Dynamic blood pressure load and nephropathy in the ZSF1 (falfa<sup>cp</sup>) model of type 2 diabetes

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Griffin KA, Abu-Naser M, Abu-Amarah I, Picken M, Williamson GA, Bidani AK. Dynamic blood pressure load and nephropathy in the ZSF1 (falfa<sup>cp</sup>) model of type 2 diabetes. Am J Physiol Renal Physiol 293: F1605–F1613, 2007. First published August 29, 2007; doi:10.1152/ajprenal.00511.2006.—Diabetes and increased blood pressure (BP) are believed to interact synergistically in the pathogenesis and progression of diabetic nephropathy. The present studies were performed to examine if there were differences in BP load and/or protective renal autoregulatory capacity between the obese diabetic Zucker fatty / spontaneously hypertensive heart failure F1 hybrid (ZSF1) (falfa<sup>cp</sup>) rats and their lean controls. By ~26 wk of age, ZSF1 (n = 13) but not their lean controls (n = 16) had developed substantial proteinuria (180 ± 19 vs. 16 ± 1.4 mg/24 h) and glomerulosclerosis (19 ± 2.4 vs. 0.6 ± 0.2%; P < 0.001). However, average ambient systolic BP by radiotelemetry (12–26 wk of age) was modestly lower in ZSF1 than in lean controls (130 ± 1.4 vs. 137 ± 1.7 mmHg, P < 0.002), although the 24-h BP power spectra showed a mild increase at frequencies <0.1 Hz in the ZSF1. Autoregulatory capacity under anesthesia in response to step changes in perfusion pressure between 100 and 140 mmHg was similarly well preserved in both ZSF1 and lean controls at 16–18 wk of age [autoregulatory indexes (AI) <0.1]. Similarly, differences were not observed for dynamic autoregulation in conscious rats [transfer functions between BP (input) and renal blood flow (output) using chronic Transonic flow probes]. Collectively, these data indicate that the pathogenesis of nephropathy in the ZSF1 model of type 2 diabetic nephropathy is largely independent of differences in systemic BP and/or its potential renal transmission. However, these data do not exclude the possibility that the diabetic milieu may alter the glomerular capillaries in the ZSF1, such that there is an enhanced local susceptibility to injury with even normal glomerular pressures.

radiotelemetry; autoregulation; glomerulosclerosis; proteinuria; obesity

THE PATHOGENETIC MECHANISMS involved in the development and progression of diabetic nephropathy, the leading cause of end-stage renal disease, continue to be the subject of intense investigation. The experimental model most frequently employed has been that of streptozotocin (STZ)-induced type 1 diabetes (2, 10, 12, 28, 29, 47, 48, 68–70). Although a large number of potential pathogenic pathways have been identified using this model, the interpretations and conclusions are limited by the fact that significant histological nephropathy with glomerular capillary loss is very slow to develop and is fairly modest in its severity (2, 10, 12, 47, 59). In contrast to the relative resistance to nephropathy in type 1 diabetes models, several recently described models of type 2 diabetes develop more substantial nephropathy with heavy proteinuria and glomerulosclerosis (GS) (4, 12, 21, 43, 50, 60–62, 67).

The diabetic Zucker fatty (ZDF)/spontaneously hypertensive heart failure (SHHF) F1 hybrid (ZSF1) model of type 2 diabetic nephropathy (GMI, Charles River) was developed by crossing rat strains with two separate leptin receptor mutations (fa and fa<sup>cp</sup>), the lean female ZDF rat (+fa) and the lean male SHHF rat (+fa<sup>cp</sup>), derived from the obese spontaneously hypertensive rat carrying the coprulent fa<sup>cp</sup> gene (21, 50, 60–62). Unlike the progenitor ZDF rat (fa<sup>cp</sup>), the ZDF/SHHF F1 hybrids (ZSF1 rats; fafa<sup>cp</sup>) do not seem to exhibit a proclivity to develop spontaneous hydronephrosis (50, 63), but do exhibit many of the characteristics that are common to human type 2 diabetes, including obesity, hyperglycemia, insulin resistance, moderate hypertension, and severe dyslipidemia (50, 61, 62). The obese animals die at an early age (~12 mo) with symptoms of end-stage renal failure, accompanied by marked cardiac hypertrophy (50, 61). The heterozygotes for the leptin receptor mutations, while they are not technically truly lean rats, are nonobese and nondiabetic and have been referred to and used as lean controls in the present and previous studies as they exhibit minimal nephropathy (21, 50, 61). Given that substantial clinical and experimental evidence supports a synergistic interaction between diabetes and hypertension in the pathogenesis and progression of diabetic nephropathy (2, 3, 8, 10, 18, 29–32, 34, 42–44, 53, 54, 68), the present studies were performed using blood pressure (BP) radiotelemetry to examine if there were significant differences between the ZSF1 and its lean control rats with respect to the ambient BP and/or its potential transmission to the renal microvasculature due to differences in renal autoregulatory capacity.

METHODS

Male ZSF1 obese diabetic rats and their lean controls were obtained at ~6–8 wk of age (GMI, Charles River) and placed on the recommended diabeticogenic diet (Purina no. 5008). The animals were cared for in accordance with the National Institutes of Health Guide For The Care And Use Of Laboratory Animals, and the studies were approved by the Institutional Animal Care and Use Committee of Loyola University and Hines Veterans Administration Hospital. Three sets of studies were performed in separate groups of ZSF1 diabetic rats and their lean controls to characterize 1) BP load and the course of nephropathy; 2) steady-state autoregulatory capacity after step changes in renal perfusion pressure (RPP); and 3) assessments of “dynamic” autoregulation in conscious rats using transfer function analysis between BP (input) and renal blood flow (RBF) (output). 1.  

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BP load and course of nephropathy. These studies were addressed to defining the relationship of the radio-telemetrically assessed ambient BP load to the temporal evolution of nephropathy in this model. At 10 wk of age, ZSF1 rats and their lean controls underwent placement of radiotransmitters for continuous BP monitoring, as previously described (6, 10, 24–27). The rats were followed up to ~24–28 wk of age, by which time the ZSF1 diabetic rats have been reported to develop substantial nephropathy. BP was recorded for 5 s at 10-min intervals for the entire duration of the follow-up.

BP load was additionally assessed in the ZSF1 diabetic and their lean control rats using power spectral analysis. Such analysis can be used to estimate the separate BP power (energy/unit time) as consisting of two primary components: that due to its mean value (direct current BP power) and that due to its fluctuations from the mean due to heartbeat (HB) and other slower neurohormonal mechanisms [alternating current (AC) BP power] (7, 9, 10). For such analysis, BP signals were acquired at a sampling rate of 200 Hz for 24 h. One to two such recordings were obtained at each time point in each rat. The 24-h recordings are then resampled to a 20-Hz sampling rate after low-pass filtering to remove signal components at >10 Hz. The 24 recordings are broken into ~100 overlapped segments of 32,768 samples at the 20-Hz rate (with 50% overlap of segments), and the BP power spectra were determined using Welch’s average periodogram method (10, 52) (with a fast Fourier transform applied to each segment after detrending and multiplication by a Hanning window). BP power content was quantitated separately for the individual frequency bins 0.0006–0.1 Hz, 0.1–1 Hz, and 1–3 Hz, and at the HB frequency, as most of the AC BP power is present at frequencies <0.1 Hz or at the HB frequency (1, 9, 24, 41). The HB frequency was determined from the BP power spectral density at the frequency at which maximal BP power is reached in the 4- to 8-Hz frequency range. For computation of BP power, the HB frequency was then identified as the set of frequencies lying under the peak, with its edges defined by the points where the spectral density drops to 1% of the maximum. The results from the separate recordings in each rat were averaged before calculating the mean data for the individual groups.

Proteinuria (mg/24 h) was measured at multiple time points during the course using the sulfsalicylic acid method, as described previously (6, 10, 23–27). A few ZSF1 rats (3/16) had to be killed prematurely before the planned data were obtained because of technical and/or infection problems with the radio-telemetry transmitter, and their data are not included in the presented results.

Steady-state renal autoregulation. As noted above, 14–18 wk-old obese ZSF1 diabetic rats and their lean controls were anesthetized with inactin (100 mg/kg ip) and surgically prepared for steady-state autoregulation studies, as described previously (9, 11, 23–26). In brief, a tracheostomy was performed using polyethylene (PE-200) tubing, and a carotid and a femoral artery were cannulated with PE-50 tubing and connected to a Windograf recorder (model 40–8474, Gould, Glen Burnie, MD) for continuous recording of RPP. The femoral vein was cannulated with PE-50 tubing, and a 150 mM NaCl bolus equal to 1% of the body weight was administered, followed by a continuous maintenance infusion of 150 mM NaCl at 0.055 ml/min for replacement of surgical and ongoing fluid losses. An ultrasonic transit time (1RB, Transonic Systems, Ithaca, NY) flow probe was placed around the left renal artery for measurement of RBF by a flowmeter, and autoregulation studies were performed using aortic mini-clamps positioned above and below the left renal artery to lower or raise RPP measured via the femoral or the carotid artery catheter. Flow probes were validated as previously described (1, 9, 23–26). The RBF was allowed to stabilize for 1–2 min at each pressure before RBF measurements were made. Initial step reductions of RPP were followed by pressure steps in the reverse order. As no significant or consistent differences were observed in the RBF responses for each pressure step (in both directions), these responses were then averaged for the calculation of AI (23–26). AI were calculated as previously described for each pressure step (fractional change in RBF/fractional change in RPP) (1, 9, 11, 23–26). An AI of zero indicates perfect autoregulation, whereas an AI of one indicates that the vessels act as passive conduits for blood flow.

Dynamic renal autoregulation. Additional 14–18 wk-old ZSF1 and lean control rats underwent placement of radiotransmitters and chronic renal perivascular transonic flow probes (left renal artery) under pentobarbital sodium anesthesia, as previously described (1, 9, 24). In brief, under pentobarbital sodium anesthesia, each rat had a BP sensor (model T111PA-C40; Data Sciences) inserted into the aorta via the right femoral artery and advanced into the aorta to a position below the level of the renal arteries, and the transmitter was fixed to the peritoneum. An ultrasonic transit time flow probe (1RB) was placed around the left renal artery and packed in Dacron mesh to ensure proper alignment of the probe and vessel. The probe cable was secured to back muscles, routed subcutaneously, and exteriorized at the back of the neck. After rats were allowed to recover for ~1 wk (7–9 days), the flow probes were connected to a transonic flowmeter (T106, Transonic Systems), and simultaneous recordings of BP and RBF were obtained for 1–2 h at a sampling rate of 200 Hz in conscious, unrestrained ZSF1 and lean control rats between 10:00 AM and 3:00 PM. Two recordings were obtained in each rat at intervals of at least ~24 h, and the results were averaged for each rat.

Subsegments of 30 min in duration from each recording that were free of noise or other artifacts were selected from each data record. These 30-min signals were resampled to a sampling rate of 20 Hz using a low-pass anti-aliasing filter to remove variations in the signals of >10 Hz. Each time sequence of 36,000 data points was then subjected to linear trend removal. The transfer functions of the dynamic relationship between BP (input) and RBF (output) were analyzed using standard methods, as previously described (1, 9, 24).

The BP and RBF power spectra estimates were determined using the fast Fourier transformation-based Welch’s averaged periodogram method (50% overlap of 7 segments of 8,192 samples), and a Hanning window was applied. Input and output autopower spectra and cross-power spectra were calculated for each segment and then averaged. The admittance function was computed as the ratio of cross-spectrum to BP power spectrum. The coherence function was also computed from the cross- and autopower spectra. Fractional gain in admittance (FGA) was obtained by normalizing admittance gain by the conductance computed over the entire 30-min record. The natural frequencies of the myogenic and the tubuloglomerular feedback mechanisms (TGF) were determined from their characteristic signature resonance peaks in fractional gains between 0.1 and 0.3 Hz and between 0.025 and 0.05 Hz, respectively, by inspection of individual records, and then averaged across each record. For quantitative analysis of the transfer functions, the BP and RBF power content was computed for the relevant individual frequency bins, as described earlier for BP power and in previous publications (1, 9, 24).

Histology. Transverse sections through the papilla were cut at a thickness of ~3–4 μm and stained with hematoxylin and eosin and periodic acid Schiff. At least 100 glomeruli were examined in each animal, and the percentage of glomeruli exhibiting clear histological evidence of segmental or global GS was estimated in a blinded fashion using standard morphological criterion, as previously described (6, 10, 23–27).

Statistical analysis. Results are means ± SE. Statistical comparisons between the groups were performed using the Student t-test, with
Welch’s correction as indicated. For comparison within a group, repeated-measures ANOVA was used, followed by the Student-Newman-Keuls test. A P value of <0.05 was considered significant (66).

RESULTS

BP load and course of nephropathy. Significant differences in body weight were present between obese diabetic ZSF1 rats (n = 13) and their lean controls (n = 16) from the very outset of the studies at 8 wk of age (345 ± 11 vs. 281 ± 14 g; P < 0.001). These significant differences in body weight between the groups persisted throughout the course until the rats were killed at 24–28 wk of age (ZSF1 727 ± 12.5 g vs. lean controls 564 ± 5.5 g; P < 0.0001). By contrast, at 8 wk of age, the blood glucose values in the ZSF1 were more variable (109 ± 15.2 mg/dl) compared with lean controls (80 ± 2.3 mg/dl), and, therefore, the differences did not reach statistical significance (P = 0.08). However, by ~16 wk of age at the time when the step and dynamic autoregulatory studies were performed, the blood glucose had increased substantially in the ZSF1 rats and achieved a consistent separation from their lean controls (230 ± 25 vs. 78 ± 7 mg/dl; P < 0.0001). These differences in blood glucose persisted at ~22 wk (364 ± 22 vs. 70.4 ± 3.7 mg/dl) and at ~28 wk (242 ± 19.4 vs. 80 ± 4.0 mg/dl) (P < 0.0001 for both comparisons).

Figure 1, A and B, illustrates the course of systolic BP in an individual lean control and an obese diabetic ZSF1 rat, respectively, while Fig. 2 depicts the aggregate systolic BP course (2-wk averages) for the two groups after 12 wk of age. As can be noted, the average systolic BP was modestly but significantly lower in the ZSF1 rats than in their lean controls at most time points during the course. Consistent with these data, the overall average systolic BP during the entire course (the average of all 12,000–16,000 systolic BP readings obtained through the course in an individual animal) was also significantly lower in the ZSF1 rats (Fig. 3). Figure 2 also shows that, in contrast to the relatively stable systolic BP in both groups during the observed time course, proteinuria showed a progressive increase after the 12th wk in the ZSF1 but not in the lean control rats. Similarly, Fig. 3 also shows that substantially greater GS had developed in the ZSF1 rats compared with the lean control rats by the time of death at 24–28 wk of age, despite their lower average systolic BP during the course.

The quantitative data obtained for the BP power spectral analysis in all ZSF1 and lean control rats at ~16, 22, and 28 wk of age are provided in Table 1, and the 22-wk comparison is illustrated in Fig. 4A. The most notable difference between ZSF1 and their lean controls was the increased BP power at the very low frequencies (VLF) (<0.1 Hz) (15, 33) that was observed in the ZSF1 rats at all three time points compared with the lean controls. Also of interest, the heart rate was significantly slower at all time points in the ZSF1 rat, as is evident from the BP power spectra comparison illustrated in Fig. 4A. Figure 4A also shows that, by contrast, the respiratory rate was faster in the obese ZSF1, as indicated by the location of the peak associated with the respiratory frequency (~1–1.5 Hz). But a significant increase in the BP power at the HB frequency was only seen at the 28-wk time point. Figure 4, B

Fig. 1. Illustration of the course of radiotelemetrically recorded systolic blood pressure (BP) in an individual lean control (A) and obese diabetic Zucker fatty/spontaneously hypertensive heart failure (ZSF) rat (B) from ~10 to ~28 wk of age. BP was recorded every 10 min, and each point represents the average of ~60 systolic BP readings over a 5-s period.

Fig. 2. The course of 2-wk averages of radiotelemetrically recorded systolic BP in lean controls and obese diabetic ZSF rats after 12 wk of age. The BP data for the last two time points are for 10 ZSF rats only. Also shown are proteinuria data (mg/24 h) over the course of observation for the two groups of rats. *P < 0.03 maximum.
and C, shows the temporal changes in BP power spectra for those 10 lean and 5 ZSF1 controls rats in whom these BP recordings at 200 Hz for analysis of power spectra were obtained at each of the 16-, 22-, and 28-wk time points. While no significant differences were observed for any of the parameters over the time course of the studies in the lean control rats, the ZSF1 rats demonstrated a significant increase in average BP and in AC BP power at the HB frequency by 28 wk and a progressive increase in the BP power at the VLF (0.0006–0.1 Hz) at both 22 and 28 wk (Table 1).

Steady-state renal autoregulation studies. Figure 5 presents the results of these studies performed under anesthesia at ~14–18 wk of age. As can be noted, RBF autoregulation in response to step changes in perfusion pressure was fairly well preserved in both ZSF1 and lean control rats without a significant difference in autoregulatory capacity (AI) between the groups.

“Dynamic” autoregulation studies. Figure 6 depicts the results of dynamic autoregulatory studies in the ZSF1 and their lean controls. Analysis of the transfer functions between BP (input) and RBF (output) (Fig. 6a) did not reveal any significant difference in coherence (Fig. 6B) or in the dynamic autoregulatory compensation, as assessed by FGA at frequencies < 0.01 Hz (Fig. 6C). Similarly, significant differences were also not observed for the phase peaks associated with the myogenic or TGF mechanisms, with phase angles that were confined between ±90° (Fig. 6D). But differences were noted in the signature resonance peaks of the two autoregulatory control systems, suggesting possible differences in the relative contribution of the two systems to the observed autoregulatory compensation (Fig. 6C). A significantly enhanced TGF resonance peak (arrowhead) was present in the ZSF1 compared with the lean rats (1.3 ± 0.14 vs. 0.8 ± 0.08, \( P < 0.02 \)). By contrast, the myogenic resonance peak was relatively blunted/broadened in the ZSF1 rats (arrow). Precise estimation of the FGA at the myogenic frequency was rendered difficult because of the often merging of the myogenic resonance peak with an additional resonance peak between 0.4 and 0.6 Hz that was present in the ZSF1 but not the lean control rats.

**DISCUSSION**

Despite much investigation, the pathogenesis of diabetic nephropathy remains incompletely resolved and controversial. Several lines of evidence have suggested that, while hyperglycemia is a necessary condition for the development of overt nephropathy, it is not sufficient in either human or experimental diabetes. For instance, only 20–40% of patients with type 1 and/or type 2 diabetes develop nephropathy (14, 22, 47, 54, 59). Similarly, several rat and mouse models of type 1 and type 2 diabetes are relatively resistant to the development of overt nephropathy, despite substantial hyperglycemia and its associated downstream pathogenetic pathways (10, 12, 13, 28, 51, 57–59, 70). Such data have suggested that the development of overt diabetic nephropathy requires the presence of additional permissive and/or facilitative mechanisms, such as an enhanced intrinsic genetic susceptibility (10, 12, 14, 47, 51, 54, 59, 70). In this context, it is of note that the obese Zucker rat (OZR), considered to be a model of metabolic syndrome, develops proteinuria and GS by 6–8 mo of age, even in the absence of overt diabetes (17, 36, 46, 55). However, available data suggest that the severity of nephropathy is somewhat more modest than that observed in previous and present studies in the overt diabetic strains derived from the OZR (ZDF, ZSF1) (4, 21, 28, 50–62). Such data suggest that the obese Zucker strain carries an enhanced genetic susceptibility to the development of nephropathy, which is further magnified by the superimposition of diabetes. But it is of note that the same seems to be true of mouse models with the leptin receptor mutation (the obese db/db mouse), although the precise intermediate pathogenetic mechanisms remain poorly defined (12). It is also of interest that, by contrast, the obese ob/ob mouse with the leptin mutation per se does not exhibit a similar substantially increased susceptibility to GS. As it is possible that all of the splice variants of the mutated leptin receptor may not be equally dysfunctional in the different tissues, it has been

**Table 1. Analysis of BP power spectra**

<table>
<thead>
<tr>
<th>Time point, wk</th>
<th>Lean (( n = 15 ))</th>
<th>ZSF (( n = 13 ))</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Average (mean) BP, mmHg</td>
<td>Average (mean) BP, mmHg</td>
</tr>
<tr>
<td>16</td>
<td>118 ± 1.5</td>
<td>116 ± 1.6</td>
</tr>
<tr>
<td>22</td>
<td>117 ± 2.7</td>
<td>113 ± 2.0</td>
</tr>
<tr>
<td>28</td>
<td>110 ± 1.9*†</td>
<td>120 ± 4.5*†</td>
</tr>
<tr>
<td>Heart rate, beats/min</td>
<td>327 ± 3.8</td>
<td>318 ± 3.8</td>
</tr>
<tr>
<td>16</td>
<td>307 ± 3.7</td>
<td>288 ± 2.2*</td>
</tr>
<tr>
<td>22</td>
<td>297 ± 3.4*</td>
<td>268 ± 3.1*</td>
</tr>
<tr>
<td>28</td>
<td>258 ± 3.4*†</td>
<td>214 ± 19.0*†</td>
</tr>
<tr>
<td>AC BP power @ heartbeat frequency, mmHg²</td>
<td>133 ± 5.5</td>
<td>151 ± 5.5</td>
</tr>
<tr>
<td>16</td>
<td>154 ± 7.6</td>
<td>134 ± 9.3</td>
</tr>
<tr>
<td>22</td>
<td>156 ± 6.7</td>
<td>214 ± 19.0*†</td>
</tr>
<tr>
<td>28</td>
<td>214 ± 19.0*†</td>
<td>214 ± 19.0*†</td>
</tr>
<tr>
<td>AC BP power @ 0.0006-0.1 Hz, mmHg²</td>
<td>33 ± 1.5</td>
<td>35 ± 1.6</td>
</tr>
<tr>
<td>16</td>
<td>38 ± 1.6*†</td>
<td>44 ± 1.7*†</td>
</tr>
<tr>
<td>22</td>
<td>51 ± 2.2*†</td>
<td>51 ± 2.2*†</td>
</tr>
<tr>
<td>28</td>
<td>51 ± 2.2*†</td>
<td>51 ± 2.2*†</td>
</tr>
</tbody>
</table>

*Technically, satisfactory blood pressure (BP) recordings at 200 Hz were obtained in 15/16 lean control rats at 16 and 22 wk. The data at the 28-wk time point are for 10 lean controls and 5 diabetic Zucker fatty/spontaneously hypertensive heart failure (ZSF) rats only. AC, alternating current. *\( P < 0.05 \), maximum compared with its lean control group at the same time point. †\( P < 0.05 \), compared with the other time points in the same rats by repeated-measures ANOVA. Statistically significant differences were not observed for the other frequency bins (0.1–1 Hz and 1–3 Hz).
suggested that leptin may play a direct role in the pathogenesis of GS, at least in some of these models (65).

In any event, there is strong evidence that hypertension plays a facilitative role in the development and/or progression of diabetic target organ damage, including nephropathy, at least in genetically susceptible strains and/or individuals. For instance, the superimposition of even moderate hypertension seems to counteract the relative resistance to the development of overt nephropathy in several rodent diabetic models. To illustrate, the development of GS is accelerated when STZ diabetes is induced in hypertensive compared with normotensive rats (10, 19). Similarly, the Goto-Kakizaki rats with type 2 diabetes do not develop overt nephropathy (GS) or only develop mild glomerular changes spontaneously, but when deoxycorticosterone acetate + salt hypertension is superimposed, substantial GS is observed (32). Conversely, and consistent with such concepts, antihypertensive therapy slows the course of both clinical and experimental diabetic nephropathy (2, 3, 8, 11, 14, 18, 30, 34, 44, 47, 51, 54). Accordingly, aggressive BP reductions, to levels lower than those considered acceptable for nondiabetic patients, are currently recommended for patients with diabetes (3, 34). Nevertheless, despite the general recognition of the enhanced vulnerability of diabetic patients to hypertension, the underlying mechanistic basis for this synergistic interaction remains to be definitively established. But available data do indicate that there is an enhancement of BP transmission to the renal microvasculature in diabetes and that it is the increase in pressures locally in the renal circulation, rather than systemically, that is the primary determinant of these adverse effects (7, 8, 10, 29, 44, 68, 69). For instance, reduction in intrarenal pressures by a renal artery or aortic clip protects the ipsilateral kidney against STZ-induced diabetic nephropathy but accelerates it in the contralateral kidney exposed to the higher pressures (42). Analogous effects of renal artery stenosis have been observed in diabetic patients (5).

Fig. 4. A: comparison of the 24-h BP power spectra obtained in the lean controls \(n = 15\) and the obese diabetic ZSF rats \(n = 13\) at 22 wk of age. B and C: the temporal changes in BP power spectra in 10 lean and 5 ZSF rats, respectively, in whom 24-h BP recordings at 200 Hz were obtained for such analysis at each of the 16-, 22-, and 28-wk time points. The standard error data are not shown for the sake of clarity, but the relevant quantitative data are provided in Table 1. See text for methodological details.

Fig. 5. Steady-state autoregulation of renal blood flow (RBF) in response to “step” changes in perfusion pressure under anesthesia in lean control and obese diabetic ZSF rats at 16–18 wk of age. The mean autoregulatory indexes for each change in perfusion pressure are also shown. The absolute RBF was significantly greater in the ZSF compared with their lean controls at all perfusion pressures, \(P < 0.05\). However, when corrected for body weight differences and expressed as ml·min\(^{-1}\)·kg\(^{-1}\), the differences were no longer significant.
Accordingly, an increased susceptibility to hypertensive renal damage in diabetes can potentially result from an increased ambient BP load, an enhanced fractional transmission of the ambient BP load to the renal microvasculature, and/or an increased local tissue susceptibility to barotrauma (7, 8). However, the present results indicate that neither of the first two mechanisms can be implicated in the ZSF1 model of type 2 diabetic nephropathy. Also, in fact, although the ZSF1 has been considered a hypertensive model (61), the average systolic BP during the course, the BP parameter that has shown the closest correlation with clinical and experimental hypertensive renal damage (27, 41), was modestly but significantly lower in the ZSF1 than in their lean controls. However, in terms of the absolute BP, the BP is indeed modestly higher than that observed in normotensive rat strains by BP radiotelemetry (6, 10, 25). It is also of interest that an assessment of “BP load” using power spectral analysis showed that the component of BP power (energy) that is generated by the slower BP fluctuations (at frequencies <0.1 Hz) corresponding to the VLF band (15, 33), was modestly but significantly increased in the ZSF1 rats at all time points. By contrast, a significant increase in BP power at the HB frequency (pulse pressure) was only seen in ZSF1 rats at 28 wk after nephropathy had developed and may reflect the combined effects of a slower heart rate and the possible development of vascular stiffness and decreased compliance (35, 61). It is possible that the slower heart rate, as also observed in the STZ-induced diabetes model (10), and the trends for somewhat reduced BP power but increased fractional gain in ZSF1 rats in the frequency range of 0.3–0.6 Hz (Figs. 4A and 6C), reflect a lower sympathetic activity (20). This frequency range, corresponding to the low-frequency band, is considered to reflect the sympathetic activity to the resistance vessels (15, 20, 33, 35). In any event, such differences may only be of limited relevance to the development of nephropathy in the ZSF1 strain, as the renal autoregulatory capacity to buffer BP transmission to the renal microvasculature seems to be equally well preserved in both ZSF1 and lean control rats.

Previous studies have suggested that, even in the absence of increased systemic pressures, impairment of renal autoregulatory capacity is associated with an enhanced susceptibility to hypertensive glomerular injury and GS (7–10, 25–27, 41). While there is some controversy as to the precise parameters of BP transmission buffering that are assessed by current steady-state or dynamic methodologies and/or their adequacy in assessing all aspects of such BP transmission (1, 9, 20, 24, 35), no significant differences between the two strains were noted with either of these two methods for assessing autoregulatory...
efficiency and/or capacity. AI for steady-state RBF responses to step changes in RPP were <0.1 in both strains, indicating a normal magnitude of the steady-state autoregulatory response. Similarly, while some differences were observed during dynamic autoregulatory studies, it is unlikely that these differences reflect differences in dynamic autoregulatory capacity to buffer BP fluctuations. For instance, the differences in the resonance peaks of the myogenic and TGF mechanisms were not associated with differences in peak phases, although the peak phase angle of nearly 90° associated with the TGF mechanism (0.025–0.05 Hz) in both groups is somewhat higher than usually observed (9, 20, 24). In any event, these differences do not seem to be associated with evidence of an enhanced glomerular BP transmission, as the transfer functions between BP (input) and RBF (output) failed to show differences at most frequencies, including frequencies <0.01 Hz, the parameter that has conventionally been used to assess dynamic autoregulatory compensation. This can also be inferred from the BP and RBF spectra per se (Fig. 6A), showing, if anything, reduced RBF power in the ZSF1 rats, despite almost identical BP power (Fig. 6C). However, it needs to be acknowledged that, for reasons that remain to be defined, fractional gain at frequencies <0.01 Hz may not provide a reliable index of dynamic glomerular BP transmission and that optimal methods for such an assessment as yet remain to be established (1, 9, 20, 24, 35). Moreover, such autoregulatory studies primarily assess the responses of the preglomerular vasculature to BP changes, and elevation of ambient glomerular pressure may also result from an increased afferent arteriolar resistance, although such measurements also have some intrinsic limitations (7, 8, 10). Such considerations suggest that, although the presence of elevated glomerular pressures cannot be completely and definitively excluded, such elevated glomerular pressures, if present in this model, are unlikely to be due to impaired autoregulatory capacity, at least as can be assessed by current methods.

Collectively, the present data, therefore, indicate that the pathogenesis of nephropathy in the ZSF1 model of type 2 diabetic nephropathy may be largely independent of differences in systemic BP or its ambient glomerular transmission. A BP-independent pathogenesis is also suggested by the renoprotection that has been observed with several pharmacological interventions, including statins, antioxidants, and peroxisome proliferation-activated receptor agonists in these OZR and derived strains, usually without significant BP changes, at least with the tail-cuff method (4, 36, 46, 51, 55). Similarly, although agents that block the renin-angiotensin system do lower BP in these models, the achieved protection has been largely ascribed to BP-independent mechanisms (4, 18, 36, 40, 47, 48, 51, 57, 64). However, such data do not necessarily exclude the possibility that pressure-dependent mechanisms may, nevertheless, contribute to the progression of nephropathy in this type 2 diabetes model. It is possible that the diabetic milieu and the associated glomerular hypertrophy may lead to local alterations in structure and/or function of the glomerular capillaries that render them susceptible to the adverse effects from exposure to even normal pressures (7, 8, 19), possibly because of an alteration in mechatrontransduction pathways (19, 31, 32, 53, 56). For instance, recent data have noted significant podocyte abnormalities in these strains (17, 28), similar to that described in other experimental diabetes models, as well as in human diabetic nephropathy (12, 32, 47, 51, 54, 63). While such studies have generally emphasized the potential importance of these alterations in the pathogenesis of proteinuria, there is evidence that the podocyte may also have a critical mechanical structure-stabilizing role in counteracting the relatively higher pressures in the glomerular capillaries (8, 37, 38, 49). Accordingly, it is possible that such podocyte structural and functional changes may render the glomerular capillaries more vulnerable to barotrauma and enhanced the susceptibility to GS. Therefore, even with a predominantly BP-independent pathogenesis of nephropathy in the ZSF1 model, the glomerular capillaries may still exhibit an increased sensitivity to local pressure changes and benefit from BP reduction. Future studies will need to formally test such a postulate.

In this context, it may also be important to note that the glomerular pathology phenotype in this as in most other rat and mouse models differs from the human diabetic nephropathy phenotype. The predominant glomerular lesion observed in the ZSF1 was that of segmental GS. Although some mesangial expansion was observed, it was not disproportionate or severe enough to lead to the development of diabetic nodular lesions, which were not seen. These histological findings are similar to that noted in most rat and mouse models of diabetes (2, 4, 12, 17, 18, 22, 32, 43, 47, 59–63, 67–70). The disproportionate mesangial matrix expansion with loss of glomerular capillary surface (diffuse GS) with the frequent presence of nodular lesions that is characteristically observed in both type 1 and type 2 human diabetic nephropathy is rarely observed in these experimental models (12, 14, 22, 47, 59). Conversely, segmental GS lesions that are characteristic of rodent diabetic models are infrequently observed in human diabetes (15, 47, 59). The underlying reasons for these differences between human and rodent diabetic nephropathy phenotypes as yet also remain to be elucidated.

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