Functional and Structural Changes in the Kidney in the Early Stages of Obesity

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Abstract. The purpose of this study was to examine the histologic and functional changes that occur in the kidney in the early stages of obesity caused by a high-fat diet. Lean dogs (n = 8) were fed a standard kennel ration, and obese dogs (n = 8) were fed the standard kennel ration plus a supplement of cooked beef fat each day for 7 to 9 wk or 24 wk. Body weights were 58 ± 5% greater and kidney weights were 31 ± 7% greater in obese dogs, compared with the average values for lean dogs. Plasma renin activity and insulin concentrations were both 2.3-fold greater in obese dogs, compared with lean dogs. Obesity was associated with a mean arterial pressure increase of 12 ± 3 mmHg, a 38 ± 6% greater GFR, and a 61 ± 7% higher renal plasma flow, compared with lean dogs. The glomerular Bowman’s space area was significantly greater (+41 ± 7%) in dogs fed the high-fat diet, compared with lean animals, mainly because of expansion of Bowman’s capsule (+22 ± 7%). There was also increased mesangial matrix and thickening of the glomerular and tubular basement membranes and the number of dividing cells (proliferating cell nuclear antigen-stained) per glomerulus was 36 ± 8% greater in obese dogs, compared with lean dogs. There was also a trend for glomerular transforming growth factor-β1 expression, as estimated by semiquantitative immunohistochemical analysis, to be elevated with the high-fat diet. Therefore, a high-fat diet caused increased arterial pressure, hyperinsulinemia, activation of the renin-angiotensin system, glomerular hyperfiltration, and structural changes in the kidney that may be the precursors of more severe glomerular injury associated with prolonged obesity.

Previous studies have provided compelling evidence for close associations among obesity, essential hypertension, and metabolic disorders such as type II diabetes mellitus (1). However, obesity may also be an important cause of renal disease. The two most important causes of end-stage renal disease (ESRD) are diabetes mellitus and hypertension, both of which are closely associated with obesity (2). Type II diabetes mellitus, which is usually preceded by chronic obesity, accounts for at least 80 to 90% of diabetes mellitus in the United States and most of the ESRD caused by diabetes mellitus. For example, in a study of a triethnic population in Texas, type II diabetes mellitus was the cause of 84 to 93% of diabetic ESRD among African Americans and Mexican Americans and approximately 60% of that among Caucasians (3). Although the two main risk factors for ESRD are closely linked to excess body weight, obesity is currently not listed as a cause of ESRD in the United States Renal Data Systems Survey Report (2).

There have been only a few studies of renal structure and function in obesity, and those generally examined kidneys after prolonged obesity and the development of marked renal dysfunction. Those studies suggested that kidneys from obese subjects exhibit substantial focal glomerulosclerosis and other morphologic changes similar to those observed in diabetic nephropathy (4–6). However, the mechanisms that initiate these changes are poorly understood, and renal histologic and functional changes in the early stages of obesity have, to our knowledge, not been reported. Therefore, in this study we examined the early changes in renal structure and function in obese dogs chronically fed a high-fat diet. Our previous studies suggested that this model of obesity closely mimics the neurohumoral and hemodynamic changes observed in obese human subjects (7,8).

Materials and Methods

Experiments were performed with chronically instrumented mongrel dogs (n = 16) that were conditioned before the study. All experimental protocols were approved by the Institutional Animal Care and Use Committee of the University of Mississippi Medical Center and were performed according to the National Institutes of Health Guide for the Care and Use of Laboratory Animals and the guidelines of the Animal Welfare Act.

Surgical procedures were performed in dogs under isoflurane anesthesia, using aseptic techniques. Tygon (Norton Plastics, Akron, OH) catheters were implanted in the femoral arteries and veins for measurement of arterial BP and blood sampling, respectively. All catheters were tunneled subcutaneously, exteriorized in the scapular region, and filled with heparin solution (1000 USP U/mL). After surgery, the dogs were permitted to recover, antibiotics were admin-
istered daily, and rectal temperatures were monitored to ensure that the dogs were afebrile throughout the studies.

After a recovery period of 1 to 2 wk, the dogs were placed in individual metabolic cages, in a quiet, air-conditioned room with a 12-h light/dark cycle, and fitted with harnesses containing a pressure transducer (Argon, Athens, TX) at the level of the heart. Arterial pressure signals were recorded on a polygraph (model 7D; Grass Instruments, Quincy, MA), sent to an analog-to-digital converter, and analyzed with a digital computer, using software developed in our laboratory. Analog signals from the polygraph were sampled in bursts of 12 s/min for 24 h/d, and digitized data were processed with the computer for determination of systolic, diastolic, and mean arterial BP and heart rates. The average arterial BP and heart rate for each day were calculated from values recorded during an 18-h period between 2 p.m. and 8 a.m. All routine care of the dogs (including feeding and cage cleaning), studies of renal function, and blood sampling were performed between 8 a.m. and 2 p.m.

One of the venous catheters was connected to a roller infusion pump (Wiz pump; Isco Instruments, Lincoln, NE), which delivered approximately 450 ml/d of sterile isotonic saline solution. The saline solution was pumped through a disposable filter (0.22 µm, Cathive; Millipore Corp., Bedford, MA), to prevent air bubbles, contaminants, and bacteria from passing into the infusion catheter. The infusion tubing and cables from the pressure transducers and flow probes were protected by a flexible vacuum hose attached to the harness, which permitted the dogs to move freely in their cages.

Throughout the study, dogs were fed two cans/d (447 g/can) of a sodium-deficient diet (H/D; Hill’s Pet Products, Topeka, KS), which provided approximately 7 mmol/d sodium and 65 mmol/d potassium, while blood samples were being obtained from the arterial catheters with 3% H2 O2 solution for 30 min. Nonspecific binding was prevented by incubation of the sections with horse serum. The sections were then incubated with 150 µl of anti-TGF-β1 (0.375 mg/ml, 2 h; Chemicon) or anti-PCNA (1:2000, 30 min; Dako Corp., Carpinteria, CA) antibody. After three rinses with PBS, the sections were incubated with biotinylated secondary antibody, rinsed with PBS, and then incubated with avidin-labeled peroxidase (VectaStain Elite ABC kit; Vector Laboratories, Burlingame, CA). Positive labeling was observed using diaminobenzidine. Another set of slides were simultaneously processed as negative controls, using all of the steps described above but with no primary antibody incubation.

**Experimental Protocol**

After the dogs were placed in metabolic cages and the intravenous infusions were started, periods of 10 to 14 d were allowed for the dogs to achieve sodium balance and for stable control measurements to be acquired. During those periods, the dogs were trained to lie quietly while blood samples were being obtained from the arterial catheters and studies of renal function were being performed. After 1 wk with stable control measurements, cooked beef fat (0.5 to 0.9 kg) was added to the regular diet of one group of dogs (n = 8, obese). When the dogs receiving the high-fat diet achieved a 50% increase in body weight, the amount of fat in the diet was reduced, to maintain a stable body weight. For four of the dogs, the high-fat diet was continued for 7 to 9 wk while systemic hemodynamic parameters were continuously monitored and blood sampling and renal function studies were conducted; for the other four dogs, the high-fat diet was continued for 24 wk. A second group of dogs (n = 8, lean) was fed the standard kennel diet of one group of dogs (8, lean) was fed the standard kennel diet of one group from which they originated. For immunohistochemical analyses, sections were deparaffinized and rehydrated, and endogenous peroxidase activity was quenched with 3% H2O2 solution for 30 min. Non-specific binding was prevented by incubation of the sections with horse serum. The sections were then incubated with 150 µl of anti-TGF-β1 (0.375 mg/ml, 2 h; Chemicon) or anti-PCNA (1:2000, 30 min; Dako Corp., Carpinteria, CA) antibody. After three rinses with PBS, the sections were incubated with biotinylated secondary antibody, rinsed with PBS, and then incubated with avidin-labeled peroxidase (VectaStain Elite ABC kit; Vector Laboratories, Burlingame, CA). Positive labeling was observed using diaminobenzidine. Another set of slides were simultaneously processed as negative controls, using all of the steps described above but with no primary antibody incubation.

**Glomerular Measurements**

The glomerular area was quantified using hematoxylin- and eosin-stained sections. Only glomeruli containing visible efferent and afferent arteriolar stalks were used for area measurements, to ensure that the glomeruli measured were sectioned in similar planes. Glomerular images were digitized using a color video camera attached to a Leica microscope. After digitalization, Bowman’s capsule and the glomerular tuft were traced and the areas were calculated using image analysis software (Optimas 5.1; Optimas Corp., Seattle, WA). Thirty cortical glomeruli and 30 juxtamedullary glomeruli were measured in each histologic sample for each animal. Bowman’s capsule and glomerular tuft areas from cortical glomeruli from each dog were statistically compared with the areas from juxtamedullary glomeruli; because no differences were observed, the glomeruli from the two regions were grouped and averaged for each dog.

The glomeruli with expanded Bowman’s capsule spaces were counted in one hematoxylin- and eosin-stained section from each animal and that number was divided by the total number of glomeruli within the section, to obtain the fraction of glomeruli with expanded Bowman’s capsule spaces.

**Analytical Methods**

GFR and effective renal plasma flow were estimated from the total clearances of [125I]iothalamate (Glofil; Cypros Pharmaceutical Corp., Carlsbad, CA) and [131I]iodohippurate (Hippuran; Syncom International Corp., Chatworth, CA), respectively, as described previously (7). The distribution space of [125I]iothalamate was used as an index of extracellular fluid volume (7).

Plasma renin activity was measured by RIA using 125I-labeled angiotensin I (AngI) (New England Nuclear, Boston, MA) and polyclonal rabbit anti-human antibody (Arnel Products, Inc., New York, NY). Plasma insulin concentrations were measured by RIA (Diagnostic Products).

**Histological Analyses**

Kidneys were removed and immediately placed in ice-cold phosphate-buffered saline (PBS). A coronal cross-section containing the hilus was removed from the right kidney, fixed in neutral buffered formalin, and embedded in paraffin. Sections (5-µm thick) of formalin-fixed, paraffin-embedded tissue were mounted on glass slides and stained with the following: (1) hematoxylin and eosin, for general histologic assessment; (2) Sirius red F3BA (in saturated picric acid), for detection of collagen; (3) methenamine silver, for detection of the mesangial matrix and basement membrane; (4) immunohistochemical reagents for detection of proliferating cell nuclear antigen (PCNA); or (5) immunohistochemical reagents for detection of transforming growth factor-β1 (TGF-β1). All tissues were evaluated without investigator knowledge of the group from which they originated.

For immunohistochemical analyses, sections were deparaffinized and rehydrated, and endogenous peroxidase activity was quenched with 3% H2O2 solution for 30 min. Non-specific binding was prevented by incubation of the sections with horse serum. The sections were then incubated with 150 µl of anti-TGF-β1 (0.375 mg/ml; 2 h; Chemicon) or anti-PCNA (1:2000, 30 min; Dako Corp., Carpinteria, CA) antibody. After three rinses with PBS, the sections were incubated with biotinylated secondary antibody, rinsed with PBS, and then incubated with avidin-labeled peroxidase (VectaStain Elite ABC kit; Vector Laboratories, Burlingame, CA). Positive labeling was observed using diaminobenzidine. Another set of slides were simultaneously processed as negative controls, using all of the steps described above but with no primary antibody incubation.

**Cell Proliferation**

Glomerular cell proliferation was quantified by counting the number of PCNA-positive cells within a glomerulus. Forty glomeruli (20 cortical and 20 juxtamedullary) were chosen for cell counting, using
the same criteria as used for glomerular size. The number of dividing cells in cortical glomeruli from all dogs was statistically compared with the number of dividing cells in juxtamedullary glomeruli; because no differences were observed, cell numbers from the two regions were grouped and averaged for each dog.

Mesangial Matrix

For assessment of the amount of glomerulosclerosis, glomeruli from sections of kidneys stained with periodic acid-Schiff (PAS) stain were scored for the percentage of each glomerulus that was sclerotic. As previously described by Raij et al. (9), glomeruli were assigned scores as follows: 1, 1 to 25%; 2, 25 to 50%; 3, 50 to 75%; 4, 75 to 100% matrix in the glomerular area.

TGF-β

For assessment of the amount of glomerular TGF-β1, glomeruli from sections of kidneys stained with anti-TGF-β1 antibody were scored for the percentage of each glomerulus that contained visible TGF-β1 staining. As described for mesangial matrix (9), glomeruli were assigned scores as follows: 1, 1 to 25%; 2, 25 to 50%; 3, 50 to 75%; 4, 75 to 100% TGF-β1 in the glomerular area.

Statistical Analyses

Control hemodynamic and renal function data obtained for dogs before initiation of the high-fat diet period were compared with data obtained for the same dogs after the high-fat diet period by using ANOVA and Dunnett’s t test for multiple comparisons (10). Data for dogs fed the high-fat diet for 7 to 9 wk were compared with data for dogs fed the high-fat diet for 24 wk by using the t test. Histologic data for obese and lean dogs were compared by using the t test. Statistical significance was considered at a value of P < 0.05.

Results

Hemodynamic and Hormonal Data

Table 1 summarizes the hemodynamic, renal function, hormonal, and weight data for dogs fed a high-fat diet for 7 to 9 wk or 24 wk, as well as dogs fed a normal diet. Body weights increased 64 and 52%, respectively, in the 7- to 9-wk and 24-wk obese dogs. Because there were no major differences in arterial pressure or renal function for the 7- to 9-wk obese group, compared with the 24-wk obese group, the data for all obese dogs were pooled for statistical comparison with data for lean dogs. The mean arterial BP increased approximately 12 mmHg in dogs fed a high-fat diet, compared with lean dogs. Kidney weights were an average of 31 ± 7% greater in dogs fed a high-fat diet, compared with kidneys from dogs fed a normal diet (Table 1). GFR and effective renal plasma flow were 38 ± 6% and 61 ± 7% greater, respectively, in obese dogs, compared with lean dogs (Table 1). Plasma renin activity and plasma insulin levels were both 2.3-fold higher in the high-fat diet group, compared with the normal diet group (Table 1).

The duration of high-fat diet feeding (7 to 9 wk versus 24 wk) had no major effect on body weight (38.6 ± 2.1 versus 35.9 ± 0.4 kg), mean arterial BP (103.5 ± 2.7 versus 100.3 ± 5 mmHg), GFR (109 ± 4 versus 98 ± 7 ml/min), plasma renin activity (0.59 ± 0.11 versus 1.22 ± 0.37 ng of AngI/ml per min), or plasma insulin levels (40.4 ± 12.9 versus 37.5 ± 9.0 mU/ml). Although the plasma renin activity after 24 wk of the high-fat diet was twice that after 7 to 9 wk, the difference failed to reach statistical significance. The kidney weight was significantly less after 24 wk of the high-fat diet, compared with 7 to 9 wk of the high-fat diet (69.0 ± 2.9 versus 57.1 ± 4.1 g, P <

Table 1. Weight, hemodynamic parameters, renal function, hormone levels, and plasma concentrations of electrolytes for obese and lean dogs

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Lean (n = 8)</th>
<th>7- to 9-Wk Obese (n = 4)</th>
<th>24-Wk Obese (n = 4)</th>
</tr>
</thead>
<tbody>
<tr>
<td>BW (kg)</td>
<td>23.6 ± 1.7 (18.8 to 30.4)</td>
<td>38.6 ± 2.1b (33.8 to 40.5)</td>
<td>35.9 ± 0.4b (34.9 to 36.9)</td>
</tr>
<tr>
<td>MAP (mmHg)</td>
<td>90 ± 4</td>
<td>104 ± 3b</td>
<td>100.3 ± 5.0b</td>
</tr>
<tr>
<td>HR (beats/min)</td>
<td>81 ± 2</td>
<td>104 ± 3b</td>
<td>101 ± 3b</td>
</tr>
<tr>
<td>GFR (ml/min)</td>
<td>75 ± 4</td>
<td>109 ± 4b</td>
<td>97.8 ± 6.7b</td>
</tr>
<tr>
<td>ERPF (ml/min)</td>
<td>179 ± 14</td>
<td>313 ± 10b</td>
<td>264 ± 17b</td>
</tr>
<tr>
<td>KW (g)</td>
<td>48.0 ± 2.8</td>
<td>69.0 ± 3.2b</td>
<td>57.1 ± 4.1b</td>
</tr>
<tr>
<td>PNa (mEq/ml)</td>
<td>148.8 ± 0.4</td>
<td>148.6 ± 0.1</td>
<td>146.8 ± 0.4c</td>
</tr>
<tr>
<td>PK (mEq/ml)</td>
<td>4.50 ± 0.13</td>
<td>3.79 ± 0.10b</td>
<td>4.01 ± 0.16b</td>
</tr>
<tr>
<td>PIns (mg/dl)</td>
<td>113 ± 1.3</td>
<td>106 ± 0.7b</td>
<td>101 ± 2.5b</td>
</tr>
<tr>
<td>Pgluc (mU/ml)</td>
<td>17.1 ± 3.4</td>
<td>40.4 ± 12.9b</td>
<td>37.5 ± 9.0b</td>
</tr>
<tr>
<td>Pprot</td>
<td>6.8 ± 0.2</td>
<td>7.7 ± 0.1b</td>
<td>6.9 ± 0.4c</td>
</tr>
<tr>
<td>PRA (ng AngI/ml per min)</td>
<td>0.36 ± 0.10</td>
<td>0.59 ± 0.11</td>
<td>1.07 ± 0.42b</td>
</tr>
<tr>
<td>Hct (%)</td>
<td>37 ± 2</td>
<td>32 ± 0.01b</td>
<td>30 ± 2b</td>
</tr>
</tbody>
</table>

* Data are means ± SEM. BW, body weight; MAP, mean arterial pressure; HR, heart rate; GFR, glomerular filtration rate; ERPF, effective renal plasma flow; KW, kidney weight; PNa, plasma sodium concentration; PK, plasma potassium concentration; PIns, plasma insulin concentration; Pprot, plasma protein concentration; PRA, plasma renin activity; AngI, angiotensin I; Hct, hematocrit.

b P < 0.05 versus lean.

c P < 0.05 versus 7- to 9-wk obese.
0.05). The effective renal plasma flow and plasma sodium level were significantly greater after 7 to 9 wk of the high-fat diet, compared with 24 wk of the high-fat diet (313 ± 10 versus 264 ± 17 ml/min and 148.6 ± 0.1 versus 146.8 ± 0.4 mEq/ml, respectively; \( P < 0.05 \)).

Histological Analyses

Analysis of hematoxylin/eosin- and PAS-stained kidney sections revealed that most glomeruli in lean dog kidneys exhibited normal glomerular histologic features, with patent capillary loops, normal basement membranes, a delicate mesangium, and small Bowman’s space (Figure 1). The most striking glomerular characteristic in hematoxylin/eosin- and PAS-stained sections from dogs fed a high-fat diet was a marked expansion of Bowman’s capsule space (Figure 1). The percentage of glomeruli with Bowman’s capsule space expansion similar to that demonstrated in Figure 1 was 83 ± 10% in obese dogs, compared with 12 ± 4% in lean dog kidneys. Measurement of the Bowman’s capsule area and glomerular tuft area revealed that the 41 ± 7% increase in the Bowman’s capsule area in obese dogs resulted mainly from an increase in the Bowman’s capsule area, with a small but statistically insignificant increase (+16%) in the glomerular tuft area (Figure 2). Similar expansion of Bowman’s capsule space was found in the 7- to 9-wk high-fat diet group and the 24-wk high-fat diet group (\( P = 0.20 \), NS).

Cell proliferation was quantified in the glomerular tufts of obese and lean dog kidneys. The number of PCNA-positive cells was 36 ± 8% greater in the glomeruli of obese dogs, compared with lean dogs, indicating an increase in cell proliferation. The number of PCNA-positive cells was not significantly different between the 7- to 9-wk and 24-wk high-fat diet groups. The proliferative response seemed to include mesangial cells and capillary endothelial cells; however, no attempt was made to quantify the cell types using a definitive method.

Analysis of silver methenamine-stained sections revealed glomerular basement membrane thickening and mesangial expansion in kidneys of obese dogs, compared with lean dogs (Figure 3). Not all glomeruli exhibited mesangial expansion, and those that did seemed to be distributed throughout the cortex. There was also a generalized thickening of the tubular basement membrane in the cortex and outer medulla of kidneys from obese dogs, compared with lean dogs. Sections stained with Sirius red indicated no effect of the high-fat diet on fibrillar collagen (collagens I and III) in the cortex and medulla.

The glomerulosclerosis score for obese dog kidneys was not statistically different from that for lean dog kidneys (1.16 ± 0.03 versus 1.17 ± 0.02, respectively; \( P = 0.67 \), \( n = 8 \) in each group). There was no significant difference in the amount of detectable glomerulosclerosis for the 7- to 9-wk and 24-wk obese dogs (7- to 9-wk high-fat diet score, 1.19 ± 0.04; 24-wk high-fat diet score, 1.12 ± 0.03; \( P = 0.57 \), \( n = 4 \) in each group).

Immunohistochemical analyses for TGF-\( \beta1 \) suggested that kidneys from obese dogs exhibited more TGF-\( \beta1 \) than did kidneys from lean dogs. There was increased staining in the glomeruli and in the interstitium between the tubules in the
cortex of obese dog kidneys, compared with lean dog kidneys (Figure 4). Similar to mesangial expansion and basement membrane thickening, the increase in TGF-β1 levels was not observed in all glomeruli. Glomeruli staining positively for TGF-β1 were usually observed in groups of three to six. There was also TGF-β1 staining in the outer and inner medullae in both obese and lean dog kidneys. This staining was primarily in the epithelial cells of the thin loops of Henle and the collecting ducts. Not all thin loops and collecting ducts stained positively; if the loop or duct stained positively, however, then all of the cells in that section of the loop or duct stained positively. The glomerular TGF-β score after 7 to 9 wk of the high-fat diet was 3 times greater than that in lean dogs (14.3 ± 5.1 versus 4.3 ± 1.4), but this difference did not reach statistical significance (P = 0.11). There was no significant difference between the TGF-β1 score after 7 to 9 wk of the high-fat diet and that after 24 wk of the high-fat diet.

**Discussion**

This study demonstrates that a high-fat diet, fed for as little as 7 to 9 wk, caused significant histologic, biochemical, and functional changes in the kidney. These changes included expansion of Bowman’s capsule, cell proliferation in the glomeruli, thickening of glomerular and tubular basement membranes and increased mesangial matrix in many glomeruli, and increases in TGF-β1 expression in the cortex. These histologic changes were associated with marked glomerular hyperfiltration and increased renal plasma flow, modest increases in arterial BP, increased plasma renin activity, and hyperinsulinemia.

Previous studies of renal structure in obesity mainly examined kidneys from patients with long-term obesity and severe renal disease. Such studies reported focal glomerulosclerosis or changes similar to those observed in diabetic nephropathy (4–6). It has been estimated that 90 to 95% of diabetic patients have type II diabetes mellitus, which is closely associated with obesity. Therefore, many of the patients in those studies, who had been obese for a long time, probably also were diabetic or had severe glucose intolerance. The obese dogs fed a high-fat diet in our studies exhibited 2.3-fold increases in plasma insulin concentrations, probably secondary to insulin resistance. We also previously demonstrated, using the euglycemic insulin-clamp technique, that insulin sensitivity is reduced by approximately 35% in obese dogs, compared with lean dogs (8). However, at this early stage of obesity the dogs are not diabetic and plasma glucose levels remain in the normal range. However, the hemodynamic and structural changes that occur in the early stages of obesity in dogs have some similarities to changes observed in early type II diabetes mellitus in human patients. First, there is marked glomerular hyperfiltration, as well as increased arterial BP, in the early stages of obesity. Second, there is an increase in the fasting plasma insulin concentration and a decrease in insulin sensitivity. Third, similar to diabetes mellitus, there are early increases in kidney size and in mesangial matrix and thickening of the glomerular and tubular basement membranes. In obese dogs, there was also a 41% increase in Bowman’s capsule space, which was mainly attributable to an increase in the Bowman’s space area, rather than the glomerular tuft area (which was increased by only 16%, a change that was not statistically significant). The mechanisms responsible for the enlargement of Bowman’s space in obesity are uncertain but may be related to the marked glomerular hyperfiltration; it is interesting that the GFR was increased by almost the same percentage (38%) as the Bowman’s space increase. In this study, there was minimal overall glomerulosclerosis, although the structural changes we observed early in obesity may be precursors to more serious renal damage with the persistence of obesity.

Previous studies of structural changes in the kidneys of obese animals primarily used the obese Zucker rat model. Obese Zucker rats, even at very young ages, exhibit major renal damage, including glomerulosclerosis and tubular necro-
sis leading to tubular casts (11). However, the Zucker rat model of obesity may not be representative of the structural and functional changes observed in obese human subjects, because the model does not mimic the neurohumoral changes of obese humans and the metabolic abnormalities are much more severe in Zucker rats than in most obese human patients. Arterial BP is increased slightly in obese, compared with lean, Zucker rats, and the obese rats are moderately resistant to insulin. However, plasma renin activity is decreased in obese Zucker rats, in contrast to obese humans, for whom plasma renin activity is usually increased (11). Also, Zucker rats spontaneously become obese while receiving normal rat chow, because of a mutation of the leptin receptor (a genetic abnormality that is rarely observed in obese humans) (12).

Although these studies were not designed to elucidate the specific mechanisms by which a high-fat diet causes structural changes in the kidney, there are several hemodynamic and hormonal alterations in obese dogs fed a high-fat diet that may contribute to the renal changes. For example, we observed that a high-fat diet caused increased arterial pressure, glomerular hyperfiltration, and increased renal plasma flow and that these hemodynamic alterations were sustained for up to 24 wk of study. The combination of increased arterial pressure and renal vasodilation likely causes marked glomerular hypertension, which stretches the mesangium and initiates a complex cascade of biochemical and histologic changes (13,14). Using isolated glomeruli, Cortes et al. (13,14) demonstrated that an acute increase in glomerular pressure stretched the mesangium and stimulated production of collagen IV, collagen I, and laminin and that increased extracellular matrix formation was associated with increased TGF-β1 expression. In a subsequent study, Riser et al. (15) observed that cyclic stretching of isolated mesangial cells caused significant expression of TGF-β1. Although these observations may partly explain the structural changes observed in glomeruli after a high-fat diet, the contribution of glomerular hyperfiltration to the production of biochemical and structural changes in the renal cortex in obesity remains to be elucidated.

In addition to the hemodynamic alterations, there are also hormonal changes that could contribute to renal remodeling in obesity. Two possibilities include elevated plasma AngII levels and hyperinsulinemia, both of which occur in this model of obesity as well as in obese human subjects. Plasma renin activity and plasma insulin concentrations increased 2.3-fold in dogs fed a high-fat diet. Evidence that elevated plasma AngII and insulin levels may contribute to glomerular structural changes is derived primarily from in vitro studies of mesangial cell cultures. For example, in isolated mesangial cells, AngII increases TGF-β1 production and insulin has a modest stimulatory effect. Moreover, the effects of insulin and AngII on mesangial cell TGF-β1 production seem to be additive (16). However, these effects have been observed in vitro using very high concentrations of AngII (usually 10^{-6} M or higher) and insulin. In vivo studies with animals and humans suggest that blockade of AngII may reduce the decrease in renal function associated with severe renal disease, but AngII blockade also reduces arterial and glomerular hydrostatic pressures. Also, it is not clear whether AngII blockade prevents or ameliorates the changes in renal structure and function that occur early in obesity, before severe renal injury. Therefore, the roles of increased AngII levels and hyperinsulinemia in mediating the renal changes associated with a high-fat diet and obesity remain to be determined.

TGF-β1 has been suggested to promote renal structural changes and fibrosis in several kidney disorders, including diabetic nephropathy and experimental nephritis, by increasing the production and deposition of extracellular matrix (17–26). In fact, blockade of TGF-β with TGF-β-binding proteins markedly attenuated renal scarring in experimental nephritis (27). In this study, glomerular TGF-β1 levels, as assessed in immunohistochemical analyses, tended to be increased after 7 to 9 wk of a high-fat diet. These observations are consistent with the possibility that, in the early stages of obesity, elevated TGF-β1 levels may contribute to increased mesangial matrix in glomeruli from obese dogs, but more definitive experiments are needed to test this hypothesis.

Our laboratory previously reported that, early in obesity, there was a two- to threefold increase in hyaluronic acid content in the inner medulla (28), whereas other laboratories reported that TGF-β1 stimulated hyaluronan synthesis in fibroblasts and synovial lining cells (25). Therefore, TGF-β1 may be partly responsible for the increased renal medullary hyaluronic acid levels in obesity, although this hypothesis remains to be tested.

In summary, dogs fed a high-fat diet for 7 to 9 wk or 24 wk exhibited increased GFR and renal plasma flow, elevated arterial BP, hyperinsulinemia, and increased plasma renin activity. After 7 to 9 or 24 wk of the high-fat diet, there was an expansion of Bowman’s capsule, increased cell proliferation in the glomerulus, thickening of the mesangial matrix and Bowman’s capsule basement membranes, and indications of increased glomerular TGF-β expression. Although the precise mechanisms for these structural changes remain to be elucidated, the changes may be precursors of more severe renal injury in chronic obesity.

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