Detrimental Effects of a High Fat Diet in Early Renal Injury Are Ameliorated by Fish Oil in Han:SPRD-cy Rats\textsuperscript{1,2}

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ABSTRACT Dietary fish oils containing (n-3) fatty acids can modulate renal inflammatory injury. We previously demonstrated that a high fat (HF) diet worsens early renal disease progression in the Han:SPRD-cy rat model of polycystic kidney disease (PKD). Therefore, using HF (20 g/100 g diet) and low fat (LF; 5 g/100 g diet) diets, we compared the effects of menhaden oil (MO), soybean oil (SO) and cottonseed oil (CO) on renal function and histology in male Han:SPRD-cy rats fed the diets for 6 wk in the early stages of renal disease. Overall, rats fed HF compared with those fed LF diets had larger kidneys, more renal fibrosis and lower creatinine clearance (main effects of fat level). Rats fed MO rather than CO and SO diets had significantly lower kidney weights, kidney water content, cyst volumes and serum cholesterol and triglyceride concentrations (main effects of fat type). Rats fed MO diets also had less renal fibrosis than those fed CO diets, but the least fibrosis was in rats fed SO diets. Analysis of simple effects (due to interactions between fat level and type) revealed that HF diets increased renal inflammation in rats fed CO diets, but reduced inflammation was present in those fed SO and MO diets; HF diets also increased compared with LF diets serum urea nitrogen concentrations in rats fed the MO and CO diets, but not the SO diet. These results confirm that high dietary fat worsens early disease progression in this model of renal disease, and further demonstrate that diets with oils containing (n-3) fatty acids ameliorate some of the detrimental effects of a high fat diet.


KEY WORDS: \hspace{1em} dietary fat level \hspace{1em} fish oil \hspace{1em} early renal disease \hspace{1em} rats

There have been many attempts to modify the progression of chronic renal diseases with diet, including altering the types and levels of dietary fat. We demonstrated recently that a high dietary fat intake using soybean oil as the fat source increases early kidney disease progression, evidenced by greater kidney size, fluid accumulation and cyst growth, and worsened renal function in the Han:SPRD-cy rat model of polycystic kidney disease (PKD)\textsuperscript{3} (1). In separate studies, we also demonstrated that feeding flaxseed, enriched in 18:3(n-3), ameliorates early disease progression in Han:SPRD-cy rats with PKD through moderation of the associated chronic interstitial nephritis (2). Whether these beneficial effects of dietary (n-3) fatty acids in flax seeds also occur with fish oil, however, is not known. In DBA/2FG-pcy/pcy mice, feeding fish oil containing 20:5(n-3) and 22:6(n-3) was beneficial in a short-term early feeding study (3) but detrimental in a survival study (4). Diets containing (n-3) polyunsaturated fatty acids (PUFA) appear to be renoprotective in rats with immune and/or inflammatory mediated renal disorders (5).

PKD is a common genetic renal disease in humans; it is more prevalent than the combined incidences of cystic fibrosis, muscular dystrophy, hemophilia, sickle cell anemia and multiple sclerosis. It encompasses a variety of inherited or acquired cystic disorders characterized primarily by abnormal renal growth and cystogenesis (6). However, even with the current understanding of the genetic defects that cause PKD, there is no known therapeutic intervention available for this disease. Treatment of PKD patients is confined to alleviation of the secondary complications such as hypertension. The rate of decline in renal function and the age of onset of detectable cysts in patients with PKD demonstrates considerable variability, with approximately half of the patients with the most common form of PKD developing end stage renal disease by the age of 58 y (7). This indicates that factors other than the
primary genetic defect influence the progression of the disease. A growing number of reports demonstrate that diet influences disease progression in animal models with this renal disorder, particularly in the early stages of disease (2,8–13).

Whether the previously observed detrimental effect of a high fat diet in Han:SPRD-cy rats is dependent on the type of dietary fat is not known. Therefore, we used cottonseed oil, rich in (n-6) fatty acids, and menhaden oil, rich in very long-chain (n-3) fatty acids, as well as the previously utilized soybean oil to determine whether the effects of dietary fat level in the early stages of renal disease in Han:SPRD-cy rats with PKD are influenced by the type of fat in the diet. This model of renal disease is an excellent system with which to study how diet can modify early renal injury because it is characterized by progressive dilation of nephrons and marked renal interstitial inflammation and fibrosis in young rats. The results demonstrate that the effects of fat level on renal injury are influenced by the type of fat in the diet.

**MATERIALS AND METHODS**

**Animals, diets and experimental protocol.** 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. Han:SPRD-cy rat model was obtained from breeding colony derived from animals provided by Dr. B. D. Cowley (University of Kansas Medical Center). The Han:SPRD-cy rat model is a well-documented animal model of PKD that mimics the main form of PKD in humans (14). Rats were weaned at 4 wk of age and individually housed at a temperature of 23 ± 2°C, relative humidity of 60–65%, and a 12-h light:dark cycle. Male offspring from heterozygous parents were randomly assigned to dietary groups. Homozygous (cy/cy) rats do not survive to weaning, and the surviving rats are either normal (+/+) or diseased (cy/+). Random assignment of 19 rats to each dietary group resulted in the distribution of 11–14 diseased rats in the dietary groups; the remaining rats in each group were normal. Only rats with renal disease were used for analyses and the specific numbers in each group are detailed in Tables 2 and 4.

Male weanling rats (4 wk of age) were given free access to water and to either a high (HF) or a low fat (LF) diet containing 20 or 5 g/100 g of diet using cottonseed oil (CO), menhaden oil + soybean oil (4:1, g/g) (MO), or soybean oil (SO). The diets were based on the AIN 93G diet, with alteration of the oil level and type, and adjustment for several reasons, i.e., they are virtually undetectable in healthy kidney, the transient population from normal circulating cells is small, they are invariably present in the chronic interstitial inflammation seen in this model and they are easily and specifically detected by a robust immunohistochemical technique. The use of macrophage infiltration to assess renal infiltration has been previously described by others and by our laboratory (2,12,14,15). Briefly, macrophages were first identified using a primary monoclonal antibody against a 90- to 100-kDa protein that is expressed on lysosomal membranes and has many characteristics of the human CD68 antigen (Chemicon MAB 1435, Chemicon, Temecula, CA). Sections were incubated at a dilution of 1:100 for 60 min. Secondary detection was performed by using a Vectastain Elite kit with rat absorbed anti-mouse immunoglobulin G derived in goat and with peroxidase-diaminobenzidine color development (Vector Laboratories, Burlingame, CA). Nonspecific binding of goat protein was blocked by a 60-min preincubation with full strength goat serum. Negative controls included both sections without primary or without secondary antibody incubation and normal kidney, which contains very few cells of this lineage. Rat colon, which has a resident macrophage/monocyte population, was used as a positive control. Macrophage numbers were counted using module 2500 of the Imagemenu software package (Phoenix Technology, Seattle, WA). The counts were reported as a mean per high power video field (40X microscope objective) with at least 50 randomly selected, nonoverlapping fields counted. Because cystic lumens contained no cellular elements, results were corrected for the extent of cystic change in each kidney and thus represented counts per solid tissue area. This correction used the following formula:

\[
\text{Count}_{\text{adjusted}} = \frac{\text{Count}}{1 - \text{cyst lumen area}} \times \text{total image area}
\]

Sections for quantitative analysis of fibrosis were stained using aniline blue as an adaptation of Mason’s trichrome stain (12,16).

**TABLE 1**

<table>
<thead>
<tr>
<th>Fatty acid content of the experimental diets</th>
<th>CO</th>
<th>MO</th>
<th>SO</th>
</tr>
</thead>
<tbody>
<tr>
<td>g/100 g total fatty acids</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>14:0</td>
<td>1.26 ± 0.22b</td>
<td>9.38 ± 0.50</td>
<td>0.26 ± 0.11</td>
</tr>
<tr>
<td>16:0</td>
<td>20.32 ± 0.22</td>
<td>21.07 ± 0.14</td>
<td>11.04 ± 0.09</td>
</tr>
<tr>
<td>18:0</td>
<td>3.12 ± 0.28</td>
<td>3.08 ± 0.10</td>
<td>4.29 ± 0.12</td>
</tr>
<tr>
<td>18:1                                      (−)1</td>
<td>0.94 ± 0.02</td>
<td>2.96 ± 0.01</td>
<td>3.14 ± 0.01</td>
</tr>
<tr>
<td>18:2                                      (−)1</td>
<td>17.98 ± 0.12</td>
<td>11.34 ± 0.15</td>
<td>20.33 ± 0.04</td>
</tr>
<tr>
<td>18:3                                      (−)1</td>
<td>49.84 ± 1.17</td>
<td>11.86 ± 0.20</td>
<td>52.88 ± 0.49</td>
</tr>
<tr>
<td>18:4                                      (−)2</td>
<td>0.46 ± 0.05</td>
<td>2.06 ± 0.05</td>
<td>7.78 ± 0.07</td>
</tr>
<tr>
<td>20:5                                      (−)3</td>
<td>3.08 ± 0.26</td>
<td>12.97 ± 0.26</td>
<td>1.98 ± 0.09</td>
</tr>
<tr>
<td>22:5                                      (−)3</td>
<td>3.18 ± 0.18</td>
<td></td>
<td>14.18 ± 0.18</td>
</tr>
</tbody>
</table>

1 Values are means ± SEM of two determinations of the major fatty acids (>0.5 g/100 g total fatty acids present in any diet).

2 Abbreviations: CO, cottonseed oil; MO, menhaden oil; SO, soybean oil.

3 Not detected (<0.1 g/100 g total fatty acids).
This adaptation demonstrates a perfect concordance of staining with an immunofluorescent detection of collagen type III. Renal fibrous volume was determined using a 2X objective and using the proportion of section areas that had taken up aniline blue stain. The product of the proportion and the reference renal volume, derived from renal weight assuming an average tissue density of 1 g/L, corrected to body weight, gives the final volume occupied by either renal cyst volume or renal fibrous tissue.

Sections for analysis of cyst volume were stained with hematoxylin and eosin as described previously (12). Measurement of the portion of the tissue section occupied by tubular lumen or cysts was performed through a 2X objective, moving stepwise from a random starting point until 50 measurements were made from each of four separate whole kidneys. Results were expressed as the ratio of the area identified as tubular lumen or cyst by hue and intensity characteristics by an automated measurement subroutine to the total image area, excluding any area of image beyond the renal capsule. An average of 50 measurements from 3–5 different sections was used to determine the cyst area ratio. Cyst volume was calculated from this ratio as described for renal fibrosis.

Statistical analyses. All data were expressed as least-square means and the pooled SEM. Data were analyzed by two-way ANOVA using JMP statistical software (SAS, Cary, NC). Contrasts were used to determine differences between main effects of oil type, or if there were significant interactions between fat level and type, contrasts were used to determine simple effects. Differences were considered significant if $P < 0.05$.

RESULTS

All rats thrived on the different diets and body weights were not different among the groups (Table 2) from wk 4 through 10. Rats fed the LF diets ate more than those fed the HF diets, resulting in similar energy intakes in all groups. The feeding study occurred in the period of most rapid early cyst growth in this animal model, with death from renal failure not typically occurring until 6–10 mo of age (14,16,17).

Fatty acid compositions of renal tissues. Generally, the fatty acid compositions of kidneys reflected the compositions of the diets (Tables 1 and 3). The incorporation of very long-chain (n-3) PUFA, especially 20:5(n-3) and 22:6(n-3), was highest in kidneys from rats fed MO. These rats also had the lowest levels of renal (n-6) fatty acids. Rats fed CO had the highest levels of 20-carbon and 22-carbon (n-6) fatty acids, whereas renal tissues from rats fed SO had the highest contents of 18:2(n-6) and 18:3(n-3), reflecting the relative enrichment of these fatty acids in the diets. Saturated fatty acid compositions were affected less by these diets than were unsaturated fatty acids.

Fat level effects. Overall, HF compared with LF diets resulted in larger kidneys ($P = 0.0479$) (Table 2), higher serum creatinine ($P < 0.0001$), reduced creatinine clearance ($P = 0.0021$) and larger renal fibrous volumes ($P = 0.0351$) (Table 4). Serum lipids were not affected by fat level (Table 4). Fat level effects on SUN concentrations and renal inflammation were influenced by dietary oil type, as evidenced by significant interactions between fat level and type (Table 4). HF diets compared with LF diets had higher SUN levels in rats fed CO or MO diets, but not in rats fed SO diets (Table 4). As a histological effect, HF diets increased renal fibrosis in rats fed CO diets (Table 4). Interestingly, HF diets increased renal inflammation in rats fed CO, but caused less inflammation in those fed MO or SO diets (Table 4, Fig. 1).

Fat type effects. There were also overall dietary fat type effects on disease progression in Han:SPRD-cy rats (Tables 2 and 4). Rats fed MO rather than CO and SO diets had significantly lower kidney weights, kidney water content, serum cholesterol, triacylglycerol concentrations and cyst volumes (Tables 2 and 4). Rats fed all three types of oils had different degrees of renal fibrosis, with those fed the CO diets having the highest and those fed the SO diets having the lowest fibrous volume (Table 4). Fat type did not affect creatinine levels or creatinine clearance. Fat type effects on SUN concentrations and renal inflammation were influenced by the level of dietary oil. SUN concentrations were lower in rats fed MO and CO compared with SO only in those fed the LF diets (Table 4). The effect of oil type on renal inflammation was not different in LF-fed rats, but in rats fed HF diets, those fed the CO diet had more renal inflammation than those fed the MO and SO diets (Table 4, Fig. 1).

### Table 2

**Food and energy intake, body and renal weights of Han:SPRD-cy rats fed diets containing 5 or 20 g of cottonseed (CO), menhaden (MO) or soybean oil (SO)/100 g for 6 wk$^{1,2}$**

<table>
<thead>
<tr>
<th>Fat type</th>
<th>CO</th>
<th>MO</th>
<th>SO</th>
<th>Pooled ANOVA</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fat level</td>
<td>LF</td>
<td>HF</td>
<td>LF</td>
<td>HF</td>
</tr>
<tr>
<td>n</td>
<td>13</td>
<td>13</td>
<td>13</td>
<td>13</td>
</tr>
<tr>
<td>Food intake, g/d</td>
<td>17.6</td>
<td>15.1</td>
<td>18.3</td>
<td>16.0</td>
</tr>
<tr>
<td>Energy intake, kJ/d</td>
<td>287.0</td>
<td>290.8</td>
<td>298.7</td>
<td>307.9</td>
</tr>
<tr>
<td>Body weight, g</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Initial</td>
<td>83.8</td>
<td>87.4</td>
<td>87.1</td>
<td>87.4</td>
</tr>
<tr>
<td>End of study</td>
<td>329.2</td>
<td>326.1</td>
<td>344.9</td>
<td>333.2</td>
</tr>
<tr>
<td>Total kidney weight/body weight, g/100 g</td>
<td>2.76</td>
<td>2.92</td>
<td>2.54</td>
<td>2.61</td>
</tr>
<tr>
<td>Kidney water$^3$, g</td>
<td>3.67</td>
<td>3.72</td>
<td>3.47</td>
<td>3.42</td>
</tr>
<tr>
<td>Kidney water content, $^3$ g/100 g</td>
<td>89.1</td>
<td>89.5</td>
<td>88.9</td>
<td>88.9</td>
</tr>
</tbody>
</table>

$^1$ Values are least-square means.

$^2$ Abbreviations: CO, cottonseed oil; MO, menhaden oil; SO, soybean oil; LF, low fat; HF, high fat; NS, $P > 0.05$.

$^3$ Data from right kidney only.

$^4$ Symbols denote main effects: * MO differs from SO and CO; ** MO differs from SO; † All oil type means differ from one another.
Fibrous volume, mL/kg body

The most damaging effects of the HF diet were observed in rats fed the CO diet, which is virtually devoid of (n-3) fatty acids. In contrast, the MO diet, containing a high amount of very long-chain (n-3) fatty acids, ameliorated the renal injury in rats fed the MO diet within the same fat level. Furthermore, this study also demonstrates that the detrimental effects of the HF diet were observed in rats fed the CO diet, which is virtually devoid of (n-3) fatty acids. In contrast, the MO diet, containing a high amount of very long-chain (n-3) fatty acids, ameliorated the renal injury in rats fed the MO diet within the same fat level. This study confirms that a high fat diet accelerates early renal disease progression in Han:SPRD-cy rats, consistent with our previous study in which SO was the sole dietary lipid (1). Furthermore, this study also demonstrates that the detrimental effects of a HF diet are modulated by the source of dietary lipid. The most damaging effects of the HF diet were observed in rats fed the CO diet, which is virtually devoid of (n-3) fatty acids. In contrast, the MO diet, containing a high amount of very long-chain (n-3) fatty acids, ameliorated the renal injury observed in rats fed the HF diet. When MO was the dietary lipid source, not only was the negative effect of the HF diet abrogated, but renal inflammation was actually lower when a different diet was fed.

### TABLE 3

Fatty acid compositions of kidneys of Han:SPRD-cy rats fed diets containing 5 or 20 g of cottonseed (CO), menhaden (MO) or soybean oil (SO)/100 g diet for 6 wk.

<table>
<thead>
<tr>
<th>Fat type</th>
<th>CO</th>
<th>MO</th>
<th>SO</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fat level</td>
<td>LF</td>
<td>HF</td>
<td>LF</td>
</tr>
<tr>
<td>Fat level</td>
<td>4</td>
<td>4</td>
<td>4</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Fat</th>
<th>Level Type Interaction</th>
</tr>
</thead>
<tbody>
<tr>
<td>14:0</td>
<td>0.46</td>
</tr>
<tr>
<td>16:0</td>
<td>0.89</td>
</tr>
<tr>
<td>18:0</td>
<td>15.64</td>
</tr>
<tr>
<td>18:1(n-7)</td>
<td>2.50</td>
</tr>
<tr>
<td>18:1(n-9)</td>
<td>8.29</td>
</tr>
<tr>
<td>18:2(n-6)</td>
<td>9.55</td>
</tr>
<tr>
<td>18:3(n-3)</td>
<td>0.02</td>
</tr>
<tr>
<td>20:4(n-6)</td>
<td>23.67</td>
</tr>
<tr>
<td>22:4(n-6)</td>
<td>2.98</td>
</tr>
<tr>
<td>18:0</td>
<td>15.64</td>
</tr>
<tr>
<td>16:1(n-7)</td>
<td>0.89</td>
</tr>
</tbody>
</table>

1 Values are least square means of fatty acids present at a level >0.5 g/100 g total fatty acids in kidneys of at least one diet group.
2 Abbreviations: CO, cottonseed oil; MO, menhaden oil; SO, soybean oil; LF, low fat; HF, high fat; NS, P ≥ 0.05.
3 Letters denote simple effects: a Different from LF diet within the same fat type; b Different from CO diet within same fat level; c Different from MO diet within same fat level.
4 Symbols denote main effects: * MO differs from CO and SO; ** All oil type means differ from one another.
5 Letters denote simple effects: a Different from LF diet within the same fat type; b Different from MO diet within same fat level; c Different from SO diet within the same fat level.

### TABLE 4

Serum chemistry and renal pathology of male Han:SPRD-cy rats fed diets containing 5 or 20 g of cottonseed (CO), menhaden (MO) or soybean oil (SO)/100 g for 6 wk.

<table>
<thead>
<tr>
<th>Fat type</th>
<th>CO</th>
<th>MO</th>
<th>SO</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fat level</td>
<td>LF</td>
<td>HF</td>
<td>LF</td>
</tr>
<tr>
<td>Fat level</td>
<td>13</td>
<td>13</td>
<td>13</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Fat</th>
<th>Level Type Interaction</th>
</tr>
</thead>
<tbody>
<tr>
<td>Serum cholesterol, mmol/L</td>
<td>3.24</td>
</tr>
<tr>
<td>Serum triacylglycerol, mmol/L</td>
<td>1.12</td>
</tr>
<tr>
<td>Serum urea nitrogen, mmol/L</td>
<td>88.4</td>
</tr>
<tr>
<td>Creatinine clearance, mL/(min · 100 g body)</td>
<td>0.204</td>
</tr>
<tr>
<td>Serum urea nitrogen, mmol/L</td>
<td>8.1</td>
</tr>
<tr>
<td>Inflammation, macrophage cells/high power video field</td>
<td>30.9</td>
</tr>
<tr>
<td>Fibrous volume, mL/kg body</td>
<td>0.83</td>
</tr>
<tr>
<td>Cyst volume, mL/kg body</td>
<td>7.08</td>
</tr>
</tbody>
</table>

1 Values are least-square means.
2 Abbreviations: CO, cottonseed oil; MO, menhaden oil; SO, soybean oil; LF, low fat; HF, high fat; NS, P ≥ 0.05.
3 Corrected to solid renal tissue to eliminate differences due to variation in extent of cystic change.
4 Letters denote simple effects: a Different from LF diet within the same fat type; b Different from MO diet within the same fat level; c Different from CO diet within the same fat level.
5 Symbols denote main effects: * MO differs from CO and SO; ** All oil type means differ from one another.

DISCUSSION

This study confirms that a high fat diet accelerates early renal disease progression in Han:SPRD-cy rats, consistent with our previous study in which SO was the sole dietary lipid (1). Furthermore, this study also demonstrates that the detrimental effects of a HF diet are modulated by the source of dietary lipid. The most damaging effects of the HF diet were observed in rats fed the CO diet, which is virtually devoid of (n-3) fatty acids. In contrast, the MO diet, containing a high amount of very long-chain (n-3) fatty acids, ameliorated the renal injury observed in rats fed the HF diet. When MO was the dietary lipid source, not only was the negative effect of the HF diet abrogated, but renal inflammation was actually lower when a higher level of MO fat was fed. The effects of the SO diet,
containing a lower level of (n-3) fatty acids, on the progression of early renal injury varied depending on the marker of disease progression, but were generally intermediate between the effects of CO and MO.

This study adds to the growing number of reports that demonstrate that dietary interventions in rodent models of PKD can alter the early progression of renal injury. Although progress is being made toward understanding the underlying genetic defects in PKD, no known therapeutic interventions for this disease currently exist, and treatment is confined to alleviation of secondary disorders. Dietary manipulations in animal models that retard early disease progression without compromising growth include reducing dietary protein to a low, yet growth-maintaining level, reducing dietary fat, substituting soy protein for casein, adding flaxseed to the diet, and adding citrate to drinking water (1,2,9,12,13,18,19). Of note in the current study is that reducing the dietary fat level and including a source of (n-3) fatty acids in the diets are dietary interventions that maintain normal body growth and have positive effects on early disease progression. The high and low fat levels consumed by the rats are comparable to very low fat (~10% of total energy from fat) and high fat (~40% of total energy from fat) human diets. The level of (n-3) fatty acid in the MO diet would be difficult to achieve in human diets, and the optimal level of (n-3) fatty acids in the diet remains to be determined. However, the improvement in some markers of renal injury in rats consuming the SO diets containing a small amount of 18:3(n-3) suggests that inclusion of even small amounts of (n-3) fatty acids retards early renal disease progression. Intervention studies with purified fatty acids are required to address this issue. These studies are needed to confirm that the beneficial effects of the MO and SO diets are indeed due to their (n-3) fatty acid content and to identify which fatty acids are responsible for these effects.

The Han:SPRD-cy rat model, first described by Kaspareit-Rittinghausen et al. (17) in 1989, inherits PKD as an autosomal dominant trait and expresses pathologic features consistent with the most common form of PKD in humans. PKD in Han:SPRD-cy rats is characterized by renal inflammation and fibrosis associated with the development of cystic disease, azotemia and protooncogene expression (14). Because these features are common in most forms of renal injury, Han:SPRD-cy rats comprise an excellent animal model not only of PKD, but also of chronic renal injury in general.

The mechanisms by which dietary fat can influence PKD progression in Han:SPRD-cy rats remain to be determined. However, the current study and a number of our recent studies suggest that eicosanoids may be important in this renal disorder. Dietary flaxseed enriched in 18:3(n-3) and soy protein retard PKD progression in Han:SPRD-cy rats. Both of these interventions alter the fatty acid composition of the kidneys, thus altering the potential pools of eicosanoid precursors (2,15). Furthermore, the steady-state levels of the rate-limiting enzymes in eicosanoid synthesis, phospholipase A2, cyclooxygenase-1 and cyclooxygenase-2 are altered in the Han:SPRD-cy rat and pcy mouse models of PKD (20,21).

Functional changes in glomerular filtration rate and renal plasma flow in animals consuming high protein diets, which worsen experimental renal injury, also are accompanied by increased kidney synthesis and excretion of eicosanoids (22–

**FIGURE 1** Renal interstitial inflammation in male Han:SPRD-cy rats fed 5 or 20 g of cottonseed (CO), menhaden (MO) or soybean oil (SO)/100 g diet. Hematoxylin and eosin stain, magnification 100X. Arrows point to areas of interstitial inflammation (macrophage infiltration): a, low fat (LF)-CO; b, high fat (HF)-CO; c, LF-MO; d, HF-MO; e, LF-SO; f, HF-SO. Reduced inflammation is evident as fewer and smaller foci of interstitial inflammatory cells, with concomitant lesser expansion of the renal interstitial space.
Although the role of eicosanoids in normal renal physiology appears to be minor, in the diseased kidney, eicosanoids appear to play a crucial role in maintaining glomerular filtration rate as well as being involved in inflammatory processes in response to renal injury (22,24). In some types of renal disease, a reduction in eicosanoid formation is associated with amelioration of the disease process, whereas in others, it appears to have a protective effect (22–24). Increased levels of dietary fat are known to increase eicosanoid synthesis and may be involved in the mechanism by which the HF diets increased disease progression in Han:SPRD-cy rats in this study. The amelioration of renal injury in rats fed the HF diets by (n-3) fatty acids may be due to the fact that the 3-series eicosanoids produced from 20:5(n-3) are generally less bioactive. The fatty acids may be due to the fact that the 3-series eicosanoids amelioration of renal injury in rats fed the HF diets by (n-3) fatty acids and previously in the mouse model because in this model the disease progresses more slowly. It is important, therefore, to determine whether the apparent beneficial effects of fish oil in the early stages of the disease are mitigated by a detrimental effect in the latter stages, and whether the potential benefit of dietary fish oil in PKD is limited to the early stages of disease progression.

LITERATURE CITED

5. Holub, B. J. (1992) Effects of dietary protein restriction and oil type on the early formation and alteration of the charge selectivity in glomerular basement membrane (36). Although hyperlipidemia initially damages the glomerulus, it is evident that alterations in glomerular function ultimately influence tubulointerstitial injury, inflammation, and fibrosis. Tubular function correlates most closely with renal function, even in nephropathies that are traditionally considered to be glomerular diseases (37). Probucol, a cholesterol-lowering agent prevents the accentuation of kidney enlargement, renal fibrosis, and azotemia in BDF1-pcy mice with PKD (38). Similarly, the administration of lovastatin, another cholesterol-lowering agent, diminishes the rate of kidney enlargement in male Han:SPRD-cy rats (39). Therefore, the beneficial effect of (n-3) fatty acids in the MO diet could be due in part to its ability to lower serum lipid levels (40).

The results of this study are consistent with our previous studies with a high fat diet in which the early progression of disease was examined in growing Han:SPRD-cy rats (1). The only exception was the SUN concentrations, which were higher in the HF-fed rats in the previous study, but not different in the current study. The reason for this is not clear, but SUN is less reliable than serum creatinine, which was significantly elevated in HF-fed rats in the current study. The detrimental effect of the HF diet is also in agreement with our findings in adult pcy mice with PKD (41). The effect of the menhaden oil in this study is consistent with the retarding effects of dietary flaxseed (2,42) on disease progression in growing Han:SPRD-cy rats. In pcy mice, previous studies also demonstrated that dietary fish oil marginally slows early cyst formation (3). However, we found no benefit when fish oil was fed during both the growing and adult stages in pcy mice, and that long-term feeding of dietary fish oil fed accelerated disease progression (4,8,41). The reason for this remains to be elucidated, but in addition to effects possibly related to developmental stage, it may also reflect the lesser degree of inflammation in the pcy mouse model because in this model the disease progresses more slowly. It is important, therefore, to determine whether the apparent beneficial effects of fish oil in the early stages of the disease are mitigated by a detrimental effect in the latter stages, and whether the potential benefit of dietary fish oil in PKD is limited to the early stages of disease progression.

Given the current lack of treatment modalities for PKD, and the evidence from animal studies that demonstrate that this disease is very sensitive to dietary treatments, it is imperative to delineate when and what dietary treatments have the most beneficial effects.
20. Aukema, H. M. & Jiang, J. J. (2000) Steady-state levels of renal COX-1 and COX-2 are higher and lower, respectively, in two animal models of PKD. J. Am. Soc. Nephrol. 11: 368A (abs.).