Long-Term High Intake of Whole Proteins Results in Renal Damage in Pigs

Yong Jia, Sun Young Hwang, James D. House, Malcolm R. Ogborn, Hope A. Weiler, Karmin O, and Harold M. Aukema

Abstract

Despite evidence of potential antiobesity effects of high-protein (HP) diets, the impact of consuming diets with protein levels at the upper limit of the acceptable macronutrient distribution range (AMDR) on kidney health is unknown. To test whether HP diets affect renal health, whole plant and animal proteins in proportions that mimicked human diets were given to pigs, because their kidneys have a similar anatomy and function to those of humans. Adult female pigs received either normal-protein (NP) or HP (15 or 35% of energy from protein, respectively) isocaloric diets for either 4 or 8 mo. The higher protein in the HP diet was achieved by increasing egg and dairy proteins. Although there were initial differences in body weight and composition, after 8 mo these were similar in pigs consuming the NP and HP diets. The HP compared with NP diet, however, resulted in enlarged kidneys at both 4 and 8 mo. Renal and glomerular volumes were 60–70% higher by the end of the study. These enlarged kidneys had greater evidence of histological damage, with 55% more fibrosis and 30% more glomerulosclerosis. Renal monocyte chemoattractant protein-1 levels also were 22% higher in pigs given the HP diet. Plasma homocysteine levels were higher in the HP pigs at 4 mo and continued to be elevated by 35% at 8 mo of feeding. These findings suggest that long-term intakes of protein at the upper limit of the AMDR from whole protein sources may compromise renal health. J. Nutr. 140: 1646–1652, 2010.

Introduction

Although the acceptable macronutrient distribution range (AMDR) for protein has been set at 10–35% of energy, the Institute of Medicine (IOM) Committee for the Reference Intakes for Macronutrients indicates that there is insufficient data on the long-term safety of the upper limit of this range (1). Despite this, high-protein (HP) diets are increasingly being recommended as one of the management strategies for weight control in overweight and obese individuals (2,3). HP diets appear to be effective with respect to reductions in appetite, body mass, fat mass, and retention of lean mass, at least in the short term (4,5). However, in view of the high prevalence of obesity, type 2 diabetes, and metabolic syndrome (6,7), it is important to understand the effect of high levels of dietary protein on health. This is particularly important for the kidney, because these populations are characterized by renal hyperfiltration and increased risk of kidney disease (8–10).

Consumption of HP diets in the short term leads to hemodynamic changes, increased renal workload, and an increase in glomerular filtration rate (GFR) (11–13). However, to our knowledge, there are no reports of long-term effects of HP diets at the upper end of the AMDR on human kidneys, and the effects of moderately HP diets on GFR in normal kidneys are unclear. Brandle et al. (14) examined individuals who had been consuming elevated protein diets for at least 4 mo and reported that protein intake was correlated to creatinine clearance. However, creatinine clearance does not necessarily reflect GFR, as demonstrated in a 2-wk HP intervention study in which the HP diet increased creatinine clearance but not inulin clearance (15). In overweight individuals consuming moderately HP diets (25% of energy) for 6 mo, GFR was higher compared with those consuming a reduced protein diet but not compared with controls who maintained their normal protein intake (16). Whether the adverse effect of dietary protein levels at the upper end of the AMDR (35% of energy) on GFR persists in the long term is not known.

Because changes in renal pathology take place before changes in renal function occur, evidence of dietary HP effects on renal pathology is limited to studies in rodents. These studies have provided evidence for adverse effects of HP diets on the normal kidney in the long term. Rats and mice exposed to HP diets have a greater prevalence of developing nephropathy, including glomerular hypertrophy, glomerulosclerosis, tubulo-interstitial...

1 Supported by the Canadian Institutes of Health Research grant MOP 67034
3 Supplemental Table 1 and Figures 1–3 are available with the online posting of this paper at jn.nutrition.org.
4 Abbreviations used: AMDR, acceptable macronutrient distribution range; GFR, glomerular filtration rate; HP, high protein; IOM, Institute of Medicine; MCP-1, monocyte chemoattractant protein-1; NP, normal protein.
5 To whom correspondence should be addressed. E-mail: aukema@umanitoba.ca.
fibrosis, tubule regeneration, and chronic inflammatory cell infiltration (17–20). However, one of the reasons that the IOM rejected these data as a basis for setting the AMDR for protein was the belief that evidence from rodent models was not applicable to human health. Furthermore, most of these studies have used a single source of purified protein, which do not reflect the human diet and may have unique effects on pathological changes in the kidney. Specific protein sources and amino acids have been shown to have unique effects on GFR (13).

The pig has been proposed as a model to study renal function in humans, because, like primates and unlike rodent kidneys, nephrogenesis is complete before birth. Pig kidneys also have similar anatomy, physiology and ability to handle fluid volume, osmolarity, and metabolites such as urea, creatinine, ammonia, and electrolytes (21,22), although there are some dissimilarities between human and porcine physiology (23,24). In addition, the pig kidney is the animal of choice for possible renal xenotransplantation in humans (25) and is a model to study metabolic pathways in the regulation of glomerular inflammatory and hemodynamic events (26).

Therefore, the long-term effect of dietary protein intake at the upper limit of the AMDR on kidney health was examined in adult pigs, providing them with diets of mixed whole protein origin in proportions that reflected human diets.

Materials and Methods

**Pigs and diet.** Adult female (Genesus) nonpregnant pigs at 7 mo of age (175 ± 1.5 kg) were obtained from the Glenlea Swine Research Unit at the University of Manitoba. The pigs were randomized to receive isocaloric diets containing either 15% [normal-protein (NP) diet] or 35% of energy as protein (HP diet) (Table 1). These levels were selected, because 15% mimics the mean human protein intake in Canada and the US (1,27) and 35% represents the upper end of the AMDR (1). Whole protein sources were used in both diets and were derived from animal proteins in the form of poultry meal, pork meal, egg albumin, and skim milk powder, and plant proteins came from wheat and barley. The animal:plant protein ratio was 2:1 in the NP diet, which represents the typical protein intake (27). HP diets were achieved by increasing egg albumin and skim milk content, because poultry and dairy products are common sources of increased protein intake by individuals. To maintain isocaloric diets, energy from carbohydrate was decreased by reducing sucrose and cornstarch content in the HP diet. Furthermore, lactose was included in the NP diet to make the lactose content equal in both diets, because the increased content of skim milk contributes added lactose in the HP diet. The same amount of canola was added into both diets and lard was included in the NP diet to provide the same amount of fat with comparable fatty acid composition (Supplemental Table 1). All ingredients were analyzed for nutrient composition and Ca, P, K, Na, and Zn levels and were balanced with supplements to achieve the same amounts of these minerals in both diets. Both diets met the nutritional requirements of pigs (28).

Pigs were given free access to feed and water. Feed disappearance was recorded every week and body weights every 2 wk. One week prior to termination, pigs were placed in metabolic crates and feed intake for individual pigs was recorded for 6 d. There were 30 pigs receiving each diet at the beginning of this study, but 8 from the NP group and 5 from the HP group were removed from the study because of foot health problems associated with flooring problems in a new facility. Eight pigs receiving each diet were terminated after 4 mo and 14 from the NP diet and 17 from the HP diet were terminated after 8 mo. All procedures were approved by the University of Manitoba Animal Care Committee and followed the guidelines of the Canadian Council on Animal Care.

**Renal function.** Two days prior to termination, pigs were lightly sedated, a foley catheter was introduced into the bladder for urine collection, and a 22-ga 25-mm cannula was introduced into an ear vein for inulin infusion. After 1 d, urine was collected for 24 h and weighed to calculate the volume and a sample was frozen at −80°C for further analysis. On the day of termination, a primed (60 mg/kg) continuous (2 mg/kg-1 min-1) infusion of inulin was initiated to evaluate GFR (29). After allowing 2 h to reach steady state, timed urine was collected to calculate urine flow. At the end of the collection, a blood sample was obtained from the ear vein. After infusion, pigs were terminated with an overdose of xylazine/azaperone (2 mg/4 mg/kg body weight-1).

<table>
<thead>
<tr>
<th>Ingredient</th>
<th>NP</th>
<th>HP</th>
</tr>
</thead>
<tbody>
<tr>
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<td>310</td>
</tr>
<tr>
<td>Lipid</td>
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<tr>
<td>Carbohydrate</td>
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<td>Calcium</td>
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<tr>
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<td>5.4</td>
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<tr>
<td>Zinc</td>
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<td>0.19</td>
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<tr>
<td>Lard</td>
<td>80</td>
<td>80</td>
</tr>
<tr>
<td>Canola oil</td>
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<tr>
<td>Corn starch</td>
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<td>96</td>
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<td>Lactose</td>
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<tr>
<td>Low ash poultry meal</td>
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<td>44</td>
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<tr>
<td>Wheat</td>
<td>304</td>
<td>304</td>
</tr>
</tbody>
</table>

**TABLE 1** Composition of experimental diets

1. The Puratone Corporation, Niverville, MB.
2. Rothsay, Winnipeg, MB.
3. Micheal Foods, Minnetonka, MN.
4. Parmalat, St. Claude, MB.
5. Upper Canada Mill, Burlington, ON.
6. Casco Inc., Etobicoke, ON.
7. Davisco, Eden Prairie, MN. The lactose content of both diets was 72.8 g/kg diet.
8. White Cap Frozen Foods, Winnipeg, MB.
9. Landmark Feeds, Winnipeg, MB, containing the following (mg/kg mix): sodium, 300; chloride, 8200; potassium, 600; magnesium, 1200; sulfur, 6700; zinc, 72,032; manganese, 16,036; iron, 30,021; copper, 10,003; iodine, 601; cobalt, 0.03; selenium, 120; retinyl acetate, 1100; cholecalciferol, 15; α-tocopheryl acetate, 16,000; menadione, 750; vitamin B-12, 7.5; thiamine, 400; riboflavin, 2000; pantothenic acid, 8000; niacin, 10,000; folic acid, 252; biotin, 80; pyridoxine, 402; choline, 52,099.
10. Landmark Feeds, Winnipeg, MB, containing the following (mg/kg mix): sodium, 300; chloride, 8200; potassium, 600; magnesium, 1200; sulfur, 6700; zinc, 72,032; manganese, 16,036; iron, 30,021; copper, 10,003; iodine, 601; cobalt, 0.03; selenium, 120; retinyl acetate, 1100; cholecalciferol, 15; α-tocopheryl acetate, 16,000; menadione, 750; vitamin B-12, 7.5; thiamine, 400; riboflavin, 2000; pantothenic acid, 8000; niacin, 10,000; folic acid, 252; biotin, 80; pyridoxine, 402; choline, 52,099.
11. Calcium carbonate, dicalcium phosphate, potassium chloride, and sodium chloride were added as necessary to balance these minerals.

Morphology. At termination, kidneys were removed, weighed, and measured. The left kidney was used for all measurements and analyses. Renal volume was estimated by measuring the length, width, and thickness of the kidney and applying the ellipsoid formula as described (30). A transverse incision from the middle portion was made to allow optimal examination of the renal pelvis, renal papilla, and the junction with the ureter. An integrated section of kidney from the upper pole of the left kidney was sampled and included portions of both the cortex and medulla. Kidney tissues were fixed in 10% formalin prior to embedding in paraffin and were sectioned at 5 μm. Sections were stained with hematoxylin and eosin or Masson’s Trichrome to evaluate glomerular volume and kidney cortical fibrosis, respectively. Image Pro version 6.0 software was used to measure the largest diameter of each glomerulus and glomerular volume was calculated as described (32,33).
To evaluate fibrosis, the collagen density was determined as described (34). In brief, images were randomly captured from renal cortex using the 10× objective to obtain images for total cortical fibrosis and the 40× objective for glomerulosclerosis. Analyses were done in Adobe Photoshop CS3 Extended program. All blue colors in the picture were selected using the magic wand tool and the density was measured.

Body composition. After organ removal, pig carcasses were bisected along the sagittal plane and the left and right sides weighed. The right carcass half was further dissected into 4 primal cuts: ham, belly, loin, and the head-shoul der cuts, as described (35). The right ham from each pig was analyzed for composition by dual energy X-ray absorptiometry (Lunar BX-1 L-8743, GE Healthcare). The ham was put in the leg position on the scanning bed and the “human” module was used for scanning. The software used to analyze composition was Encore 2005 (GE Healthcare).

Biochemical analysis. A portion of cortex sampled from the upper pole of the left kidney was lyophilized and homogenized in Triton X-100 as described (36). Renal monocyte chemoattractant protein-1 (MCP-1) (KHC1012, BioSource International) levels were determined by ELISA following the kit instructions. Urinary protein concentrations were assayed using the Bradford protein method (37). Urinary and serum creatinine concentrations were determined using a modified Jaffe method based on the creatinine-picrate reaction (38). Plasma total homocysteine and cysteine were analyzed by reverse-phase HPLC (39,40). Concentrations were determined based on external standard curves with inter- and intra-assay CV < 2%.

Statistical analysis. Data were analyzed by 2×2 ANOVA, with diet and time as factors, using the GLM procedure (SAS, version 9.1). Normality of the data was assessed using the Shapiro-Wilk’s Statistic (W > 0.05). If the data did not follow a normal distribution, transformation was used to achieve normality of the data. If data could not be normalized, the MIXED procedure was used. Main effects were considered significant if P < 0.01. If significance was marginal (i.e. 0.01 < P < 0.05), or if interactions were present (P < 0.05), Tukey-Kramer comparisons were used to test differences among groups.

Results

Body weight and composition. After 4 mo of feeding, pigs given HP diets had 14% lower body weights than those given NP diets; however, the effect did not persist and after 8 mo, body weights of pigs given HP and NP diets were similar (Table 2). Food disappearance data showed that food intake initially was lower in pigs given the HP compared with the NP diet, but food disappearance was similar after 1 mo (Supplemental Fig. 1). Feed intake data determined in pigs in metabolic cages just prior to the 4- and 8-mo termination points confirmed that feed intake was not different after the initial period. The differences in body weight appeared to be due to lower levels of adipose, as suggested by the composition of the hams in pigs given the HP diet. At 4 mo, estimated fat mass was 26% (2 kg) lower in the hams from pigs given the HP compared with the NP diets, whereas the amount of lean and bone mineral mass was similar. This resulted in 9% higher and 20% lower proportions of lean and adipose tissue, respectively, in the hams from pigs given HP diets compared with those from pigs given NP diets. By 8 mo, however, none of these variables differed (Table 2).

Morphologic studies. Gross examination of kidneys indicated that kidneys from pigs given the HP diet tended (P = 0.0793) to have greater mass than those given the NP diet. When adjusted for body size, this difference was significantly different with pigs given the HP diet having greater renal mass and volume compared with pigs given the NP diet. Results were similar when kidney weight was expressed relative to lean body weight (Table 3).

Under microscopic examination, it was observed that in kidneys from pigs given the HP diet, some glomeruli had obviously enlarged diameters and the Bowman’s space was occupied by enlarged glomeruli (Supplemental Fig. 2). Hypercellular glomeruli also were detectable in kidneys from pigs given the HP diet at 4 and 8 mo, and open capillary loops were not noticeable in many kidneys from pigs given the HP diet at 8 mo. Obvious collagen deposition around the glomerular and tubular areas and chronic inflammatory cell infiltration in the tubulointerstitial area also were observed in kidneys from pigs given the HP diets (Supplemental Fig. 3). There was evidence of scattered chronic inflammatory cell infiltration at 4 mo in kidneys of pigs given the HP diet; at 8 mo, chronic inflammatory cell foci in the tubulointerstitial area were observed in kidneys from pigs given the HP diet. Because of these observations, glomerular volume and cortical fibrosis were quantified. In kidneys from pigs given the HP diet, glomerular enlargement was evident by 4 mo of feeding and was even greater after 8 mo. At 8 mo, glomeruli were 70 or 90% larger in kidneys from pigs given the HP diets when expressed relative to kidney or body weight, respectively (Table 3). Quantification of the density of collagen staining revealed that total cortical fibrosis was 55% higher and glomerulosclerosis was 30% higher in kidneys from pigs given HP compared with NP diets for 8 mo (Table 3).

Cysts with clear yellow fluid and smooth walls were observed in the kidney cortex from pigs given both HP and NP diets, with

### Table 2: Body weight and ham composition of pigs that consumed NP or HP diet for 4 or 8 mo

<table>
<thead>
<tr>
<th></th>
<th>NP</th>
<th>HP</th>
<th>NP</th>
<th>HP</th>
<th>Effects Diet Time</th>
</tr>
</thead>
<tbody>
<tr>
<td>n</td>
<td>8</td>
<td>8</td>
<td>14</td>
<td>17</td>
<td></td>
</tr>
<tr>
<td>Body weight, kg</td>
<td>254 ± 10</td>
<td>219 ± 10*</td>
<td>274 ± 8</td>
<td>270 ± 14</td>
<td>0.0004</td>
</tr>
<tr>
<td>Feed intake2, kg/d</td>
<td>2.7 ± 0.4</td>
<td>2.2 ± 0.3</td>
<td>2.8 ± 0.1</td>
<td>2.5 ± 0.2</td>
<td>0.0482</td>
</tr>
<tr>
<td>Ham lean, %</td>
<td>68.4 ± 1.7</td>
<td>74.8 ± 2.1*</td>
<td>62.7 ± 1.6</td>
<td>63.8 ± 1.5</td>
<td>0.0004</td>
</tr>
<tr>
<td>Ham lean mass, kg</td>
<td>16.82 ± 0.71</td>
<td>16.09 ± 0.47</td>
<td>15.89 ± 0.32</td>
<td>16.10 ± 0.52</td>
<td>0.0004</td>
</tr>
<tr>
<td>Ham fat, %</td>
<td>31.6 ± 1.7</td>
<td>25.2 ± 2.1*</td>
<td>37.3 ± 1.6</td>
<td>36.3 ± 1.5</td>
<td>0.0001</td>
</tr>
<tr>
<td>Ham fat mass, kg</td>
<td>7.80 ± 1.78</td>
<td>5.79 ± 1.87*</td>
<td>9.74 ± 2.88</td>
<td>9.32 ± 2.78</td>
<td>0.0001</td>
</tr>
<tr>
<td>Ham bone mineral content, g</td>
<td>605 ± 21</td>
<td>661 ± 25</td>
<td>738 ± 21</td>
<td>766 ± 32</td>
<td>0.0001</td>
</tr>
<tr>
<td>Ham bone mineral density, g/cm²</td>
<td>1.40 ± 0.03</td>
<td>1.45 ± 0.03</td>
<td>1.46 ± 0.03</td>
<td>1.49 ± 0.04</td>
<td>0.0001</td>
</tr>
</tbody>
</table>

1 Values are means ± SEM. *Different from NP group at same time point, P < 0.05.
2 Data obtained from pigs in metabolism cages 1 wk prior to termination points (n = 6, 4, 8, 12, respectively).
11 pigs given HP and 9 given NP diets having such cysts. Under microscopic examination, the walls of the cysts were lined with flattened epithelium, indicating renal simple cysts, which are not associated with diseased kidneys (41).

Renal function. Renal function as determined by inulin or creatinine clearance or proteinuria was not affected by protein level in the long term, with no significant differences at 8 mo. Inulin clearance was initially higher at 4 mo, but this change did not persist, and creatinine clearance and proteinuria were not different (Table 4).

Biomarkers. The total amount of kidney MCP-1 was 22% higher in kidneys from pigs given the HP compared with NP diet (Table 4). Plasma homocysteine concentrations were 36% higher overall in pigs given the HP compared with the NP diet, whereas cysteine concentrations were not altered by diet.

Discussion

This study demonstrates that long-term dietary intake of whole proteins at the upper range of the AMDR for protein may have detrimental effects on renal health. In adult female pigs free of disease, the HP compared with NP diet resulted in renal and glomerular hypertrophy, higher renal glomerulosclerosis, fibrosis, and MCP-1 content, and elevated plasma homocysteine levels.

These results are consistent with previous studies in rodent models that also exhibit renal enlargement and histological damage with HP diets in the long term (42–44). However, studies in rodents have been criticized because of renal differences compared with the human kidney and because these studies have primarily used purified single protein sources. The current results in pigs also are consistent with a human study in overweight or obese but otherwise healthy individuals that demonstrated a kidney volume increase after 6 mo of a moderately HP diet and a correlation between kidney volume and dietary protein intake (16). Thus, although the pig kidney is not a complete mimic of the human kidney (23,24), similar effects are observed in human, rat, and now pig studies with varying degrees of purification of the protein sources in the diet. However, because the increased protein in this study came primarily from egg albumen and milk powder, studies with other types of protein are warranted to determine whether these effects on the kidney are generalizable to other protein sources.

One of the early signs of kidney disease is glomerular hypertrophy associated with initially increased GFR (hyperfiltration) (45). This is observed in diabetic individuals as well as those who are overweight and have metabolic syndrome, population groups with an increased risk for development of renal disease (8–10). In humans, HP diets appear to increase GFR in the short term (13,46,47) and possibly the long term (14,16). In the current study, the higher inulin clearance in HP pigs was marginally significant (P = 0.0495) and post hoc analysis revealed that the effect was primarily due to the difference at 4 mo. This is analogous to the natural history of diabetic or obesity-associated nephropathy in which an early period of increased GFR is followed by a period of normal GFR but the beginnings of renal histological damage and proteinuria (9,48). The presence of higher renal fibrosis in the current study in the absence of proteinuria may reflect a very early stage of renal damage in pigs given the HP diets.

### Table 3

<table>
<thead>
<tr>
<th>Characteristics of kidneys of pigs that consumed NP or HP diet for 4 or 8 mo</th>
<th>4 mo</th>
<th>8 mo</th>
<th>Effects</th>
</tr>
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<tbody>
<tr>
<td>NP</td>
<td>HP</td>
<td>NP</td>
<td>HP</td>
</tr>
<tr>
<td>n</td>
<td>8</td>
<td>8</td>
<td>14</td>
</tr>
<tr>
<td>Kidney weight, g</td>
<td>302 ± 13</td>
<td>331 ± 26</td>
<td>314 ± 13</td>
</tr>
<tr>
<td>Kidney weight, g/kg body weight</td>
<td>1.25 ± 0.04</td>
<td>1.60 ± 0.14</td>
<td>1.15 ± 0.03</td>
</tr>
<tr>
<td>Kidney weight, g/kg lean tissue</td>
<td>1.94 ± 0.10</td>
<td>2.21 ± 0.19</td>
<td>1.96 ± 0.08</td>
</tr>
<tr>
<td>Kidney volume, cm³/kg body weight</td>
<td>0.57 ± 0.05</td>
<td>0.70 ± 0.03</td>
<td>0.56 ± 0.03</td>
</tr>
<tr>
<td>Glomerular volume, cm³/kg body weight</td>
<td>16.3 ± 1.4</td>
<td>22.8 ± 1.5*</td>
<td>18.6 ± 1.1</td>
</tr>
<tr>
<td>Glomerular volume, cm³/kg kidney</td>
<td>13.5 ± 1.1</td>
<td>15.4 ± 1.4</td>
<td>17.0 ± 1.3</td>
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<tr>
<td>Total cortical fibrosis, pixels x 10⁶</td>
<td>4.57 ± 0.65</td>
<td>5.32 ± 0.35</td>
<td>3.28 ± 0.28</td>
</tr>
<tr>
<td>Glomerulosclerosis, pixels x 10⁶</td>
<td>0.67 ± 0.04</td>
<td>1.00 ± 0.05</td>
<td>0.99 ± 0.02</td>
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</table>

1 Values are means ± SEM. *Different from NP group at same time point, P < 0.05.
2 n = 4, 6, 11, 10 for NP diet at 4 mo, HP diet at 4 mo, NP diet at 8 mo, HP diet at 8 mo, respectively.
The initial events of renal hyperfiltration and glomerular hypertrophy in the early stages of kidney damage lead to renal inflammation, tubular atrophy, and kidney fibrosis. Exposure of rodents to long-term HP diets results in glomerular hyperfiltration, glomerular hypertrophy, and a greater prevalence of renal pathological changes (17–20). Renal inflammation is induced via release of proinflammatory chemokines, such as MCP-1, which plays an important role in the recruitment of inflammatory cells into the kidney (49,50). Infiltrating inflammatory cells interact with renal cells, causing them to synthesize excessive extracellular matrix, ultimately resulting in the development of kidney fibrosis (49–52). In the present study, glomerular hypertrophy and abnormalities, histological changes, infiltration of inflammatory cells (especially at 8 mo), and higher MCP-1 levels in pig kidneys were observed with HP feeding. This was accompanied by more cortical fibrosis (including total fibrosis and glomerulosclerosis) in pigs given the HP diets, indicating that renal histological damage was incurred by long-term consumption of HP diets from whole protein sources.

Elevated homocysteine also can induce glomerular injury via enhanced MCP-1 expression and monocyte chemotaxis (53). We have previously demonstrated a direct link between hyper-homocysteinemia and an inflammatory response in rat kidneys, suggesting that increased chemokine expression may represent one of the mechanisms that contribute to renal injury in patients with hyperhomocysteinemia (54). In addition to water-soluble vitamins (55,56), the intake of dietary methionine, and protein in general, has been shown to influence plasma homocysteine concentration in neonatal piglets (57) and in some but not all human studies (58–60). In the current study, methionine and cysteine levels were much higher in the HP diet (8.52 vs. 2.42 and 6.31 vs. 1.91 g/kg diet, for HP vs. NP diets, respectively) and likely contributed to the elevated homocysteine levels. These elevated levels may be one of the contributing factors to the elevated MCP-1 levels and/or renal pathology.

Pigs given HP diets initially exhibited improved body composition, with a lower amount of fat in their hams while maintaining their lean mass, but these differences no longer were present after 8 mo. The initial changes may have been due to early alterations in metabolism or simply to the fact that it required ~2 wk longer for them to adapt to the experimental diets and consume as much as those given the NP diet. This initial weight difference is consistent with many human studies that demonstrate that an initial benefit to body size and composition can occur with HP diets but that long-term benefits are more challenging to achieve (4,61–65).

Despite the lack of evidence of safety for the kidney in particular, however, the popularity of HP diets is high, with many organizations and Web sites touting the benefits of HP diets. The American Diabetes Association recommends that prediabetic or diabetic individuals consider HP diets as a strategy of weight control (2) and the Canadian Clinical Practice Guidelines for the Management and Prevention of Obesity recommend that HP diets within the AMDR for 6–12 mo is a reasonable treatment option for obese individuals (3). When organizations such as these make these recommendations and the IOM sets an AMDR with a HP upper range, there is a perception that this level of dietary protein is safe, despite the fact that these recommendations are always made using cautious language. Furthermore, a significant portion of the population is obese or diabetic or has metabolic syndrome. Among other things, these disorders are characterized by glomerular hyperfiltration, hyperhomocysteinemia, inflammation, and increased risk of renal disease (8–10,66). Increases in GFR, homocysteine, and MCP-1 by HP diets might further increase the risk of the development of renal disease in these populations. Past studies in rodents with purified protein sources indicated (17–20), and the current pig study utilizing whole protein sources indicates, that there may be a potential risk of HP diets to the normal kidney. Because neither pig nor rat kidney completely mimics human kidney, this must be confirmed in human studies. A logical population to test this in would be in those with marginally compromised kidneys. Up to 10% of the population is considered to have chronic kidney disease (67) and as many as one-half of individuals with kidney disease are not aware of their marginal chronic kidney disease status (68). Higher dietary protein in humans with marginally compromised kidneys is associated with greater decline in GFR rate in the long term (69). Therefore, reexamination of the AMDR upper limit of 35% for protein intake in the “normal” population appears to be warranted.

Acknowledgments

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Literature Cited


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