Elevated Sympathetic Activity Contributes to Hypertension and Salt Sensitivity in Diabetic Obese Zucker Rats

Scott H. Carlson, Jonathon Shelton, C. Roger White, J. Michael Wyss

Abstract—Zucker rats are a useful model in which to define the mechanisms that link obesity to diabetes and associated cardiovascular disease. The present study tests the hypothesis that diabetic obese (compared with nondiabetic lean) Zucker rats are hypertensive and display a further increase in arterial pressure when fed a high salt diet. Male, nondiabetic lean and diabetic obese Zucker rats were chronically instrumented with telemetry probes and fed a basal salt diet for 3 weeks followed by exposure to a high salt diet for 11 days. On the basal diet, obese (vs lean) rats had significantly higher arterial pressures (≈13 mm Hg), and the high salt diet significantly elevated mean arterial pressure (MAP) in obese (but not lean) Zucker rats (≈12 mm Hg). Blockade of the sympathetic nervous system with hexamethonium caused a significantly larger decrease in MAP in obese (vs lean) Zucker rats fed the basal diet (51 vs 33 mm Hg), but the high salt diet did not increase the hexamethonium-induced reduction in arterial pressure in obese rats. Acute blockade of angiotensin receptors with losartan resulted in similar decreases in MAP in both groups on either diet. Acetylcholine-induced vasodilatory capacity of the carotid artery was significantly less in the obese (vs lean) Zucker rats. Together these data indicate that increased sympathetic nervous system activity and decreased vascular reactivity may contribute to elevated arterial pressure in type 2 diabetic, obese Zucker rats, but the sympathetic nervous system does not appear to contribute to the dietary salt-sensitive hypertension in this model. [Hypertension. 2000;35(part 2):403-408.]

Key Words: carotid artery ● diabetes mellitus ● angiotensin II ● circadian rhythm

A pproximately 25 million individuals in the United States have diabetes mellitus,1 which results in numerous pathological changes, including nephropathy, retinopathy, neuropathy, and cardiovascular disease. Of these factors, cardiovascular disease is the single largest cause of death in diabetes mellitus, accounting for an estimated 80% of deaths in diabetic individuals.2 The risk of cardiovascular disease in diabetic individuals is increased by concomitant hypertension, which occurs in an estimated 70% of type 2 diabetic patients.3 Results from the Framingham4 and MRFIT5 trials indicate that the coexistence of the 2 diseases triples the risk of cardiovascular disease. Moreover, the presence of hypertension probably accelerates diabetic nephropathy, which in turn exacerbates the hypertension, creating a vicious feed-forward cycle that contributes to the extremely high occurrence of end-stage kidney failure in type 2 diabetic patients.6

The mechanisms underlying type 2 diabetes and its associated pathologies have begun to be elucidated through the use of several animal models, including obese Zucker rats, in which a mutation of the leptin receptor–encoding gene impairs the ability of leptin to suppress food intake. Thus Zucker rats are obese, dyslipidemic, and hyperinsulinemic; however, it is unclear whether they are hypertensive. Several reports indicate that obese (compared with lean) Zucker rats are hypertensive.7–13 but other studies suggest that they are not.14–18 These differing findings may relate to the common use of hyperinsulinemic obese Zucker rats that display normal (or only slightly elevated) plasma glucose concentrations.11,14,18–21 Only a few studies have used the Zucker rat as a model that were hyperglycemic,9,22–24 a more appropriate model for type 2 diabetes.

Telemetric monitoring has proved to be a powerful technique for analyzing arterial pressure, heart rate, and activity in animals.25,26 Rats remain unrestrained and untethered throughout the experiment, thereby removing potential stress associated with other methods. The present study used telemetric recording to test the hypothesis that hyperglycemic, diabetic obese (compared with normoglycemic, nondiabetic lean) Zucker rats have elevated arterial pressure and that a high salt diet further elevates arterial pressure in obese (but not lean) Zucker rats. The present study also tested the hypotheses that the sympathetic nervous system,17,22 the renin-angiotensin system,7,11 and changes in vascular reactivity8,20,21,27–30 contribute to the elevated arterial pressure observed in Zucker rats.

Methods

Nine-week-old male obese Zucker rats that have a genetically induced form of type 2 diabetes with severe hyperglycemia and
nondiabetic and nonobese lean Zucker rats were used (Genetic Models Inc). All rats were housed in individual cages in a sound-attenuated room at constant humidity (60±5%), temperature (24±1°C), and light cycle (6 AM to 6 PM) and were allowed ad libitum access to either basal (0.6%; diet No. 8746, Teklad) or high salt (8%; diet No. 5008, Teklad) diet and water. All protocols for the use of animals were approved by the University of Alabama at Birmingham’s Institutional Animal Care and Use Committee in accordance with the NIH guide on The Humane Treatment of Experimental Animals.

**Surgical Procedures**

Rats were chronically instrumented with telemetry probes (TA11-PA40; Data Sciences International) for continuous monitoring of mean arterial pressure (MAP) and heart rate (HR), as described previously. Briefly, rats were anesthetized with isoflurane (inhalant); by a midline abdominal incision, the descending aorta was exposed, and the flexible tip of the telemetry probe was inserted into the aorta just below the renal arteries. The transmitter was surgically sutured into the abdominal wall, the incision was closed, and the rat was returned to its home cage and allowed a 7-day recovery period.

At the conclusion of the telemetry experiments, rats were anesthetized (isoflurane) and chronically instrumented with an indwelling Silastic femoral venous catheter, as described previously. On recovery from anesthesia, rats were returned to their home cage and recovered for 3 days before initiation of further experiments. The catheters were flushed daily with 100 μL of heparinized saline (50 U/mL).

**Experimental Protocol**

MAP and HR were monitored while rats were on a basal salt diet for 3 weeks and a high salt diet for 11 days. Thereafter they were returned to the basal salt diet for 2 weeks, and changes in MAP were measured in response to angiotensin (AT1) receptor blockade with losartan (10 mg/kg body wt IV) and (24 hours later) in response to ganglionic blockade with hexamethonium (10 mg/kg body wt IV). Rats were then fed a high salt diet for 1 week, and the blockade experiments were repeated.

At the conclusion of the study, rats were deeply anesthetized with sodium pentobarbital (100 mg/kg body wt), and the thoracic cavity was opened. A blood sample (2 mL) was quickly taken in a chilled, heparinized syringe through cardiac puncture, and portions of the right common carotid artery were excised, cut into individual ring segments (2 to 3 mm in width), and suspended from a force-displacement transducer (Radnotti) in a tissue bath containing a bicarbonate-buffered Krebs-Henseleit solution. Vascular reactivity was then determined with capacitative force transducers, as described previously. Briefly, changes in isometric tension were measured in response to graded concentrations of phenylephrine (~10⁻³ to 10⁻⁷ mol/L), acetylcholine (10⁻⁷ to 10⁻⁵ mol/L) or sodium nitroprusside (1 nmoL to 30 nmoL/L).

**Data Acquisition and Analysis**

For the telemetry experiments, data were collected and analyzed as described previously. Circadian rhythm analysis of the individual hourly MAP and HR data were performed with the nonlinear, least-squares fitting program PHARMFIT, and the subprogram SYNOPS, as described previously. All analyses were based on data for 4 consecutive days, and the MESOR (24-hour average), amplitude, and acrophase (clock time of peak amplitude) of the 24-hour adjusted rhythm were determined. Daily peak and nadir values (highest and lowest arterial pressure for each rat) for MAP and HR were calculated from the curve-fit data.

For analysis of ganglionic and AT1 receptor blockade, MAP and HR were sampled continuously every 2 seconds for a 10-minute control period, at which time the drug was administered, and MAP and HR were recorded until they returned to control levels. For measurement of vascular reactivity, real-time data were acquired and digitized with the use of a PC computer and stored for later analysis using Workbench for Windows (Strawberry Tree, Inc). Dose-response profiles for different experimental conditions were analyzed and tested to determine differences in contraction and relaxation responses.

**Analysis of Blood Samples**

Blood samples were centrifuged at 4°C, and the plasma was saved for subsequent analysis. Plasma glucose levels were measured colorimetrically with the use of a commercially available kit (Sigma). Obese Zucker rats were included in the present study only if their plasma glucose levels exceeded 200 mg/dL. Two rats were excluded on the basis of this criteria. The serum lipid profile was measured by the Outreach Pathology Laboratory at the University of Alabama Hospital.

**Statistical Analysis**

All data were evaluated by ANOVA (significance criteria of P<0.05) with appropriate post hoc tests (Newman-Keuls) to determine the source of main effects and interactions.

**Results**

Obese (compared with lean) Zucker rats were significantly heavier throughout the study (initial body weights 326±6.7 g vs 266.5±4.7 g; final body weights 437.8±12.8 g vs 373.7±7.2 g). Obese rats also displayed significantly higher plasma glucose, cholesterol, triglyceride, and very-low-density lipoprotein concentrations (Table).

Baseline MAP was significantly elevated in obese (compared with lean) rats maintained on the basal salt diet (Figure 1 and Table), and obese rats also had significantly higher nighttime peak and daytime nadir arterial pressures (Figure 1 and Table). Both obese and lean Zucker rats displayed a significant 24-hour circadian MAP rhythm with similar amplitude, although the acrophase of the rhythm occurred earlier in the lean animals (Figure 1 and Table).

Exposure to the high salt diet significantly elevated MAP in the obese (but not lean) Zucker rats (Figure 1 and Table). Peak arterial pressures were significantly elevated in both obese and lean Zucker rats (Figure 1 and Table), whereas nadir arterial pressures did not significantly change in either group (Figure 1 and Table). Both peak and nadir pressures in obese rats were significantly higher than in the lean rats. The high salt diet increased the amplitude of arterial pressure in lean rats and shifted its acrophase in both groups (Figure 1 and Table).

Baseline blood pressure was significantly higher in obese (vs lean) rats for both the ganglionic blockade (106.4 vs 91.9 mm Hg) and losartan (108.4 vs 97.4 mm Hg) studies. Ganglionic blockade with hexamethonium led to a significantly greater decrease in MAP in the obese (compared with lean) rats fed the basal salt diet, but AT1-receptor blockade with losartan produced similar decreases in MAP in both groups (Figure 2). After exposure to the high salt diet, arterial pressure was significantly higher in obese (vs lean) rats for both the ganglionic blockade (125.9 vs 88.0 mm Hg) and losartan (120.5 vs 97.2 mm Hg) studies. Hexamethonium produced a significantly greater decrease in MAP in the obese (compared with lean) rats (Figure 2), but the absolute reduction in MAP was not significantly different from the reduction in obese rats fed the basal diet. The high salt diet did not significantly affect the reductions in MAP after losartan infusion in either group of rats (Figure 2).
Throughout the study, HR was significantly lower in obese rats (Figure I and Table). Exposure to the high salt diet led to a significant reduction in heart rate in lean animals, whereas HR remained unchanged in the obese group (Figure I and Table).

There was no significant difference between obese and lean rats in the contractile response (ie, the maximum force generating capacity and the ED_{50}) of carotid ring segments to phenylephrine (data not shown). Similarly, vasodilatory responses to the endothelium-independent dilator sodium nitroprusside were not significantly different in lean compared with obese Zucker rats (data not shown). In contrast, ring segments from obese (compared with lean) Zucker rats displayed a diminished response to cumulative addition of acetylcholine (Figure 3).

**Discussion**

These data demonstrate that diabetic obese (vs nondiabetic lean) Zucker rats have increased arterial pressure and suggest that the sympathetic nervous system and changes in vascular reactivity contribute to this elevation. Conversely, increased vascular sensitivity to circulating angiotensin does not appear to contribute to the elevation in arterial pressure. Furthermore, the results demonstrate that diets high in salt raise arterial pressure in diabetic Zucker obese (but not lean) rats. This salt-sensitive response is not dependent on the sympathetic nervous system but may relate to impaired vascular reactivity.

Previous studies of the arterial pressure control in Zucker obese rats have yielded contradictory results, describing Zucker obese rats as both hypertensive^{7–13,19,21} and normotensive^{14–18}. The design of the present experiments shed light on this dispute. This study is the first to use telemetry to chronically compare arterial pressure in diabetic obese and nondiabetic lean Zucker rats. This method facilitates an accurate assessment of arterial pressure that is not confounded by the stress of tethering or a limited number of sampling times. The only previous study that chronically monitored arterial pressure (tethered system for a 5-day average) in lean and obese Zucker rats found that MAP was significantly higher (14 mm Hg) in the latter rats. The present study confirms these observations and demonstrates that both the daytime (normal sleep phase) and the nighttime (normal awake phase) arterial pressures are elevated in the obese rats. Second, this study focused on Zucker rats that were severely hyperglycemic, therefore more closely approximating the physiological conditions observed in type 2 diabetes.

Both hyperinsulinemia and increased blood glucose levels probably contribute to hypertension in type 2 diabetes, and elevated arterial pressure has been observed in all previous studies in which obese Zucker rats had either mild^{11} or moderate hyperglycemia. In contrast, the majority of studies using normoglycemic, hyperinsulinemic obese Zucker rats have found no increase in arterial pressure. These results support the hypothesis that hyperglycemia contributes to hypertension in obese Zucker rats. It should be noted that most studies examining arterial pressure control in obese Zucker rats have not reported blood glucose concentrations, thus precluding an estimation of the contribution of glycemic levels to elevated arterial pressure.

The present results demonstrate that ganglionic blockade leads to a significantly greater reduction in MAP in the obese Zucker rats, whereas blockade of AT_{1} receptors with losartan produced similar decreases in MAP in both groups. These observations confirm previous reports suggesting that obese Zucker rats have elevated sympathetic nervous system activity and that the vasculature is not more sensitive to circulating angiotensin II. However, other studies indicate that obese Zucker rats display increased vascular responsive-
ness to circulating angiotensin II. Similar to the present report, Zemel et al have demonstrated that obese Zucker rats have higher arterial pressure, but their results indicate that the sympathetic nervous system does not contribute to this differential. In contrast, they report that responsiveness to angiotensin II is enhanced in the obese Zucker rats, but they also saw a similar increase in sensitivity to norepinephrine, suggesting a general impairment in vascular reactivity.7 Likewise, Alonso-Galicia et al have reported that chronic losartan treatment leads to a greater reduction in MAP in obese Zucker rats, but their data demonstrated no differential response after 1 time point during the daytime, whereas Alonso-Galicia et al analyzed these variables over a 24-hour period.

Figure 1. Obese (n=7) compared with lean (n=6) Zucker rats display elevated MAP (A) on a basal (0.6%) salt diet and further increased MAP on a high (8%) salt diet. B and C display the circadian rhythms (averaged for 4 days) of MAP (B) and HR (C) in obese and lean Zucker rats on the basal salt diet and after 11 days on a high salt diet.

**Figure 2.** Intravenous hexamethonium infusion induced a greater decrease in MAP in obese (compared with lean) Zucker rats on a basal (0.6%) salt diet (top), but this differential did not increase when the rats were on a high (8%) salt diet. In contrast, intravenous losartan infusion (bottom) had no differential effect in obese (compared with lean) Zucker rats on either diet. *P<0.05 vs lean group.

**Figure 3.** Isolated carotid arteries of obese (compared with lean) Zucker rats display impaired dilatory responses to acetylcholine (ACh). *P<0.05 vs lean group.
It should be noted that the present study did not assess differences in food intake in the lean and obese rats. Thus a greater intake of dietary salt in the obese (compared with lean) Zucker rats may result in a higher plasma sodium concentration, which could in turn activate central osmoreceptors and thereby reflexly increase sympathetic nervous system activity.

Impaired endothelial cell function also may contribute to hypertension in obese Zucker rats in the present study. These results demonstrate for the first time that vasodilatory responses to acetylcholine are impaired in carotid arteries of obese Zucker rats. These data also indicate that isolated carotid arteries of obese Zucker rats do not have an enhanced sensitivity to phenylephrine or nitric oxide. Together this suggests that endothelially mediated vasodilation is impaired in diabetic obese Zucker rats. Isolated blood vessels from both type 1 and 2 diabetic patients\(^{36,37}\) and hyperglycemic animal models\(^{38}\) exhibit increases in vessel tone. Thus a reduction in nitric oxide signaling from endothelial cells to vascular smooth muscle may contribute to the elevation in MAP in obese Zucker rats and in diabetic patients.

When the Zucker rats are placed on a high salt diet, MAP significantly increases in the obese but not the lean rats. This confirms previous reports that obese Zucker rats are salt sensitive.\(^{16}\) It is unclear what mechanisms account for this mild salt sensitivity in the obese rats, although the reduction in vasodilatory capacity probably contributes to it. First, the present ganglionic blockade results indicate that the sympathetic nervous system does not contribute to the differential response. Second, HR in response to the high salt diet is significantly decreased in lean (but not obese) Zucker rats, thus perhaps limiting any rise in MAP. Interestingly, HR appears to already be suppressed in obese rats fed the basal salt diet, potentially limiting the ability for further decrease after exposure to the high salt diet. Third, impaired baroreflex responses\(^{4,4}\) and increased plasma volume also may contribute to the dietary salt-induced rise in arterial pressure in obese Zucker rats.\(^{39,40}\) Thus the nervous system mechanisms that contribute to the elevated arterial pressure in obese (compared with lean) Zucker rats on a basal salt diet are distinct from the mechanisms that underlie the dietary salt-induced elevation of arterial pressure in these rats.

In summary, the present results demonstrate that diabetic obese (compared with nondiabetic lean) Zucker rats display elevated arterial pressure that appears to be due, at least in part, to sympathetic nervous system overactivity and impaired vasodilatory capacity. Furthermore, arterial pressure in obese Zucker rats is sensitive to dietary salt excess, but the salt-induced increase in arterial pressure appears to be independent of enhanced sympathetic nervous system activity. Finally, these results suggest the usefulness of hyperglycemic obese Zucker rats as a model in which to study the interaction of mechanisms that contribute to type 2 diabetes.

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References


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