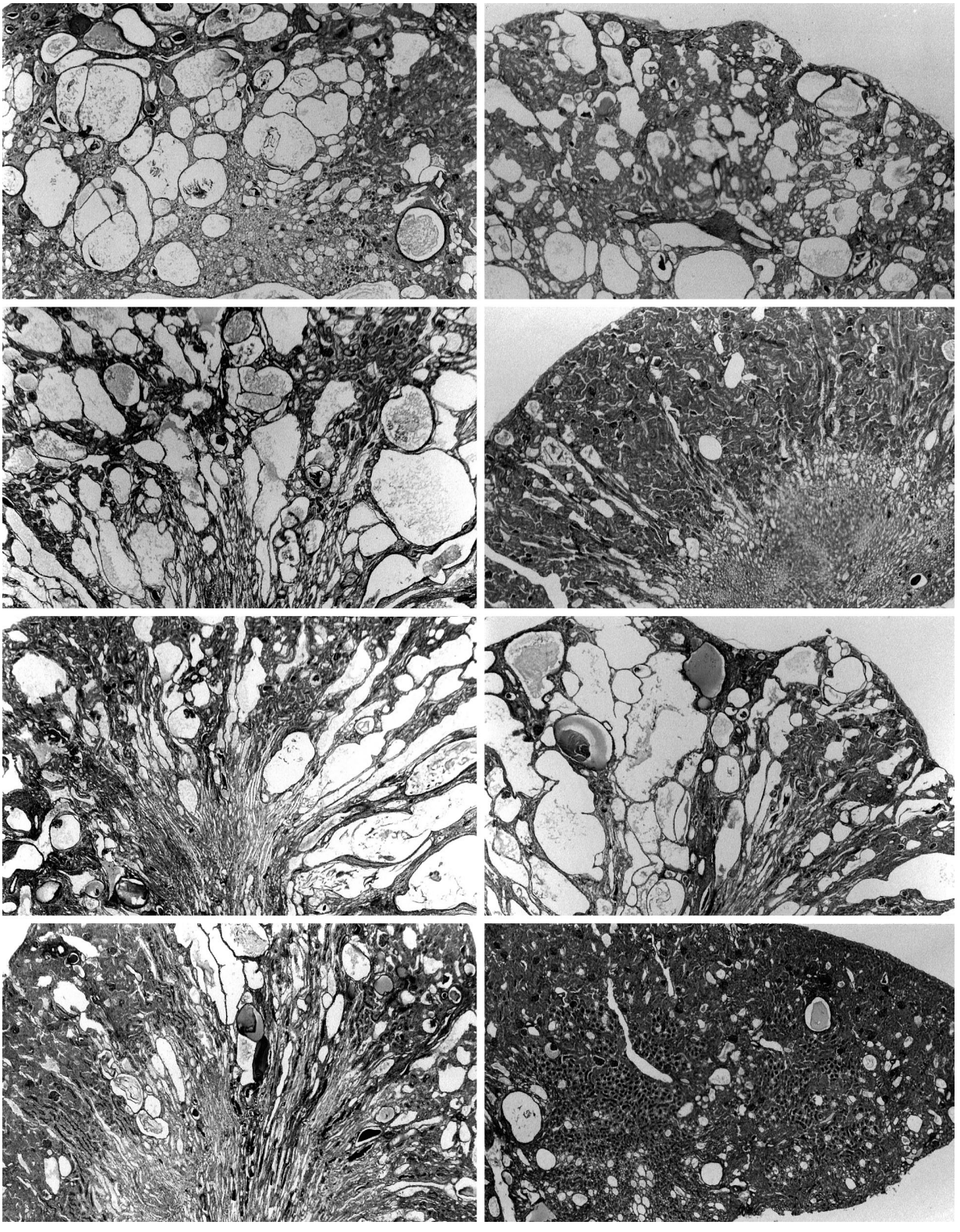

Figure 5. Four-micrometer kidney sections (periodic acid-Schiff, ×25) from male (A through D) and female (E through H) CD1-*pcy*/*pcy* mice. (A and E) Casein-fed animals on the normal protein diets. (B and F) Casein-fed animals on the low protein diets. (C and G) Soy-fed animals on the normal protein diets. (D and H) Soy-fed animals on the low protein diets.

Discussion

Reducing dietary protein intake has been shown to retard the progression of PKD in the *pcy* mouse and in the Han:SPRD-*cy* rat and lengthen survival in the *pcy* mouse (7–10). The present study suggests not only that protein reduction is beneficial in female *pcy* mice, but also that the effects of dietary soy protein

on kidney and cyst size may be greater in female *pcy* animals. Other dietary manipulations that influence the progression of PKD differently in male and female animal models have been reported. Substituting dietary fish oil for sunflower seed oil reduced the life span of male *pcy* mice, but had no effect on their female counterparts (21). In Han:SPRD-*cy* rats, NH_4Cl loading had a greater impact on relative kidney weights and SUN concentrations in female compared with male animals (7).

For humans, adherence to a low protein dietary regimen while maintaining sufficient energy intake can be difficult, but is achievable with only modest changes in the level of dietary satisfaction (31). Many individuals choose to follow vegetarian eating patterns or use specialty low protein products to maintain a reduced level of dietary protein. Potentially beneficial effects of dietary soy protein have been shown in normal and diseased kidneys in humans (13–15), as well as in animal studies (16,17). In addition, long-term feeding of dietary soy protein compared with casein increases life span and decreases renal pathology in the rat (32). The beneficial effect of soy protein on renal enlargement and cyst changes observed in this study therefore is of interest. These findings are consistent with recent studies with male animal models of PKD (11,12). A study using the *pcy* mouse showed that dietary soy protein compared with casein reduced relative kidney size in male mice when given soy protein at a level of 12% of the diet (11). In the present study, dietary soy effects on kidney size in males were significant in the animals fed 6% protein (LP diets), but not in the animals fed 17.4% protein (NP diets), indicating that dietary soy effects may be greater when the level of protein consumed is closer to the dietary requirement. In a study using the male Han:SPRD-*cy* rat, in which a protective effect of soy protein was demonstrated, animals were younger and the level of protein also was closer to the dietary protein requirement for the growing rat. Taken together, these studies indicate that the beneficial effects of the soy protein diet are diminished when the level of dietary protein exceeds the dietary protein requirement by a significant amount.

The mechanism(s) by which soy protein retards kidney enlargement and cyst growth are unclear. The true digestibilities of casein and soy protein are both high (97 to 98%), so N intake on these two diets is similar (33). In addition, the soy protein was heat-treated to inactivate soybean trypsin inhibitor activity. The AIN-93G diet that was used in this study also includes supplementation with sulfur amino acids (29), since both casein and soy protein are relatively deficient in these amino acids. In dietary studies, it is often desirable to include a pair-fed group. However, in agreement with a study using male *pcy* mice fed soy protein (11), food (and caloric) intake was not different in casein-fed compared with soy-fed animals. A pair-fed group given the same amount of food as was consumed by the soy-fed animals, therefore, would be identical to the casein-fed (control) group.

SUN levels in *pcy* mice do not increase above normal levels until later in the progression of cystic disease in these mice. An earlier study that characterized the *pcy* mouse model reported that SUN values (mg/dl) were marginally higher at 18 wk of age (28 ± 2.5) and markedly higher at 30 wk of age (122 ± 33.4) in DBA/2-*pcy/pcy* mice compared with normal DBA mice at these ages (22.5 ± 1.5), all given laboratory chow diets (27). The *pcy* animals given purified diets in the present study were 23 wk of age at sacrifice, and SUN values in the groups of mice ranged from 19 to 34 mg/dl. The lower SUN values in mice on the soy protein diets indicate a delay in the progression of the disease at this relatively early time point in the disease process. Although the lower SUN values in animals on the LP diets may be explained by lower N intakes, this does not explain the lower SUN values in the animals fed soy protein compared with casein. Differences in SUN levels between groups were consistent with changes in cyst score, renal size, and water content in all groups, except the males fed the NP diets. This suggests that other factors (which remain to be elucidated) in addition to renal function also may influence SUN levels. Lower SUN values in soy- compared with casein-fed animals are consistent with recent studies with *pcy* mice (11) and Han:SPRD-*cy* rats (12). In contrast to the study with Han:SPRD-*cy* rats, however, serum creatinine levels were not different in *pcy* mice fed soy compared with casein in both the previous study (11) and in the present study. This is likely due to the fact that serum creatinine levels are altered in Han:SPRD-*cy* rats at the age studied (12), but are not altered in *pcy* mice compared with healthy animals at the ages examined in this study and in the previously published study (11).

The estrogen-like compounds present in soy may be involved in the mechanisms by which soy exerts its effects on disease in this model of PKD and may help explain why effects in females are more prominent. Soy products contain isoflavonoids, including genistein and daidzein, which have estrogenic activity (34,35). Steroid hormone effects on PKD are implicated, as renal failure occurs approximately 5 yr later in women than in men (22–25), although not all investigators have detected gender differences (26). In addition, having three or more pregnancies appears to accelerate cyst development (25). In the Han:SPRD-*cy* rat model, cyst development is much slower in females compared with male animals (36). Castration of the males results in a slower progression of the disease, as seen in the female animals. Conversely, administration of testosterone to females causes the disease to progress more rapidly (19,20). As models for PKD, glucocorticoids have been used to induce cysts in rodents, although the steroids must be administered within the first neonatal week (37). In contrast, the glucocorticoid methylprednisolone has been shown to retard cyst progression in several models of PKD when administered later in life (5). A recent study demonstrated that lovastatin given at a dose that reduces serum cholesterol concentrations in obese Zucker rats can delay the development of cysts in male Han:SPRD-*cy* rats (38). Lovastatin is a 3-hydroxy-3-methyl-glutaryl CoA reductase inhibitor and, in addition to other effects, may influence the cellular level of cholesterol and its steroid metabolites. Finally, the Ke6 gene is downregulated in several models of PKD, including *pcy*, *cpk*, and *jck* mice (18). Although the precise function of the Ke6 protein is not known, sequence homology and expression pattern similarities suggest that it is a member of the steroid

dehydrogenase family, which is involved in hormone inactivation. Whether the phytoestrogens in soy influence disease in *pcy* mice by altering the hormonal balance remains to be elucidated.

Abnormalities in extracellular matrix metabolism and basement membrane components may alter epithelial cell growth in PKD, and appear to cause interstitial inflammation and fibrosis (1–3). In this regard, an anti-inflammatory effect of soy protein was recently demonstrated in the Han:SPRD-*cy* rat model of PKD (19). Inhibition of inflammation appears to reduce cyst growth, as has been shown with methylprednisolone in the Han:SPRD-*cy* rat and the *pcy* mouse (5). Soy isoflavones may have anti-inflammatory effects since some, such as genistein (39), can inhibit tyrosine kinase activity associated with cytokine and growth factor activation, and can inhibit the proliferation of inflammatory cells (40). Tyrosine phosphorylation events are important regulators of renal phosphoinositide metabolism events (41,42); this intracellular signaling system is altered in the *pcy* mouse (43–45). On the other hand, a recent study indicated that addition of genistein to the diets of male *pcy* animals given a high level of dietary protein does not affect disease progression (11). Therefore, it is unclear whether dietary genistein or other isoflavones play a role in the reported anti-inflammatory effects of dietary soy protein.

It has been suggested that the amino acid L-arginine may mediate the protective effect of dietary protein restriction in renal disease (46), and that the higher level of L-arginine in soy protein may confer its protective effect on renal function (16). L-Arginine is a precursor for several products involved in tissue injury and repair, *i.e.*, nitric oxide, polyamines, and proline. However, not all studies have demonstrated a protective effect of L-arginine in renal disease (reviewed in reference (47)), and we have found that adding L-arginine to a low protein diet for 3 mo does not affect kidney size in adult male or female *pcy* mice (our unpublished observations).

Previous studies using casein-based diets and male animals demonstrated significant effects of protein reduction on kidney size and water content and PKD progression in *pcy* mice (8,9), and in Han:SPRD-*cy* rats (7,10). The effects of protein reduction in male casein-fed animals in the present study, although showing similar trends, were not consistent. This may be due to the fact that the difference in dietary protein level was 11.4% (6 *versus* 17.4 g/100 g diet) in the present study, whereas in previous studies with *pcy* animals (8,9) the difference was 15.5% (6 *versus* 21.5 g/100 g diet). A smaller difference in protein levels may result in smaller differences in cyst growth. The level of protein in the present study for the NP level was used because it is the level present in the standard AIN-93G-purified diet for laboratory rodents (29). In addition, the Han:SPRD-*cy* rat studies (7,10) and one of the *pcy* mouse studies (8) were carried out earlier in the disease process. Reducing protein intake in the earlier stages of the disease may increase the efficacy of this treatment, although a drawback of this strategy is that the protein reduction also negatively affected body growth in these studies. Another factor that may explain the differences between the current study and previous studies with *pcy* mice is that previously, the DBA strain of the *pcy* mouse was used, whereas in this study the CD1 strain of *pcy* mouse was used. Results from breeding experiments on *pcy* mice demonstrate that disease progression is strongly influenced by modifier genes (48). Whether a higher level of protein in the NP diet or examining protein reduction effects at an earlier stage of the disease would result in cyst growth changes similar to previous studies, or whether the gender effects of soy protein would be present in the DBA strain of the *pcy* mouse or the Han:SPRD-*cy* rat remains to be elucidated.

Human studies indicate that restriction of dietary protein may provide a protective effect in established, late renal disease (49,50). With respect to PKD specifically, a study of patients in the advanced stages of renal disease showed that over an average of 3.5 yr, the increase in serum creatinine was significantly lower for patients on low protein diets compared to those consuming normal protein diets (51). Likewise, in a study of a small number of PKD patients examined for up to 3.3 yr, serum creatinine concentration increased more slowly in patients on low protein diets supplemented with keto acids compared with control subjects (52). On the other hand, in shorter term studies, no evidence for a protective effect of protein restriction during late chronic renal insufficiency (including patients with PKD) was found in renal patients on low protein diets followed for up to 2 yr (53), or for an average of 2.2 yr (Modification of Diet in Renal Disease [MDRD] study) (54). When the MDRD data were reanalyzed to examine the effect of actual (as opposed to prescribed) protein intake, however, dietary protein restriction appeared to slow the progression of moderate renal disease (55). This reanalysis showed that although GFR decline was initially more rapid in patients consuming lower protein levels, the subsequent rate of decline slowed significantly compared to that of control subjects. This supports the notion that potential beneficial effects of reducing dietary protein intake may not be detected unless observations are made over a longer period of time.

The mechanism by which soy protein exerts beneficial effects in *pcy* mice, especially in females, and whether this translates into improved survival remains to be determined. The current study, however, confirms that reduction of renal and cyst size in PKD can be achieved in animal models of the disease, using dietary strategies that preserve normal growth. It also indicates that effects of diet therapy on disease may be significantly different in males and females. Although these effects must be confirmed in human studies before any recommendations can be made, evidence that protein source has a significant impact on disease in this model raises the possibility of an additional mode of diet therapy.

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