Obesity is the major contributor to vascular dysfunction and inflammation in high-fat diet hypertensive rats

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ABSTRACT

Obesity and hypertension are the two major risk factors that contribute to the progression of end-stage renal disease. To examine whether hypertension further exacerbates oxidative stress and vascular dysfunction and inflammation in obese rats, four groups of male Sprague–Dawley rats were fed either a normal (7% fat) or high-fat (36% fat) diet for 6 weeks and osmotic pumps were implanted to deliver ANG (angiotensin II) or vehicle for an additional 4 weeks. Treatment with the high-fat diet did not alter ANG-induced hypertension compared with the normal diet (174 ± 6 compared with 170 ± 5 mmHg respectively). Treatment with the high-fat diet increased body weight gain and plasma leptin levels and induced insulin resistance in normotensive and ANG-induced hypertensive rats. Plasma TBARS (thiobarbituric acid-reacting substances), a measure of oxidative stress, were elevated in high-fat diet-fed rats compared with controls (11.2 ± 1 compared with 8.4 ± 1 nmol/ml respectively) and was increased further in ANG-induced hypertensive rats fed a high-fat diet (18.8 ± 2.2 nmol/ml). Urinary nitrite excretion was also decreased in rats fed a high-fat diet without or with ANG infusion compared with controls. Afferent arteriolar relaxation to acetylcholine was impaired in rats fed the high-fat diet without or with ANG infusion. Renal cortical TNF-α (tumour necrosis factor-α), COX-2 (cyclo-oxygenase-2) and phospho-IKK (inhibitor of nuclear factor κB kinase) expression increased in high-fat diet-fed rats compared with normal diet-fed rats. The increases in phospho-IKK and COX-2 expression were elevated further in ANG-induced hypertensive rats fed the high-fat diet. These results suggest that ANG-induced hypertension exacerbates oxidative stress and renal inflammation without further impairment in vascular dysfunction in high-fat diet-induced obesity.

INTRODUCTION

Obesity is one of the biggest global health problems nowadays [1]; however, the aetiology of obesity remains unclear. The danger of obesity over time is that the increase in body weight will cause insulin resistance, diabetes, dyslipidaemia, atherosclerosis and hypertension, a cluster of factors also referred to as the metabolic syndrome [2,3]. Obesity and hypertension have been identified as independent risk factors for the development of vascular dysfunction and renal disease [4]. Interestingly, hypertension and diabetes account for...
70% of patients with end-stage renal disease [4,5]. Studies have shown that obese Zucker and obese SHR (spontaneous hypertensive rats) develop renal inflammation and endothelial dysfunction [6–8]; however, the mechanisms of vascular dysfunction and renal inflammation are not clear and require further elucidation.

Oxidative stress plays a major role in the pathogenesis of endothelial dysfunction and inflammation in cardiovascular disease [9]. The increase in oxidative stress and its impact on NO metabolism in rats with diet-induced metabolic syndrome are well established [10,11]. Previous studies have shown that NADPH oxidase is the major source of superoxide production in high-fat diet-fed rats [10,12]. Oxidative stress is also an important trigger for insulin resistance [13]. Increased superoxide production decreases NO bioavailability and activates inflammatory responses in obese animal models that could contribute to the incidence of renal injury [14–16]. Inflammatory cytokines also play a critical role in the incidence of insulin resistance in obese subjects [17]. For example, a positive correlation has been found between serum TNF-α (tumour necrosis factor-α) concentrations and both SBP (systolic BP (blood pressure)) and insulin resistance in subjects with a wide range of adiposity [18]. TNF-α expression is increased in patients with weight gain and is reduced following weight loss [19]. Likewise, neutralizing TNF-α in obese mice improved insulin sensitivity, suggesting that elevated TNF-α levels in obesity induce vascular insulin resistance by impairing insulin signalling [20,21]. TNF-α has been shown to activate the nuclear transcription factor NF-κB (nuclear factor κ B) [22,23], which then translocates to the nucleus to activate downstream inflammatory signalling, such as the COX-2 (cyclo-oxygenase-2) gene, perturbing the inflammatory signal and this could be a possible mechanism for obesity-induced vascular dysfunction and renal inflammation. It is not clear whether the increase in BP or body weight gain is the main cause of obesity-induced vascular dysfunction and inflammation. Thus we hypothesized that obese hypertensive rats have exacerbated vascular dysfunction and inflammation compared with obese or hypertensive rats alone. Therefore, in the present study, we investigated the effect of chronic treatment with the high-fat diet on vascular function in control and ANG (angiotensin II)-induced hypertension, and examined the potential mechanisms involved in the development of vascular dysfunction in obesity and hypertension.

**MATERIALS AND METHODS**

**Animals**

All animal studies were approved by the Medical College of Georgia Institutional Review Committee according to the National Institutes of Health guidelines for the care and use of laboratory animals. Male 10–11-week-old Sprague–Dawley rats (Charles River) were used in the present study. Rats were fed either a normal rodent chow diet (Bio-Serv #F3028) or a high-fat diet (Bio-Serv #F2685) for 10 weeks. The normal chow diet contains 11.9 % kcal (where 1 kcal≈4.184 kJ) as fat and a total of 3.3 kcal/g, and the high-fat diet contains 58.3 % kcal as fat and a total of 5.4 kcal/g. After 6 weeks of treatment with the normal or high-fat diet, osmotic mini-pumps (Alzet model 2004) were used to deliver ANG (60 ng/min; Phoenix Pharmaceuticals) subcutaneously for the last 4 weeks of experiment, and rats were maintained on either the normal or high-fat diet until the end of the experimental period. Four groups of rats (n = 6–8) were used as follows: Normal (normal diet), Normal/ANG (normal diet with ANG infusion), High-Fat (high-fat diet) and High-Fat/ANG (high-fat diet with ANG infusion). Body weight gain, blood glucose levels from a tail vein using a glucometer and SBP using tail-cuff plethysmography were determined weekly in all groups. Rats were placed in metabolic cages (Nalgene) for 24-h urine collection at the end of the 10 week experiment. After 10 weeks, rats were killed by an intraperitoneal injection of sodium pentobarbital (50 mg/kg of body weight), and plasma was separated and used to determine lipid profiles and other hormonal and metabolite levels. For example, plasma insulin (Mercodia), cholesterol (Wako Chemicals), triacylglycerols (triglycerides; Wako Chemicals), LDL (low-density lipoprotein)-cholesterol (Wako Chemicals), leptin (Linco Research) and TBARS (thiobarbituric acid-reactive substances; Zeptometrix), and urinary nitrite excretion (Cayman Chemical) were determined. Renal cortical tissues were dissected and snap-frozen in liquid nitrogen for Western blotting.

**Measurement of insulin resistance**

The development of insulin resistance was identified in the four groups (n = 3–4) using the hyperinsulinaemic–euglycaemic clamp method as described previously [8]. Briefly, rats were anaesthetized and placed on a heating pad to maintain body temperature at 37 °C. Jugular and femoral veins were catheterized and used as intravenous injection lines. The carotid artery was catheterized for blood collection. Before starting the clamp, two arterial blood samples were taken for blood glucose determination. Insulin was then infused at a rate of 0.01 unit/min. Blood glucose readings were taken every 5 min for 20 min. A glucose solution (10%) was infused and the rate was adjusted until blood glucose readings averaged 125 mg/dl for the last 30 min. The rate of glucose infusion during the last 20 min determines insulin sensitivity.

**In vitro juxtamedullary nephron preparation**

This technique was used to evaluate endothelial function in the Normal, Normal/ANG, High-Fat and High-Fat/ANG groups (n = 5) as described previously [8]. Rats
were anaesthetized with sodium pentobarbital (50 mg/kg of body weight intraperitoneally). The right kidney was isolated and, after a midline laparotomy, the right renal artery was cannulated through the superior mesenteric artery. The kidney was immediately perfused with Tyrode’s solution containing 6 % (w/v) albumin and a mixture of l-amino acids. After the microdissection procedures were completed, the renal artery perfusion pressure was set to 100 mmHg. The tissue surface was superfused continuously with Tyrode’s solution containing 1 % (w/v) albumin. After a 20-min equilibration period, an afferent arteriole was chosen for study, and baseline diameter was measured. After the control period, the afferent arteriole was constricted with phenylephrine and the endothelium-dependent relaxation was assessed using increasing concentrations of acetylcholine (0.01–10 μmol/l). The afferent arteriole diameter changes in response to acetylcholine were monitored for 3 min at each concentration. The steady-state diameter in response to acetylcholine was attained by the end of 2 min, and the average diameter at 3 min was used for statistical analysis. Endothelium-independent relaxation was also assessed at the end of the experiment using sodium nitroprusside.

Renal cortical expression of TNF-α, COX-2 and IKKα/β (IκB (inhibitor of NF-κB) kinase α/β)

Frozen kidney cortex was sliced very thinly and homogenized in complete lysis buffer for preparation of whole-cell extracts using a nuclear extract kit (Pierce). The homogenate was centrifuged at 9000 g for 10 min at 4 °C, and then supernatant was aliquoted and stored at −80 °C. The protein concentration was determined using the bicinchoninic acid protein assay kit (Pierce). Protein samples (50 μg) were separated by SDS/PAGE on a 10 % (w/v) Tris/glycine gel, and proteins were transferred electrophoretically on to a PVDF membrane. Non-specific binding sites were blocked by incubating the membranes overnight at 4 °C in TBS (Tris-buffered saline) containing 5 % (w/v) non-fat dry milk and 0.1 % Tween 20 in addition to the primary antibody. The primary antibodies used were rabbit anti-TNF-α, phospho-IKKα (Ser180)/IKKβ (Ser181), -IKK and -COX-2 (Cell Signaling Technology). The membranes were then washed in TBS/0.1 % Tween 20 and were incubated with the secondary antibody (goat anti-rabbit; 1:5000 dilution) conjugated to HRP (horseradish peroxidase) for 1 h at room temperature (25 °C). Detection was accomplished using enhanced chemiluminescence. Band intensity was measured densitometrically and the values were factored for β-actin. For IKK, the band intensity for both phospho-IKKα and phospho-IKKβ was also measured densitometrically and the values were factored for IKK.

NF-κB transcription factor assay

Cortical whole-cell extract (20 μg) was used for the determination of NF-κB activity using the TransAM NF-κB p65 transcription factor assay kit (Active Motif), according to the manufacturer’s instructions.

Pair-fed experiment

Initially, average daily food consumption was measured in male Sprague–Dawley rats and total caloric intake was calculated. Rats were then divided into two groups (n = 4) and fed either the normal diet or pair-fed a high-fat diet (the amount of food intake for the high-fat diet was limited to the same caloric intake of the normal diet) for 10 weeks. The amount of food intake for the two groups was adjusted each week to ensure the same caloric intake. Body weight gain and blood glucose were determined weekly in the two groups. Rats were killed after 10 weeks and plasma insulin levels were determined. Afferent arteriolar relaxation in response to acetylcholine was also assessed.

Statistical analysis

All values are presented as means ± S.E.M. Data for SBP, percentage body weight gain and percentage afferent arteriolar relaxation were analysed by ANOVA for repeated measurements. All other data were analysed using one-way ANOVA, followed by Tukey’s post-hoc test for multiple group comparisons. Differences were considered statistically significant with P < 0.05 compared with the control. Analyses were performed using GraphPad Prism version 4.0 software (GraphPad).

RESULTS

BP and body weight gain

Figure 1(A) shows the effect of long-term treatment of the high-fat diet on BP. SBP increased slightly in the High-Fat group compared with the Normal group, but this increase was only significant at the end of the 9 weeks of treatment (149 ± 3 compared with 132 ± 2 mmHg). ANG infusion did not potentiate the increase in BP in the High-Fat/ANG group compared with the Normal/ANG group (179 ± 7 compared with 176 ± 6 mmHg), suggesting that the high-fat diet did not exacerbate ANG-induced hypertension. Figure 1(B) shows that body weight gain increased significantly in the High-Fat and High-Fat/ANG groups compared with the Normal group, and there was no difference in body weight gain in the High-Fat and High-Fat/ANG groups. However, the Normal/ANG and High-Fat/ANG groups did not gain as much weight as the Normal or High-Fat groups respectively. In fact, the Normal/ANG group started to lose weight after ANG infusion, which could be attributed to the decrease in food intake during ANG infusion. The average food intake for the Normal and High-Fat groups
Figure 1  SBP (A) and percentage body weight (B) in rats fed a normal or high-fat diet for 10 weeks without or with ANG infusion in the last 4 weeks of the treatment
Values are means ± S.E.M. (n = 7–8). *P < 0.05 compared with the Normal group; ̸=P < 0.05 compared with the Normal/ANG group.

Table 1  Plasma lipid profile and blood glucose in rats fed the normal or high-fat diet without or with ANG infusion

<table>
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<td>58 ± 2</td>
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<td>19 ± 1</td>
<td>30 ± 3*</td>
<td>31 ± 5 ̸=</td>
</tr>
<tr>
<td></td>
<td>Blood glucose (mg/dl)</td>
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<td>100 ± 2</td>
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</table>

was 30.4 ± 1.5 and 20 ± 1 g/day respectively. Infusion of ANG resulted in a 14 % decrease in food intake in the Normal/ANG group and a 20 % decrease in the High-Fat/ANG group at the end of 10 weeks of treatment.

Blood glucose levels and insulin sensitivity
Random blood glucose levels, plasma insulin and leptin levels, and insulin sensitivity were assessed in rats fed the normal and high-fat diets for 10 weeks without or with ANG infusion. Although there were no differences in blood glucose levels during treatment with the high-fat diet or at the end of the 10 week study period between all of the groups (Table 1), plasma insulin increased significantly by approx. 65 % in the High-Fat and High-Fat/ANG groups compared with the Normal group (Figure 2A). Hyperinsulinaemic–euglycaemic clamp experiments were carried out for 10 weeks to monitor peripheral insulin sensitivity in the normal and high-fat diet groups without or with ANG treatment (Figure 2B). The High-Fat and High-Fat/ANG groups had a 60–70 % reduction in the glucose infusion rate compared with the Normal group, indicating the development of insulin resistance (P < 0.05). Plasma leptin levels were also significantly increased in both the High-Fat and High-Fat/ANG groups compared with the Normal group, and ANG infusion decreased plasma leptin levels in the Normal/ANG group (Figure 2C).

Afferent arteriole endothelial function
Afferent arteriole endothelial function was assessed using the in vitro juxtaglomerular preparation and the results are shown in Figure 3(A). Afferent arteriole relaxation in response to acetylcholine was significantly impaired in Normal/ANG, High-Fat and High-Fat/ANG groups compared with the Normal group (P < 0.05). Although the high-fat diet had some tendency to exacerbate this impairment in ANG-treated rats, this effect did not reach a statistical difference (Figure 3A). Endothelium-independent relaxation to sodium nitroprusside was not different between the groups (results not shown), indicating that only the endothelium-dependent dilatory response is impaired by the high-fat diet and/or ANG in this model.

Rats fed the caloric-restricted high-fat diet for 10 weeks gained as much weight as the normal group (body weight, 540 ± 30 compared with 543 ± 24 g respectively). The impairment in afferent arteriolar relaxation in response to acetylcholine that was observed previously in the High-Fat group was abolished when rats were fed the caloric-restricted
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Figure 2  Plasma insulin levels (A), glucose infusion rate (B) and plasma leptin levels (C) in rats fed a normal or high-fat diet for 10 weeks without or with ANG infusion in the last 4 weeks of the treatment

Values are mean ± S.E.M. In (B), the glucose infusion rates are from hyperinsulinaemic–euglycaemic clamp experiments (n = 3–4). In (A) and (C), n = 6–8.

∗P < 0.05 compared with the Normal group.

Figure 3  Afferent arteriole vascular response to acetylcholine

Values are means ± S.E.M. (A) Afferent arteriole vascular response to acetylcholine (n = 5) in rats fed a normal or high-fat diet for 10 weeks without or with ANG infusion in the last 4 weeks of the treatment. ∗P < 0.05 compared with the Normal group. (B) Afferent arteriole vascular responses to acetylcholine in rats fed a normal diet or caloric-restricted high-fat diet with the same amount of calories as the normal diet for a 10 week treatment period (n = 4).

high-fat diet (Figure 3B). There was no difference in plasma insulin levels in rats fed the caloric-restricted high-fat diet compared with the normal diet group (3.6 ± 0.5 compared with 4.3 ± 0.1 μg/l respectively). Together, these findings suggest that the impairment of renal endothelial function and the induction of insulin resistance is due to obesity and not to the content of the high-fat diet.

Plasma lipid levels

Table 1 shows the plasma lipid profile in rats fed the normal or high-fat diet without or with ANG infusion. There was a 50% increase in plasma cholesterol, 30% increase in plasma triacylglycerol and 35% increase in plasma LDL-cholesterol levels in the High-Fat group compared with the Normal group. The same trend was also shown in the High-Fat/ANG group compared with the Normal/ANG group (Table 1). However, plasma cholesterol and triacylglycerol levels were lower in the High-Fat/ANG group compared with High-Fat group and, again, this could be due to the decrease in food intake, as the High-Fat/ANG group consumed 20% less food daily than the High-Fat group.

Oxidative stress and inflammatory markers

Plasma TBARS were assessed as a measure of oxidative stress in rats fed the normal or high-fat diet. The High-
Fat group had increased plasma TBARS compared with the Normal group, and the increase in plasma TBARS was exacerbated further in the High-Fat/ANG group (Figure 4A). Nitrite excretion increased in the Normal/ANG group; however, urinary nitrite excretion was decreased in the High-Fat and High-Fat/ANG groups compared with the Normal and Normal/ANG groups (Figure 4B). These findings suggest that treatment with the high-fat diet increases oxidative stress and subsequently decreases NO bioavailability in obese rats.

Next, we assessed the protein expression levels of some inflammatory cytokines in the kidney cortex of rats fed the normal or high-fat diet without or with ANG infusion. Renal cortical TNF-α expression increased significantly in the High-Fat and High-Fat/ANG groups compared with the Normal group (Figure 5A). Renal cortical phospho-IKKα/β was elevated significantly in the High-Fat group compared with the Normal group and was increased further in the High-Fat/ANG group (Figure 5B). We then verified the activation of NF-κB by measuring renal cortical p65 NF-κB. No significant change in renal cortical p65 NF-κB was observed in the High-Fat group compared with the Normal group (0.06 ± 0.008 compared with 0.1 ± 0.03 pg/mg respectively); however, cortical p65 NF-κB was elevated significantly in the High-Fat/ANG compared with the Normal or Normal/
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ANG groups (0.2 ± 0.03 compared with 0.1 ± 0.03 or 0.05 ± 0.006 pg/mg respectively). Likewise, cortical COX-2 expression increased significantly in the High-Fat group compared with the Normal group, and this increase was exacerbated further in the High-Fat/ANG group compared with the High-Fat group (Figure 5C).

DISCUSSION

The incidence of obesity has dramatically increased in the United States over the past two decades [24]. Studies have demonstrated a close association between obesity, hypertension and inflammation, although the exact mechanisms remain to be elucidated [4]. In the present study, we tested whether hypertension exacerbates vascular dysfunction, oxidative stress and inflammation in rats fed a high-fat diet. Although ANG infusion did not potentiate the elevation in BP and impairment in vascular function in rats fed a high-fat diet compared with a normal diet, it exacerbated the increase in plasma TBARS in high-fat diet-fed rats. The high-fat diet increased renal TNF-α, phospho-IKK and COX-2 expression, and COX-2 expression was increased further in ANG-induced hypertensive rats fed a high-fat diet. These results suggest that ANG-induced hypertension exacerbates oxidative stress and inflammation without worsening vascular dysfunction in high-fat diet-induced obesity.

Consistent with our previous findings [8], body weight gain was significantly higher in rats fed a high-fat diet than those fed a normal diet. Rats fed a normal diet tended to lose weight during ANG infusion. This could be attributed to the decrease in food intake in rats fed a normal diet during ANG infusion. Our findings are in agreement with results published previously [25–27]. For example, Brink et al. [26] reported that ANG-infused hypertensive rats lost 18–26% of body weight after 1 week compared with the sham controls, and pair-feeding experiments indicated that 74% of this loss was due to a reduction in food intake.

Although studies have demonstrated that a long-term high-fat diet increased BP in rats [28–30], our present study showed that there was only a slight increase in BP in rats fed the high-fat diet and that this increase was only significant after 9 weeks of treatment with the high-fat diet. Because rats fed a high-fat diet for 10 weeks did not develop hypertension, we postulated that ANG infusion would potentiate the elevation in BP in high-fat diet-fed rats. Surprisingly, there was no difference in BP between ANG-induced hypertensive rats fed the high-fat diet compared with the normal diet. These results are in agreement with our previous findings in normotensive WKY (Wistar–Kyoto) rats and SHRs. Treatment with the high-fat diet for 10 weeks did not alter SBP, but increased MCP-1 (monocyte chemoattractant protein-1) excretion, a marker of renal inflammation in WKY rats and SHRs; however, high fat only increased microalbuminuria and protein excretion, markers of renal injury, in SHRs [8]. The increase in renal injury without a change in BP suggests that a high-fat diet impairs renal function via pressure-independent mechanisms. We also published recently that there was a difference in SBP between obese (db/db) mice and lean (db/m) mice, and that obesity did not potentiate the elevation in BP upon DOCA (deoxycorticosterone acetate)-salt treatment [31]. Collectively, these findings suggest that treatment with a high-fat diet for 10 weeks increases body weight gain without inducing hypertension or exacerbating ANG-induced hypertension.

Interestingly, we observed significant endothelial dysfunction after 10 weeks of the high-fat diet in both normotensive (High-Fat) and ANG-induced hypertensive (High-Fat/ANG) rats, as well as in ANG-induced hypertensive rats fed the normal diet (Normal/ANG). The endothelium-independent relaxation in response to sodium nitroprusside was not different among the groups, suggesting that the impairment in afferent arteriolar response to acetylcholine was a result of endothelial and not smooth muscle dysfunction. These results are consistent with our previous findings that afferent arteriolar responses were impaired in SHRs compared with WKY rats and that treatment with a high-fat diet for 10 weeks also induced impairment in afferent arteriolar responses to acetylcholine in both WKY rats and SHRs without affecting smooth muscle function [8]. To test the possibility that the incidence of endothelial dysfunction is due to the content of the diet, we fed rats either a normal or a caloric-restricted high-fat diet for 10 weeks. Pair-feeding rats the caloric-restricted high-fat diet abolished the increase in body weight gain and the impairment in afferent arteriolar relaxation in response to acetylcholine. These results suggest that the incidence of endothelial dysfunction is due to obesity or hypertension and is not linked to the diet content, and ANG-induced hypertension did not increase further the impairment in endothelial function in high-fat diet-fed rats.

Previous studies have shown that a high-fat diet or ANG infusion increased oxidative stress, and the increase in superoxide levels could result in endothelial dysfunction via scavenging of NO and decreasing NO bioavailability [10,28,32–34]. However, superoxide-induced endothelial dysfunction may not be the only mechanism for decreased NO bioavailability. Chalupsky and Cai [35] demonstrated that, during oxidative stress, uncoupling of NOS (NO synthase) occurs through the oxidation of BH4 (tetrahydrobiopterin), an important cofactor in NO synthesis, and hence NOS produces superoxide rather than NO. We have demonstrated recently that obesity is the main trigger for increased oxidative stress and renal injury in db/db mice and

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that the increase in oxidative stress is exaggerated with the co-existence of DOCA-salt hypertension in db/db obese mice [31]. In the present study, a high-fat diet increased plasma TBARS, which was exacerbated further in ANG-induced hypertensive rats fed the high-fat diet. NO is metabolized via intermediates to nitrate and nitrite, which are excreted in the urine. Therefore the excretion of nitrate/nitrite has been used as an index of NO generation [36]. In agreement with the previous findings of Dobrian et al. [28], demonstrating that a long-term high-fat diet increased oxidative stress and decreased urinary and plasma nitrate/nitrite [28], increased oxidative stress upon treatment with a high-fat diet was paralleled by a decrease in nitrite excretion. Surprisingly, nitrite excretion significantly increased in ANG-induced hypertensive rats fed the normal diet (Normal/ANG), although plasma TBARS were elevated compared with the normal diet control. Deng et al. [36] demonstrated that acute ANG infusion increased nitrite/nitrate excretion in rats, and this could be attributed to increased shear stress. Nitrite/nitrate excretion was also higher in rats chronically infused with ANG, although these changes were not significant, suggesting that shear-stress-induced increases in renal NO generation are lost during prolonged ANG infusions [36]. Rajapakse et al. [37] have also shown that plasma nitrate/nitrite increased in ANG-infused rats, and that this increase was exacerbated further in the presence of exogenous L-arginine. On the basis of these findings, we have postulated that the increase in nitrite excretion after ANG infusion is due to the compensatory up-regulation of the NO system in ANG-induced hypertensive rats fed a normal diet, and this effect was abolished during treatment with a high-fat diet. This could also explain why a high-fat diet failed to exacerbate the BP elevation and vascular dysfunction in ANG-induced hypertensive rats even though oxidative stress was elevated further in ANG-induced hypertensive rats fed the high-fat diet. In addition, ANG infusion could have maximized the superoxide-induced BP increase and, hence, ANG infusion failed to produce any further elevation in BP when combined with treatment with the high-fat diet. Collectively, these results suggest that increased oxidative stress could be a potential mechanism for high-fat diet-induced hypertension and vascular dysfunction and ANG infusion only exacerbates oxidative stress without further impairment in vascular dysfunction in high-fat diet-induced obesity.

Another potential mechanism for high-fat diet-induced vascular dysfunction is the incidence of insulin resistance. Previous studies have suggested that increased oxidative stress is an important trigger for insulin resistance [13]. For example, Park et al. [38] have recently shown a positive correlation between oxidative stress biomarkers and insulin resistance in non-diabetic young adults. Matsuzawa-Nagata et al. [39] have also demonstrated that increased oxidative stress preceded the onset of high-fat diet-induced obesity and insulin resistance in high-fat diet-fed mice. In the present study, plasma insulin levels were significantly higher in rats fed a high-fat diet compared with a normal diet, suggesting that long-term treatment with a high-fat diet induced insulin resistance. The incidence of insulin resistance was confirmed using the hyperinsulinaemic–euglycaemic clamp, where a reduction in peripheral insulin sensitivity after 10 weeks of treatment with the high-fat diet was observed. These results are consistent with our recent findings, where 10 weeks of treatment with a high-fat diet also increased plasma insulin levels in WKY rats and SHRs [8]. Insulin resistance has been shown to contribute to the development of endothelial dysfunction [24,40], as high insulin levels induce renal haemodynamic changes, glomerular hypertrophy and mesangial cell proliferation [41,42]. Taken together, these results suggest that high-fat diet-induced insulin resistance and ANG-induced hypertension did not potentiate this effect.

Previous studies have suggested a clear link between visceral obesity and the development of insulin resistance and inflammation via increasing inflammatory cytokines and adipokines, such as leptin [4,8,43,44]. Cytokines produced by adipose tissue in obesity, such as TNF-α and MCP-1, have been associated with the progression of vascular dysfunction and inflammation [45–47]. We have observed previously that endothelial dysfunction precedes renal injury in WKY rats and SHRs fed a high-fat diet, and hypertension combined with obesity induced powerful inflammatory responses and disruption of the renal filtration barrier [8]. Plasma leptin levels also increased in WKY rats and SHRs fed a high-fat diet for 10 weeks, and this increase was significantly higher in WKY rats compared with SHRs [8]. Consistent with our previous finding, plasma leptin levels were elevated in rats fed a high-fat diet without or with ANG infusion compared with rats fed a normal diet in the present study. In contrast with the results published previously by Kim et al. [48], showing that ANG increased leptin secretion from cultured adipose cell lines and human adipose tissue in vitro, ANG infusion in vivo decreased plasma leptin levels in our present study. Studies have demonstrated that high leptin levels reduced acetylcholine dilatory responses and this could be attributed to the ability of leptin to increase oxidative stress and decrease NO bioavailability [49,50]. Renal TNF-α expression was also elevated in rats fed a high-fat diet, which may have contributed to the development of vascular dysfunction observed as a result of treatment with the high-fat diet. TNF-α is a pro-inflammatory adipokine that is secreted by adipose tissue. TNF-α plays a primary role in stimulating the production of leptin and other inflammatory cytokines [51–53]. Plasma TNF-α increased in obese patients [54], and expression of TNF-α increased with weight gain and decreased with weight.
loss [19]. Neutralizing TNF-α in obese mice improved insulin sensitivity, suggesting that elevated TNF-α levels in obesity induce vascular insulin resistance [20,21]. Collectively, our present study suggests that the incidence of insulin resistance and the increase in leptin and TNF-α upon treatment with the high-fat diet could be potential mechanisms for the induction of renal dysfunction and inflammation, and ANG-induced hypertension did not exacerbate the increase in leptin and TNF-α in obese rats.

The increase in oxidative stress and/or TNF-α also activates the intracellular transcription factor NF-κB, which then translocates from the cytoplasm to the nucleus to activate downstream inflammatory cytokines perturbing the inflammatory cycle [4,5,55,56]. Normally, NF-κB proteins are composed of two subunits that are usually present in the cytoplasm as inactive heterodimers bound to the inhibitory protein IκB (inhibitor of NF-κB). The main enzyme responsible for NF-κB activation is the kinase IKK, which phosphorylates the IκB subunit and is then degraded [22,23]. This allows the activation of NF-κB in the cytoplasm and its translocation to the nucleus to regulate the transcription of many inflammatory genes such as COX-2 and MCP-1 [8,23]. Thus NF-κB promotes the synthesis and release of inflammatory cytokines that recruit monocytes and macrophages to vessel wall, and this could be another potential mechanism for endothelial dysfunction. Our previous findings have demonstrated that 10 weeks of a high-fat diet in combination with hypertension resulted in marked inflammatory responses manifested by increased MCP-1 excretion in WKY rats and SHRs [8]. This combination also strongly stimulated the up-regulation of inflammatory cytokine mRNA compared with a high-fat diet or hypertension alone. In the present study, renal cortical phospho-IKK increased in high-fat diet-fed rats compared with normal diet-fed rats and was elevated further in ANG-induced hypertensive rats fed a high-fat diet. Renal p65 NF-κB activity was also elevated in ANG-induced hypertensive rats fed a high-fat diet compared with the normal diet-fed rats. Renal COX-2 expression increased in rats fed a high-fat diet compared with those fed a normal diet, and ANG infusion exacerbated this increase. These findings suggest that inflammatory cytokines could play a role in the incidence of endothelial dysfunction upon treatment with the high-fat diet, and the coincidence of hypertension with obesity does exacerbate the increase in inflammation, but not the vascular dysfunction in obese rats.

In summary, ANG-induced hypertension did not exacerbate insulin resistance or vascular dysfunction; however, it potentiated oxidative stress and inflammation in obese rats. Oxidative stress and inflammation are clearly associated with vascular dysfunction in obesity. The incidence of BP elevation cannot be overstated, yet the growing increase in hypertension worldwide and its impact on renal function, especially with the coexistence with obesity, will need further elucidation. Future study will address the use of antioxidant and anti-inflammatory drugs in slowing the progression of vascular dysfunction in obese rats.

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REFERENCES


