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Extended Longitudinal Analysis of Arterial Pressure and Heart Rate Control in Unanesthetized Rats with Type 1 Diabetes

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Abstract

We recorded arterial pressure (BP) and heart rate (HR) in type-1 diabetic rats versus controls for 6 months. Diabetic rats (DIAB) were maintained on insulin from the day glucose > 250 mg/dl ("Day 0"). Weight was similar between groups until ~3 weeks before Day 0 when the weight in DIAB transiently lagged the controls (CONT); this difference was maintained throughout the study, but both groups otherwise gained weight in parallel. Plasma glucose attained 371 ± 109 (SD) mg/dl by day 1 in DIAB. Mean BP was similar across groups, and declined through the initial 4–6 months in both the CONT (at -0.06 ± 0.04 mm Hg/day) and in the DIAB (at -0.14 ± 0.21 mm Hg/day; NS vs. CONT). HR in the CONT (Month 1: 341 ± 13 bpm) exceeded DIAB (325 ± 25 bpm) through ~6 months after Day 0, and also decreased progressively over this period in CONT (-0.19 ± 0.14 beats/day) and DIAB (-0.29 ± 0.23 bpm/day; NS vs. CONT) before leveling. The BP power within 0.35–0.45 Hz changed during the 90 minutes before vs. after the transition from dark to light, and light to dark; there were no between group differences. The slope of the log-log linear portion of the BP power spectrum between 1.0/hr to 1/min was similar across groups, and increased in both from month 1 to month 6. Regulatory mechanisms maintain similar profiles in BP and HR in diabetic vs. control animals through the initial half year of the disease.

Keywords

Cardiovascular system; autonomic nervous system; weight gain; plasma glucose; dysautonomia; slope β (beta)

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INTRODUCTION

Diabetes and its complications are known to affect the autonomic control of cardiovascular, neural, renal, gastrointestinal, sexual and other functions (reviewed in American Diabetes Association and American Academy of Neurology, 1988; Vinik, et al., 2003; Vinik and Ziegler, 2007; Freeman, 2003), and diabetic cardiovascular autonomic neuropathy is “one of the most overlooked of all serious complications of diabetes...” (Vinik and Ziegler, 2007). The disease may damage small fibers, large fibers or both; the sympathetic and parasympathetic systems may each be affected by the disease process (Hosking, et al., 1978; Hilsted, 1982; Pfeifer, et al., 1982.). Autonomic neuropathy (AN) causes widespread disturbances in visceral function, particularly including that of the heart and blood vessels. A study of 605 patients followed for 9 years revealed that individuals with diabetes and low autonomic function had approximately a doubled risk of mortality (Gerritsen, et al., 2001). Finally, hypertension is common in diabetic patients (e.g., Czupryniak, 2006). Even if it is granted that in many cases cardiovascular disease co-exists with diabetes, it is difficult to demonstrate from observations in humans any causal relationships between diabetes and associated cardiovascular co-morbidities. It is clear, however, that knowledge of arterial pressure *per se* is important at all developmental stages in diabetes (ACCORD Study Group, 2010).

Many previous animal studies in experimental diabetes used alloxan or streptozotocin administration to kill the insulin-secreting beta cells of the subject's pancreas. Alternatively, the Bio-Breeding Labs bred the BB^{DP}/Wor rat, or ‘BB’ rat, which spontaneously develops an autoimmune, insulin-dependent diabetes that resembles the human disease in many ways. In particular, the pancreas of the affected BB animals shows lymphocytic insulinitis, fibrosis and the absence of beta cells (Chappel and Chappel, 1983). Autonomic and sensory neuropathies have been described in the BB rat. Alterations in nerve collagens can be detected by 4 months (Wang, H, et al., 2003.). Nerve conduction velocity slows progressively, declining by 17% at 14 months (Sima, et al., 2000.). At 4 and 8 months the BB^{DP}/Wor shows a progressive redistribution of nodal Na⁺ channels across the paranodal and internodal regions that are associated with the conduction slowing (Cherian, et al., 1996). Axonal dystrophic changes in sympathetic fibers, which are the hallmark of AN in human diabetics, are consistently evident in the BB rat after ~8 months of diabetes (Yagihashi and Sima, 1985a,b; Schmidt, et al., 2003). The main structural abnormality consists of expanded axons containing a variety of normal and abnormal subcellular structures, prompting the investigators to conclude that “dystrophic and degenerative axonopathy is a reproducible structural hallmark of diabetic sympathetic neuropathy” in the BB rat (Yagihashi and Sima, 1985b). BB rats diabetic for 28 weeks have a 57% loss relative to controls in ventricular myocardial sympathetic nerve fibers and varicosities and, in atrial tissue, a 42% loss in sympathetic axon profiles containing varicosities (Addicks, et al., 1993).

The cardiovascular consequences in the BB rat of the degenerative changes in autonomic nerves that occur over time are of obvious interest. Multiple standard analyses, such as comparison of absolute mean values across groups, are insightful, but power spectral analysis of a given variable quantifies the dynamics of that ‘signal’, and helps delineate those regulatory mechanisms responsible for a given fluctuation of the variable. For example, in dog heart rate (HR) changes that characteristically repetitiously recur with a period of ~25 seconds in the resting animal appear as a concentration of power in the computed HR power spectrum within the range of ~0.003 to 0.09 Hz (i.e., averaging ~1 cycle per 50 sec. or 0.02 Hz). The power within the HR spectrum resultant from this recurring ‘periodicity’, classically attributed to vasomotor activity (e.g., Akselrod, et al., 1981), is significantly attenuated, but not eliminated, by selectively surgically eliminating

the parasympathetic innervation of the SA-node, while leaving sympathetic innervations intact; it is further decreased by the addition of pharmacologic β -adrenergic blockade (Randall, et al., 1991). Conversely, the peak in the HR power spectrum at ~ 0.32 Hz, that is tightly linked to respiration in the resting dog, is virtually eliminated by that same selective SA-nodal parasympathectomy (Randall, et al., 1991). More recent recordings of sympathetic nervous activity (SNA) and changes in arterial blood pressure (BP) in unanesthetized rat revealed concentrations of power in both spectra centered around ~ 0.4 Hz; these recordings further demonstrated that fluctuations in BP and SNA are tightly coupled, or 'coherent', at this frequency (Brown, et al., 1994; Burgess, et al., 1999). This portion of the 'mid-frequency' spectrum represents primarily, though not exclusively, harmonic power that can be modeled as resultant from the natural frequency of the baroreflex (e.g., Cerutti, et al., 1994; Burgess, et al., 1997). These observations suggest that assessment of differences in the BP power spectra of diabetic vs. age-matched control rats centered within the mid-frequency range would be informative. Sanyal, et al., 2002 and Zhang, et al., 1990 have, in fact, examined other aspects of BP variability in the BB rat associated with autonomic function. Conversely, no one has described how mid-frequency rhythm may change across a 24-hour, light:dark cycle. That is, the 'dynamics' of the 0.4 Hz rhythm itself have not been examined.

One additional feature of the BP power spectrum is noteworthy: the log of BP spectral power increases linearly as the log of frequency decreases. This inverse 'log-log linear' behavior is seen widely in nature (e.g., Voss, 1989) and, more particularly, is evident in HR and BP spectra (e.g., Butler, et al., 1994). This so-called ' $1/f$ ' (or $1/f^\beta$, see below) character is indicative of 'fractal' or 'self similar' behavior. That is, the statistical characteristics of a signal possessing self-similarity look remarkably similar irrespective of the 'scale' being examined: the undulations of a coastline look comparable when examined across meters, kilometers or hundreds of kilometers. With respect to biological signals that display this phenomenon, the persistent and beguiling tenet is that such remarkable behavior must tell us something truly fundamental about the forces that shape that variable's behavior, though, admittedly, this ultimate 'meaning' remains elusive. The use of BP telemetry would now allow the extended recording of arterial pressure required for such broad frequency measurements in diabetic vs. non-diabetic rat. In sum, frequency domain analyses provide useful means to quantify BP 'dynamics' and point towards the underlying autonomic control of those rhythms.

The purposes of this study were, first, to document changes in weight, plasma glucose, mean arterial BP (mBP) and HR in diabetic animals (DIAB) and age-matched controls (CONT) during the months following the former animals' becoming type 1 diabetic. We tracked arterial pressure and HR extending up to 6 months or more to determine if there were differences between the two groups which might be attributable to the diabetic state and/or dysautonomia. Second, we also quantified any differences between the two groups in the dynamics of the harmonic variability of mBP and of its fractal power. In particular, we documented any differences in mBP power within the range 0.35 – 0.45 Hz between DIAB and CONT, any changes in such power across a 24 hour period, and the value of β computed between 1.0/hr to 1/min. Finally, we interpreted our data in so far as possible in terms of the effects of dysautonomia known to accompany extended periods of diabetes in the BBWP/Wor rat. A preliminary report of these findings has been published (Anigbogu, et al., 2005).

MATERIALS AND METHODS

Animals

A total of 34 diabetes prone (BBDP/Wor) and 26 diabetes resistant (control; BBDR/Wor) rats participated in one or more stages of this study. Three additional diabetes prone animals

never became diabetic; data from these latter animals are not included in the analyses reported here. All animals were obtained from the Biomedical Models Inc. (Rutland MA). The diabetes prone rats spontaneously develop an autoimmune, abrupt onset type 1 diabetes mellitus between 50 – 120 days of age characterized by polydipsia, polyuria and hyperglycemia (Chappel and Chappel, 1983). The rats were obtained from the vendor at 31 – 45 days of age. The animals were housed in an isolated, sound shielded, limited access room where the temperature was controlled at 72 F°, 56% humidity, and a 12/12 hour light/dark cycle. The rats were fed on standard rat chow (Harlan Teklad 2018, Madison WI) and had access to water ad-libitum. The study was approved by the University of Kentucky Animal Care and Use Committee.

The diabetes prone animals were weighed each morning, including weekends, and blood glucose was measured in a drop of blood from the saphenous vein using the One-Touch Ultra glucometer (LifeScan Inc., Milpitas CA). The control animals were weighed each Friday, and their plasma glucose determined, also from prick of the saphenous vein.

Surgery

All surgery was performed under anesthesia (sodium pentobarbital; 65mg/kg, IP) with procedures appropriate for rodent survival surgery. Surgery was performed prior to the diabetes prone animals becoming diabetic, and at a similar age in the matched-controls. The abdominal aorta was accessed via a laparotomy. The sensory element of a Data Sciences International (DSI) probe (model TA11PA-C40) was placed into the aorta in 12 diabetes prone and 12 control rats via a puncture. The probe's 'catheter' was secured in place with surgical glue. The body of the probe that contains the sensor, transmitter and battery was sutured to the interior abdominal wall. The incision was closed and the rat monitored until it aroused from the anesthetic. The animal was returned to the home cage once it had aroused and was self grooming.

Experimental Protocol

The day the animal first showed a morning blood glucose level above 250 mg/dl was taken as onset of diabetes mellitus and designated "Day 0". The duration of diabetes, in days, was calculated from this time. The diabetic rats were maintained on an insulin dose schedule developed by the breeder to manage blood glucose levels appropriately. This involved giving PZI insulin 0.9U/100g/day, subcutaneously. The dose was increased or reduced by 0.2 units per day, depending on weight gain or loss and plasma glucose level. Blood pressure was monitored beat-by-beat via telemetry, optimally over two week uninterrupted intervals each month, though the actual schedule varied somewhat in individual animals from this standard. The battery was turned off when not in use. The goal was to maintain an animal for 6 months or more following conversion to the diabetic state (and in the age-matched controls), though this goal was not achieved in all subjects.

Data acquisition and analysis

Blood pressure recording commenced two to three days after surgery. The telemetry data were obtained using the PhysioTel RPC 1 receiver. The received signal was fed into a DSI Data Exchange Matrix which was connected to an ambient pressure reference. The output from the matrix was fed, via a Dataquest PCI card, into the Pentium IV computer based workstation running a Dataquest A.R.T system software program. The output from this computer was cross fed into an analog-output Data Exchange Matrix (DEM). The analog output of the DEM was passed through a BNC-2110 A-D converter (National Instruments), sampled at 500 Hz, and passed into an analysis and output computer for further processing or display. The data were analyzed using an in-house developed computer program (ViiSoftware, Lexington KY) running on a Pentium IV based computer. Heart rate was

computed from the pulsatile blood pressure signal. Spectral power was computed either for successive 248 second, or for 51 second, intervals (see “Results”) using a fast Fourier transform. The slope of the log-log relationship between power and frequency (i.e., β) was calculated, as described previously (Brown, et al., 2006), between approximately 1.0 cycles per hour and 1 cycle per minute. We have previously found this frequency range to present the most consistent linear log-log relationship (Brown, et al., 2006). The time series data were manually edited to remove artifacts (see ‘Results’) prior to computation of the spectrum. Data are presented as mean $\pm$ SD. A mixed two-factor between group analysis of variance (ANOVA) was applied to the strains (DIAB and CONT) with repeated measures across time (months). One way within subjects ANOVA was used to test for changes across time for the DIAB and CONT subjects with appropriate post-hoc tests. Significance was taken as $P < 0.05$.

RESULTS

Blood Glucose and Body Weight Changes in Diabetic and Control rats

Daily monitoring of blood glucose and body weight of the rats commenced one week after receipt from the supplier. The top panel of Figure 1 shows the blood glucose level from blood drawn each morning prior to administration of daily insulin (i.e., in the diabetic rats after Day 0) in both the diabetes prone (thick line) and diabetes resistant (thin line) rats. In the DIAB rats, therefore, this is essentially the peak daily blood glucose level. Forty days before the onset of diabetes, blood glucose levels were similar in the diabetes prone (98 ± 15 mg/dl) and diabetes resistant controls (97 ± 14 mg/dl) rats. However, divergence between groups appeared prior to Day 0 so that two weeks prior to the diabetic rats' conversion, blood glucose level in the diabetes prone (108 ± 20 mg/dl; $n=31$) was significantly ($t_{55} = 2.767$) greater than in the control animals (95 ± 16 mg/dl; $n=26$). One week prior to Day 0 the morning plasma glucose in DIAB was 112 ± 18 mg/dl and 98 ± 15 mg/dl in CONT ($t_{40} = 2.438$). Figure 2 documents plasma glucose across 29 animals for the ten days immediately prior to their conversion to the diabetic state (i.e., morning plasma glucose > 250 mg/dl). For these ten days' data there was a significant change in glucose ($F_{9,252} = 23.24$) across time, with the observations on days -1 and -2 (i.e., 1 and 2 days before conversion) significantly exceeding the value at Day -10 (Bonferroni). This gradual rise gave way to an abrupt sharp rise in blood glucose levels reaching 371 ± 109 mg/dl at day 1; morning levels of plasma glucose in these animals remained markedly elevated over the control animals throughout the remainder of the study (Figure 1). Conversely, the blood glucose level in the control rats remained consistent throughout the study (Figure 1). The protocol for determining the daily insulin dose required plasma glucose readings (only) once in the morning, but we also determined this value in 6 DIAB rats over a period of 4 days (i.e., starting ~ 75 days after their conversion) at about 9 am, noon and 4 pm; the respective values were 280 ± 42 , 81 ± 29 and 73 ± 26 mg/dl.

Table 1 summarizes the body weight findings at fifty day intervals, except, as for glucose, the pre-conversion data are given for at -40 days. The weights were significantly different for all times excepting the initial values. The bottom panel of Figure 1 indicates, in fact, that the growth curves started to diverge prior to the diabetic rats' conversion. Two weeks prior to conversion (i.e., Day -14) the diabetic animals had a significantly ($t_{56} = 4.263$) lower body weight (257 ± 33 g; $n=32$) than the resistant rats (297 ± 39 g; $n=26$). Starting from about this time the growth curves remained separate over the course of the study, though the rate of weight increase otherwise remained similar across the groups such that the two curves advanced essentially in parallel.

Blood Pressure and Heart Rate Changes during development of Diabetes

Figure 3 shows 16 days of mean arterial pressure and HR from on-going, beat-by-beat recordings from individual age-matched control (top) and diabetic (bottom) rats moving freely within their individual home cage. The insert shows ~two seconds of data for a diabetic rat recorded 90 days after it converted to illustrate the nature of the raw recordings; the telemetry unit had been implanted 10 days before this DIAB rat converted. The phasic characteristics of the pressure signal are evident.

Figure 4 shows mBP (filled circles; scale at right) and HR (open circles; scale at left) for a control rat (top panel; DOB: 3-24-06) and a diabetes prone partner (bottom panel; DOB: 3-21-06). Each datum represents a beat-by-beat average of the respective variable recorded over 24 hours. The diabetes prone animal converted to the diabetic state at chronological age of 81 days (morning plasma glucose: 320 mg/dl); the glucose values for the previous five days were 96, 130, 127, 159, and 197 mg/dl, respectively. These data are presented to illustrate circumstances encountered with this portion of the study and as specific examples of changes in mBP and HR across time observed in all animals. Note, therefore, in both panels that HR declined rather precipitously through the initial set of recordings, probably during the animals' progressive recuperation from surgery (see "Discussion"). Over the very last week mBP in the control rat declined to a seemingly non-physiological range, but its HR remained on a continuing, slow downward projection despite the change in mBP (see Discussion). Finally, HR increased sharply in the diabetic animal during the week immediately prior to its death at a diabetic age of 201 days (extreme right, bottom panel); its morning plasma glucose became unusually labile concomitant with the tachycardia, ranging from a low value of 52 to a high of 508. The major physiological lesson in Figure 4 is the steady, gradual, downward decline in both mBP and HR over time in both rats. For the entire control group for those animals with at least three months of data ($n=12$) mBP declined at 0.06 ± 0.04 mm Hg/day; HR decreased at a rate of 0.19 ± 0.14 beats/day. Corresponding data for the diabetic subjects ($n=9$; duration of recordings in 3 animals < 3 months) mBP decreased at -0.14 ± 0.21 mm Hg/day and HR at -0.29 ± 0.23 bpm/day. Neither between-group difference was significant.

Figure 5 shows monthly averages $\pm$ SD for mBP (top panel) and for HR (bottom panel) recorded from diabetic (solid line) and control rats (dashed line) for the period starting one month prior to conversion (pre) and extending through 6 months after the BBDP/Wor animals became diabetic. Findings for a subgroup of diabetic ($n=5$) and of control ($n=7$) rats that were each included across six monthly observations are given in Table 2. A mixed analysis of variance (control/diabetic, months post-conversion) for mBP revealed no statistically significant between group differences, or a significant group $\times$ time (months) interaction. Conversely, the main effect for time (i.e., months) was significant ($F_{5,50} = 5.23$). A subsequent one-way ANOVA for repeated measures on the arterial pressure findings for the control subjects across the 6 months was significant ($F_{5,30} = 2.50$; $p=0.053$); likewise, the corresponding computation for the diabetic subjects was significant ($F_{5,20} = 3.25$). The overall ANOVA for the control and diabetic HR data reported both a significant between group effect ($F_{1,10} = 4.83$) and an effect of time ($F_{5,50} = 24.03$). Both the lower HR in the diabetic rats, and the decline in HR over time in both groups are evident in Figure 5 and Table 2. The subsequent one-way ANOVAs for repeated measures on the HR data for both the control subjects ($F_{5,30} = 13.31$) and for the diabetic subjects ($F_{5,20} = 11.77$) were significant. The HR decline generally paralleled a gain in the subject's weight. The average linear correlation coefficient between monthly average weight and HR in the CONT was -0.80 ± 0.11 (range: -0.94 to -0.60) with an average p value of 0.06 ± 0.07 (range: 0.01 to 0.21). Corresponding data for the DIAB were -0.85 ± 0.06 (range: -0.96 to -0.79) with a p value of 0.04 ± 0.03 (range: 0.003 to 0.08).

It is obvious that HR and mBP cannot continue their downward trends indefinitely, and, in fact, the curves in Figure 5 flatten over the last 2–3 months. We tracked two control rats for 12 months plus another for 11 months. Likewise, we tracked four diabetic rats for 7 months, and two of these for 9 months. Figure 6 shows the monthly average $\pm$ SD HR for these animals over these times. One-way ANOVAs for repeated measures were significant for both the control (months 1–11, $F_{1,20} = 6.79$) and diabetic (months 1–6, $F_{5,15} = 9.3$) groups. The initial downward trend and the leveling of the HR during the final months of recording are obvious in each group. In addition, the two curves converge with time, affirming that the initial relative tachycardia in the control animals relative to the diabetic rats eventually disappears.

Power Spectral Analysis of mBP

Figure 7A plots mBP spectral power in the third, or ‘Z’, dimension for a control rat over a day during Month 1 for a frequency range extending from 0.27 (left margin) to 0.51 Hz (right margin); the area in red is centered upon 0.4 Hz, extending from 0.35 to 0.45 Hz, that region wherein changes in BP and SNA are tightly coupled (Brown, et al., 1994). Note, therefore, that the ‘strength’ of the power is indicated by the height of the ‘mountain peaks’ and that this height quantifies the variation in mBP at the indicated frequency as computed across the individual 248 second long recording intervals that are successively ‘stacked’ together as time progresses along the axis projecting into the plane of the figure. The figure includes two segments of data where the back panel starts at midnight and extends forward to noon; the front panel starts at noon and extends forward to the next midnight. The gray segments denote ‘lights-off’ and the green ‘lights-on’. It is visually obvious in the rear panel that, on the average, power within the 0.35–0.45 Hz range is higher during the dark, and lower when the lights are on. The diminution of power is particularly stark at the transition between dark and light at 6 am. Likewise, in the front panel power within this range increases after ‘lights out’ and, in fact, this increase appears to begin somewhat in advance of the 6 pm transition, perhaps as the rats ‘anticipate’ the event.

Figure 7B focuses upon the 90 minute intervals before and after the transition from dark to light and *vice versa*. It plots the average power (ordinate scale that is given in the middle of the figure) within 0.35 – 0.45 Hz (red range in Figure 7) on a moment-to-moment basis on a single day starting (leftmost, darker background) 90 minutes before the transition between dark to light (left half) and (right half) starting 90 minutes before the transition between light and dark. The value given in the center of each 90 minute segment is the average power centered on 0.4 Hz for each respective 90 minute segment (e.g., 0.3404 mm Hg²). Power is clearly larger during the dark periods than during light periods for these recordings for this animal. Table 3 provides the average $\pm$ SD power across the control and diabetic subjects within 0.35 – 0.45 Hz for the 90 minute periods immediately before and after the transition from dark to light (left 2 rows) and light to dark (right 2 rows), as given, for example, by the numerical values in Figure 7. Data are given for months one and six for the diabetic animals (n=3) and their controls (n=4). An overall 3 variable, mixed ANOVA [Group (control/Diabetic), Condition (dark, light, light, dark), Month (1 and 6)] reported significant effect of dark-light, but no significant group effect or any significant interaction. For the Controls during Month 1 the powers were significantly different (one way repeated measures ANOVA; MO1: $F_{3,9} = 28.2$; MO6: $F_{3,9} = 9.65$) across the transitions as indicated in the table. The corresponding data for the diabetic animals were significant for Month 6 ($F_{3,6} = 14.4$).

Figure 8 shows a log-log spectrum for mBP power (ordinate) vs. frequency (abscissa) for a control rat based upon 5 days of continuous, beat-by-beat recordings; these data were recorded during the first month following the age-matched diabetic rat’s conversion. Note the unusually broad frequency range covered by this spectrum, extending from cycles that

exceed one per day in length (left margin) to a relatively fast cycle that repeats every ~5 seconds (right margin). The ordinate is given in actual (i.e., non-normalized) power (mm Hg^2). For computation of this spectrum 0.5 percent of the data was edited to remove artifacts. The red line is the best fit line whose inverse-slope characterizes the log-log linear, or fractal, portion of the spectrum (Brown, et al., 2006). The inverse-slope for this trial is 1.485. β for the DIAB at 1 month (1.298 ± 0.133 ; $n=3$) did not differ from their age-matched CONT (1.269 ± 0.145 ; $n=3$) at one month. At 6 months after conversion there was, again, no between group difference in β (DIAB: 1.456 ± 0.028 ; CONT: 1.396 ± 0.207), but the slope had increased (i.e., became more negative) across time ($F_{1,4} = 9.23$).

DISCUSSION

The BB rat has been widely used to study cardiovascular function in diabetes. Using this model of type 1 diabetes we report here that (1) a lag in weight gain occurs in the diabetes prone animals relative to their age-matched controls prior to the onset of the diabetic state, as defined in terms of a plasma glucose exceeding 250 mg/dl, and that this early difference in weight persists at least through the year following conversion. Conversely, after this initial event the two groups gained weight at essentially the same rate. (2) The twenty-four hour, beat-by-beat averaged mean arterial blood pressure did not differ between groups, and declined in parallel across the first ~4–5 months of the study in both the controls and diabetic subjects. (3) The diabetic animals' HRs were modestly, but significantly, lower than the controls' during the initial months, but, as with mBP, HR in both groups declined in parallel during the initial ~4–5 months and converged thereafter. (4) The 'harmonic' power within the range 0.35–0.45 (i.e., centered around 0.4 Hz), decreased significantly across the transition between dark to light, but there were no significant between group differences through 6 months of diabetes vs. control. (5) The slope (i.e., β) of the log-log linear portion of the mBP power spectrum, which characterizes the dynamics of the mBP signal within the frequency range of 1 cycle/hr to 1/min., did not differ between groups, but increased (i.e., became more negative) across the general time period during which mBP was declining.

Use of the DSI commercially available telemetry device is, by now, well-established. Tests by others (Van Vliet, et al., 2000) of the stability in the offset (i.e., with respect to atmospheric pressure) and sensitivity of the DSI unit (TA11PA-C40) revealed no systematic (i.e., tending either to greater or lower pressures) changes over time since manufacture exceeding 100 weeks with tight correlation with values reported by implanted catheter. We did observe fairly large decreases in HR and/or BP in the initial days and weeks after implantation (left most observations in Figure 4), which we attribute to continuing recuperation from the abdominal surgery required to implant the telemetry unit. We also observed changes in HR or BP in some animals with extended time after implantation to seemingly non-physiologic values which we attribute to a compromise in the position of the sensitive element of the telemetry unit (e.g., BP at extreme right for control subject, Figure 4, though pulsations were sufficiently large to calculate HR reliably) or a physiological deterioration of the animal (e.g., HR at extreme right for diabetic subject, Figure 4). We ceased using such data in computations prior to the onset of any such deterioration. Otherwise, we observed progressive downward trends in both HR and mBP (e.g., period from ca. Day 0 to Day 150, Figure 4) that we attribute to a physiologically real consequence of a given rat's maturation. That is, while we cannot reject the possibility of artifacts, we think it unlikely that non-physiological phenomena would result in such consistent changes in both variables across a sustained period in most animals in both groups. In short, we are comfortable in reporting (a) the rates of change in HR and mBP per day given in "Results;" (b) in noting any differences, or lack thereof, across groups; and, (c) in providing the 24-hr, beat-by-beat averages in Table 2.

The spectral findings are not subject to any shifts in the 'DC' value of the mBP across time. We now report for the first time that the power centered around 0.4 Hz changes significantly with the transition between dark to light and light to dark: this frequency-domain quantification of the dynamics of the mBP signal is itself dynamic. This is of interest within the present context primarily because the 0.4 Hz rhythm corresponds to the 'resonance frequency' of the baroreflex in rat, which is expressed in associated oscillation of SNA (Brown, et al., 1994; Burgess, et al., 1997, 1999; Cerutti, et al., 1994). In effect, the rhythm expresses itself so strongly in both mBP and SNA because the resonance is 'stationary' across time (i.e., stable over time). This mBP / SNA rhythm, by its very nature, therefore, is a prime example of 'harmonic power' (Brown, et al., 1994; Burgess, et al., 1997). The transition from light to dark is less abrupt than from dark to light, probably because the animals 'anticipate' the onset of the dark phase of the day when, as primarily nocturnal creatures, they are most active. It is noteworthy, therefore, that no between group differences, or interactions, were apparent through 6 months of diabetes duration. Interestingly, the 0.4 Hz rhythm conforms to the same self-similar behavior characteristic of lower frequencies after interruption of sympathetic outflow via spinal cord transection between T₄ and T₅ (Randall, et al., 2005). From this we concluded "that 1) an intact sympathetic nervous system endows that portion of the power spectrum centered around ~0.4 Hz with properties (e.g., a periodicity) that differ significantly from the self-similar behavior that characterizes the lower frequencies and 2) even within the relatively high frequency range at 0.4 Hz self-similarity is the 'default' condition after sympathetic influences have been eliminated" (Randall, et al., 2005). If so, it appears that sympathetic control of the circulation was functionally intact through 6 months of diabetes in this animal model, even during the behavioral changes attendant to transitioning from light to dark and *vice versa*. We do report elsewhere, however, that with extended diabetes (10 months) there are both similarities and differences in the mBP and HR responses of the two groups of animals to a sudden, short-duration behavioral stress (Randall, et al., 2011).

In marked contradistinction to the localized concentrations of harmonic power discussed immediately above, non-harmonic power appears throughout the HR and mBP spectra (e.g., Butler, et al., 1994). One characteristic of non-harmonic, 'self-similar' or 'fractal' power is the log-log linear increase in power as frequency decreases, which can be quantified in terms of the inverse slope, or β . A beta of zero results from a white noise process, whereas a beta of two corresponds to a random walk (see Brown, et al., 2006, Table 2 and Figure 5 for demonstration). Processes with a beta of one are often referred to as '1/f noise'. This results in a self-similarity relationship producing wavelet-like waveforms at various scales and amplitudes (Wornell, 1993). The presence of such scaling relationships indicates that the underlying phenomena occur over a wide range of time scales. That is, a 1/f relationship in power spectra exists in dynamic systems that have multiple control mechanisms with different time constants. A steeper slope suggests a less complex control system (Wang S, et al., 2012). If so, our present findings suggest that the complexity of the sympathetic regulation of BP diminished between months 1 and 6 in both groups, possibly reflecting a diminution of sympathetic regulation of the heart and vasculature as animals in both groups matured.

These same findings can be considered from another perspective. We previously reported a very broad frequency range power spectrum of mBP in freely-moving rat, quantified this non-harmonic, log-log fractal power in terms of β , and tested a mathematical model that offered an explanation of the etiology of several features of the spectrum in terms of control mechanisms (Brown, et al., 2006). The model posited that pressure changed from beat-to-beat at ~2 mm Hg/beat (i.e., a value chosen to closely match the empirically observed average value of 1.87 ± 0.01 mm Hg) governed exclusively by the dynamics of a random walk. Only one additional restraint was imposed upon mBP's random 'wandering': pressure

must remain within 85–115 mm Hg (i.e., as though a ‘baroreflex’ did not permit pressure to fall below 85 or rise above 115 mm Hg). Nonetheless, the model’s predicted value for β was influenced by this restriction, which, in turn, is a function of reflex regulation. In fact, the predictions were remarkably close to the actual mBP spectra from the rat across the very wide range of frequencies assessed. For example, the value the model predicted for β within the frequency range 1–6/hr (1.71) was notably close to the actual value within this frequency range (1.80 ± 0.16). What’s more, the model predicted a ‘shoulder’ (i.e., a region where mBP power was nearly unchanged across frequency, as is seen in Fig. 8 between 1 per day and 1 per hr). Again, the model’s predicted slope (0.21) for the shoulder (i.e., range 0.083 – 1 /hour) nearly matched the empirical value (0.32 ± 0.28). Note, again, that no autonomic control of mBP was required to match the predicted and empirical spectra beyond the action of a ‘baroreflex’. If so, just as we now report, one would predict that β would be similar for the control and diabetic rats, provided baroreflex function remained relatively intact across 6 months of the diabetic condition.

The present findings indicate that the combined effects of low plasma insulin (i.e., in type 1 diabetes) and high glucose, at least as sustained over a period up to 6 months in rats maintained on daily insulin injections, do not result in differences in mBP vs. diabetes resistant controls. In fact, arterial BP in type 1 diabetic children is not generally reported to exceed that of like-aged non-diabetic controls (e.g., Boysen, et al., 2007; Krause, et al., 2009; Pozza, et al., 2007). For example, BP in diabetic children aged 7 – 18 years (116/67 mm Hg; average duration of diabetes = 62 months; range = 8 – 156) did not differ in magnitude from that of control children (117/69 mm Hg) aged 5 – 17 years (Krause, et al., 2009). Such observations demonstrate that, during the early stages of the diabetic state, those mechanisms regulating cardiovascular function are capable of maintaining a normal arterial pressure. In this regard, in an earlier study (Fitzovich and Randall, 1990) we reduced endogenous insulin secretion to negligible levels (alloxan) in chronically instrumented dogs (arterial BP, left ventricular pressure (LVP)) and then maintained a euglycemic state by exogenous insulin infusion. Over the period of the next several weeks, we examined the effects of acute, controlled variations in plasma insulin and plasma glucose on the cardiovascular response to bilateral carotid occlusion (i.e., baroreflex activation) and dobutamine infusion. We found no significant differences in short-term baseline BP or HR measurements over five acute conditions: (1) normal insulin (NI) and normal glucose; (2) 1/5 NI with glucose allowed to vary; (3) NI and high glucose (~300 mg/dl); (4) 5 times NI with glucose held at normal levels; or (5) 5 times NI with high glucose. (The peak of the first time derivative of LVP, $d(LVP)/dt_{\max}$, in the basal condition was, however, significantly lower in the $5 \times$ NI with normal glucose than it was in all other states except NI and high glucose.) Moreover, there were no statistically significant differences across insulin and glucose states in the BP and HR responses to bilateral carotid occlusion or to dobutamine across the various experimental conditions.

Studies of diabetic children have described differences in spectral indices of sympathetic function (e.g., ‘low frequency’ HR spectral power or the ratio of low frequency to high frequency power) and parasympathetic function (high frequency power) as compared to control (Boysen, et al., 2007; Krause, et al., 2009; Pozza, et al., 2007); alterations in parasympathetic function may precede those in sympathetic function (Javorka, et al., 2005). We note, in fact, that while our own canine experiments at the functional level, as above, detect no gross effects of the consequences of acute alterations in plasma glucose and/or insulin *per se*, we have published a preliminary report of subtle effects upon baroreflex changes in sympathetic nerve activity in anesthetized diabetic rats when tested > 1 year after conversion (Burgess, et al., 2009). Moreover, other reports have linked alterations of cardiovascular and pulmonary function to autonomic dysfunction in type 1 diabetes in man

(e.g., Ewing, et al., 1985; Kahn, et al., 1987), and in other diseases (e.g., Anigbogu and Adigun, 1996; Morfis, et al., 1997).

Insight into arterial BP behavior is particularly important in diabetes since hypertension is a common co-morbidity in the disease. Clinical trials indicate that antihypertensive treatment of diabetic patients suppresses the incidence of untoward cardiovascular events (UK Prospective Diabetes Study Group, 1998). Type 1 patients reportedly typically develop this condition some years after the diagnosis of diabetes (Mancia, 2005). In most clinical cases diabetic hypertension is secondary to the onset of more advanced kidney disease, and long-term survivors of diabetes without nephropathy reportedly rarely have hypertension (Kaufman, 2008). The question arises, therefore, as to the precise role of the diabetic state and of plasma insulin and glucose levels on the etiology of hypertension in type 1 diabetes, both in the short term and longer term. Our spontaneously diabetic rats were not hypertensive vs. the controls over the initial ~6 months of the diabetic state. Perhaps the most analogous study with which to compare our findings for reliability in this regard is that of Bidani, et al. (Bidani, et al., 2007) who studied HR and BP in “partially insulin-treated streptozotocin (STZ)-diabetes” rats via radio-telemetry for ~38 weeks. Their Sprague-Dawley rats were 8 weeks of age at the start of the study, approximately the same age as our own; insulin was delivered via osmotic minipumps at a rate calculated to maintain blood glucose at 300–400 mg/dl, approximately the morning value for our animals. They report that “systolic BP was significantly lower in the STZ-diabetic rats at most time points during the course of the study” as compared to the control rats, though the pressures in the two groups appear to converge towards the end (i.e., by ~30 weeks). More specifically, 24-hr mean BP (DC BP power from spectral analysis) in their diabetic rats (92.9 ± 2.3 mm Hg (SEM)) was lower than the control’s average (100.6 ± 2.5 mm Hg). Irrespective of any differences in our studies, neither our spontaneously diabetic strain, nor this STZ-treated Sprague-Dawley strain was hypertensive, arguing that type 1 diabetes per se is not intimately associated with elevated BP within the first few months of the development of the condition. Conversely, HR in the STZ-diabetic rats was lower (312 ± 8.6 bpm), as in our study, than in their control animals (344 ± 7.4 bpm), at least indirectly suggesting that at the earlier stages of the development of diabetic pathology parasympathetic function is still sufficiently robust to afford a lower HR. Studies such as these continue to unravel the pathophysiological consequences of chronic diabetes mellitus.

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Figure 1.

(Top Panel). Plasma glucose measured in the morning for control (thin line) and diabetic rats (thick line). The numbers immediately above the abscissa scale give the number of diabetic animals / number of control animals at 50 day intervals, excepting the pre-conversion data are for 40 days before conversion. Starting on Day 0, which designates the onset of diabetes (blood glucose >250mg/dl), insulin was administered to diabetic rats after blood glucose measurement; glucose value given here, therefore, is at or near daily peak value. (Bottom panel). Body weight of diabetic and resistant rats. Measurements commenced one week after receipt from vendor. Note between-group divergence in weights prior to conversion which was sustained over ensuing ~year.

Figure 2.

Mean $\pm$ SD morning plasma glucose in BB diabetes prone rats ($n = 29$) over ten days immediately before they became diabetic (morning plasma glucose > 250 mg/dl). Gradual rise in plasma glucose culminated in statistically significant ($* < 0.05$) difference at two days and one day prior to conversion vs. that at ten days before conversion (i.e., Time = -10 days).

Figure 3.

Heart rate (HR) and mean arterial blood pressure (mBP) recordings for a control (top) and a diabetic (bottom) subject recorded over 16 day period. Insert shows 2 sec. (see scale, abscissa) of phasic arterial BP recording in a diabetic rat 90 days after it converted to diabetic state; signal showed clear systole and diastole. HR and mBP data reported in this study are from beat-by-beat averages over 24 hours or longer from recordings such as these.

Figure 4.

Heart rate (HR, open circle; scale at left) and mean arterial blood pressure (mBP, solid circle; scale at right) in individual diabetes resistant (Control, top panel) and age-matched diabetes prone (Diabetic, bottom panel) rats. The diabetes prone animal 'converted' to diabetic state at Day 0 (abscissa). Each data point is 24 hour, beat $\times$ beat average. The telemetry probe was implanted in the control and diabetic animals when they were 43 and 47 days of age, respectively. The first data point shown for the control animal was taken 11 days after probe implantation; the first for the diabetic animal was 4 days post-surgery. Lines in each graph represent least-squares average over the period represented by the length of the line; computations were limited to that portion of the data set during which recordings

were reasonably regular from day-to-day, as delineated by the length of the individual lines. Note gentle downward trend in both HR and mBP for each animal. In the control rat the best-fit linear slope over this range for HR was -0.15 bpm/day ($r^2 = 0.53$; 95% confidence limits = -0.190 to -0.119); the corresponding rate of decline in mBP was -0.05 mm Hg/day ($r^2 = 0.50$; 95% confidence limits = -0.065 to -0.040). Similarly, in the diabetic animal HR declined at -0.42 bpm/day ($r^2 = 0.83$; 95% confidence limits = -0.475 to -0.379) and mBP at -0.09 mm Hg/day ($r^2 = 0.79$; 95% confidence limits = -0.105 to -0.081).

Figure 5.

Monthly averages $\pm$ SD of mean arterial pressure (mBP, top panel) and heart rate (HR, bottom panel) for diabetic (solid line) and age-matched control (broken line) rats. "Pre" represents observations taken during month prior to conversion of diabetic animals and concurrent observations in control rats. Number of diabetic / number of control animals contributing to each monthly average is indicated immediately above abscissa. No meaningful differences between groups in mBP are obvious, though HR in the control rats appears to be higher; statistical tests on a subset of rats recorded for each of the six months (see Table 2 and text) confirm these observations. Gentle declines in both mBP and HR over

the initial months, with an apparent leveling during the latter months, are apparent for both control and diabetic animals.

Figure 6.

Mean $\pm$ SD heart rate (HR) for three control rats that were studied for 11–12 months ($n=2$ for month 12) and for 4 diabetic rats studied for 6 ($n=4$) or 9 months ($n=2$). Each data point is based upon 24 hour beat $\times$ beat averages for multiple days each month. Observation for month 7 was missing for one diabetic rat. Declines in HR observed in Figure 5 are apparent during initial months, as is higher HR in control animals, but both curves flatten and converge by end of observation period. * = $p < 0.05$ vs. month 1 for control and diabetic; # = $p < 0.05$ vs. month 2 for control.

Figure 7.

Panel A. Mean arterial blood pressure power spectra centered upon 0.4 Hz (i.e., red tone, extending from 0.35 to 0.45 Hz) for a single 24 hour day for a single control rat during Month 1. Successive, juxtapositioned spectra, each computed from 248 second long intervals, were chained to yield this 24 hour plot. The earliest spectrum starts at midnight ('rear-most' portion of plot in back panel); individual spectra extend progressively forward to noon (N; bordering on blue area in middle). The front panel extends from noon to last panel at (next) midnight (M). Dark period is shown in gray shading, while lights-on period is given in green. In this animal spectral power decreased noticeably as soon as the lights turned on at 6 am, though there were individual spectra showing transient increases in 0.4 Hz power. Spectral power within the 0.35–0.45 Hz band appeared to increase in 'anticipation' of lights-on, and was clearly increased during the first 90 minutes of dark.

Panel B. Left half: Average spectral power from 0.35 to 0.45 Hz in a rat over last 90 minutes

of dark (gray shading, left-most panel) immediately prior to lights-on (i.e., at '6 am'), and for first 90 minutes after lights were turned on (left-most non-shaded panel). Right half: Average spectral power during final 90 minutes of lights-on (non-shaded panel) immediately prior to lights-off (i.e., at '6 pm'), and during first 90 minutes of dark (right-most shaded panel). The individual spectra were computed from a 51 second long interval such that the power shown in the plot 'marches' from left towards right at successive 51 sec. intervals. Numerical values in each panel are average power over the respective 90 minute intervals. Spectral power centered on 0.4 Hz was dynamic: higher during periods of dark than periods of light (see Table 3). Power appears to increase prior to lights-out, perhaps as rats 'anticipate' the coming 'night'.

Figure 8.

Broad frequency range mBP power spectrum for control subject computed from 5 days of continuous recording during month 1. Logarithm of power (absolute units) is on ordinate and logarithm of frequency is on abscissa. Red line is best fit over range for which beta is computed β (i.e., ~1.0 cycles/hr and 1 cycle/min). Beta was similar in control and diabetic rats, but increased from month 1 to month 6 of diabetes duration.

Table 1Weight (grams) $\pm$ SD at designated times pre- and post-conversion for diabetic and age-matched control rats

Days from Conversion	Diabetic	Control
-40	196 $\pm$ 46	198 $\pm$ 43
0	283 $\pm$ 31 *	337 $\pm$ 37
50	356 $\pm$ 23 *	412 $\pm$ 23
100	387 $\pm$ 35 *	454 $\pm$ 25
150	415 $\pm$ 34 *	478 $\pm$ 25
200	440 $\pm$ 31 *	500 $\pm$ 28
250	467 $\pm$ 36 *	520 $\pm$ 31
300	483 $\pm$ 42 *	520 $\pm$ 40
350	482 $\pm$ 42 *	538 $\pm$ 31

*
p < 0.05, diabetic vs. control

Table 2

Monthly Averages $\pm$ SD Mean Arterial Blood Pressure and Heart Rate for Control (n=7) and Diabetic (n=5) Rats

month	<u>Control</u>		<u>Diabetic</u>	
	mBP (mm Hg)	HR (bpm)	mBP (mm Hg)	HR (bpm)
1	98.2 $\pm$ 3.0	341 $\pm$ 13	99.4 $\pm$ 5.4	325 $\pm$ 25
2	96.8 $\pm$ 3.1	334 $\pm$ 18	97.0 $\pm$ 11.5	305 $\pm$ 13
3	93.5 $\pm$ 4.6	319 $\pm$ 20 *	95.2 $\pm$ 5.8	306 $\pm$ 15 [†]
4	91.7 $\pm$ 4.4	318 $\pm$ 18 *	92.2 $\pm$ 4.9	293 $\pm$ 16 *
5	93.1 $\pm$ 7.1	307 $\pm$ 19 [‡]	92.9 $\pm$ 8.6	287 $\pm$ 26 *
6	93.5 $\pm$ 8.9	310 $\pm$ 17 [‡]	90.3 $\pm$ 7.1 *	285 $\pm$ 23 *

The value for month 3 for one diabetic rat was the average of that rat's value for months 2 and 4; for another diabetic animal the value for month 5 was the average of months 4 and 6.

* significant vs. 1 month;

[†] significant vs month 6;

[‡] significant vs. month 2

Table 3

Average $\pm$ SD mBP Power centered on 0.4 Hz over 90 minutes immediately before transition from dark to light and light to dark in control and diabetic rats at one month and 6 months

<u>Control</u>				<u>MO6</u>			
<u>MO1</u>		<u>light</u>		<u>dark</u>		<u>light</u>	
dark	light	light	dark	dark	light	light	dark
0.34 $\pm$ 0.06 *	0.15 $\pm$ 0.02	0.21 $\pm$ 0.01 *	0.38 $\pm$ 0.08	0.34 $\pm$ 0.13	0.16 $\pm$ 0.08	0.29 $\pm$ 0.14	0.47 $\pm$ 0.26
<u>Diabetic</u>							
0.28 $\pm$ 0.08	0.14 $\pm$ 0.04	0.21 $\pm$ 0.01	0.30 $\pm$ 0.10	0.18 $\pm$ 0.10	0.14 $\pm$ 0.06	0.18 $\pm$ 0.06 *	0.30 $\pm$ 0.11

* P < 0.05, transition from dark to light, or light to dark