Progression of coronary and mesenteric vascular dysfunction in Zucker obese and Zucker diabetic fatty rats

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Submitted 8 December 2005; accepted in final form 18 May 2006

Progression of coronary and mesenteric vascular dysfunction in Zucker obese and Zucker diabetic fatty rats. *Am J Physiol Heart Circ Physiol* 291: H1780–H1787, 2006. First published May 19, 2006; doi:10.1152/ajpheart.01297.2005.—We investigated the progression of vascular dysfunction associated with the metabolic syndrome and Type 2 diabetes in Zucker obese and Zucker diabetic fatty (ZDF) rats. Responses of aorta and small coronary and mesenteric arteries were measured to endothelium-dependent and -independent vasodilators. Indices of oxidative stress were increased in serum and vascular tissues from Zucker obese rats. Our data suggest that diabetes enhances the progression of vascular dysfunction. Increases in indices of oxidative stress precede the development of dysfunction and may serve as a marker of endothelial damage.

The ZDF rat was derived from the Zucker obese rat by inbreeding for the hyperglycemia phenotype, which occurred in a subpopulation of the Zucker rat when fed a high-fat diet. Therefore, the genetic backgrounds for these rats are very similar. In the ZDF rat model, all fatty males become hyperglycemic by 10 wk of age when fed a high-fat diet, and glucose remains elevated throughout their life span (18). Initially, at 10–13 wk of age, ZDF rats are hyperinsulinemic. However, by 22–42 wk of age, serum insulin levels decline to below levels of insulin in age-matched lean control rats (18). A similar decrease in insulin levels is observed in human Type 2 diabetics, which is thought to be caused by pancreas β-cell exhaustion. Throughout their life span, free fatty acids, triglycerides, and cholesterol levels are significantly higher in the euglycemic Zucker obese and hyperglycemic ZDF rats compared with lean littermate controls (18).

Previous studies have shown vascular dysfunction associated with the metabolic syndrome and Type 2 diabetes using these rat models. Stepp and colleagues showed augmented adrenergic vasoconstriction (23) and enhanced myogenic responses (7) in arteries from the gracilis muscle in the Zucker obese rat. Attenuated acetylcholine (ACh) responses have been shown in mesenteric (2) and cerebral (19) arteries of Zucker obese rats. Furthermore, Coppey et al. (5) have shown ACh-mediated responses are attenuated in epineurial arterioles of the sciatic nerve from ZDF rats. There has also been considerable evidence demonstrating enhanced reactive oxygen species as pathological factors responsible for the impaired vasomotor function in these rats (6, 9, 13, 20). However, these studies did not examine the development and progression of vascular dysfunction. To examine progression and development of vascular dysfunction associated with the metabolic syndrome and Type 2 diabetes, we designed a longitudinal study that was performed every 4 wk in rats from 8 to 40 wk of age. We measured indices of oxidative stress in serum and vascular tissues from Zucker diabetic fatty rats that are insulin resistant, hypertensive, and dyslipidemic (18); 2) Zucker diabetic fatty (ZDF) rats that have similar characteristics of the Zucker obese rats and are also hyperglycemic; and 3) lean littermate control rats that are euglycemic, normotensive, and have normal lipid metabolism.

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lar reactivity in aorta and coronary and mesenteric small arteries.

METHODS

Rat models. All animal experiments were approved by the Institutional Animal Care and Use Committee of the Iowa City Veterans Administration Medical Center, an American Association of Laboratory Animal Care-accredited animal facility.

Zucker obese, ZDF, and lean littermate rats were obtained from Charles River Laboratories (Wilmington, MA) at 6 wk of age. Food (Harlan Teklad, Madison, WI), no. 7001 (4% fat content, normal chow) for Zucker obese and lean rats or no. 7013 (6% fat content) for ZDF rats, and water were provided ad libitum.

On the day of the experiment, rats were weighed and anesthetized with pentobarbital sodium (Nembutal, 50 mg/kg ip; Abbott Laboratories, North Chicago, IL), and a catheter was inserted into the carotid artery to measure blood pressure. A blood sample was collected for determining nonfast serum glucose (Lifescan, Milpitas, CA), and a catheter was inserted into the carotid artery. At 8–12 wk of age and ZDF rats, respectively (P = NS). Various vascular beds have altered sensitivity to many vasoactive agents. A single dose of papavérine (10–4 M) was given at the end of each experiment to determine maximal diameter. To evaluate the role of vascular superoxide, some vessels were pretreated with 1 mM tiron for 30 min before constricture with U-46619. The following criteria were required for an acceptable microvessel experiment: 1) microvessels had to demonstrate no leaks and maintain tone under 40 mM; 2) microvessels had to constrict >50% to 75 mM KCl and >30% to U-46619; and 3) microvessels had to dilate by >80% to 10–4 M papaverine.

Solutions and drugs. Krebs-Henseleit solution contained (in mM) 120.0 NaCl, 4.7 KCl, 2.5 CaCl2, 1.2 MgSO4, 23.0 NaHCO3, 1.2 KH2PO4, 11 glucose, and 0.025 EDTA. Solutions were aerated with 20% O2-5% CO2-75% N2 and maintained at 37°C with pH at 7.4. U-46619 was obtained from BioMol (Plymouth Meeting, PA). All other salts, chemicals, and vasoactive agents were purchased from Sigma Chemical (St. Louis, MO). All solutions and vasoactive agents were prepared fresh on the day of the experiment.

Statistical analysis. Data are expressed as means ± SE. Vascular responses were grouped according to age of the rats. Results are expressed as 8–12 wk, 16–24 wk, and 28–40 wk of age. Analysis revealed no significant differences among these age groups. All concentration-response curves were evaluated for differences by using two-way repeated-measures ANOVA followed by the Fisher least significant difference correction for multiple comparisons using SigmaStat software (Jandel Scientific). Significance of differences among mean values of body weight, blood glucose, and plasma insulin levels was assessed with a one-way ANOVA. Differences with P < 0.05 were considered significant.

RESULTS

Metabolic parameters. Lean animals consistently gained weight throughout the 40 wk of the study (Fig. 1A). When compared with lean rats, Zucker obese rats were heavier by 8 wk of age and continued to gain weight throughout the study. Weights of ZDF animals were similar to lean rats throughout the first 24 wk but stabilized at 28 wk and for the remainder of the study were significantly less than lean rats. This time point corresponds to a significant decrease in serum insulin levels in ZDF rats compared with lean rats (Fig. 1C).

At 6 wk of age, rats in all groups had blood glucose values in the normal range (~100 mg/dl). Glucose levels in lean rats remained around 100 mg/dl throughout the study (Fig. 1B). Glucose values in Zucker obese rats were similar to lean animals for the first 36 wk but were increased slightly at 40 wk, which was preceded by a decrease in serum insulin levels (Fig. 1C). ZDF rats were hyperglycemic at 8 wk, and glucose values remained elevated throughout the 40 wk.

Insulin levels in lean rats remained steady throughout the study (Fig. 1C). Plasma insulin levels in Zucker obese rats were markedly elevated early in the study but consistently declined after 16 wk of age, as the rats became progressively insulinopenic. At 36 wk of age, insulin levels in Zucker obese rats were no longer elevated compared with lean rats. Insulin levels in ZDF rats decreased at 28 wk of age, which is the same time point that these rats began to lose weight.

Mean arterial blood pressure was determined by using a catheter inserted in the carotid artery. At 8–12 wk of age and
36–40 wk of age, the mean arterial blood pressure was 120/110 \( \pm \) 4 and 117/106 \( \pm \) 6 in lean animals, 129/114 \( \pm \) 4 and 146/125 \( \pm \) 5 (\( P < 0.05 \) vs. lean) in Zucker obese rats, and 129/113 \( \pm \) 3 and 139/120 \( \pm \) 2 mmHg (\( P < 0.05 \) vs. lean) in ZDF rats, respectively. This shows that aortic phenylephrine-induced contractile responses were attenuated at the earliest time points in Zucker obese and ZDF rats when compared with responses from lean rats. Similar to oxidative stress results, vascular dysfunction was observed in aorta from the diabetic model before the time point of dysfunction in the model of the metabolic syndrome.

Endothelial function was evaluated in thoracic aortic segments by performing ACh concentration-response curves on phenylephrine-preconstricted vessels. Maximal relaxation response to 1 \( \mu \text{M} \) ACh in aorta segments at 8–12 wk was not different in lean (59.3 \( \pm \) 2.1%), Zucker obese (58.1 \( \pm \) 2.2%), and ZDF (59.6 \( \pm \) 1.4%) rats (\( P = \text{NS} \)). ACh-induced relaxation was not altered by increasing age in aorta from lean rats (Fig. 3B), and minimal dysfunction was observed in aorta from Zucker obese animals. ACh-induced relaxation was not altered at 8–12 or 16–24 wk but was attenuated in aorta from ZDF animals in the 28–40 wk group.

SNP concentration-response curves were performed to evaluate smooth muscle-dependent relaxation in segments of thoracic aorta. SNP produced complete relaxation of the phenylephrine-induced constriction in aorta from all groups of rats. In the 28–40 wk groups 10 \( \mu \text{M} \) SNP produced 93.3 \( \pm \) 1.2, 98.4 \( \pm \) 0.4, and 92.2 \( \pm \) 1.2% relaxation in aorta segments from lean, Zucker obese, and ZDF rats, respectively (\( P = \text{NS} \)).

Vascular responses in small coronary and mesenteric arteries. Intraluminal pressurized diameter was 167 \( \pm \) 16, 141 \( \pm \) 12, and 127 \( \pm \) 10 \( \mu \)m in coronary arteries from lean, Zucker obese, and ZDF rats, respectively, in 28- to 36-wk-old animals (\( P = \text{NS} \)). Vasomotor responses in coronary arteries from 40-wk-old rats are not included due to decreased ability of the arterioles to dilate.

Fig. 2. Formation of lipid peroxide as determined by thiobarbituric acid reactive substance (TBARS) assay in serum. Data are presented as means \( \pm \) SE; \( n = 11–20 \) rats. *\( P \leq 0.05 \) vs. lean rats.
vessels to constrict to KCl or maintain a stable constriction to U-46619. In mesenteric arteries, intraluminal pressurized diameter was 161 ± 11, 151 ± 8, and 159 ± 6 μm from 28- to 40-wk-old lean, Zucker obese, and ZDF rats, respectively; P = NS. ACh-induced endothelium-dependent relaxation in rat coronary arteries. ACh-induced relaxation was not altered in coronary arteries from lean rats (Fig. 4A). In coronary arteries from Zucker obese rats (Fig. 4B), relaxation to ACh was not altered at 16–24 wk; however, it was nearly abolished in the 28- to 36-wk age group. In coronary arteries from the ZDF rats (Fig. 4C), ACh-induced dilation was attenuated by 16–24 wk of age and remained decreased throughout the study. Thus diabetic rats demonstrated endothelial dysfunction in coronary arteries before Zucker obese rats without hyperglycemia.

In arteries obtained from the mesenteric circulation, ACh-induced relaxation was not altered in lean (Fig. 4D) or Zucker obese (Fig. 4E) rats with aging. However, vessels from ZDF rats (Fig. 4F) showed attenuation of ACh-induced relaxation at high concentrations from 16 to 40 wk of age.

In a comparison of data from the same aged animals, ACh responses were not altered in lean versus Zucker obese and ZDF rats in coronary vessels in the 8- to 12-wk age groups. At 16–24 wk, ACh responses in coronary vessels were attenuated in the ZDF group compared with responses from lean animals (Fig. 4, A and C). In coronary vessels from 28- to 36-wk-old rats, ACh-induced dilator responses were nearly abolished in vessels from Zucker obese and ZDF rats (Fig. 5A).

ACh responses were not altered in lean versus Zucker obese and ZDF rats in mesenteric vessels from the 8- to 12-wk age groups. In mesenteric vessels from 28- to 40-wk-old Zucker obese and ZDF rats, ACh responses were attenuated compared with responses from lean rats (Fig. 5B).

The role of superoxide in impaired endothelium-dependent vasodilation was examined in the presence of tiron (1 mM), a nonspecific free radical scavenger. Treatment of coronary arteries with tiron completely restored the attenuated vasodilator response to ACh from Zucker obese (Fig. 6A) and ZDF (Fig. 6B) rats similar to dilation observed in vessels from lean rats. In mesenteric arteries, improved ACh-induced dilation was observed in Zucker obese (82.6 ± 8.5 and 52.0 ± 10.1%) and ZDF rats (58.7 ± 11.4 and 47.7 ± 4.7% relaxation) in tiron versus untreated vessels, respectively. These data indicate that
superoxide produces significant injury to nitric oxide (NO)-mediated vasodilation in coronary and mesenteric arteries from Zucker obese and ZDF rats.

SNP concentration-response curves were performed to evaluate smooth muscle relaxation responses in small coronary and mesenteric arteries. In coronary arteries, SNP-induced relaxation was not altered by the age of rats (lean data not shown) or the progression of disease [Zucker obese (Fig. 7A), and ZDF (Fig. 7B) rats]. In mesenteric arteries, SNP responses were also not altered in the lean animals (data not shown) or in vessels from ZDF rats (Fig. 7D). In mesenteric vessels from Zucker obese rats, SNP responses were potentiated in the 16- to 24-wk age and 28- to 40-wk age groups compared with the 8- to 12-wk age group (Fig. 7C).

**DISCUSSION**

Several studies have examined vascular function associated with the metabolic syndrome (2, 12, 19, 20, 23); however, these studies have not examined the progression of vascular dysfunction associated with the metabolic syndrome.
dysfunction as the disease develops. This is the first longitudinal study that focuses on the microcirculation during development of the metabolic syndrome and Type 2 diabetes. The major findings of this study are 1) increased levels of oxidative stress were found in serum from Zucker obese and ZDF rats compared with lean animals, 2) ACh-induced relaxation was attenuated in coronary arteries from Zucker obese and ZDF rats, 3) oxidant stress is involved in the attenuated ACh response, 4) attenuated ACh response was observed in mesenteric arteries from ZDF rats but not Zucker obese rats, 5) smooth muscle responses were preserved in coronary arteries, and 6) altered responses occur earlier in ZDF rats than in Zucker obese rats. These responses include measurement of oxidative stress, aortic constriction, and endothelium-dependent responses in coronary arteries.

The metabolic syndrome, as defined by National Heart, Lung, and Blood Institute and the American Heart Association, consists of six major components: abdominal obesity, atherogenic dyslipidemia (elevated triglycerides and low HDL cholesterol), hypertension, insulin resistance/glucose intolerance, a proinflammatory state, and a prothrombotic state (8). The metabolic syndrome encompasses Type 2 diabetes, and estimates are that there will be 200–300 million cases in Western society by 2010 (1). All charac-

Fig. 6. Effect of free radical scavenger (1 mM tiron) on ACh-induced dilation in coronary arteries from Zucker obese (A) and ZDF (B) rats. Data are presented as means ± SE; n = number of rats. *P < 0.05, tiron vs. untreated.

Fig. 7. Sodium nitroprusside (SNP)-induced dilation of coronary (A and B) and mesenteric (C and D) arteries from Zucker obese (A and C) and ZDF (B and D) rats. U-46619-induced constriction was similar in all groups. Data are presented as means ± SE; n = number of rats. *P < 0.05, 8–12 vs. 16–24 wk; #P < 0.05, 8–12 vs. 28–40 wk.
teristics of the metabolic syndrome are known to be significant risk factors for cardiovascular disease.

The Zucker obese and ZDF rat strains have been well characterized as models of the metabolic syndrome and Type 2 diabetes (7, 13, 18, 22). The lean rats in this study were normotensive and euglycemic, had normal insulin values, and gained small amounts of weight throughout the 40 wk of the study. Zucker obese animals were also euglycemic; however, they developed hypertension, were insulin resistant, and gained a substantial amount of weight throughout the study. ZDF rats were hypertensive, insulin resistant, and hyperglycemic and lost weight after 28 wk of age, corresponding with reduced serum insulin levels.

Increased oxidative stress has been implicated in several disease models, including the metabolic syndrome and diabetes. We found enhanced formation of lipid peroxide in serum from Zucker obese and ZDF rats. When compared with samples from lean rats, serum from ZDF rats showed increased lipid peroxide throughout the study, whereas values from Zucker obese rats showed increased formation of lipid peroxide at later stages of disease progression. Katakam et al. (9) showed increased reactive oxygen species in coronary arteries from Zucker obese rats. Phillips et al. (19) and Frisbee et al. (7) showed increased superoxide production in cerebral and femoral arteries from Zucker obese rats, respectively. Our studies suggest that hyperglycemia contributes to superoxide formation in ZDF rats. Oxidative stress, as measured by serum TBARS, increases with age, as does vascular dysfunction in the coronary arteries.

We observed an attenuated response to phenylephrine-induced constriction with increasing age in aorta from all three groups of rats (lean, Zucker obese, and ZDF). This appeared to be an age-related effect, as responses from lean rats were also attenuated. In our studies, we found an attenuated response to phenylephrine in Zucker obese and ZDF aorta compared with aorta from lean rats at 8–12 wk, suggesting early dysfunction in contractile properties. Potential mechanisms for the decreased phenylephrine response include receptor desensitization or signaling pathways or alteration in calcium sensitivity/handling mechanisms. In contrast, Subramanian et al. (24) showed no changes in norepinephrine- or endothelin-1-induced constriction in aorta from 20- and 32-wk-old Zucker obese rats. This difference could be due to the vasoconstrictors used between these studies.

ACh-induced relaxation was attenuated in aorta from 28- to 40-wk-old ZDF rats. Subramanian et al. (24) found an increase in ACh response in aorta from 32-wk-old Zucker obese rats, which was not observed in the current study. Zhang et al. (25) showed decreasing endothelial function over time (3–9 mo) in ZDF rat aorta. Smooth muscle responses as determined by SNP were not altered in aorta from Zucker obese or ZDF rats in our study. We found limited impairment in the vasoactive properties of aorta from Zucker obese and ZDF rats. Thus conduit vessels, such as the aorta, appear more resistant to vascular damage than do the smaller arteries from coronary and mesenteric beds in Zucker obese and ZDF rats.

Progression of endothelium-dependent vascular dysfunction in coronary and mesenteric arteries was examined by performing ACh concentration-response curves in vessels from lean, Zucker obese, and ZDF rats. ACh-induced dilation was not altered in coronary and mesenteric vessels from 8- to 40-wk-old lean rats. Thus there was not an effect of age on ACh-induced dilation in these rats. ACh-induced relaxation in coronary arteries from 8- to 12-wk-old and 16- to 24-wk-old Zucker obese rats was normal; however, vasodilation to ACh by vessels from 28- to 36-wk-old Zucker obese rats was severely impaired. Data from the younger Zucker obese rats in this study are consistent with Katakam et al. (9), who showed that ACh responses were not altered in larger (225 um) coronary arteries from 12-wk-old Zucker obese rats. Normal relaxation was observed in mesenteric vessels from the same rats, which suggests that endothelial dysfunction occurs earlier in the coronary circulation than in some other microvascular beds in Zucker obese rats. Thus vessels from the coronary bed may be more susceptible to the effects of the metabolic syndrome or diabetes.

In coronary arteries from ZDF rats, ACh-induced responses were attenuated in 16- to 24-wk-old and 28- to 36-wk-old rats compared with 8- to 12-wk-old rats. The attenuated ACh response was also observed in mesenteric arteries from 28- to 40-wk-old ZDF rats, again suggesting that endothelium-dependent responses were altered in the coronary vascular bed before dysfunction in vessels from the mesenteric bed in ZDF rats. It is not surprising that endothelial dysfunction occurs at different times during the progression of the disease states; because these vessels are subjected to different stresses, they may have diverse receptor and ion channel populations and the prominence of NO and endothelium-derived hyperpolarizing factor pathways may be different in coronary and mesenteric vascular beds.

The mechanism of attenuated ACh response in Zucker obese and ZDF rats is controversial. A leading hypothesis in several vascular beds is that endothelium-dependent dilation is altered by increases in oxidative stress (7, 9, 20). Our data support this concept, because endothelial dysfunction observed in coronary vessels follows observed increased oxidative stress in the serum. Also, our data with a free radical scavenger show that oxidant stress is involved in the attenuated ACh response observed in coronary and mesenteric arteries from Zucker obese and ZDF rats.

SNP responses were not altered by age or disease state in coronary arteries from lean, Zucker obese, or ZDF rats. This has also been observed in larger coronary arteries (225 μm) from Zucker lean and Zucker obese rats (9). This suggests that the effect of the metabolic state of these animals does not alter smooth muscle relaxation in the coronary bed. SNP responses were not altered in mesenteric arteries from lean or ZDF rats. In mesenteric arteries from the Zucker obese animals, we found an enhanced SNP response. This may be due to an altered NO response, which could increase guanylate cyclase activity (3, 11).

In summary, vascular function was evaluated in aorta, coronary, and mesenteric arteries of rats by using isolated vessel techniques. These various vessels react differently to a variety of stimuli, as they have different functions and diverse receptor and ion channel populations. Our results suggest that vascular dysfunction in Zucker obese and ZDF rats progresses at a different rate of time in coronary and mesenteric arteries and precedes changes in conduit (aorta) arteries. An option for decreasing the late-stage complications of the metabolic syndrome and diabetes may be intervening earlier in the disease process before vascular dysfunction occurs.
REFERENCES


GRANTS

This work was supported by Veterans Affairs Merit Reviews (to C. L. Oltman and M. A. Yorek).