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Original Research

Urinary MCP1 and Microalbumin Increase Prior to Onset of Azotemia in Mice with Polycystic Kidney Disease

Naomi A Kirby,[1] Aaron M Stepanek,[1] Andyna Vernet,[1] Sarah M Schmidt,[1] Carrie L Schultz,[2] Nicola MA Parry,[3] Steven M Niemi,[1,4] James G Fox,[3] and Diane E Brown[1,4,*]

Urinary biomarkers may offer a more sensitive and less invasive means to monitor kidney disease than traditional blood chemistry biomarkers such as creatinine. CD1$^{pcy/pcy}$ (*pcy*) mice have a slowly progressive disease phenotype that resembles human autosomal dominant polycystic kidney disease with renal cyst formation and inflammation. Previous reports suggest that dietary protein restriction may slow disease progression in mice and humans with polycystic kidney disease. Accordingly, we fed *pcy* mice either a standard chow (22.5% protein) or a protein-restricted (11.5% soy-based protein) diet from weaning until 34 wk of age. Every 6 wk we measured markers of kidney disease, including serum creatinine, BUN, and serum albumin as well as urinary monocyte chemoattractant protein 1 (MCP1), microalbumin, and specific gravity. Progression of kidney disease was equivalent for both diet groups despite dietary protein restriction. Urinary biomarkers proved useful for early detection of disease, in that urinary microalbumin was elevated as early as 22 wk of age and urinary MCP1 was increased by 28 wk of age, whereas increases in serum creatinine and BUN were detected later (at 34 wk of age) in both diet groups. Thus, urinary microalbumin and MCP1 analyses provided earlier, noninvasive indicators for detection of kidney disease and disease progression in *pcy* mice than did serum creatinine and BUN.

Abbreviations: ADPKD, autosomal dominant polycystic kidney disease; MCP1, monocyte chemoattractant protein 1; PE diet, protein-restricted experimental diet.

Autosomal dominant polycystic kidney disease (ADPKD) is one of the most common heritable diseases in people and is the most frequently inherited nephropathy in North America.[19] Mouse models of ADPKD have been described, in which mutant phenotypes result from spontaneous mutations or gene-specific targeting in mouse orthologs of human polycystic kidney disease genes.[8] CD1$^{pcy/pcy}$ (*pcy*) mice, which have a mutated *NPHP3* gene, develop similar renal pathology to human ADPKD including cyst development, interstitial nephritis, and fibrosis.[8] The disease is transmitted as an autosomal recessive trait, and 100% affected offspring can be achieved by intercrossing homozygous *pcy* mice.[24] The murine *pcy* phenotype recapitulates human ADPKD, with renal cyst location along the entire nephron and slow disease progression.[8] Restricted protein diets have been reported to modulate the progression of polycystic kidney disease in humans and *pcy* mice.[8,14] Compared with standard casein-based diets, soy-protein–based diets attenuated the disease course in one mouse study, in which feeding a low concentration of soy protein (6%) resulted in lower kidney weights, lower cyst scores (% cyst area times relative kidney weight), and reduced renal cyst growth in *pcy* mice at 23 wk of age.[2] In addition, dietary fat type can influence kidney injury; for example, low or high amounts (7% or 20%) of flaxseed, a rich source of ω3 fatty acid and phytoestrogens, reportedly slowed early fibrosis progression in *pcy* mice, compared with diets containing either corn oil (rich in linoleic acid, an ω6 fatty acid, 18:2n-6) or an oil rich in docosahexaenoic acid, an ω3 fatty acid (22:6n-3).[20]

Compared with traditional serum biomarkers such as creatinine and BUN, urinary microalbumin, creatinine, and monocyte chemoattractant protein (MCP1) are well-described renal biomarkers and early predictors of kidney disease progression in humans with polycystic kidney disease.[26] Urinary biomarkers can provide an adjunct to traditional renal biomarkers to assess disease such as glomerular or tubular damage.[12,16,28] Increased urinary albumin and MCP1 excretion are detected earlier than are altered glomerular filtration rate and azotemia in human ADPKD patients,[28] and microalbuminuria is associated with disease progression.[12,16] To assess the use of urinary biomarkers as a potentially more sensitive and less invasive means of monitoring and comparing kidney disease progression in different diet treatment groups, we fed *pcy* mice either a standard or protein-restricted diet and measured urinary microalbumin and MCP1 excretion from weaning until 34 wk of age, near end-stage kidney disease. These values were compared with concurrent serum creatinine, BUN, and albumin data. In addition, body weight and urine

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[1]Center for Comparative Medicine, Massachusetts General Hospital, Boston, Massachusetts; [2]Purina LabDiet, St Louis, Missouri; [3]Division of Comparative Medicine, Massachusetts Institute of Technology, Boston, Massachusetts; [4]Department of Pathology, Massachusetts General Hospital, Harvard Medical School, Boston, Massachusetts.
[*]Corresponding author. Email: dbrown31@partners.org

specific gravity were measured serially at the same time points, and CBC results and morphologic pathology were evaluated at the end of study.

Materials and Methods

Animals. All protocols were approved by the Committee on Animal Care of the Massachusetts Institute of Technology and adhered to the NIH's *Guide for the Care and Use of Laboratory Animals*.[10] CD1$^{pcy/pcy}$ mice were bred inhouse, derived from animals generously provided by Dr Harold Aukema (University of Manitoba, Winnipeg, Manitoba, Canada). All mice were housed and maintained in an AAALAC-accredited facility under controlled temperature, humidity, and light (12:12-h light:dark cycle). Rederivation of mice was accomplished by natural mating followed by embryo transfer.[17] Breeding female mice (age, 4 to 6 wk) were fed a standard diet (Purina Prolab RMH 3000 Rodent Diet, 5P00, PMI Nutrition International, St Louis, MO) and water ad libitum. At 5 wk of age, 20 *pcy* pups of similar weight (mean, 23 ±1.7 g) were randomly allocated into 2 groups of 10 mice (5 male, 5 female) each and fed 2 diets of different protein contents and fatty-acid ratios for 29 wk (Table 1). In addition, 10 CD1 control mice (5 male, 5 female) that had been bred inhouse were housed similarly to the *pcy* mice and were analyzed concurrently as a nonrenal disease control group. At the beginning of the study, all mice were weighed and ear-punched for identification; thereafter they were weighed weekly until study termination (at 34 wk of age).

Diets and experimental protocol. To assess the use of urinary biomarkers as a noninvasive means for early detection and monitoring of kidney disease in *pcy* mice, 20 *pcy* mice were randomly allocated into 2 dietary groups; these groups were fed 1 of 2 different diets (Table 1) from weaning until 34 wk of age. Both diets contained soy protein concentrate as the primary source of protein. Group 1 (Chow, $n = 10$) *pcy* mice and CD1 control ($n = 10$) mice were fed a standard rodent diet (Purina Prolab RMH 3000 Rodent Diet, 5P00, PMI Nutrition International [LabDiet]; 22.5% protein), with an ω6:ω3 fatty-acid ratio of 5.0. Primary fat sources were soy oil and pork animal fat. Group 2 (PE, $n = 10$) *pcy* mice were fed a purified low-protein experimental (PE) growth diet (TestDiet 5AVJ, PMI Nutrition International; 11.5% soy protein) from 5 to 10 wk of age and then were switched to a maintenance diet containing 6.0% protein (TestDiet 5AVK, PMI Nutrition International) until 34 wk of age. The maintenance and growth PE diets contained a combination of soy, fish, and flaxseed oils to provide an ω6:ω3 fatty-acid ratio of 1.24. The fish oil used for the PE diet was from menhaden (eicosapentaenoic acid:docosahexaenoic acid = 13:13; eicosapentaenoic acid + docosahexaenoic acid = 23%; Sigma, St Louis, MO).

Urine collection and analyses. Urine samples were collected every 6 wk from 16 to 34 wk of age, for a total of 4 collection time points. Urine was collected from individual mice in metabolic cages at 16, 22, 28, and 34 wk of age. While in the metabolic cages for 12 h (decreased to 6 h at week 34 for all mice due to polyuria in the *pcy* mice), mice were fasted and provided with free access to water. Urine was collected into 15-mL centrifuge tubes on dry ice packs and ice and insulated within a polystyrene box. Urine specific gravity was measured and recorded immediately, and samples were aliquoted and stored at −80 °C for later testing.

Urine samples were thawed once prior to analyses. Urinary microalbumin and creatinine were measured by using the DCA Vantage Analyzer according to the manufacturer's instructions (sample volume, 40 µL; Siemens Healthcare Diagnostics, Tarrytown, NY) and quantified by using a calibration curve of absorbance compared with albumin or creatinine concentration, respectively. Microalbumin:creatinine ratios were then calculated and reported. Quality controls were performed for each lot number. Urine creatinine results from the DCA analyzer (Benedict–Behre method) were compared with those from the Architect ci8200 (sample volume, 74 µL; kinetic alkaline picrate method; Abbott Diagnostics, Abbott Park, IL). Urine creatinine methods were compared by statistical testing; the Spearman correlation coefficient between methods was 0.90. According to the results from Bland–Altman difference plot analysis and Wilcoxon signed-rank testing, the measurements from the 2 instruments were determined to be identical within the inherent imprecision of both methods.[11] Therefore, for samples below the lower limit of detection on the DCA analyzer at 34 wk due to polyuria ($n = 9$), urine creatinine results from the Architect system were used for calculation of the microalbumin:creatinine ratio. All urinary microalbumin values were normalized to urine creatinine concentration.

Urinary MCP1 excretion was measured by using a mouse-specific immunoassay (Multi Array System, Mouse MCP1 Ultra-Sensitive Kit, MesoScale Discovery, Gaithersburg, MD). Method evaluation studies included comparisons of dilutions and spiked recoveries for mouse serum and urine and modified urine pH (data not shown). Performance was as expected for interpolation of serum and urine samples, with acceptable intraassay coefficient of variation (less than 15%). In light of information from the preliminary reproducibility and recovery studies, urine from control and *pcy* mice (sample volume, 25 µL) was analyzed in duplicate according to the manufacturer's instructions, which were designed for the use of serum. Standard curves, run in parallel and in duplicate, were used to interpolate the concentration (pg/mL) of the analyte. Plates for all kits were incubated (MTS 2/4 Digital Plate Shaker, IKA, Wilmington, NC), washed manually, and scanned (Sector Imager 2400, MesoScale Discovery); data were analyzed by using Discovery Workbench (3.0) software (MesoScale Discovery). For each plate, the lower level of detection was calculated as 2.5 SD above the signal for the zero calibrator; the upper level was set to the highest calibrator (10,000 pg/mL). All urinary MCP1 values were normalized to urine creatinine concentration.

Blood collection and analyses. Blood samples were collected every 6 wk from 16 to 34 wk of age, for a total of 4 collection time points. Submandibular blood collection was performed using a 4 mm lancet as previously described in conscious mice.[7] Approximately 130 µL blood was collected into serum microtainer collection tubes (Becton Dickinson, Franklin Lakes, NJ), allowed to clot at room temperature, and centrifuged for serum separation. Serum concentrations of albumin, BUN, and creatinine were measured by using a veterinary chemistry analyzer (Dri-Chem 7000, Heska, Loveland, CO). Terminal blood collection was performed in CO_2-anesthetized mice at 34 wk via cardiocentesis, and in addition to measuring serum concentrations of albumin, BUN, and creatinine, CBC analyses were performed (950FS Hemavet, Drew Scientific, Miami Lakes, FL) by using blood collected into K_3EDTA microtainer tubes (Sarstedt AG, Nümbrecht, Germany). Fresh blood films were made and stained with Wright–Giemsa for later review.

Histopathology. At 34 wk of age, final body weights were taken, and mice were euthanized by CO_2 inhalation. The spleen was

Table 1. Composition of the experimental diets

	Purina ProLab 5P00 RMH 3000 chow diet	Purified experimental growth diet[d]	Purified experimental adult diet[e]
Protein, %[a]	22.5	11.5	6.0
Fat, %	5.4[b]	7.2[c]	7.2[c]
ω3 fatty acids, %	0.34	1.69	1.69
ω6 fatty acids, %	1.7	2.10	2.10
ω6:ω3 ratio	5.0	1.24	1.24
Carbohydrate (%)	52.0	69.4	77.7
Energy (Kcal/g)	3.46	3.88	3.96
Energy from, %			
Protein	25.9	11.9	6.0
Fat	14.0	16.6	16.1
Carbohydrate	60.0	71.5	77.9

[a]Protein in all diets is primarily supplied by soy vegetable protein.
[b]Fat provided by soybean oil and porcine animal fat.
[c]Fat provided by soybean, fish, and flaxseed oil.
[d]Purina TestDiet 5AVJ.
[e]Purina TestDiet 5AVK.

removed and weighed. Both kidneys were removed and weighed, transversely sectioned, and photographed. Tissues were fixed in 10% neutral buffered formalin, paraffin-embedded, sectioned at 5 µm, and stained with hematoxylin and eosin according to accepted histologic technique. In addition, kidney sections were stained with Masson's trichrome for evaluation of tissue fibrosis by light microscopy. Renal cortical fibrosis was graded semiquantitatively according to the following scale: 0, less than or equal to 5% fibrosis; 1, 5% to 25%; 2, 25% to 50%; 3, 50% to 75%; and 4, greater than or equal to 75%.[23] Damage to individual glomeruli and the distribution of the damaged glomeruli were combined in a grading system according to the following definitions: grade 0 (none), no glomeruli affected; grade 1 (mild), single glomeruli affected; grade 2 (moderate), multiple glomeruli affected and multiple dilated Bowman capsules present; grade 3 (marked), many glomeruli affected and many dilated Bowman capsules present; grade 4 (severe), majority of glomeruli affected and general dilation of all Bowman capsules in all areas. A similar semiquantitative scheme was applied to assess tubulointerstitial damage.[21] All grading was performed by a single investigator (NAK) without knowledge of the identity of the slides.

Kidney images stained with hematoxylin and eosin were acquired under bright-field microscopy; cortical cystic burden was quantified by using image analysis software (Image J version 1.47, NIH, Bethesda, MD). Digital images (model BX50, Olympus, Center Valley, PA) of 6 nonoverlapping (300 × 200 pixel) fields at one pole of each kidney were photographed by using a 10× objective and QImaging MicroPublisher RTV 5.0 (JH Technologies, Fremont, CA).[13,22]

Statistical analysis. Differences between diet groups were evaluated for significance by using nonparametric one-way ANOVA (Kruskal–Wallis) and Dunn posthoc tests for multiple comparisons[18] (Prism 5, GraphPad Software, San Diego, CA). Data are reported as medians and ranges, with P values of less than 0.05 in a 2-sided test considered to be significant.

Results

All *pcy* mice survived to the study endpoint (that is, 34 wk of age), except for one female mouse (17 wk of age) on the standard chow diet and 2 male mice (28 and 33 wk of age) on the protein-restricted experimental (PE) diet. Of the 3 mice that died, tissues suitable for analysis were obtained only from the 2 mice on the PE diet. Histopathologic evaluation of tissues from these 2 mice showed severe pyelonephritis and advanced polycystic kidney disease.

All *pcy* mice failed to gain body weight over time. All pcy mice in both diet groups failed to gain body weight over the course of study compared with CD1 controls (Figure 1). However, *pcy* mice fed the PE diet initially maintained a higher ($P < 0.05$) average body weight compared with the *pcy* mice on the standard chow, likely due to the higher energy content of that diet (Table 1).

Clinical and morphologic pathology. In both diet groups, *pcy* mice became azotemic, as indicated by increased serum BUN (Figure 2 A) and creatinine (Figure 2 B) concentrations at 34 wk compared with controls. In addition, *pcy* mice in both diet groups had significantly ($P < 0.05$) decreased serum albumin concentrations by 34 wk of age (Figure 2 C) and became polyuric over time, indicating loss of urine concentrating ability (Figure 3). In light of the findings of azotemia, decreased serum albumin, and polyuria, *pcy* mice fed the protein-restricted (PE) diet with a lower ω6:ω3 fatty acid ratio did not show delayed kidney disease progression or clinical improvement over the course of the study.

Urinary biomarkers were increased in* pcy *mice. Urinary microalbumin and MCP1 excretion were measured and normalized to urine creatinine concentration, thereby providing an indication of protein loss in relation to glomerular filtration rate.[26] Increased urinary microalbumin in *pcy* mice from both diet groups was detected earlier in the study (22 wk of age) than were the traditional serum biomarkers of creatinine and BUN (34 wk of age) and progressively increased over the time course of the study (Figure 4) , compared with those in CD1 control mice. Therefore, urinary microalbumin normalized to urinary creatinine provided a noninvasive, early indicator of kidney disease and disease progression for *pcy* mice. There were no significant differences in urinary microalbumin concentrations between the 2 *pcy* diet groups.

Increased urinary MCP1 excretion was detected as early as 22 wk in both *pcy* diet groups and increased over the course of the

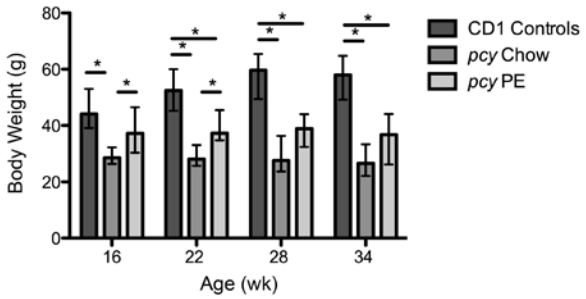

Figure 1. Body weight over time in mice on a standard grain-based rodent diet (chow diet; CD1 control and *pcy* mice: $n = 10$ in each group at 16 wk and $n = 9$ in each group at 22–34 wk) or protein-restricted purified experimental (PE) diet (*pcy* mice: $n = 10$ at 16 and 22 wk, $n = 9$ at 28 wk, and $n = 8$ at 34 wk). Compared with control mice, *pcy* mice on both diets failed to gain body weight over time (*, $P < 0.0001$).

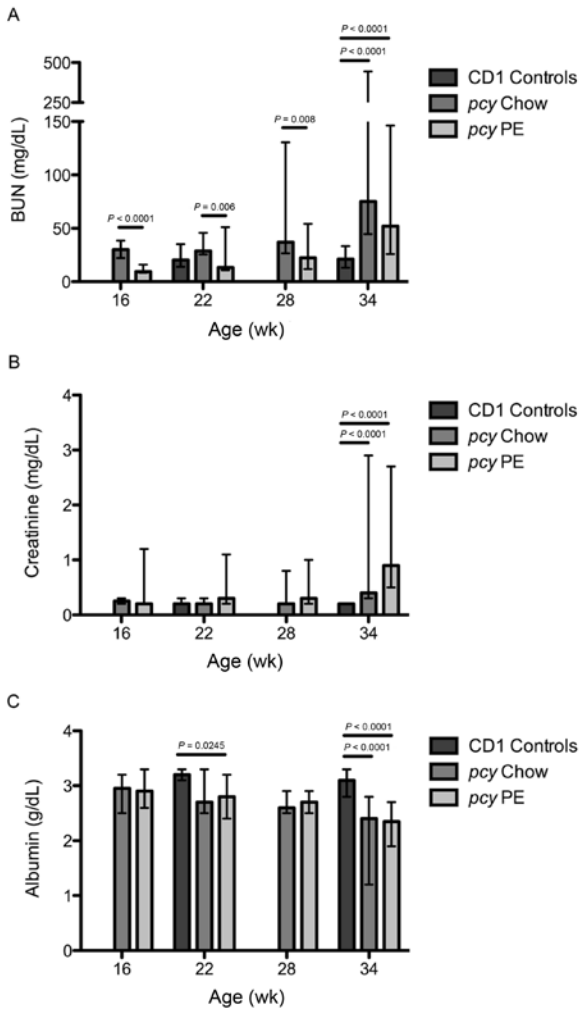

Figure 2. Serum (A) BUN, (B) creatinine, and (C) albumin in CD1 control and *pcy* mice fed a standard grain-based rodent diet (chow diet; CD1 control mice: $n = 5$ at 22 wk and $n = 10$ at 34 wk; and *pcy* mice: $n = 10$ at 16 wk and $n = 9$ at 22–34 wk) or protein-restricted purified experimental (PE) diet (*pcy* mice: $n = 10$ at 16 and 22 wk, $n = 9$ at 28 wk, and $n = 8$ at 34 wk).

study, compared with levels in CD1 control mice. This urinary biomarker was significantly ($P < 0.05$) increased in both *pcy* diet groups compared with CD1 control mice by 28 wk of age (Figure 5).

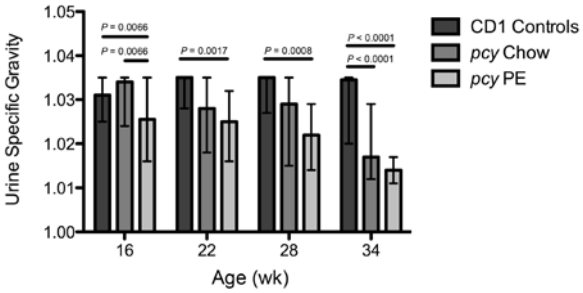

Figure 3. Urine specific gravity for mice on a standard grain-based rodent diet (chow diet; CD1 control mice, $n = 10$; *pcy* mice, $n = 9$) or protein-restricted purified experimental (PE) diet (*pcy* mice: $n = 10$ at 16 and 22 wk, $n = 9$ at 28 wk, and $n = 8$ at 34 wk). Compared with control mice, *pcy* mice on both diets became polyuric over time.

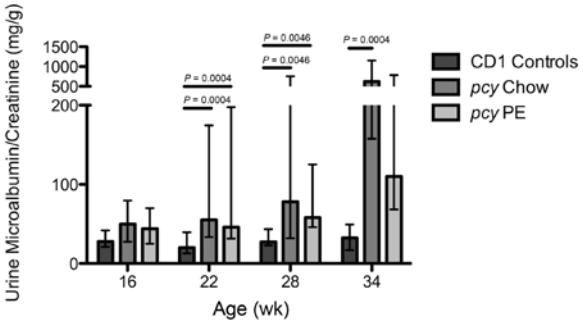

Figure 4. Urinary microalbumin:creatinine ratios for mice on a standard grain-based rodent diet (chow diet; CD1 control mice, $n = 6$; *pcy* mice: $n = 7$ at 16 and 28 wk and $n = 8$ at 22 and 34 wk) or protein-restricted purified experimental (PE) diet (*pcy* mice, $n = 8$). On both diets, *pcy* mice had increased urinary microalbumin as early as 22 wk of age.

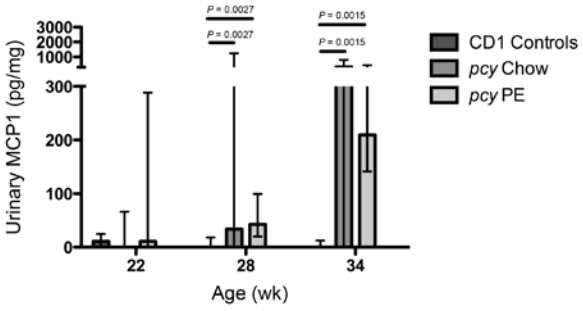

Figure 5. Urinary excretion of monocyte chemoattractant protein 1 (MCP1) normalized to urine creatinine for mice on a standard grain-based rodent diet (chow diet; CD1 control mice, $n = 7$ at 22 and 28 wk and $n = 8$ at 34 wk; *pcy* mice: $n = 9$ at 22 wk, $n = 7$ at 28 wk, and $n = 8$ at 34 wk) or protein-restricted purified experimental (PE) diet (*pcy* mice, $n = 9$ at 22 and 28 wk and $n = 7$ at 34 wk). On both diets, *pcy* mice had increased urinary MCP1 excretion as early as 28 wk of age.

There were no significant differences in urinary MCP1 concentrations between the 2 *pcy* diet groups. Therefore, MCP1 is a useful early biomarker of kidney disease in *pcy* mice.

***Pcy* mice on both diets were anemic.** To provide hematologic and morphologic correlation of kidney disease with the serum and urinary biomarkers for kidney disease, hematology and histopathology were performed at the study endpoint. In both diet groups, *pcy* mice developed microcytic anemia, as demonstrated

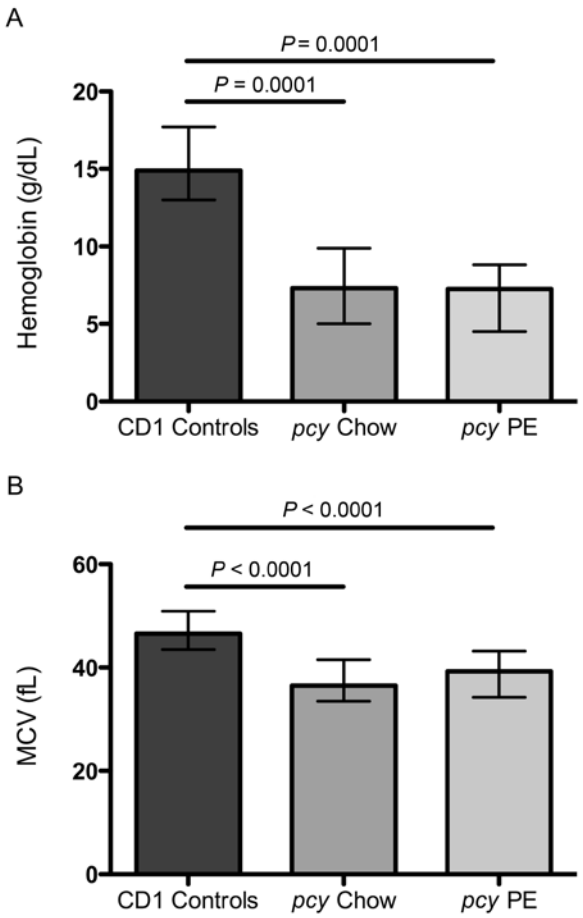

Figure 6. *Pcy* mice become anemic, regardless of diet. (A) Blood hemoglobin and (B) MCV at 34 wk in CD1 control and *pcy* mice fed a standard grain-based rodent diet (chow diet; CD1 control mice, $n = 10$; *pcy* mice, $n = 9$) or protein-restricted purified experimental (PE) diet (*pcy* mice, $n = 8$).

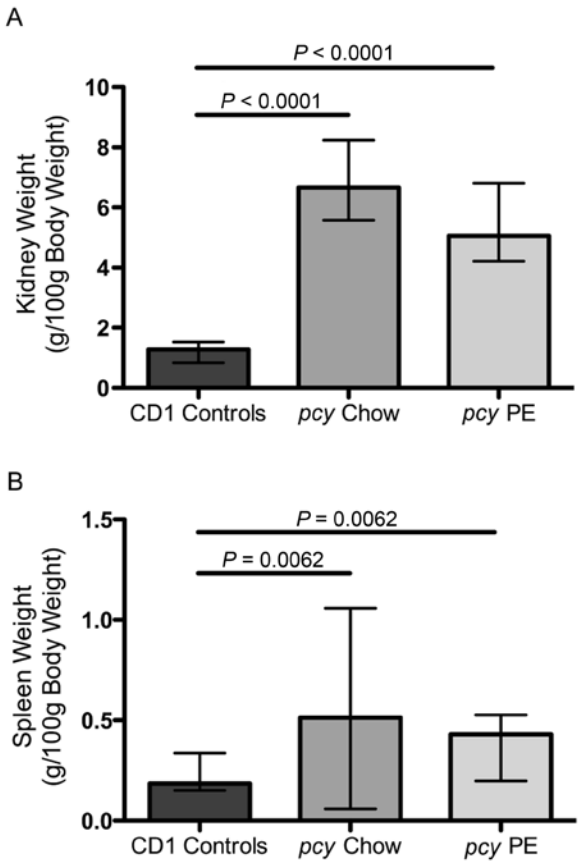

Figure 7. Increased (A) kidney weight:body weight and (B) spleen weight:body weight ratios at 34 wk in *pcy* mice. CD1 control and *pcy* mice were fed a standard grain-based rodent diet (chow diet; CD1 control mice, $n = 10$; *pcy* mice, $n = 9$) or protein-restricted purified experimental (PE) diet (*pcy* mice, $n = 8$). Weights of both kidneys were combined.

by decreased blood hemoglobin (anemia) and decreased erythrocyte MCV (that is, microcytosis; Figure 6 A and B) as compared with parameters in CD1 control mice. This finding is consistent with the anemia and microcytosis previously described in a mouse model of polycystic kidney disease.[15] There were no significant differences in hemoglobin or MCV values between the 2 *pcy* diet groups.

Gross and histopathology confirmed advanced polycystic kidney disease in both pcy diet groups. To provide morphologic correlation of kidney disease with the live-phase serum and urinary biomarkers, gross and histopathologic examinations were performed at the end of the study (34 wk). Kidneys were enlarged and cystic in both *pcy* diet groups, compared with CD1 controls (Figure 7 A) . In addition, splenomegaly was present in both *pcy* diet groups compared with CD1 controls (Figure 7 B), in part due to an extramedullary hematopoietic response to anemia (Figure 6 A), as is expected for mice.[4,5]

Kidney abnormalities consistent with previously described renal pathology of *pcy* mice[1,8,24,27] were observed equally frequently in both *pcy* diet groups. Renal architecture was markedly effaced—the capsular surface was irregularly undulated, and tubules (primarily the distal tubules) within both the cortex and medulla were expanded; were variably, cystically dilated; and often compressed the adjacent parenchyma. The lined epithelium was attenuated, and the luminae sometimes contained eosinophilic material or blood, with occasional cellular debris. The tubules were markedly atrophic and fibrotic, with interstitial nephritis. The glomeruli had shrunken tufts with variably dilated and sometimes cystic Bowman's capsule spaces. In addition, there was marked dilation of the renal pelvis, and hydronephrosis was present in both diet groups. Furthermore, pyelonephritis was an uncommon finding, present only in 2 early-death male PE mice. Renal fibrosis, demonstrated by Masson's trichrome staining, occurred in both *pcy* diet groups. At 34 wk, *pcy* mice in both diet groups compared with CD1 controls had significant renal interstitial fibrosis, renal tubular destruction, shrunken glomeruli, and dilation of the Bowman capsule space (Figure 8, Table 2). There was no apparent disease-sparing effect of the PE diet on the kidney disease of *pcy* mice on the basis of histopathology, consistent with all other clinical and clinical pathologic findings.

Renal cystic index was increased in both *pcy* diet groups. To assess whether diet influenced stasis or regression of renal cyst growth, the renal cystic index was measured for both *pcy* diet groups. The cystic burden (cysts measuring 166 to greater than 1000 µm) at 34 wk for both *pcy* diet groups was significantly ($P < 0.05$) increased compared with that in CD1 control mice but did not differ between *pcy* diet groups (Table 2). In summary, there was no significant difference or reduction in cystic burden between

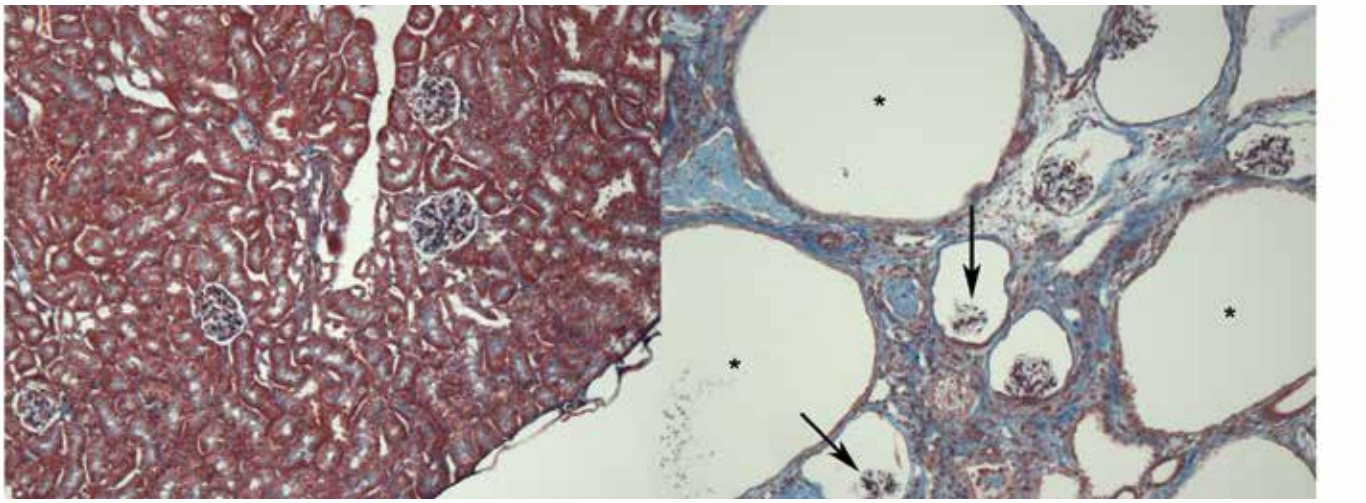

Figure 8. Kidney sections from a 34-wk-old CD1 control female mouse fed a standard grain-based (chow) rodent diet (left panel) and 34-wk-old *pcy* female mouse fed the protein-restricted purified experimental (PE) diet (right panel). Note the increased interstitial fibrosis (blue), shrunken glomeruli (arrows), dilated Bowman spaces, and renal cysts (*) in the *pcy* mouse. Masson trichrome stain for collagen; original magnification, 100×.

Table 2. Semiquantitative evaluation (median [range]) of renal cortical fibrosis, Bowman capsule and renal tubule dilation, and percentage cystic renal tissue at the end of the study (34 wk of age)

	CD1 control mice (n = 10)	*pcy* mice on chow diet (n = 9)	*pcy* mice on PE diet (n = 8)
Renal fibrosis (score, 0 to 4)	0	2 (1–4)[a]	4 (1–4)[a]
Bowman capsule dilation (score, 0 to 4)	0	2 (1–4)[a]	3 (1–4)[a]
Renal tubular dilation (score, 0 to 4)	0	3 (1–4)[a]	4 (2–4)[a]
Renal cystic tissue (%)	6.0 (3.2–9.2)	56.3 (43.1–69.2)[b]	57.9 (49.9–74.7)[b]

PE, purified experimental.
Value is significantly ([a]$P < 0.0001$; [b]$P = 0.0001$) different from that for CD1 control mice.

the *pcy* diet groups, and the PE diet did not demonstrate a kidney-sparing effect.

Discussion

Urinary microalbumin, creatinine, and MCP1 are well-described renal biomarkers and early predictors of kidney disease progression in humans with polycystic kidney disease when compared with traditional serum biomarkers, such as BUN and creatinine.[26] As a chemokine expressed by endothelial cells, renal tubular epithelial cells, and macrophages secondary to inducers including IL1α and TNFα, MCP1 signals monocyte recruitment and activation.[6] Activated monocytes, in turn, stimulate tubular epithelial cells or endothelial cells to produce chemokines, which may result in cellular expression of MCP1 and increased urinary excretion of that chemokine.[6] A mouse-specific MCP1 serum kit evaluated for microvolume urine samples successfully detected and measured urinary MCP1 in the *pcy* mice. Increased urinary MCP1 excretion in *pcy* mice with polycystic kidney disease was detected by 28 wk of age—6 wk prior to azotemia—in the current study. In addition, increased urinary microalbumin was detected by 22 wk of age—12 wk prior to azotemia—and progressively increased throughout the study in *pcy* mice. Therefore, measurement of urinary MCP1 excretion and urinary microalbumin—both normalized to urine creatinine—appears to provide a useful means for early, noninvasive detection of kidney disease in *pcy* mice. Furthermore, urinary MCP1 and microalbumin were more sensitive tests for the early detection of kidney disease than were serum creatinine and BUN for *pcy* mice.

Dietary protein intake can modulate kidney function, and a high-protein diet is known to decrease glomerular filtration rate.[9,14] Reducing dietary protein intake previously was shown to retard the progression of polycystic kidney disease and to prolong survival in *pcy* mice.[2,3,20,25] The present study suggests severely restricting the soy protein content coupled with decreasing the dietary ω6:ω3 fatty-acid ratio and adding long-chain fatty acids, may not be beneficial in improving survival time or maintaining kidney function in *pcy* mice, as indicated by all of the biomarker and pathologic measures of kidney disease performed. There were no significant differences in the development of anemia, polyuria, azotemia, proteinuria, or renal cyst development between *pcy* mice fed the modified protein- and fatty-acid–diet compared with those fed the standard chow. Important differences between our study and other published *pcy* mouse studies that reported clinical improvement after dietary protein restriction[2,3,20] include our study's extended duration (5 to 34 wk of age) and the variety in the dietary lipid content. Feeding high concentrations of combined ω3 fatty acids (flaxseed and fish oil) and a decreased dietary ω6:ω3 fatty-acid ratio for this extended period of time may have led to the different outcome for our study compared with others.

In conclusion, our results suggest that a diet severely restricted in soy protein (PE diet) was not protective in regard to preserving renal function in *pcy* mice. Urinary biomarkers were useful for

monitoring disease progression in mice with polycystic kidney disease. Measurement of urinary microalbumin concentration and urinary MCP1 excretion offer noninvasive means, by using microvolume test methods, for early detection of kidney disease in *pcy* mice and provide sensitive new biomarkers for sequential monitoring of experimental therapeutic interventions in this mouse model of polycystic kidney disease.

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