The present study tested the hypothesis that altered vascular regulation of arachidonic acid enzymes in obese Zucker rats contributes to renal damage. Protein expression of CYP450 (cytochrome P450) and COX (cyclo-oxygenase) enzymes in renal microvessels was studied in obese and lean Zucker rats at 20–21 weeks of age. Body weight and blood glucose averaged 649 ± 13 g and 142 ± 10 mg/dl in obese Zucker rats compared with 437 ± 10 g and 111 ± 5 mg/dl in age-matched lean Zucker rats. Renal microvascular CYP4A and COX-2 protein levels were increased and CYP2C protein levels decreased in obese Zucker rats. TX (thromboxane) B2 excretion was 2-fold higher and PG (prostaglandin) E2 excretion significantly lower in obese Zucker rats. Additional studies investigated the ability of the COX-2 inhibitor, rofecoxib, to slow the progression of renal injury in obese Zucker rats. Rofecoxib treatment decreased urinary PGF2α and 8-isoprostane levels in obese Zucker rats. Renal microvessel mRNA expression of pro-inflammatory chemokines was decreased in COX-2-inhibitor-treated obese Zucker rats. Urinary albumin excretion, an index of kidney damage, averaged 95 ± 11 mg/day in vehicle-treated and 9 ± 1 mg/day in rofecoxib-treated obese Zucker rats. Glomerulosclerosis, characterized by mesangial expansion, tubulo-interstitial fibrosis and extracellular matrix accumulation, was prominent in obese Zucker rats compared with a lack of damage in age-matched lean Zucker rats and rofecoxib-treated obese Zucker rats. These results suggest that altered vascular arachidonic acid enzymes contribute to the renal damage, and that COX-2 inhibition decreases glomerular injury in obese Zucker rats.

INTRODUCTION

The number of ESRD (end-stage renal disease) patients maintained on dialysis is projected to double between 2000 and 2010. A major contributing factor to this alarming increase in ESRD is the disorder named ‘syndrome X’ or ‘insulin resistance syndrome’ [1–3]. Syndrome X is a polygenic disorder that involves the clustering of metabolic and cardiovascular risk factors, such as obesity, insulin resistance, Type II diabetes, hypertension, dyslipidaemia and endothelial dysfunction [2–4]. Obese Zucker rats have metabolic characteristics similar to those observed in humans with Type II diabetes, and these changes develop during the first 20 weeks of...
life [5–9]. These rats have impaired glucose tolerance and increased blood glucose levels, endothelial dysfunction, and die of renal failure [5–9]. The mechanisms responsible for renal injury in syndrome X patients and the obese Zucker rats are not well understood.

Arachidonic acid metabolites of the COX (cyclooxygenase) and CYP450 (cytochrome P450) pathways are generated in the kidney and contribute to vascular and tubular function [10–12]. It is also recognized that during renal and cardiovascular disease states there are changes in the regulation and activity of COX and CYP450 enzymes [10–12]. There are two COX isoforms expressed in the kidney, COX-1 and COX-2, which convert arachidonic acid to PG (prostaglandin) H$_2$ and subsequently to PGD$_2$, PGE$_2$, PGF$_{2\alpha}$, PGF$_{3\alpha}$ and TX (thromboxane) A$_2$ via synthetases [10]. CYP450 enzymes generate EET (epoxyeicosatrienoic acid) and 20-HETE (20-hydroxyeicosatetraenoic acid) [10,12]. Similar to the COX enzymes, two main CYP450 enzymes contribute to the renal vascular EET and 20-HETE generation. CYP2C enzymes are primarily responsible for EET production, and CYP4A enzymes primarily produce 20-HETE. In addition, hydration of the epoxide group of the EET to a diol, forming the corresponding DHET (dihydroxyeicosatrienoic acid), is mediated by SEH (soluble epoxide hydrolase) [10]. The balance between COX and CYP450 metabolites can influence renal vascular function, and an improper balance during disease states could contribute to the progression of nephropathy. Although the role of arachidonic acid metabolites in the pathogenesis of diabetic nephropathy has been suggested in a number of clinical and experimental studies, evidence for the involvement in obesity-related kidney damage has not been addressed [13–16]. Therefore, the present study tested the hypothesis that altered renal vascular regulation of COX and CYP450 enzymes occurs in obese Zucker rats and could contribute to renal damage. As these studies demonstrated an increase in COX-2 protein expression, we also evaluated the ability of COX-2 inhibition to slow the progression of renal injury in obese Zucker rats.

**METHODS**

**Animals**

All experiments involving animals were carried out according to the guidelines of the Medical College of Georgia Institutional Animal Care and Use Committee. Male obese Zucker rats (ZDF/GmiCrl-fa/fa) and lean Zucker rats (Charles River Laboratories, Wilmington, MA, U.S.A.) were used for this study. The rats were housed in separate cages and maintained in a temperature- and light-controlled room. Throughout the experimental period, animals had access to standard chow and drinking water. Obese Zucker rats were further studied to determine the effect of the COX-2 inhibitor, rofecoxib, on renal injury. These rats were divided into two groups: vehicle- and rofecoxib-treated groups. Rofecoxib was administered via the drinking water for three weeks (Vioxx, 10 mg/kg per day; Merck) [16,17]. Surgical procedures were performed with animals under pentobarbital anaesthesia (40 mg/kg, intraperitoneal) and in sterile conditions. Animals were killed at 20–21 weeks of age for tissue harvesting.

**Blood glucose and cholesterol measurements**

Blood glucose levels were measured, using a commercially available kit (Roche), from the tail vein of the rats after overnight intake of food and after 3–4 h of food deprivation. Total serum cholesterol was measured using a colorimetric assay (Wako Diagnostics, Richmond, VA, U.S.A.).

**Isolation of renal microvessels**

Renal microvessels were isolated according to a method described previously [18]. Animals were anaesthetized and a midline abdominal incision was made. The abdominal aorta below the renal arteries was cannulated and the superior mesenteric and aorta above the renal arteries ligated. The kidneys were cleared of blood by perfusion of the isolated aortic segment with ice-cold PSS (physiological saline solution).

The kidneys were removed, decapsulated and the renal medulla removed. Renal cortical tissue was pressed with a spatula on a 180-μm pore size stainless sieve and the retentate rinsed several times with ice-cold PSS. The vascular tissue remaining was drawn through an 18-gauge needle four times to shear off attached glomeruli. Renal microvessels, with some attached tubules, were gently agitated in a PSS solution, containing 0.2 mg/ml each of dithiothreitol, collagenase type II (200–300 units/mg), soya-bean trypsin inhibitor type I-S (10 000 units/mg of protein) and albumin, and incubated for 1–1.5 h at 37°C in 20 ml of a mixture of 95 % O$_2$/5 % CO$_2$. After collagenase digestion, the incubation media was discarded and the vessels suspended in 20 ml of ice-cold PSS, placed on to a nylon membrane (100 μm mesh) in a vacuum filtration apparatus and rinsed several times with ice-cold PSS. Microvessels retained on the nylon membrane were collected under a stereomicroscope to ensure that they were free of tubular contamination. Renal microvessels (80 mg of tissue) from rats were quickly frozen in liquid nitrogen and stored at −80°C until used.

**CYP450, SEH and COX protein expression in renal microvessels**

Protein extracted as described above was homogenized in lysis buffer and concentration determined by the method of Lowry et al. [19]. Renal microvessels were separated.
by SDS/PAGE on a 10 % Tris/glycine gel, and proteins were transferred electrophoretically on to a 0.4 µm nitrocellulose membrane. Molecular mass markers (20–120 kDa) were used to determine the approximate molecular masses. The non-specific binding was blocked by incubating the membranes at 4 °C in a blocking solution containing 20 % non-fat dried milk in PBST (PBS containing 0.3 % Tween-20). The membranes were washed with PBST and incubated with primary antibodies [goat anti-rat CYP4A (1:2000, Gentest), goat anti-rat CYP2C11 (1:500, Gentest), rabbit anti-rat CYP2C23 (1:5000; a gift from Dr Jorge H. Capdevila, Division of Nephrology, Vanderbilt University, Nashville, TN, U.S.A.), rabbit anti-mouse SEH (1:2000, a gift from Dr Bruce D. Hammock, Department of Entomology, University of California at Davis, Davis, CA, U.S.A.), goat anti-mouse COX-1 (1:100, Santa Cruz) and rabbit anti-mouse COX-2 (1:1000, Cayman Chemicals)] for 2 h at room temperature (24 °C). The blots were washed in PBST or 100 mM Tris/HCl (COX) and incubated with secondary antibodies [CYP4A, CYP2C11, CYP2C23, SEH, COX-1 and COX-2: donkey anti-goat IgG–HRP (horseradish peroxidase) (1:40 000), rabbit anti-goat IgG–HRP (1:30 000), goat anti-rabbit IgG–HRP (1:100 000), goat anti-rabbit IgG–HRP (1:40 000), rabbit anti-goat IgG–HRP (1:40 000) and goat anti-rabbit IgG–HRP (1:20 000) respectively] for 1 h. After incubation with the secondary antibodies, the membranes were washed with PBST and the bands detected using ECL (enhanced chemiluminescence) Western blotting. The densities of the band intensity were measured and the values were normalized to expression of β-actin protein expression.

Measurement of urinary COX metabolites, electrolytes and albumin
The urinary levels of COX metabolites, electrolytes and albumin from 20–21-week old obese and lean Zucker rats were measured. Animals were housed in separate metabolic cages that efficiently separated urine from food and faeces. Urine was collected in a conical tube containing 5 mg of triphenylphosphine and cooled by dry ice. Samples were stored at −80 °C until assayed. Urinary electrolyte levels were measured using ion-selective electrodes (Synchron EL-ISE, Beckman Instruments, Brea, CA, U.S.A.). The levels of 6-keto PGF1α, PGF2α, PGE2, TXB2 and 8-isoprostane in urine were measured using enzyme immunoassays in accordance with the manufacturer's instructions (Cayman Chemicals). The urinary albumin level was measured by using a competitive ELISA (Nephrat®, Exocell).

Chemokine mRNA expression in renal microvessels
Total RNA was isolated from the renal microvessels by using an ultra-pure TRIzol reagent according to the manufacturer's instructions (Gibco BRL). TGF-β1 (transforming growth factor-β1), MCP-1 (monocyte chemotactic protein-1), VCAM-1 (vascular cell adhesion molecule-1), IL-1β (interleukin-1β) and GAPDH (glyceraldehyde-3-phosphate dehydrogenase) mRNA expression in renal microvessels was assessed by RT-PCR (reverse-transcriptase PCR) using the SuperScript™-one-step RT-PCR kit (Invitrogen). The reaction mixture (50 µl) contained 1.5 mM MgSO4, 200 µM dNTPs, 0.3 µM of each primer and 0.75 µg of RNA isolated from the microvessels. The sequences of the TGF-β1, MCP-1, VCAM-1 and IL-1β primers are shown in Table 1. cDNA was synthesized by incubating the reaction mixture at 50 °C for 30 min followed by denaturation of DNA at 94 °C for 4 min. The PCR conditions used for 30 cycles were as follows: 94 °C for 30 s for denaturation, 60 °C for 30 s for annealing, 72 °C for 1 min/kb for extension. In addition, one cycle of 72 °C for 10 min was also carried out. PCR products were analysed by electrophoresis in 1.5 % agarose gels stained with ethidium bromide (0.5 µg/ml). The gel was scanned with UV illumination using Digital Imaging and Analysis (Alpha Innotech Corporation). Band intensity was measured densitometrically and the values were normalized to expression of GAPDH.

<table>
<thead>
<tr>
<th>Primer</th>
<th>Sequence</th>
</tr>
</thead>
<tbody>
<tr>
<td>VCAM-1</td>
<td></td>
</tr>
<tr>
<td>Forward</td>
<td>5′-GGAGACACTGTCATTCCCTGG-3′</td>
</tr>
<tr>
<td>Reverse</td>
<td>5′-TCTTCTCGTATGTTCTTCGG-3′</td>
</tr>
<tr>
<td>TGF-β1</td>
<td></td>
</tr>
<tr>
<td>Forward</td>
<td>5′-TATAGCAACTACCTCCTGG-3′</td>
</tr>
<tr>
<td>Reverse</td>
<td>5′-CAGAGTGCTGGAGTAGCC-3′</td>
</tr>
<tr>
<td>MCP-1</td>
<td></td>
</tr>
<tr>
<td>Forward</td>
<td>5′-CTTGAGTCGCCATCTATTGG-3′</td>
</tr>
<tr>
<td>Reverse</td>
<td>5′-CTTCTGAGCACTCCATTGG-3′</td>
</tr>
<tr>
<td>IL-1β</td>
<td></td>
</tr>
<tr>
<td>Forward</td>
<td>5′-TGAATGCCATTCTGGACAG-3′</td>
</tr>
<tr>
<td>Reverse</td>
<td>5′-GGAGTGTTCTGATGGCACAG-3′</td>
</tr>
</tbody>
</table>

Histology and GSI (glomerulosclerotic index)
For tissue preparation and histopathological evaluation, the kidneys were retrogradely perfused with cold PSS (145 mM NaCl, 6 mM KCl, 1 mM MgCl2, 10 mM Hepes and 10 mM glucose), followed by 10 % formalin, pH 7.4, and kept overnight in the same solution then routinely processed for paraffin embedding. Kidney slices were cut at 4 µm and stained with periodic acid Schiff/haematoxylin or haematoxylin/eosin according to the standard methods. All sections were mounted with cytoseal and observed using light microscopy. Eighty

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metabolic parameters in 20–21-week old obese and lean Zucker rats

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Lean (n = 12)</th>
<th>Obese (n = 18)</th>
<th>Obese plus rofecoxib (n = 6)</th>
</tr>
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<tbody>
<tr>
<td>Blood glucose (mg/dl)</td>
<td>111.4 ± 4.7</td>
<td>142.3 ± 10.3*</td>
<td>138.5 ± 9.6*</td>
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<tr>
<td>Body weight (g)</td>
<td>436.8 ± 10.2</td>
<td>649.2 ± 13.3*</td>
<td>623.3 ± 13.6*</td>
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<tr>
<td>Urine volume (ml/day)</td>
<td>12.6 ± 0.6</td>
<td>23.8 ± 2.9†</td>
<td>22.5 ± 1.1†</td>
</tr>
<tr>
<td>Urinary excretion rate (mmol/day)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Na⁺</td>
<td>0.8 ± 0.1</td>
<td>1.1 ± 0.1</td>
<td>1.6 ± 0.2†</td>
</tr>
<tr>
<td>K⁺</td>
<td>3.5 ± 0.2</td>
<td>5.2 ± 0.4†</td>
<td>6.7 ± 0.8†</td>
</tr>
<tr>
<td>Cl⁻</td>
<td>1.2 ± 0.1</td>
<td>2.0 ± 0.2†</td>
<td>2.7 ± 0.3†</td>
</tr>
<tr>
<td>Albumin (mg/day)</td>
<td>8.7 ± 3.3</td>
<td>94.5 ± 11.2*</td>
<td>9.8 ± 0.8‡</td>
</tr>
<tr>
<td>Cholesterol (mg/dl)</td>
<td>90.9 ± 5.2</td>
<td>133.7 ± 7.4*</td>
<td>140.5 ± 16.6*</td>
</tr>
</tbody>
</table>

Table 2 Metabolic parameters in 20–21-week old obese and lean Zucker rats

Values are expressed as the means ± S.E.M. *Significant difference between obese versus lean Zucker rats; †significant difference between obese Zucker versus rofecoxib-treated obese Zucker rats.

Results

Baseline parameters in obese and lean Zucker rats

Table 2 presents metabolic parameters in obese and lean Zucker rats. The 5-week old obese Zucker rats weighed 171 ± 3 g and the lean Zucker rats weighed 145 ± 3 g at the initiation of the study. At the end of 20 weeks, the obese Zucker rats had a significantly higher body weight than the lean Zucker rats. The increase in body weight with age was also associated with a significant increase in blood glucose over the 15-week period. At the end of the study, the obese Zucker rats developed a modest increase in blood glucose to levels averaging 142 ± 10 mg/dl, whereas the lean animals had blood glucose levels that averaged 111 ± 5 mg/dl. Plasma cholesterol levels were also elevated by 47% in obese Zucker rats compared with lean Zucker rats. Urinary Na⁺ and Cl⁻ excretion rates were similar between obese and lean Zucker groups. Interestingly, urinary K⁺ excretion was higher in obese Zucker rats compared with lean Zucker rats. Urinary albumin excretion, an indicator of renal damage, was increased 13-fold in the 20–21-week old obese Zucker rats compared with the lean Zucker rats.

The changes in metabolic parameters that occurred in obese Zucker rats after 3 weeks of rofecoxib treatment are also shown in Table 2. Body weight, blood glucose and plasma cholesterol levels in rofecoxib-treated obese Zucker rats were similar to untreated obese Zucker rats. COX-2 inhibition resulted in a significant increase in Na⁺ and Cl⁻ urinary excretion rates. Interestingly, urinary albumin excretion was significantly decreased in obese Zucker rats treated with rofecoxib for 3 weeks.

CYP450 and COX expression in renal microvessels of obese and lean Zucker rats

Renal microvascular CYP450 and COX enzyme protein expression is shown in Figures 1, 2 and 3. The representative Western blot and densitometric analysis of CYP4A and SEH protein expression in Zucker rats are shown in Figure 1. A significant 1.5-fold increase in CYP4A expression was observed in the renal microvessels from 20–21-week old obese Zucker rats when compared with age-matched lean Zucker rats. On the other hand, obese Zucker and lean Zucker rats had similar levels of renal microvascular SEH expression. Figure 2 depicts the results of the Western blots and densitometric analyses of CYP2C11 and CYP2C23 in Zucker rats. CYP2C11 protein expression was decreased 1.5-fold in the renal microvessels isolated from 20–21-week old obese Zucker rats when compared with lean Zucker rats. The protein expression of CYP2C23 was also decreased 2.0-fold in the microvessels isolated from obese Zucker rats when compared with lean Zucker rats. COX-1 and COX-2 protein expression in renal microvessels of obese and lean Zucker rats is shown in Figure 3. There was no change in expression of microvascular COX-1 between obese and lean Zucker rats. In contrast, a significant 1.6-fold increase in COX-2 expression was observed in the renal microvascular tissues of obese Zucker rats.

Urinary excretion of COX metabolites in Zucker rats

Urinary COX metabolite excretion rates are presented in Figure 4. The urinary excretion rate of TXB₂ significantly increased 2-fold in obese Zucker compared with lean Zucker rats. Likewise, urinary excretion of 6-keto PGF₁α was significantly higher in obese Zucker rats. Obese and lean Zucker rats excreted PGF₂α at similar rates. In contrast, the PGE₂ urinary excretion was significantly lower in obese Zucker rats compared with lean Zucker rats. Urinary excretion rates of 8-isoprostane, a marker
Obesity, cyclo-oxygenase-2 and renal injury

Figure 1 Microvascular protein expression levels of CYP4A and SEH in obese and lean Zucker rats
Upper panels. Left: microvascular CYP4A protein expression. Representative Western blots showing 52-kDa CYP4A protein bands in 20–21-week old obese (lanes 1–4) and 20–21-week old lean Zucker rats (lanes 5–8). Right: densitometric values (du) for renal microvascular levels (10 µg/lane; n = 8 per group) normalized to the expression of β-actin. Values are expressed as the means ± S.E.M. *Significant difference between obese Zucker rats versus lean Zucker rats. Lower panels. Left: microvascular SEH protein expression. Representative Western blots showing 62-kDa SEH protein bands in 20–21-week old obese (lanes 1–4) and 20–21-week old lean Zucker rats (lanes 5–8). Right: densitometric values for renal microvascular levels (10 µg/lane; n = 8 per group) normalized to the expression of β-actin. Values are expressed as the means ± S.E.M. *Significant difference between obese Zucker rats versus lean Zucker rats.

of oxidative stress, were increased 3-fold in obese Zucker rats (15.6 ± 2.1 ng/day, P < 0.05) compared with lean Zucker rats (4.8 ± 1.2 ng/day).

Obese Zucker rats administered rofecoxib for 3 weeks had significant changes in urinary COX metabolites. COX-2 inhibition decreased urinary excretion of 6-keto PGF₁𝛼 by 32 % and PGF₂𝛼 by 83 %, but did not significantly alter urinary TXB₂ or PGE₂ excretion rates. Interestingly, urinary 8-isoprostane excretion was significantly reduced in obese Zucker rats receiving 3 weeks of rofecoxib treatment (4.3 ± 0.8 ng/day, P < 0.05).

Effect of COX-2 inhibition on renal microvascular chemokine mRNA expression in obese Zucker rats
Figure 5 depicts the results of RT-PCR and densitometric analysis of TGF-β1, MCP-1, IL-1β and VCAM-1 mRNA expression relative to GAPDH in control and rofecoxib-treated obese Zucker rats. Three weeks of COX-2 inhibition significantly reduced the mRNA expression of the pro-inflammatory cytokines in obese Zucker rats. TGF-β1 expression was decreased almost 2-fold in the treated animals. MCP-1 was highly expressed in renal microvessels of obese Zucker rats and rofecoxib treatment reduced expression by 65 %. IL-1β expression was also decreased significantly in obese Zucker rats treated with rofecoxib. Lastly, rofecoxib treatment caused a significant decrease in VCAM-1 expression in obese Zucker rats.

Glomerular morphology in obese and lean Zucker rats
The obese Zucker rats were characterized by moderate glomerulosclerosis (GSI: obese, 0.57 ± 0.07 arbitrary unit; lean, 0.21 ± 0.02; P < 0.05) and tubulointerstitial fibrosis, associated with mesangial matrix expansion, thickening of glomerular and tubular basement membranes, accumulation of extracellular matrix deposits, infiltration of monocyte/macrophages, occasional tubular

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Figure 3  Microvascular protein expression levels of COX-1 and COX-2 in obese and lean Zucker rats
Upper panels. Left: microvascular COX-1 protein expression. Representative Western blots showing 68-kDa COX-1 protein bands in 20–21-week old obese (lanes 1–4) and 20–21-week old lean Zucker rats (lanes 5–8). Right: densitometric values (du) for renal microvascular levels (10 µg/lane; n = 8 per group) normalized to the expression of β-actin. Values are expressed as the means ± S.E.M. *Significant difference between obese Zucker rats versus lean Zucker rats. Lower panels. Left: microvascular COX-2 protein expression. Representative Western blots showing 72-kDa COX-2 protein bands in 20–21-week old obese (lanes 1–4) and 20–21-week old lean Zucker rats (lanes 5–8). Right: densitometric values for renal microvascular levels (10 µg/lane; n = 8 per group) normalized to the expression of β-actin. Values are expressed as the means ± S.E.M. *Significant difference between obese Zucker rats versus lean Zucker rats.

Figure 4  Urinary excretion rates of COX metabolites in 20–21-week old lean Zucker rats (n = 12), obese Zucker rats (n = 18) and rofecoxib-treated obese Zucker rats (n = 6)
Values are expressed as the means ± S.E.M. *Significant difference between obese Zucker or rofecoxib-treated obese Zucker rats versus lean Zucker rats; †Significant difference between obese Zucker versus rofecoxib-treated obese Zucker rats.

Figure 5  TGF-β1, MCP-1, IL-1β and VCAM-1 mRNA expression and densitometric analysis in untreated and rofecoxib-treated obese Zucker rats
Representative RT-PCR showing mRNA bands in renal microvessels isolated from 18-week old untreated obese (lanes 3 and 4) and rofecoxib-treated obese Zucker rats (lanes 1 and 2). Densitometric values for microvascular levels (20 µg/lane; n = 6 per group) normalized to the expression of GAPDH. Values are expressed as the means ± S.E.M. *Significant difference between obese Zucker versus rofecoxib-treated obese Zucker rats.

DISCUSSION

Obesity, which affects multitudes in the Western society, is associated with related metabolic disorders, such as dyslipidaemia, hypertension, insulin resistance and hyperinsulinaemia, that contribute to an increased risk of renal and cardiovascular diseases [1–4]. The clustering of obesity and these other metabolic and cardiovascular risk factors are characteristics of syndrome X. Interestingly,
a major contributing factor to the increasing incidence of ESRD is syndrome X and Type II diabetes [1–4]. In particular, diabetic nephropathy develops as a result of the interplay between metabolic abnormalities inherent to diabetes and abnormalities of the renal vasculature that ultimately contribute to progressive structural malformations and dysfunction of the glomerulus [3,4,21]. The present study evaluated renal damage and microvascular arachidonic-acid-metabolizing enzymes in the obese Zucker rat which has similar characteristics to syndrome X patients. We provide initial evidence that increased CYP4A and COX-2 and decreased CYP2C11 and CYP2C23 protein expression in the renal microvessels of obese Zucker rats are associated with nephropathy. Additional studies were conducted to determine the contribution of COX-2 to the renal injury, because of the increased expression of COX-2 in the obese Zucker rat. We observed that glomerular injury was decreased in obese Zucker rats receiving the COX-2 inhibitor, rofecoxib, for 3 weeks.

As expected, the present study demonstrates that there is a significant increase in blood glucose with increasing body weight in obese Zucker rats. The obese Zucker rats also had unaltered urinary excretion of Na⁺ and increased urinary K⁺ excretion. This finding is consistent with activation of the renin–angiotensin–aldosterone system which was observed previously in obese patients and animal models of obesity [22–24]. In addition to the mild hyperglycaemia, increased circulating cholesterol levels were evident in the obese Zucker rats. These parallels between the obese Zucker rat and syndrome X patients have previously been reported [3,4,21,25].

Evaluation of renal microvascular CYP450 protein expression suggested that these enzymes could contribute to renal functional changes in the obese Zucker rat. We observed increased renal vascular CYP4A protein expression in 20–21-week old obese Zucker rat. This finding is in agreement with a previous report showing increased liver CYP4A expression in obese Zucker rats and increased CYP4A2 expression in renal microsomes from STZ (streptozotocin)-induced diabetic rats [26–28]. Enriquez et al. [26] also demonstrated that obese mice had increased CYP4A10 and CYP4A14 mRNA expression in the liver. The CYP4A product, 20-HETE, is a potent vasoconstrictor and pro-hypertensive factor [10,12]. 20-HETE has also been implicated in the vascular myogenic and angiotensin responses [10,12]. Although the exact contribution of 20-HETE remains to be defined, it is interesting that the obese Zucker rats have increased skeletal muscle arteriolar myogenic responses and increased blood pressure responses to exogenous angiotensin [7]. On the other hand, the expression of epoxygenase enzymes CYP2C11 and CYP2C23 was significantly decreased in the renal microvessels of obese Zucker rats. In previous studies, a similar decrease in renal CYP2C23 expression and activity was observed in rats fed a high-fat diet and in obese Zucker rats at 12 weeks of age [29,30]. Likewise, decreased hepatic CYP2C11 levels have been shown in obese Zucker rats [31,32]. Epoxygenase metabolites possess renal and cardiovascular protective properties, including vascular relaxation, anti-inflammatory actions and inhibiting vascular smooth muscle proliferation, and decreased EET levels have been demonstrated to contribute to vascular dysfunction [10–12]. Therefore, increased CYP4A and decreased CYP2C renal microvascular expression could contribute to the renal and vascular injury observed in the obese Zucker rats. The specific renal vascular functional and structural changes attributed to the altered CYP450 expression in obese Zucker rats remain to be explored.

Several studies have linked STZ-induced diabetes to the COX pathway and, in particular, enhanced COX-2-derived prostanoid production [13–15]; however, the regulation of the renal vascular COX pathways in obesity...
is not well characterized. We examined the expression of COX enzymes in the renal microvessels of lean and obese Zucker rats. In the present study, the expression of COX-2 was increased, whereas the expression of COX-1 was unaltered in the renal microvessels of 20–21-week old obese Zucker rats. We also observed increased urinary TXB$_2$ and 6-keto PGF$_{1\alpha}$ and decreased PGE$_2$ excretion rates in 20–21-week old obese Zucker rats. These changes in renal microvessel COX-2 expression and urinary TXB$_2$ and PGE$_2$ levels have been observed as early as 12 weeks of age in the obese Zucker rat [29]. Similar changes in renal COX-2 and COX metabolite levels have been observed in humans and other diabetic rat models [13–15,33,34]. Interestingly, the Otsuka Long-Evans Tokushima Fatty strain, which is another obese rat model for Type II diabetes, has increased renal TXA$_2$ and PGI$_2$ production with progression of nephropathy compared with the lean Long-Evans Tokushima Otsuka strain [35]. STZ-induced diabetes is consistently associated with increased TXB$_2$ excretion, and urinary prostanooids are elevated early and decline later [33–35]. As COX-2 is usually associated with renal PGE$_2$ generation, the finding of decreased urinary PGE$_2$ levels and increased COX-2 levels in the obese Zucker rats cannot easily be explained. One possible explanation for this finding is that the increased COX-2 levels were unaltered or decreased in other cell types in the kidney. Moreover, the regulation of the synthetase responsible for the conversion of the COX-2 product PGH$_2$ into PGE$_2$ could be decreased in the obese Zucker rats. Nonetheless, there is ample evidence for increased urinary TXB$_2$ excretion associated with increased renal COX-2 levels [36,37]; however, our findings with rofecoxib treatment would suggest that COX-2 contributes primarily to PGF$_{2\alpha}$ and 8-isoprostanate levels in the obese Zucker rat.

We conducted additional experiments to determine the ability of COX-2 inhibition to ameliorate renal injury in the obese Zucker rats. Blood glucose and cholesterol levels were unaltered in obese Zucker rats receiving rofecoxib for 3 weeks. Rofecoxib treatment did not alter body weight, but did increase urinary electrolyte excretion. Untreated obese Zucker rats had increased albumin levels as an indicator of renal damage. Additionally, we observed increased glomerulosclerosis with mesangial expansion and collagen deposition in the obese Zucker rats. Other renal histological pathologies observed in the untreated obese Zucker rat included the presence of inflammatory infiltrates and accumulation of extracellular matrix deposits in the tubulointerstitium, tubular atrophy and the presence of proteinaceous casts. These observed histological changes observed in the obese Zucker rats also closely resemble kidney abnormalities noted in syndrome X and Type II diabetic patients [21,25,38,39]. Interestingly, obese Zucker rats treated with rofecoxib for 3 weeks had decreased urinary albumin levels and amelioration of glomerular injury. The consequences of elevated renal COX-2 expression in STZ-induced diabetes have also been studied by administering selective COX-2 inhibitors. COX-2 inhibitors reduce proteinuria, extracellular matrix deposition in glomeruli and improve renal blood flow and glomerular filtration in STZ-induced diabetes [13–15]. Thus COX-2 inhibition appears to provide renal protection in various animal models of diabetic nephropathy.

Decreased cytokine expression is one possible way that COX-2 inhibition could ameliorate renal injury in the obese Zucker rat. We demonstrated that 3 weeks of rofecoxib treatment decreased renal microvascular expression of TGF-β1, MCP-1, IL-1β and VCAM-1 in obese Zucker rats. TGF-β1 is a prosclerotic growth factor that has been shown to affect extracellular matrix turnover in renal cells exposed to elevated glucose conditions, and several manifestations of diabetic nephropathy may be a consequence of altered TGF-β1 production [40–43]. Similarly, MCP-1 is a member of the chemokine family that contributes importantly to the recruitment of monocytes/macrophages into the renal tubulointerstitium and mediates renal interstitial inflammation, tubular atrophy and interstitial fibrosis [44]. A connection between MCP-1 and obesity has been suggested, because MCP-1 mRNA expression is increased in the adipose tissue of genetically obese mice and in the kidney of Type II diabetic mice [45,46]. IL-1β is another central mediator of inflammatory reactions and acts on liver to produce the characteristic dyslipidaemia associated with the metabolic syndrome [47]. Like the other chemokines, IL-1β was decreased in obese Zucker rats treated with rofecoxib. Lastly, VCAM-1 is a chemokine that has been associated with increased oxidative stress [48]. Decreased VCAM-1 levels in the renal microvessels are an initial suggestion that oxidative stress could be decreased in COX-2-inhibited obese Zucker rats. Overall, selective COX-2 inhibition caused the down-regulation of cytokines in obese Zucker rats, indicating that a decreased inflammatory response contributed to the amelioration of glomerular injury.

Measurement of urinary metabolites revealed that rofecoxib treatment decreased 6-keto PGF$_{1\alpha}$, PGF$_{2\alpha}$, and 8-isoprostanate excretion rates. COX-2 inhibition did not alter urinary TXB$_2$ or PGE$_2$ levels in obese Zucker rats. Interactions between COX-2, 20-HETE, cytokines and diabetes were recently suggested by Ferreri et al. [49]; however, relationships amongst these factors in obesity remain unexplored. Urinary and tissue COX metabolites affected by COX-2 inhibitors that have been reported are quite variable and depend on the experimental setting, species and tissue studied. The finding of decreased PGI$_2$ and unaltered TXB$_2$ levels in the obese Zucker rats is in agreement with COX-2 inhibition studies in humans [50,51]. In addition, studies in rats have demonstrated that COX-2 inhibitors do not alter intestinal or urinary
crease 8-iso-PGF2α levels [52–54]. Although rofecoxib decreased prostacyclin levels in obese Zucker rats, this would not be expected to provide renal protection, because PGI2 is a vasodilator and platelet inhibitor [50,55]. On the other hand, decreased PGF2α and 8-isoprostane more likely contributed to the decrease in urinary albumin and glomerulosclerosis. 8-iso-PGF2α, a major F2 isoprostane and a potent vasoconstrictor, is increased in patients with diabetes and obesity and appears to contribute to platelet activation in these pathophysiological conditions [55–58]. COX-2 inhibition has also been shown to decrease 8-iso-PGF2α, lower blood pressure and decrease urinary protein excretion in hypertensive rats [59]. Thus the ability of rofecoxib treatment in obese Zucker rats to decrease oxidative stress likely contributes to the renal protection.

In conclusion, our results demonstrate that changes in the renal microvascular CYP450 and COX protein expression in 20–21-week old obese Zucker rats are associated with glomerular injury. Increased renal microvascular CYP4A and COX-2 protein expression and decreased CYP2C11 and CYP2C23 protein expression in the obese Zucker rat were observed. We further demonstrated that administering rofecoxib for 3 weeks decreased urinary albumin excretion, oxidative stress and glomerular damage. Therefore, increased COX-2 and oxidative stress could contribute to obesity-related kidney damage.

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