Cardiovascular dysfunction in Zucker obese and Zucker diabetic fatty rats: role of hydronephrosis

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The incidence of type 2 diabetes is rapidly increasing (12), as is the prevalence of cardiovascular disease resulting from diabetic complications (13). Although much of the increased risk of cardiovascular disease can be attributed to an increase in atherosclerosis, there is both clinical and experimental evidence demonstrating that diabetes leads to changes at the level of the myocardium consistent with the development of a diabetic cardiomyopathy (3). The streptozotocin-induced model of diabetes is the most commonly used model for investigating the effect of diabetes on cardiac function; however, this is a model of uncontrolled type 1 diabetes, whereas the majority of patients have type 2 diabetes. A number of animal models of type 2 diabetes are available that encompass both obesity and insulin resistance, both features of type 2 diabetes in humans (20). The Goto Kakizaki (GK) rat has impaired insulin secretion at birth, and insulin resistance later develops; however, the GK rat does not become obese without dietary intervention. The ob/ob and db/db mouse models are leptin deficient and -resistant, respectively, with the db/db mouse rapidly developing hyperglycemia and subsequent diabetes. The Zucker (fa/fa) rat is also a leptin-resistant model of obesity, and the Zucker diabetic fatty (ZDF) rat, while genetically identical to the Zucker obese animal, also develops diabetes, thus providing a complete set of lean, obese, and obese/diabetic animals.

Hyperphagia in the homozygous (fa/fa) Zucker rat is a result of a nonfunctioning leptin receptor, and the resulting obesity and hyperinsulinemia are analogous to the prediabetic state in humans. The ZDF phenotype originated from selective breeding of Zucker rats that exhibited high glucose levels in the mid-1970s, and ZDF rats typically develop diabetes at ~10 wk of age. A number of laboratories, including our own, have used obese Zucker and ZDF rats to investigate the consequences of insulin resistance and type 2 diabetes on cardiac function (9, 30, 31). Therefore, we chose these models for a recent series of experiments to examine the effect of diabetes on left ventricular (LV) remodeling in response to volume overload induced by aortocaval fistula (ACF). However, in preliminary studies, there was an unexpectedly high mortality rate shortly (i.e., within 10 days) after the creation of the ACF, particularly in otherwise healthy homozygous lean Zucker rats. In previous studies in our laboratory using the ACF in Sprague-Dawley (SD) rats, mortality rates were minimal during the same time period (unpublished data). We subsequently found bilateral hydronephrosis in the kidneys of both lean and obese Zucker and ZDF animals in the absence of any surgical intervention.

The presence of hydronephrosis in lean Zucker/ZDF rats has been reported in one previous study by Vora et al. (29) and noted in another study (4); however, a third study reported no such observations with these animals (11). Progressive renal disease is characteristic of both type 1 and type 2 diabetes (1, 21); however, diabetic kidney disease typically takes the form of glomerular and mesangial expansion, formation of tubulo-interstitial lesions with an insidious onset of proteinuria, and a progressive decline in glomerular filtration rate (17, 21). Consequently, hydronephrosis in young rats, particularly lean nondiabetic animals, is not representative of the general human population and is certainly not a typical feature of type 2 diabetes. Therefore, the goal of this study was to characterize, in detail, systolic and diastolic function in homozygous lean and obese Zucker and ZDF rats compared with SD rats and to determine whether the incidence and extent of hydronephrosis contributed to the development of LV dysfunction. We hypothesized that both systolic and diastolic dysfunction would be observed in all Zucker/ZDF groups but of increased severity in animals with marked renal pathology and/or diabetes.

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**METHODS**

**Animals.** This protocol was approved by the Institutional Animal Care and Use Committee at the University of Alabama at Birmingham. Ten-week-old male Zucker lean (+/+; n = 8), ZDF lean (+/+; n = 8), Zucker obese (ZO, fa/fa; n = 8), and ZDF obese (ZDFO, fa/fa; n = 8) rats were studied, and SD (n = 9) rats served as control animals; all animals were obtained from Charles River Laboratories (Wilmington, MA). Rats were housed two per cage, maintained on a 12:12-h light-dark cycle, and provided with food and tap water ad libitum for 4 wk until death. Zucker/ZDF rats were maintained on the Purina 5008 diet as recommended by the supplier, while SD rats received standard rat chow. There were no biochemical or morphological differences between Zucker and ZDF lean groups (data not shown); therefore, since there is also no difference in their genetic background, these animals were pooled to form one group (ZL; n = 16).

**Hemodynamic and echocardiographic measurements.** Rats were anesthetized with 2% isoflurane, and echocardiography was performed (Agilent Sonos 5500, Philips, Bothel, WA) as previously described (23). Animals were then intubated and mechanically ventilated with 2% isoflurane in 100% oxygen. Heparin (100 U) was administered through the right external jugular vein, and the right carotid artery was isolated. A 2-F combined-conductance catheter-micromanometer (Millar, Houston, TX) was advanced retrogradely to the ascending aorta, where arterial pressure waveforms were collected before the catheter was advanced into the LV. Simultaneous pressure and volume measurements were collected at 1,000 Hz both at baseline and during reduced loading conditions caused by a transient (3–5 s) occlusion of the inferior vena cava below the level of the kidneys. End-systolic and end-diastolic volumes were calculated with the Bullet formula (7) with long- and short-axis dimensions obtained with a B-mode video; these values were then used to calibrate the raw data from the conductance catheter. Pressure-volume relations were analyzed with Cardiac Pressure-Volume Analysis software (PVAN 3.0, Millar). Pulse pressure was calculated as the difference between maximum and minimum aortic pressures. Augmentation index, a measure of arterial stiffness, was calculated as the height of the reflected arterial pressure wave as a percentage of pulse pressure with customized software (SphygmoCor 8.0, AtCor Medical, Sydney, Australia).

**Blood/plasma analysis.** Glucose and glycated hemoglobin (HbA1c) levels were determined in whole blood with Accu-Chek Advantage (Roche Diagnostics, Basel, Switzerland) and DCA2000+ (Bayer Healthcare, Elkhart, IN) analyzers, respectively. Angiotensin peptide levels were determined with a combination of solid-phase extraction, reverse-phase HPLC, and radioimmunoassay as previously described (32). Plasma levels of norepinephrine and epinephrine were determined with commercially available enzyme immunoassay kits per the manufacturer’s instructions (Labor Diagnostika Nord, Nordhorn, Germany). Creatinine and blood urea nitrogen (BUN) levels were determined in plasma with the VetScan Chemistry Analyzer (Abaxis, Union City, CA).

**Morphological analysis of hydronephrosis.** Kidneys were cut along the short axis through the hilar region, immersion fixed in 10% neutral-buffered formalin, and stored in 70% ethanol until paraffin embedding. Five-micrometer sections were stained with hematoxylin and eosin, and hydronephrosis was quantitated according to the criteria defined by Vora and colleagues (29): 0 = normal, either no or minimal space between papilla and calyx; 1 = mild, narrow but definable calyceal space with normal papillary shape (e.g., Fig. 1E); 2 = moderate, dilation of the calyx, compression of the papilla but preserved papillary shape (e.g., Fig. 1F); 3 = severe, gross distension of the calyx, severe compression of lateral cortex and marked distortion of papilla (e.g., Fig. 1G).

**Immunohistochemistry.** For myocyte cross-sectional area, horizontal short-axis sections through the mid-LV were embedded in optimum cutting temperature (OCT) compound (Sakura Finetek, Torrance, CA), frozen in methylbutane with liquid nitrogen, sectioned at 5 μm, and probed for laminin (Sigma-Aldrich, St. Louis, MO). Images of tissue in cross-sectional orientation from the anterior, posterior, and free walls of the LV endocardium were acquired (×40 objective), total field size was measured, and myocytes were counted within the field to determine average area. Myocyte apoptosis was examined in formalin-fixed, paraffin embedded, 5-μm sections with terminal deoxyribonucleotide transferase-mediated dUTP nick end labeling (TUNEL; Promega, Madison, WI) per the manufacturer’s instructions. Sections were colabeled with myosin and laminin (Abcam, Cambridge, MA) to identify myocytes. All data were analyzed with epifluorescence microscopy and digital image analysis software.

**Collagen analysis.** Horizontal short-axis sections through the mid-LV and transverse sections through the hilar region of the kidney were formalin fixed, paraffin embedded, sectioned at 5-μm thickness, and stained with picric acid Sirius Red F3BA. Sections were quanti-

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**Fig. 1. Morphological evidence of hydronephrosis in Zucker/Zucker diabetic fatty (ZDF) animals. Kidneys were cut through the short axis of the hilar region (A–D), and 5-μm sections were stained with hematoxylin and eosin (E–H). A and E: Sprague-Dawley. B and F: Zucker/ZDF lean. C and G: Zucker obese. D and H: ZDF obese.**
I was more than twofold higher in ZL compared with SD rats acute response to the anesthetic and surgical procedure. ANG ZL groups were in the normal range, confirming that the statistical significance (0.05), and elevated levels in ZO animals are indicative of a statistically significant difference at end diastole (P < 0.05). HbA1c levels indicate that ZDFO animals are indeed diabetic (P < 0.05), and elevated levels in ZO animals are indicative of a prediabetic state. As expected, the HbA1c levels in the SD and ZL groups were in the normal range, confirming that the elevated plasma glucose level seen in these groups was an acute response to the anesthetic and surgical procedure. ANG I was more than twofold higher in ZL compared with SD rats (P < 0.05), and while ANG I levels were >70% higher in ZO and ZDFO groups compared with SD rats this did not reach statistical significance (P = 0.47 and 0.11, respectively). Conversely, ANG II was lower in both ZO and ZDFO compared with SD groups (P < 0.05); however, there was no difference between ZL and SD groups. Norepinephrine levels were 2.5-fold higher in ZDFO compared with SD and ZL groups (P < 0.05), whereas epinephrine levels were not different between groups.

**Kidney function and morphology.** Renal dysfunction was evident in obese animals, with higher plasma BUN levels in ZO and ZDFO compared with SD and ZL groups (Table 1; P < 0.05); however, plasma creatinine levels were not different between groups. When normalized to tibia length to account for the significant differences in body weight, left and right kidneys were significantly heavier in both ZO and ZDFO compared with SD and ZL groups (Table 2; P < 0.05). There was little evidence of hydropnephrosis in the SD group; however, there was an increased incidence in ZL rats, with more than one-third exhibiting moderate to severe levels of hydropnephrosis (Fig. 1, Table 3). An increased incidence of moderate to severe hydropnephrosis was evident in ZO and ZDFO groups in the absence of ureter and/or bladder dilation. It should be noted that the dehydration and heating steps involved in formalin fixation and paraffin embedding of the tissues are likely to have caused the small amount of tissue shrinkage and narrow calyceal spaces in SD and ZL groups. Histological examination of collagen volume percent in the cortex revealed no significant difference in fibrosis between any of the groups.

**LV morphology.** Heart and LV weights normalized to tibia length were not different in any of the groups (Table 4), suggesting that the presence of hyperglycemia, diabetes, or hydropnephrosis was not associated with the development of cardiac hypertrophy. There was no difference in LV end-diastolic or LV end-systolic dimension between groups; nevertheless, ZDFO rats exhibited significantly thinner posterior walls at LV end systole (P < 0.05), with a trend toward a statistically significant difference at end diastole (P = 0.058). Reductions in LV wall thickness can be attributed to smaller myocyte size, myocyte apoptosis, or the development of thin-

### Table 1. Whole blood and plasma parameters from fed Sprague-Dawley, Zucker/ZDF lean, Zucker obese, and ZDF obese animals at 14 wk of age

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Sprague-Dawley</th>
<th>Zucker/ZDF Lean</th>
<th>Zucker Obese</th>
<th>ZDF Obese</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glucose, mg/dl</td>
<td>385±24</td>
<td>375±19</td>
<td>533±47†</td>
<td>825±38†</td>
</tr>
<tr>
<td>HaA1c, %</td>
<td>3.43±0.07</td>
<td>3.33±0.05</td>
<td>4.41±0.31†</td>
<td>8.90±0.27†</td>
</tr>
<tr>
<td>Angiotensin I, pg/ml</td>
<td>68±11</td>
<td>163±25*</td>
<td>118±33</td>
<td>120±12</td>
</tr>
<tr>
<td>Angiotensin II, pg/ml</td>
<td>103±17</td>
<td>80±8</td>
<td>28±5†</td>
<td>40±5*</td>
</tr>
<tr>
<td>Norepinephrine, pg/ml</td>
<td>317±51</td>
<td>340±55</td>
<td>474±56</td>
<td>811±174†</td>
</tr>
<tr>
<td>Epinephrine, pg/ml</td>
<td>149±44</td>
<td>86±18</td>
<td>297±54</td>
<td>330±91</td>
</tr>
<tr>
<td>Blood urea nitrogen, mg/dl</td>
<td>20.9±0.5</td>
<td>22.8±0.9</td>
<td>29.4±1.2†</td>
<td>26.9±1.4†</td>
</tr>
<tr>
<td>Creatinine, mg/dl</td>
<td>0.56±0.09</td>
<td>0.78±0.06</td>
<td>0.83±0.08</td>
<td>0.61±0.07</td>
</tr>
</tbody>
</table>

Values are means ± SE. ZDF, Zucker diabetic fatty. *P < 0.05 vs. Sprague-Dawley; †P < 0.05 vs. Zucker/ZDF lean.

### Table 2. Kidney morphology and fibrosis

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Sprague-Dawley</th>
<th>Zucker/ZDF Lean</th>
<th>Zucker Obese</th>
<th>ZDF Obese</th>
</tr>
</thead>
<tbody>
<tr>
<td>Body wt, g</td>
<td>480±13</td>
<td>338±6*</td>
<td>560±199†</td>
<td>356±27*</td>
</tr>
<tr>
<td>TL, mm</td>
<td>50±1</td>
<td>44±0*</td>
<td>45±1*</td>
<td>44±1*</td>
</tr>
<tr>
<td>Left kidney wt/TL, mg/mm</td>
<td>28.2±0.7</td>
<td>27.2±0.7</td>
<td>39.1±1.2†</td>
<td>39.0±1.0†</td>
</tr>
<tr>
<td>Right kidney wt/TL, mg/mm</td>
<td>29.7±0.7</td>
<td>28.7±0.7</td>
<td>38.0±1.9†</td>
<td>38.5±1.5†</td>
</tr>
<tr>
<td>Kidney collagen volume, %area</td>
<td>1.2±0.2</td>
<td>0.9±0.1</td>
<td>1.1±0.3</td>
<td>1.3±0.2</td>
</tr>
</tbody>
</table>

Values are means ± SE. TL, tibia length. *P < 0.05 vs. Sprague-Dawley; †P < 0.05 vs. Zucker/ZDF lean.
ner walls during growth; however, the differences in wall thickness were not accompanied by any differences in myocyte cross-sectional area (Table 4), and there was no evidence of apoptotic cardiomyocyte nuclei in any group (data not shown). It is also possible that myocyte apoptosis occurred in ZDFO rats at a much younger age than 14 wk, possibly during the onset of either hyperglycemia or hydronephrosis, which could explain the lack of nuclei undergoing apoptosis. One consequence of this would be an increase in fibrosis; therefore we evaluated myocardial interstitial collagen volume percent and MMP-2 activity (Table 4), suggesting that the differences in posterior wall thickness are most likely due to blunted growth of the LV wall during development.

Cardiac function. LV end-diastolic and end-systolic volumes were both significantly higher in ZDFO compared with ZL rats (Table 5; P < 0.05). Mean arterial and LV end-systolic pressures were significantly increased in the ZO group (P < 0.05). Mean arterial and LV end-systolic pressures were significantly increased in the ZDFO group compared with both SD and ZL groups (Table 5; P < 0.05). However, analyses of the LV end-systolic pressure-volume relation, which is inherently load independent, revealed no differences in end-systolic volume elastance ($E_{sv}$) or maximal elastance ($E_{max}$).

The time constant of isovolumic relaxation ($\tau$) was elevated in ZDFO rats compared with both SD and ZL rats ($P < 0.05$); however, the LV chamber stiffness constant ($\beta$) did not differ between groups. LV end-diastolic wall stress was significantly lower in ZL compared with SD rats ($P < 0.05$) but in ZDFO rats was elevated compared with ZL rats ($P < 0.05$).

Relationship between diabetes, hydronephrosis, and cardiac dysfunction. An analysis of covariance was used to evaluate the relationship between the degree of renal injury and systolic function. The significant differences seen in LV ejection fraction, VCFr, and augmentation index were not altered with the use of BUN as a covariate. However, LV end-diastolic wall stress was linked to renal dysfunction, as this difference was attenuated when BUN was included in a subsequent covariate analysis. The association between LV end-diastolic wall stress and BUN was elevated compared with ZL rats ($P < 0.05$).
and BUN is shown in Fig. 2; a partial correlation controlling for genotype showed that the association between these two variables was statistically significant ($r = 0.37, P < 0.05$). The use of BUN as a covariate had no effect on the significance of any of the indexes of diastolic function; however, BUN was significantly correlated with blood glucose levels ($r = 0.52, P < 0.05$).

**DISCUSSION**

In the present study we demonstrate that, independent of obesity or diabetes, young Zucker/ZDF animals exhibit a high incidence of preexisting hydronephrosis that is absent in normal SD rats. Despite the presence of a mild renal pathology in ZL and moderate to severe pathology in ZO rats, there was little or no cardiac dysfunction in these groups; however, there was significant evidence of both hydronephrosis and cardiac dysfunction in ZDFO rats. While this could be indicative of a diabetic cardiomyopathy, increased LV end-systolic wall stress was strongly associated with renal dysfunction in diabetic animals, which suggests that the combination of hydronephrosis and hyperglycemia has an adverse effect on cardiac function. These data, coupled with abnormal plasma levels of ANG I in ZL rats and ANG II in ZO and ZDFO rats, strongly suggest that the Zucker/ZDF genotype is not an appropriate model for the study of diabetes and insulin resistance on cardiac function. Furthermore, the susceptibility of these animals to hydronephrosis is also a serious limitation in their use as a model of diabetic kidney disease.

Vora and colleagues (29) were the first to describe the presence of hydronephrosis in both lean and obese ZDF rats. Recent studies have also reported significant proteinuria in lean ZDF animals (22) and hydronephrosis in lean and obese ZDF rats that was not accompanied by glomerular or tubular injury (8). The site of obstruction leading to hydronephrosis is most likely at the pelvic-ureteric junction, since the ureter and bladder were not dilated. A more detailed histological analysis of the location, degree, and type of cellular and subcellular damage has been described previously (29); further histological examination could provide more insight into the extent and etiology of this renal pathology but is beyond the scope of this paper. The underlying cause of the hydronephrosis in this genotype has yet to be determined; however, since the pathology is found in both lean and obese homozygotes, it is unlikely that the leptin receptor defect is directly responsible. Nevertheless, the greater severity in the ZO and ZDFO groups suggests that obesity and hyperglycemia contribute to the progression of the pathology, and while we have demonstrated a significant association between BUN and blood glucose we cannot assume causation. In poorly controlled diabetes, glucosuria (2), renal glucose uptake, and utilization (15) are increased, and it is likely that the known cytotoxic effects of hyperglycemia and protein glycation then exacerbate the development of diabetic nephropathy. While we did not measure urinary glucose levels in this study, it is possible that the ability of the kidneys to shed glucose was impaired in rats with severe hydronephrosis, and this may have contributed to the cycle of renal damage through a sustained elevation of blood glucose and subsequent worsening of hydronephrosis.

It is possible that the hydronephrosis is caused by a heritable condition unrelated to the fa mutation or could be the result of an epigenetic phenomenon. An epigenetic cause for the development of hydronephrosis might also account for the variable presentation of this abnormality in different populations of Zucker rats. Advanced diabetic kidney disease does not typically include hydronephrosis (17, 21) unless a neurogenic bladder causes obstructive kidney disease. Despite these findings and the well-characterized interactions between the renal and cardiac systems, the Zucker/ZDF genotype has continued to be used as a model for obesity and Type 2 diabetes in cardiac research. Indeed, our study appears to be the first to demonstrate an association between a preexisting renal pathology and baseline cardiac and vascular dysfunction in this genotype; thus the presence of hydronephrosis in Zucker/ZDF animals compromises their suitability for studies of Type 2 diabetes and any comparisons with the human disease progression.

Previous studies have demonstrated that Type 2 diabetes is accompanied by cardiomyocyte hypertrophy and apoptosis in addition to concentric remodeling in rodents (5, 19) and humans (6, 10). We did not find any change in cardiac or LV weight, and although an increase in cardiac weight is often reported it is not a uniform finding; indeed, several papers have reported no change in heart weight in young adult obese Zucker/ZDF rats (25), particularly in the absence of insulin treatment (9). Similarly, we found no differences in myocyte size, apoptosis, or LV chamber dimension in either ZO or ZDFO rats; however, the posterior walls of ZDFO rats were thinner than those of control animals. Interestingly, two previous studies that examined the morphological characteristics of 19- to 20-wk ZDFO rats differ in their findings. Zhou and colleagues (33) reported higher end-systolic chamber dimensions with no differences in end-diastolic dimension or wall thickness; in contrast, Frederdsorf and coworkers (9) found no differences in chamber dimension but did report increases in cardiomyocyte volume, posterior wall thickness, and systolic function. In the present study, animals were examined at 14 wk of age, ~4 wk after the onset of diabetes, and it is possible that the differences reported in these earlier studies may be a consequence of longer duration of diabetes. In the earlier studies there was no assessment of renal function or morphology; consequently, differences in the incidence or severity of hydronephrosis could have been a complicating factor. Regard-
less, it is evident that LV morphology and function in ZDF rats are highly variable between studies and are not consistent with other animal models of Type 2 diabetes or with results from humans with Type 2 diabetes.

In this study, we have demonstrated lower plasma ANG II levels in both obese diabetic and obese nondiabetic rats and higher plasma ANG I levels in lean rats. These data may explain, in part, the unexpectedly high mortality rate in both lean and obese Zucker/ZDF rats following the creation of an ACF in our preliminary studies, as the cardiac reserve may have already been diminished. Interestingly, previous studies of bilateral congenital hydronephrosis in rats demonstrated a decrease in renal renin production (27) as well as decreased plasma ANG II concentrations (28). A decrease in renin production is the likely cause of lower ANG II levels in ZO and ZDFO rats; this may be a reflection of a greater loss of renal parenchyma with the more severe levels of hydronephrosis in ZO and ZDFO rats than that seen in ZL rats. Nevertheless, the lower plasma ANG II levels are not consistent with previous reports in diabetic rats with renal dysfunction (16). Importantly, the lower ANG II levels in ZDFO rats in the face of LV dysfunction and increased plasma norepinephrine levels suggest a blunted renin-angiotensin system.

We originally hypothesized that LV dysfunction would be observed during both systole and diastole in all Zucker/ZDF strains of rats as a result of a preexisting renal pathology and that this would be exacerbated by an increase in the severity of this pathology and/or the presence of diabetes. Despite an increased incidence of hydronephrosis in ZL rats, the extent of kidney damage was not as severe as that seen in the obese groups, which would explain the relatively normal indexes of cardiac function seen in ZL rats. The incidence and severity of renal pathology in ZO rats were almost equivalent to those of ZDFO rats; however, the LV dysfunction was more apparent in ZDFO rats. This is most likely due to the combined effects of hydronephrosis and hyperglycemia on both vascular resistance and myocardial contractile function. Indeed, LV systolic dysfunction manifested by a decrease in fractional shortening and VCFr in ZDFO rats could be attributed to an increase in arterial stiffness in these animals. Increased systolic load can result in decreased shortening and an increase in LV end-systolic wall stress in the normal heart. The end-systolic pressure-volume relation accounts for this problem by evaluating LV shortening at multiple loads resulting in a slope, Emax, that defines the contractile state of the heart. The fact that Emax and Ees are normal suggests that systolic contractile function is preserved and that the decreases in LV ejection fraction and VCFr are a result of abnormal vascular stiffness. Vascular compliance is not completely defined by mean arterial pressure but by a measurement of vascular impedance that combines a simultaneous assessment of pressure and flow. This is best measured in the central aorta just above the aortic valve in order to better define the load seen by the ejecting LV. Reflected arterial waves that occur during systole rather than after aortic valve closure are a result of a stiff arterial tree and impose an important resistance to LV ejection. Recent studies have shown that the augmentation index from the LV pressure-volume impedance catheter provides an accurate assessment of vascular impedance (26). The increase in arterial stiffness and abnormal diastolic relaxation in ZDFO rats are consistent with previous reports demonstrating that hyperglycemia-induced dysfunction in calcium handling (18) and increased accumulation of advanced glycation end products (14, 25) contribute to increased arterial stiffness in diabetes.

While these functional alterations are consistent with cardiac dysfunction associated with diabetes, the presence of hydronephrosis in lean, obese, and obese diabetic Zucker rats potentially complicates this interpretation. To determine whether an association exists between renal dysfunction and the LV functional abnormalities, correlations and covariate analyses were performed; the only parameter that was related to renal dysfunction was LV end-systolic wall stress. Thus, despite a relatively advanced renal pathology, LV systolic and diastolic dysfunction in young ZDF rats is most likely the result of diabetes-induced complications; however, it is probable that further development of the existing hydronephrosis would result in further systolic and diastolic dysfunction in older animals.

One limitation of this study is that we have examined animals with a preexisting renal pathology rather than inducing such damage; thus the cardiac dysfunction we have observed can only be shown to be associated with such injury. Regardless, we feel that the concomitant existence of LV dysfunction, abnormal adrenergic and angiotensin peptide levels, and hydronephrosis in Zucker/ZDF animals is an important finding that has implications for investigators who are using, or intending to use, this genotype for studies involving cardiovascular function and diabetes. Second, the heart rate and mean arterial and LV end-diastolic pressures are lower in both Zucker/ZDF and SD rats than those previously reported (9, 25). We are unable to account for these findings, although it is possible that the extended duration spent under 2% isoflurane anesthesia during the echocardiographic measurements and subsequent surgical preparation before measurement of the hemodynamic parameters (up to 1 h in total) may have contributed to the lower pressures. It is possible that the observed cardiac depression may have obscured greater differences between groups, particularly in load-dependent indexes of LV systolic function, and that higher heart rates and restoration of arterial and LV pressures may have yielded findings different from those reported.

Finally, given that ZO and ZDFO rats are widely used for studies of complications associated with insulin resistance and diabetes, it is somewhat surprising that reports of hydronephrosis in these animals are not more common in the literature. However, unless one is specifically evaluating renal morphology, these abnormalities are likely to remain undetected. Furthermore, evidence of renal dysfunction would most likely be attributed to a consequence of the underlying metabolic disease, such as diabetic nephropathy, rather than hydronephrosis. It is possible that the presence of hydronephrosis may be limited to the strains of ZO and ZDFO rats from the vendor used in this study and may not be evident in institutional colonies or animals obtained from overseas vendors. However, to our knowledge, there is only one commercial vendor for the ZDF genotype in the United States; consequently, for many investigators, particularly those within the US, the availability of strains of ZO and particularly ZDFO animals that do not exhibit hydronephrosis is likely to be very limited.

In conclusion, the presence of hydronephrosis in all Zucker/ZDF genotypes at a relatively young age is of concern in cardiovascular studies because of the cardio-renal connection. Although we did not find significantly compromised cardiac...
function in nondiabetic animals, a covariate analysis indicated that renal dysfunction was a contributing factor to LV end-systolic wall stress in ZDFO rats and there was a significant association between systolic wall stress and BUN levels when controlling for genotype. Furthermore, the presence and progression of such hydrenephrosis in the ZL animals will most likely contribute to cardiac dysfunction at a later time point. While diabetic kidney disease is a significant contributing factor to the morbidity and mortality in patients with Type 2 diabetes, it is important to emphasize that the morphological changes seen with hydrenephrosis are not characteristic of the changes normally associated with diabetes. The lack of hypertension and increased norepinephrine levels in obese diabetic animals together with the reduced ANG II production in both obese groups also do not parallel the characteristics of Type 2 diabetes in humans. Thus the combination of a preexisting kidney disease due to hydrenephrosis in the Zucker/ZDF rats independent of obesity and/or diabetes and atypical ANG I, ANG II, and norepinephrine production raises serious doubts about the use of this model for studies of Type 2 diabetes and cardiovascular function. Investigators using either Zucker or ZDF rats for such studies in the future are strongly recommended to evaluate renal function and morphology to rule out the presence of hydrenephrosis in their cohorts of animals.

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