Endothelial Dysfunction and the Development of Renal Injury in Spontaneously Hypertensive Rats Fed a High-Fat Diet

Sarah F. Knight, Jeffrey E. Quigley, Jianghe Yuan, Siddhartha S. Roy, Ahmed Elmarakby, John D. Imig

Abstract—Obesity and hypertension have been identified as cardiovascular risk factors that contribute to the progression of end-stage renal disease. To examine the mechanisms by which a high-fat diet and hypertension contribute to endothelial dysfunction and renal injury, 8-week–old male spontaneously hypertensive rats and Wistar rats were fed a high-fat (36% fat) or a normal-fat (7% fat) diet for 10 weeks. The high-fat diet increased body weight in Wistar and hypertensive rats by 25 and 31 g, respectively. Systolic blood pressure was higher in the hypertensive rats compared with Wistar rats; however, blood pressure was unaltered by the high-fat diet. Afferent arteriole response to acetylcholine was impaired in the high-fat groups after just 3 weeks. Renal macrophage infiltration was increased in the hypertensive high-fat group compared with others, and monocyte chemoattractant protein-1 excretion was increased in both of the high-fat–fed groups. Renal PCR arrays displayed significant increases in 2 inflammatory genes in hypertensive rats fed a normal diet, 1 gene was increased in high-fat–fed Wistar rats, whereas 12 genes were increased in high-fat–fed hypertensive rats. Urinary albumin excretion was increased in the hypertensive rats compared with the Wistar rats, which was further exacerbated by the high-fat diet. Glomerular nephrin expression was reduced and desmin was increased by the high-fat diet in the hypertensive rats. Our results indicate that endothelial dysfunction precedes renal injury in normotensive and spontaneously hypertensive rats fed a high-fat diet, and hypertension with obesity induces a powerful inflammatory response and disruption of the renal filtration barrier. (Hypertension. 2008;51:352-359.)

Key Words: obesity ■ inflammation ■ hypertension ■ renal disease

Obesity and hypertension are comorbid pathological conditions that have been identified as independent risk factors for the development of endothelial dysfunction and renal disease.1 These risk factors are increasing in prevalence at an alarming rate, with >30% of the US population classified as obese, and 1 in 3 adult Americans currently suffering from hypertension. Blood pressure is strongly correlated with body mass index, and in the Framingham Offspring Study, ≥78% of male hypertensive cases were attributable to obesity.2 Independently obesity increases the risk for chronic kidney disease 4-fold,3 hypertensive patients account for 25% of all chronic kidney disease patients, and obese patients with hypertension are at the greatest risk for developing chronic renal disease.4–6

Independently, hypertension and obesity have been linked with the development of insulin resistance, endothelial dysfunction, inflammation, and renal injury.7,8 However, these conditions are commonly found in combination, and it is now becoming apparent that the ensuing renal injury and vascular dysfunction are results of the combination of the 2 risk factors.9 Animal models of obesity and hypertension, such as the obese Zucker rat, have been shown to develop albuminuria, progressive glomerulosclerosis, and endothelial dysfunction; however, the mechanisms involved in the development of insulin resistance, vascular dysfunction, and renal injury are complex and still not completely understood.10–12

There is growing evidence that there is a relationship among obesity, hypertension, and increased levels of circulating proinflammatory cytokines, which have been associated with the development of endothelial dysfunction and renal injury.13,14 Activation of an inflammatory response has also been observed in animal models of obesity in addition to increased oxidative stress and lipid mediators, which can contribute to renal injury.15,16 Deficiency of the inflammatory cytokine monocyte chemoattractant protein-1 (MCP-1) receptor (Ccr2) gene has been shown to decrease weight gain in high-fat–fed mice to reduce adipose tissue macrophage infiltration and improve insulin sensitivity.17 In addition, obese MCP-1 (Ccl2) knockout mice are protected from renal inflammation and diabetic renal injury.18

In light of this evidence, we hypothesized that a high-fat diet would impair endothelial function and potentially exacerbate renal injury in spontaneously hypertensive rats. We proposed that an inflammatory response to 10 weeks of
high-fat feeding would contribute to the altered endothelial function and renal injury in this model. Therefore, in this study we aimed to investigate how a high-fat diet affects renal endothelial and glomerular function and to examine potential mechanisms involved in the development of renal injury in obesity and hypertension.

Methods

All of the animal studies were approved by the Medical College of Georgia Institutional Review Committee according to the National Institutes of Health Guidelines for Care and Use of Laboratory Animals. Eight-week–old male Wistar Kyoto (WKY) rats and spontaneously hypertensive rats (SHRs) were divided into groups (n=4 to 6) and fed ad libitum either normal rat chow containing 7% fat or a high-fat diet containing 36% fat (#F2685, BioServ). Every 7 days, rats were weighed, blood glucose measurements were taken from a tail vein using a glucometer, and systolic blood pressure was measured using tail-cuff plethysmography.

Hyperinsulinemic-euglycemic clamp experiments, enzyme-linked immunosassays, in vitro–perfused juxtamedullary nephron experiments, immunohistochemistry, immunofluorescence, and Western blots gene expression arrays were carried out. For detailed methods please see the online supplemental data at http://hyper.ahajournals.org.

Results

Metabolic Parameters

Figure 1 displays the effect of 10 weeks of a normal- or high-fat diet on body weight and mean systolic blood pressure in male WKY rats and SHRs. The WKY rats had a higher baseline mean body weight of 185±11 g compared with the baseline mean body weight of 138±6 g in the SHRs (P<0.01), a pattern that continued throughout the 10-week study that was independent of diet (Figure 1A). The 10-weeks high-fat diet resulted in a significant increase in body weight in both strains compared with those fed a normal diet (P<0.05). Mean systolic blood pressure increased over the 10-week period in the WKY rats fed a normal diet (from 128±5 mm Hg to 151±4 mm Hg; Figure 1B).

The mean systolic blood pressure of WKY rats fed a high-fat diet increased similarly (from 126±2 to 151±2 mm Hg), indicating that a high-fat diet had no effect on systolic blood pressure in the WKY rats (Figure 1B). The SHRs had a significantly higher mean systolic blood pressure than the WKY rats throughout the study period, which was unaffected by diet. These data indicate that, for ≤10 weeks, a high-fat diet had no effect on systolic blood pressure in either rat strain. The systolic blood pressures measured were at the high end of the reference range for WKY rats at the corresponding age, which may have been a result of the tail-cuff technique used, which can transiently elevate blood pressure because of increasing stress levels in the rats.

Plasma Leptin and Cholesterol Levels

Plasma leptin levels were significantly increased in both the WKY rats and SHRs fed a high-fat diet compared with the groups fed a normal diet after just 3 weeks (P<0.001; Figure 2A). Interestingly, after 6 weeks on the high-fat diet the WKY rats displayed an even greater elevation in plasma leptin compared with the similarly treated SHRs (P<0.05). This pattern continued until 10 weeks on the respective diets. Plasma cholesterol levels were 44±3 and 64±2 mg/dL in the WKY rats and SHRs, respectively, fed a normal diet for 3 weeks, and levels were increased to 68±2 and 93±4 mg/dL, respectively, by 3 weeks on the high-fat diet (P<0.05). Plasma cholesterol levels remained elevated in both WKY rats and SHRs fed a high-fat diet throughout the study.

Blood Glucose Levels and Insulin Sensitivity

Insulin sensitivity measurements are presented in Figure 2B through 2D. There were no differences in blood glucose levels observed throughout the 10-week study period between rat strains or diets (Figure 2B). Plasma insulin levels were similar between the WKY rats and SHRs after 3 and 6 weeks on the normal- and high-fat diets (Figure 2C). However, after 10 weeks, both WKY and SHR groups fed high-fat diets displayed significant increases in plasma insulin compared with those on a normal diet (P<0.05).

Hyperinsulinemic-euglycemic clamp experiments were carried out to monitor peripheral insulin sensitivity in WKY rats and SHRs fed a normal- or high-fat diet for 10 weeks (Figure 2D). In the SHRs, a 20% reduction in the glucose infusion rate was required compared with the WKY rats, indicating the development of insulin resistance in SHRs (P<0.05). Interestingly, 10 weeks of high-fat feeding did not alter peripheral insulin sensitivity in the WKY rats or exacerbate the reduction in insulin sensitivity observed in the SHRs.

Afferent Arteriole Endothelial Function

Afferent arteriole endothelial function was measured using the in vitro juxtamedullary preparation, and the results are depicted in Figure 3. Afferent arteriole endothelial dilatory responses to acetylcholine were significantly impaired in the WKY rats after just 3 weeks of high-fat feeding compared with WKY rats fed a normal diet (P<0.05). Endothelial function was also impaired in the SHRs fed a high-fat diet for 3 weeks in response to acetylcholine compared with SHRs fed a normal diet (P<0.05; Figure 3A). Endothelial function continued to be impaired in both rat strains as a result of the high-fat feeding for ≤10 weeks (Figure 3B; P<0.05). The SHR group fed a normal diet for 10 weeks displayed an impaired endothelial dilatory response to acetylcholine compared with the WKY rats fed a normal diet for 10 weeks (P<0.05). Smooth muscle cell dilatory responses to sodium
nitroprusside reached 68±10% and 47±9% of baseline in WKY rats fed a normal- and high-fat diet, respectively, for 3 weeks and 73±10% and 69±4% in the normal- and high-fat–fed SHRs. These data indicate that it is specifically the endothelial dilatory response that is impaired by high fat in this model.

**Inflammatory Markers**

Figure 4A displays representative pictures of renal glomeruli, which show that macrophages were present in the kidneys of SHRs fed a normal diet for 10 weeks. In contrast, SHRs fed a high-fat diet for 10 weeks displayed an increase in macrophage infiltration compared with the other groups (P<0.05).

Plasma levels of the inflammatory marker MCP-1 were 23±1 and 22±2 ng/mL in the WKY rats and SHRs fed a normal diet, respectively, and levels were not different at 28±2 and 18±1 ng/mL in the high-fat-fed WKY rats and SHRs. Urinary MCP-1 levels were not altered by a 3-week high-fat diet in either rat strain; in contrast, MCP-1 protein excretion was significantly increased by the 10-week high-fat diet in both strains compared with those fed a normal diet (P<0.05; Figure 4B).

**Gene Expression Profiling of Inflammatory Cytokines and Receptors**

Real-time PCR arrays were used to profile mRNA expression of 84 inflammatory cytokines and receptors in the kidney cortex. In calculating fold changes in gene expression, the normal-diet WKY rats were used as controls (baseline) for both the normal-diet SHR group and the high-fat–diet WKY group. The WKY group fed a high-fat diet was then used as a control for the SHR group fed a high-fat diet. Gene expression was considered to be significantly upregulated when fold increases were >2.5 above the respective control group and P<0.05. In the WKY group fed a high-fat diet Ccr1 (2.6) mRNA was upregulated in comparison with the normal-diet WKY group (Figure 5A). In the normal-diet SHR group, mRNAs of Ccl22 (3.2) and Ccl19 (4.1) were upregulated when compared with the normal-diet WKY group (Figure 5B). The high-fat–diet SHR group had 12 genes upregulated compared with the high-fat–diet WKY group (Figure 5C): Bcl6 (4.6), Ccl11 (3.4), Ccl25 (12.9), Cxcl1 (7.6), Cxcl2 (6.2), Il18 (2.6), Il1a (4.0), Il1f6 (18.2), Il3 (8.9), Il4 (6.2), Il5 (4.9), and Il5ra (4.7). There were several other genes...
Renal Injury Markers

Urinary albumin excretion was unchanged after 10 weeks of high-fat feeding in the WKY rats (Figure 6A). After 3 and 6 weeks of either diet, the SHRs displayed albumin excretion levels similar to those observed in the WKY rats. After 10 weeks, however, the SHRs fed a normal diet developed significantly higher albumin excretion levels than the WKY rats \((P<0.05)\). In addition, the SHRs fed a high-fat diet for 10 weeks displayed an even greater increase in urinary albumin compared with the SHRs fed a normal diet \((P<0.05)\). Urinary protein excretion followed a similar pattern to that observed for albumin excretion (Figure 6B). The SHRs fed a normal diet for 10 weeks exhibited increased protein excretion compared with the WKY groups, and 10 weeks on a high-fat diet further exacerbated this proteinuria \((P<0.05)\).

The expression of the slit diaphragm protein nephrin was evaluated qualitatively by immunofluorescence on kidney sections from WKY rats and SHRs fed a normal- or high-fat diet for 10 weeks (Figure 7A) and quantitatively by Western blot on isolated glomeruli (Figure 7B). Ten weeks on a high-fat diet significantly reduced glomerular nephrin protein expression in both the WKY rats and the SHRs compared with those fed a normal diet \((P<0.05)\). Glomerular desmin was also measured by immunofluorescence on frozen kidney sections and by Western blot on isolated glomeruli (Figure 7C and 7D). Glomerular desmin expression was significantly increased in glomeruli from SHRs fed a high-fat diet for 10 weeks compared with the other groups \((P<0.001)\). In hematoxylin/eosin-stained frozen kidney sections that were blindly scored for injury, no differences in renal morphology were observed between the groups fed a normal- or high-fat diet; however, there was a trend toward an increased injury score in the SHRs compared with the WKY rats irrespective of diet (Figure S1).

Discussion

Currently, obesity and hypertension independently affect >30% of the US population.\(^4\) There is a direct link among obesity, hypertension, and the development of chronic kidney disease, the mechanisms of which are still little understood.\(^4\) SHRs experience many of the symptoms that are present in obese humans with hypertension when fed a high-fat diet. They gain weight, develop endothelial dysfunction, and, with a continued high-fat diet, renal injury and inflammation ensue.

Interestingly, we observed significant endothelial dysfunction after just 3 weeks on the high-fat diet in both normotensive and hypertensive rats, 7 weeks before changes in albumin excretion or inflammatory cytokine expression. We observed no differences in dilatory response to sodium nitroprusside, which indicated that the impaired response to acetylcholine was a result of endothelial dysfunction and not smooth muscle dysfunction. Plasma leptin and cholesterol levels were increased as a result of 3 weeks on the high-fat diet in both rat strains, which may have contributed to the development of endothelial dysfunction observed as a result of the high-fat diet. High leptin levels have been shown to reduce the dilatory response to acetylcholine.\(^18\) A potential mechanism for leptin and cholesterol-induced vascular dysfunction is through an upregulation of reactive oxygen species generation, which can disrupt the endogenous vasoactive response to acetylcholine.\(^19,20\) There is evidence that endothelial dysfunction is linked with the development of renal injury, and the presence of endothelial dysfunction is a predictor of cardiovascular risk and severity of renal disease.\(^21\) In this study we observed endothelial dysfunction 7 weeks before the development of renal injury and renal inflammation in the SHRs fed a high-fat diet but not in the WKY rats. These data would indicate that the endothelial dysfunction is not a clear predictor of renal injury in this model, but in combination with hypertension and a high-fat diet may have contributed to the renal injury observed.

Visceral obesity has been linked with the development of insulin resistance, type-2 diabetes, and renal injury by way of increased circulating free fatty acids and adipokines.\(^22,23\) High insulin levels can induce renal hemodynamic changes, glomerular hypertrophy, and mesangial cell proliferation, and autoregulation can be disrupted, lowering the pressure thresh-
old for renal damage. An increased incidence of insulin resistance has been reported in hypertensive patients, as well as animal models of hypertension, such as the SHR. In this study, we observed a significant increase in plasma insulin levels in both WKY rats and SHRs fed a high-fat diet compared with those fed a normal diet. However, euglycemic hyperinsulinemic clamp experiments did not indicate a reduction in peripheral insulin sensitivity as a result of 10 weeks of a high-fat diet. Therefore, we conclude that the WKY rats and SHRs receiving a high-fat diet have a level of insulin resistance that may be classified as prediabetic.

There is growing support for the hypothesis that obesity, hypertension, and chronic kidney disease are all inflammatory diseases and that the renal injury observed in obesity and hypertension are contributed to by increased expression of proinflammatory cytokines. Cytokines produced by the adipose tissue in obesity, such as interleukin-1β, tumor necrosis factor-α, interleukin-6, and MCP-1, have been associated with the progression of endothelial dysfunction and renal injury. We observed no effect of 10 weeks of a high-fat diet on systolic blood pressure in either the WKY rats or SHRs. However, we did observe significant albuminuria and proteinuria in the SHR groups fed the normal diet, which was further exacerbated by high fat. The exacerbation of the renal injury in the absence of changes in blood pressure indicates that the high-fat diet impaired renal function as a result of pressure-independent mechanisms. Renal blood flow and pressure were not directly measured, and, thus, a change in blood pressure transmission to the kidney as a result of the high-fat diet cannot be ruled out. One possible mechanism responsible for the development of the renal injury observed is enhanced expression of inflammatory cytokines, which we found to be upregulated in the SHR group fed the high-fat diet.

Inflammatory cytokines are characteristically produced by macrophages. An increase in the number of macrophages present in the adipose tissue, as well as in the kidney, is apparent in animal models of obesity. In obesity, the adipose tissue recruits mature macrophages leading to the secretion of these inflammatory cytokines in higher levels than in lean animals. In animal models of hypertension, there is also often an increase in the expression of inflammatory cytokines accompanied by a reduction in renal function. We observed an increase in macrophage infiltration into the

![Figure 5](image)

**Figure 5.** Real-time PCR array data showing mean renal cortical mRNA expression of 84 inflammatory cytokines genes (n=3). A, WKY high-fat (HF) vs WKY normal diet (ND) showing changes in gene expression as a result of 10 weeks of a high-fat diet in normotensive rats. B, SHR ND vs WKY ND showing changes in gene expression as a result of hypertension. C, SHR HF vs WKY HF showing changes in gene expression as a result of 10 weeks on a high-fat diet in hypertensive rats.

![Figure 6](image)

**Figure 6.** A, Urinary microalbumin excretion in WKY rats and SHRs fed a normal or high-fat diet for 3, 6, or 10 weeks. B, Urinary protein excretion in WKY rats and SHRs fed a normal or high-fat diet for 3, 6, or 10 weeks.
kidney cortex of the SHRs fed a high-fat diet compared with other groups. The elevation in macrophage infiltration, therefore, was a result of the combination of obesity and hypertension present in the SHR high-fat–fed group.

MCP-1 production is stimulated by the presence of adipokines and is a potent chemotactic factor involved in the recruitment of monocytes to the site of inflammation. MCP-1 (CCL2) acts through its chemokine receptor CCR2 resulting in an inflammatory response. Antagonism of CCR2 has been shown to reduce renal macrophage infiltration in a model of obesity. CCR2 knockout mice fed a high-fat diet have a lower body weight, in addition to reduced adipose tissue inflammation and insulin resistance. Kanda et al have shown that adipose tissue–specific MCP-1 knockout mice have reduced macrophage infiltration into the adipose tissue. Chow et al have reported significant amelioration of renal injury in obese db/db mice lacking the MCP-1 gene compared with db/db mice with their MCP-1 gene intact, which indicates that MCP-1 plays a role in the development of renal injury. We observed increases in MCP-1 excretion in both rat strains with 10 weeks on a high-fat diet; however, plasma MCP-1 levels were unchanged, suggesting that a high-fat diet induced a subacute inflammatory response that was isolated to the kidney.

The real-time PCR array data clearly show that hypertension and obesity independently increase renal inflammatory gene expression; however, in combination, the 2 risk factors have a compound effect resulting in a far greater number of inflammatory cytokines to be markedly upregulated. In particular, in the SHR high-fat group, we observed a large increase in mRNA levels of interleukin-1α (IL1α), a gene synthesized by activated macrophages that is associated with diabetic nephropathy and end-stage renal disease. There is evidence that IL1α can activate nuclear factor κB and plays a role in the genesis of inflammation by augmenting the transcription of proinflammatory genes. IL18 (interferon-γ–inducing factor) was also upregulated in the SHR high-fat group and has been identified as a diabetes candidate gene that plays an important role in energy homeostasis and insulin sensitivity. Ccl25 (TECK), a gene that acts through the chemokine receptor CCR9, was also upregulated in the SHR high-fat group compared with the WKY high-fat group. CCR9 is a macrophage chemotactic factor that also recruits monocytes and T cells to the site of inflammation. There were a number of genes, such as Bcl6, Ccl11, Cxcl1, the interleukins Il1f6, Il3, Il4, Il5, and Il5ra, that are involved in inflammatory processes that were also significantly upregulated. In addition, genes such as chemokine ligands Ccl12 and Ccl21b; chemokine receptors Ccr3 and Ccr8; interleukins Il10, Il11, Il13, Il17b and Il1f5; and receptors Il1r2, Il8ra, and Il8rb with smaller fold increases were also upregulated. Although these genes are known to be inflammatory cytokines, some of their detailed functions are less well understood; in this study we show that they are associated with obesity and hypertension.

Disruption of the renal filtration barrier is closely associated with albuminuria. The podocyte-associated protein nephrin has been linked with filtration barrier integrity, and a downregulation in nephrin expression has been observed in animal models of hypertension, obesity, and renal injury. Nephrin ran as a doublet on the Western blot, which has been reported previously. We observed a significant reduction in glomerular nephrin protein expression as a result of 10 weeks of a high-fat diet in the WKY rats and SHRs. Our data indicate that the downregulation in nephrin contributed to the breakdown of the filtration barrier, and, in combination with the inflammation observed in the high-fat–fed SHRs, reduced the ability of the slit diaphragm to prevent the leakage of proteins into the urine.
albumin into the urine. A disassociation between the nephrin expression and albuminuria was observed, indicating that other mechanisms, eg, inflammation, may be important in the development or exacerbation of renal injury. Ten weeks of a high-fat diet with hypertension induced an increase in glomerular desmin expression, which has been reported previously in models of renal injury. The disassociation of the histological data from the albuminuria indicates that a combination of mechanisms may be responsible for the development of renal injury in this model.

Perspectives
In this study we provide evidence that high-fat diet is a powerful stimulus for renal endothelial dysfunction in both a normotensive and a hypertensive rat model and exacerbates renal injury. We show that 10 weeks of a high-fat diet in combination with hypertension results in a marked inflammatory response characterized by renal macrophage infiltration and increased MCP-1 excretion. The combination of a high-fat diet and hypertension was a strong stimulus for the upregulation of inflammatory cytokine mRNA compared with the high-fat diet or hypertension alone. The identification of the role of inflammatory genes in response to hypertension with obesity may identify potential therapeutic targets for the protection of renal function in metabolic syndrome.

Sources of Funding
This work was supported by National Institutes of Health grants HL59699 and HL074167 and an American Heart Association Established Investigator Award (to J.D.I.).

Disclosures
None.

References


Endothelial Dysfunction and the Development of Renal Injury in Spontaneously Hypertensive Rats Fed a High-Fat Diet
Sarah F. Knight, Jeffrey E. Quigley, Jianghe Yuan, Siddhartha S. Roy, Ahmed Elmarakby and John D. Imig

Hypertension. 2008;51:352-359; originally published online December 24, 2007; doi: 10.1161/HYPERTENSIONAHA.107.099499

Hypertension is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
Copyright © 2007 American Heart Association, Inc. All rights reserved.
Print ISSN: 0194-911X. Online ISSN: 1524-4563

The online version of this article, along with updated information and services, is located on the World Wide Web at:
http://hyper.ahajournals.org/content/51/2/352

Data Supplement (unedited) at:
http://hyper.ahajournals.org/content/suppl/2007/12/17/HYPERTENSIONAHA.107.099499.DC1

Permissions: Requests for permissions to reproduce figures, tables, or portions of articles originally published in Hypertension can be obtained via RightsLink, a service of the Copyright Clearance Center, not the Editorial Office. Once the online version of the published article for which permission is being requested is located, click Request Permissions in the middle column of the Web page under Services. Further information about this process is available in the Permissions and Rights Question and Answer document.

Reprints: Information about reprints can be found online at:
http://www.lww.com/reprints

Subscriptions: Information about subscribing to Hypertension is online at:
http://hyper.ahajournals.org//subscriptions/
ENDOTHELIAL DYSFUNCTION AND THE DEVELOPMENT OF
RENAL INJURY IN SPONTANEOUSLY HYPERTENSIVE RATS
FED A HIGH FAT DIET

Sarah F Knight, Jeffrey E Quigley, Jianghe Yuan, Siddhartha S Roy, Ahmed
Elmarakby & John D Imig

SUPPLEMENTAL METHODS AND RESULTS
Supplementary Methods

Eight week old Wistar Kyoto and Spontaneously Hypertensive Rats were purchased from Charles River (Wilmington, MA, USA). Animals were housed in the Animal Services Unit at MCG with a 12 hour light-dark cycle, with free access to tap water. Rats were acclimatized for 7 days before use. In all experiments a minimum of four rats per group were used apart from the gene array experiments where 2-3 rats per group were used.

After 3, 6 and 10 weeks groups of WKY and SHR fed a normal or high fat diet were placed in metabolic cages for 24 hours for the collection of urine then euthanized for the collection of plasma and kidney tissue, stored at -80°C for subsequent analysis. At the same time points separate groups of rats underwent hyperinsulinemic euglycemic clamp experiments to monitor systemic insulin sensitivity.

In Vitro Assays and Enzyme Linked Immunoassays

Urinary protein excretion was measured using the BCA assay (Pierce, Rockford, IL, USA). Plasma free cholesterol levels were measured using the commercially available kit from Wako Diagnostics (Richmond VA, USA). Urinary microalbumin was measured by enzyme-linked immuno assay (ELISA) (SPI-bio, France). Urinary and plasma MCP-1 was measured by ELISA (BD-Biosciences, USA). Plasma insulin was measured by ELISA (Mercodia, USA) and plasma leptin levels were also measured using a commercially available ELISA (Linco/Millipore Billerica, MA, USA).
Hyperinsulinemic-Euglycemic Clamp Experiments

Male WKY and SHR were fasted overnight prior to the acute clamp experiments (n=4). Blood glucose measurements were taken from the tail vein and then rats were anesthetized with Inactin (1.2mg/100g body weight i.p). The jugular vein was cannulated for the infusion of 10% glucose in saline. The left femoral vein was cannulated for the infusion of 0.01U/min insulin and the left femoral artery was cannulated for blood glucose measurements every 5 minutes using a glucometer. The glucose infusion rate was adjusted to maintain blood glucose levels at 125mg/dL for 90 minutes. The mean glucose infusion rate for the final 60 minutes was used to determine insulin sensitivity.

In Vitro Perfused Juxtamedullary Nephron Experiments

Experiments were conducted, in vitro, using the perfused juxtamedullary nephron technique, as previously described \(^1\). Male WKY rats and SHR (n=4) were anesthetized with pentobarbital sodium (40 mg/kg body weight i.p). The kidney was perfused with Tyrode buffer solution containing 5.2% BSA and a complement of \(L\)-amino acids. The perfusate was consistently perfused with 95% \(O_2\)-5% \(CO_2\). Perfusion pressure was set at 110 mmHg and monitored continuously. The inner cortical surface of the kidney was superfused with warmed (37°C) Tyrode buffer containing 1% bovine serum albumin. Vessel inner diameters were viewed by a video-microscopy and measured using an image shearing monitor. An afferent arteriole was selected and after 20 minutes equilibration the vessel was constricted using 1 mmol/L phenylephrine added to the superfusate. The kidney was removed and sectioned along the longitudinal axis, with care taken to leave the papilla intact on the dorsal two-thirds of the kidney. The vasculature was isolated as
previously described\textsuperscript{1}. The vessel diameter was measured and recorded as baseline. Acetylcholine was added to the perfusate to make a final concentration of $1 \times 10^{-8}$ mmol/L then the concentration of acetylcholine was increased to $1 \times 10^{-7}$ mmol, $1 \times 10^{-6}$ and finally $1 \times 10^{-5}$ mmol/L. Mean vessel diameter was recorded for 15 minutes at each concentration of acetylcholine. The vessel was perfused with 1% BSA for 15 minutes in the absence of acetylcholine, followed by 15 minutes incubation with sodium nitroprusside in order to exclude the contribution of the smooth muscle to any differences in dilatory response to acetylcholine.

**ED-1 Immunohistochemistry**

Five $\mu$m frozen kidney sections were cut and incubated overnight at room temperature with mouse anti-rat CD-68 primary antibody 1:100 (Serotec, Raleigh, NC, USA) followed by the secondary antibody goat anti-mouse IgG HRP 1:50 (Serotec, Raleigh, NC, USA) for 1 hour at room temperature. Slides were incubated with AEC substrate chromogen (DAKO, Carpinteria, CA, USA) for 20 minutes, rinsed and counterstained with Mayers hematoxylin for 30 seconds. Photographs were taken at 400X magnification and CD-68 positive cells were counted in a blinded fashion. The number of positive cells was calculated per square millimeter.

**Nephrin and Desmin Immunofluorescence**

Five $\mu$m frozen kidney sections were incubated over night at room temperature with goat anti-human nephrin primary antibody 1:50 (sc-19000, Santa Cruz Biotechnology, CA, USA) followed by rabbit anti-goat Cy-3 fluorescent tagged secondary antibody 1:400 for
1 hour (Zymed, CA, USA). Slides were counterstained with 300nmol DAPI (Invitrogen, CA, USA) for 1 minute and mounted using Prolong Gold anti-fade (Invitrogen, CA, USA). Desmin immunofluorescence was carried out using a 1: 50 dilution of mouse anti-human desmin primary antibody (Dako, CA, USA) followed by a 1:800 dilution of FITC tagged goat anti-mouse secondary antibody (Zymed, CA, USA). Photographs were taken at 400X magnification.

**Hematoxylin and Eosin Staining**

Five μm frozen kidney sections were cut and fixed overnight in 10% formalin solution. Sections were rinsed in deionized water then in hematoxylin (Gill II formulation, Surgipath, IL, USA) for 5 minutes. Slides were immersed in bluing solution (Scotts tap water substitute, Surgipath, IL, USA) for 30 seconds, rinsed for 15 minutes in running tap water and then immersed in 95% ethanol for 30 seconds. Then sections were immersed in eosin solution (Surgipath, IL, USA) for 2 minutes followed by 95% then 100% ethanol and finally xylene before mounting with a coverslip. Photographs were taken at 400X.

**Glomeruli Isolation and Western Blotting**

Kidneys were perfused with cold phosphate buffered saline (PBS) pH 7.4, removed and pressed through a sieve with 180μm diameter holes, filtered through a 200mm filter using a vacuum and suspended in PBS. The suspension was then filtered using a vacuum through a 70μm filter and the flow-through discarded. The tissue remaining on the filter was resuspended in PBS, centrifuged and the pellet containing glomeruli was then solubilized with RIPA buffer (Upstate, USA) containing 1% protease inhibitor. For
nephrin western blots 50μg glomerular protein was run on an 8% Tris-glycine gel for 2 hours and primary goat anti-nephrin antibody (N-20, Santa Cruz, CA, USA) was incubated 1:200 and mouse anti-rat HSP90 1:1000 for one hour. This was followed by secondary donkey anti-goat and goat anti-mouse IgG HRP antibody 1:5000 for one hour. HSP90 was used as a loading control for nephrin because of its molecular weight and the use of an 8% gel. For desmin western blots, 20μg glomerular protein was run on a 10% Tris-glycine gel for 1.5 hours and primary mouse anti-human desmin antibody (clone D33 DAKO, CA, USA) was incubated 1:100 and mouse anti rat β-actin 1:10,000 for one hour followed by secondary goat anti-mouse IgG HRP antibody 1:5000 for one hour.

Real-time Polymerase Chain Reaction (PCR) Array Gene Expression Profiling

Total RNA was extracted from 20mg kidney cortex using the RNeasy® Plus Mini kit (Qiagen, CA, USA) according to the manufacturer’s protocol. RNA concentrations were determined using absorbance at 260nm. Reverse-transcription was performed on 2μg of RNA from each sample using the RT² PCR Array First Strand Kit (SuperArray Bioscience, MD, USA). Each cDNA synthesis reaction was diluted before being added to an RT² Real-Time SYBR Green PCR Mastermix (SuperArray Bioscience, MD, USA) which was aliquoted onto a 96-well PCR Array plate, one sample per plate; each well contained a primer pair for a different gene or control. Thermal cycling and real-time detection were done with a Bio-Rad iCycler (Bio-Rad Laboratories, Hercules, CA): step 1) 95°C for 10 minutes, step 2) 95°C for 15 seconds followed by 60°C for 60 seconds (repeated 40 times). Melt-curve analysis was completed after each PCR reaction. Threshold cycle (Ct) values were normalized to a set of housekeeping genes to get a ΔCt.
value and fold-changes were calculated using the equation: \((2^{-\Delta Ct_{\text{test}}} \cdot (2^{-\Delta Ct_{\text{control}}})^{-1}\). Student’s T-test was used for statistical analysis and changes greater than ±2 and p<0.05 were considered significant. Superarray results were confirmed by real-time PCR on three genes present on the arrays, picked at random.

**Statistics**

Data presented are mean ± SEM. Data was analyzed by 2-way analysis of variance for repeated measures followed by the Bonferoni and Dunn post-hoc test. The significance of difference in experiments with just one variable is calculated using an unpaired t-test. A value of P<0.05 was considered significant.

**References**


**Supplemental Figure Legends**

**Supplemental Table S1.** Results from inflammatory gene Superarrays (n=2-3). Outlined data indicates a minimum of a two fold change in gene expression with significance (p<0.05).
Supplemental Figure S1. **A**: Representative frozen kidney sections stained with hematoxylin and eosin (400X magnification). **B**: Results of hematoxylin and eosin stained kidney sections scored blindly for the presence of injury.
## Supplemental Data: Table S1

<table>
<thead>
<tr>
<th>Gene</th>
<th>WKY HF vs WKY ND</th>
<th>SHR ND vs WKY ND</th>
<th>SHR HF vs SHR ND</th>
<th>Fold Difference</th>
<th>WKY HF vs WKY ND</th>
<th>SHR ND vs WKY ND</th>
<th>SHR HF vs SHR ND</th>
<th>Fold Difference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Abcf1</td>
<td>-1.36</td>
<td>-1.04</td>
<td>1.22</td>
<td>4.55</td>
<td>-1.30</td>
<td>1.12</td>
<td>1.54</td>
<td></td>
</tr>
<tr>
<td>Bcl6</td>
<td>-3.19</td>
<td>3.36</td>
<td>-4.55</td>
<td></td>
<td>-1.30</td>
<td>1.04</td>
<td>2.61</td>
<td></td>
</tr>
<tr>
<td>Brl1</td>
<td>-1.33</td>
<td>-1.61</td>
<td>1.24</td>
<td></td>
<td>2.45</td>
<td>2.51</td>
<td>8.50</td>
<td></td>
</tr>
<tr>
<td>C3</td>
<td>-1.42</td>
<td>-1.34</td>
<td>-1.21</td>
<td></td>
<td>-1.08</td>
<td>-1.37</td>
<td>-1.27</td>
<td></td>
</tr>
<tr>
<td>C5</td>
<td>1.28</td>
<td>1.16</td>
<td>-1.13</td>
<td></td>
<td>1.31</td>
<td>1.30</td>
<td>1.47</td>
<td></td>
</tr>
<tr>
<td>Casp1</td>
<td>-1.10</td>
<td>-1.18</td>
<td>-1.16</td>
<td></td>
<td>-1.18</td>
<td>-1.09</td>
<td>1.37</td>
<td></td>
</tr>
<tr>
<td>Cel11</td>
<td>-1.05</td>
<td>1.20</td>
<td>3.37</td>
<td></td>
<td>-1.27</td>
<td>-1.47</td>
<td>2.61</td>
<td></td>
</tr>
<tr>
<td>Cel12</td>
<td>1.77</td>
<td>5.84</td>
<td>2.80</td>
<td></td>
<td>1.17</td>
<td>2.24</td>
<td>2.56</td>
<td></td>
</tr>
<tr>
<td>Cel17</td>
<td>1.54</td>
<td>1.33</td>
<td>1.43</td>
<td></td>
<td>1.34</td>
<td>2.92</td>
<td>3.96</td>
<td></td>
</tr>
<tr>
<td>Cel19</td>
<td>1.31</td>
<td>4.13</td>
<td>2.33</td>
<td></td>
<td>-1.67</td>
<td>1.24</td>
<td>-1.46</td>
<td></td>
</tr>
<tr>
<td>Cel2</td>
<td>-1.18</td>
<td>1.02</td>
<td>-1.93</td>
<td></td>
<td>-1.60</td>
<td>-1.19</td>
<td>13.80</td>
<td></td>
</tr>
<tr>
<td>Cel20</td>
<td>-1.10</td>
<td>-2.00</td>
<td>-2.02</td>
<td></td>
<td>1.28</td>
<td>1.53</td>
<td>18.21</td>
<td></td>
</tr>
<tr>
<td>Cel21</td>
<td>1.34</td>
<td>1.76</td>
<td>2.12</td>
<td></td>
<td>1.02</td>
<td>1.32</td>
<td>2.74</td>
<td></td>
</tr>
<tr>
<td>Cel22</td>
<td>2.03</td>
<td>3.17</td>
<td>1.01</td>
<td></td>
<td>1.14</td>
<td>1.30</td>
<td>1.50</td>
<td></td>
</tr>
<tr>
<td>Cel24</td>
<td>-1.05</td>
<td>-1.63</td>
<td>-1.24</td>
<td></td>
<td>1.28</td>
<td>2.07</td>
<td>4.66</td>
<td></td>
</tr>
<tr>
<td>Cel25</td>
<td>-1.08</td>
<td>1.41</td>
<td>12.88</td>
<td></td>
<td>-1.18</td>
<td>1.24</td>
<td>1.94</td>
<td></td>
</tr>
<tr>
<td>Cel3</td>
<td>-1.16</td>
<td>1.19</td>
<td>1.37</td>
<td></td>
<td>1.86</td>
<td>1.97</td>
<td>8.90</td>
<td></td>
</tr>
<tr>
<td>Cel4</td>
<td>-1.36</td>
<td>-1.00</td>
<td>1.34</td>
<td></td>
<td>-1.97</td>
<td>1.41</td>
<td>6.15</td>
<td></td>
</tr>
<tr>
<td>Cel5</td>
<td>1.12</td>
<td>1.26</td>
<td>1.11</td>
<td></td>
<td>1.07</td>
<td>-1.15</td>
<td>4.88</td>
<td></td>
</tr>
<tr>
<td>Cel6</td>
<td>1.22</td>
<td>2.21</td>
<td>1.50</td>
<td></td>
<td>1.28</td>
<td>2.07</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cel7</td>
<td>-1.49</td>
<td>-17.59</td>
<td>-1.13</td>
<td></td>
<td>-1.18</td>
<td>1.24</td>
<td>1.94</td>
<td></td>
</tr>
<tr>
<td>Cel9</td>
<td>1.31</td>
<td>-1.94</td>
<td>-2.27</td>
<td></td>
<td>1.02</td>
<td>1.17</td>
<td>1.22</td>
<td></td>
</tr>
<tr>
<td>Cer1</td>
<td>2.56</td>
<td>2.16</td>
<td>-1.06</td>
<td></td>
<td>-2.06</td>
<td>-1.85</td>
<td>3.70</td>
<td></td>
</tr>
<tr>
<td>Cer2</td>
<td>1.65</td>
<td>1.38</td>
<td>-1.68</td>
<td></td>
<td>1.02</td>
<td>1.03</td>
<td>3.07</td>
<td></td>
</tr>
<tr>
<td>Cer3</td>
<td>-1.42</td>
<td>-1.61</td>
<td>3.00</td>
<td></td>
<td>1.09</td>
<td>1.58</td>
<td>2.08</td>
<td></td>
</tr>
<tr>
<td>Cer4</td>
<td>2.23</td>
<td>2.27</td>
<td>-1.11</td>
<td></td>
<td>-1.08</td>
<td>1.36</td>
<td>2.03</td>
<td></td>
</tr>
<tr>
<td>Cer5</td>
<td>1.04</td>
<td>1.48</td>
<td>-1.27</td>
<td></td>
<td>-1.30</td>
<td>1.58</td>
<td>7.93</td>
<td></td>
</tr>
<tr>
<td>Cer6</td>
<td>1.90</td>
<td>1.84</td>
<td>-1.03</td>
<td></td>
<td>1.22</td>
<td>1.21</td>
<td>-1.27</td>
<td></td>
</tr>
<tr>
<td>Cer7</td>
<td>1.62</td>
<td>2.07</td>
<td>-1.36</td>
<td></td>
<td>-1.49</td>
<td>-1.06</td>
<td>1.54</td>
<td></td>
</tr>
<tr>
<td>Cer8</td>
<td>-2.42</td>
<td>1.20</td>
<td>4.06</td>
<td></td>
<td>1.37</td>
<td>1.80</td>
<td>1.85</td>
<td></td>
</tr>
<tr>
<td>Cer9</td>
<td>-1.01</td>
<td>-1.91</td>
<td>1.43</td>
<td></td>
<td>-1.18</td>
<td>1.33</td>
<td>1.54</td>
<td></td>
</tr>
<tr>
<td>Crp</td>
<td>-1.16</td>
<td>2.63</td>
<td>5.07</td>
<td></td>
<td>1.02</td>
<td>1.03</td>
<td>1.01</td>
<td></td>
</tr>
<tr>
<td>Cx3el1</td>
<td>-1.16</td>
<td>1.29</td>
<td>1.14</td>
<td></td>
<td>-1.13</td>
<td>-1.11</td>
<td>-1.60</td>
<td></td>
</tr>
<tr>
<td>Cx3er1</td>
<td>-1.10</td>
<td>1.08</td>
<td>-2.92</td>
<td></td>
<td>-1.03</td>
<td>-1.14</td>
<td>-1.11</td>
<td></td>
</tr>
<tr>
<td>Cxel1</td>
<td>-2.53</td>
<td>-1.13</td>
<td>7.57</td>
<td></td>
<td>-1.21</td>
<td>-1.14</td>
<td>-1.11</td>
<td></td>
</tr>
<tr>
<td>Cxel10</td>
<td>-1.56</td>
<td>1.04</td>
<td>1.43</td>
<td></td>
<td>-1.03</td>
<td>1.21</td>
<td>1.31</td>
<td></td>
</tr>
<tr>
<td>Cxel11</td>
<td>1.31</td>
<td>1.38</td>
<td>-1.06</td>
<td></td>
<td>2.62</td>
<td>1.39</td>
<td>-1.33</td>
<td></td>
</tr>
<tr>
<td>Cxel12</td>
<td>-1.01</td>
<td>1.62</td>
<td>1.43</td>
<td></td>
<td>-1.27</td>
<td>1.06</td>
<td>1.19</td>
<td></td>
</tr>
<tr>
<td>Cxel2</td>
<td>-2.84</td>
<td>1.76</td>
<td>6.15</td>
<td></td>
<td>1.81</td>
<td>1.95</td>
<td>-1.46</td>
<td></td>
</tr>
<tr>
<td>Cxel5</td>
<td>-1.13</td>
<td>-1.96</td>
<td>-1.39</td>
<td></td>
<td>1.04</td>
<td>-1.03</td>
<td>-1.19</td>
<td></td>
</tr>
<tr>
<td>Cxel9</td>
<td>-1.01</td>
<td>1.41</td>
<td>1.09</td>
<td></td>
<td>-1.39</td>
<td>-1.11</td>
<td>-1.08</td>
<td></td>
</tr>
<tr>
<td>Cxer3</td>
<td>1.41</td>
<td>1.36</td>
<td>-1.36</td>
<td></td>
<td>-1.18</td>
<td>-1.07</td>
<td>1.14</td>
<td></td>
</tr>
<tr>
<td>Gpr2</td>
<td>1.22</td>
<td>1.39</td>
<td>1.16</td>
<td></td>
<td>1.17</td>
<td>1.04</td>
<td>-1.19</td>
<td></td>
</tr>
<tr>
<td>Ifng</td>
<td>2.23</td>
<td>1.64</td>
<td>1.25</td>
<td></td>
<td>-1.03</td>
<td>1.17</td>
<td>1.34</td>
<td></td>
</tr>
<tr>
<td>Il10</td>
<td>1.12</td>
<td>-1.23</td>
<td>2.38</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Supplemental Data: Figure S1

A

WKY Normal Diet

WKY High Fat Diet

SHR Normal Diet

SHR High Fat Diet

200 μm

B

Injury Units

 WKY

 SHR

 Normal Diet

 High Fat Diet