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Spontaneous baroreflex sensitivity is attenuated in male UCD-type 2 diabetes mellitus rats: a link between metabolic and autonomic dysfunction

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Abstract

Patients with type 2 diabetes mellitus (T2DM) have impaired arterial baroreflex function, which may be linked to the co-existence of obesity. However, the role of obesity and its related metabolic impairments on baroreflex dysfunction in T2DM is unknown. This study aimed to investigate the role of visceral fat and adiponectin, the most abundant cytokine produced by adipocytes, on baroreflex dysfunction in T2DM rats. Experiments were performed in adult male UCD-T2DM rats assigned to the following experimental groups (n=6 in each): prediabetic (Pre), diabetes-onset (T0), 4 weeks after onset (T4), and 12 weeks after onset (T12). Age-matched healthy Sprague-Dawley rats were used as controls. Rats were anesthetized and blood pressure was directly measured on a beat-to-beat basis to assess spontaneous baroreflex sensitivity (BRS) using the sequence technique. Dual-energy X-ray absorptiometry (DEXA) was used to assess body composition. Data are presented as mean ± SD. BRS was significantly lower in T2DM rats compared with controls at T0 (T2D: 3.7 ± 3.2 ms/mmHg vs Healthy: 16.1 ± 8.4 ms/mmHg; P=0.01), but not at T12 (T2D: 13.4 ± 8.1 ms/mmHg vs Healthy: 9.2 ± 6.0 ms/mmHg; P=0.16). T2DM rats had higher visceral fat mass, adiponectin, and insulin concentrations compared with control rats (all P<0.01). Changes in adiponectin and insulin concentrations over the measured time-points mirrored one another and were opposite those of the BRS in T2DM rats. These findings demonstrate that obesity-related metabolic impairments may contribute to an attenuated spontaneous BRS in T2DM, suggesting a link between metabolic and autonomic dysfunction.

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Author contributions

M.S. and M.L.H. conception and design of research; M.S., Y.H., S.C. performed experiments; R.K.M. performed and analyzed ELISA; M.S. and M.L.H. analyzed data; M.S. and M.L.H. interpreted results of experiments; M.S. prepared figures; M.S. drafted manuscript; M.S. and M.L.H. edited manuscript. All authors contributed to revising the manuscript and approved the final version. All persons designated as authors qualify for authorship, and all those who qualify for authorship are listed.

Disclosure of conflicts of interest

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Keywords

UCD-T2DM; obesity; insulin; adiponectin; baroreflex function

1. Introduction

Cardiac autonomic neuropathy (CAN), a common and early complication of type 2 diabetes mellitus (T2DM), is associated with significant adverse cardiovascular events. As an independent risk factor for silent myocardial ischemia and cardiovascular disease, CAN represents a main cause of morbidity and mortality in T2DM patients (Pop-Busui et al., 2010). Briefly, this type of diabetic neuropathy is characterized by autonomic dysfunction, which results from damage to the autonomic nerve fibers innervating the heart and blood vessels (Pop-Busui et al., 2017; Rowaiye et al., 2013). In addition to cardiovascular abnormalities such as tachycardia, exercise intolerance, and orthostatic hypotension, CAN is also associated with impaired baroreflex function in T2DM (Frattola et al., 1997; Wang et al., 2012).

The arterial baroreflex is an essential neural mechanism responsible for maintaining blood pressure (BP) homeostasis through autonomic adjustments to the cardiovascular system (Sheehan et al., 1941). It is well established that arterial baroreflex function is impaired in several diseases (Colombo et al., 1999; Farrell et al., 1992; Judy and Farrell, 1979; Man et al., 2021; Pinna et al., 2005; Sabino-Carvalho et al., 2020, p.; Souza et al., 2008), including T2DM (Bakkar et al., 2020; Chang and Lund, 1986; Cseh et al., 2020; Dall'Ago et al., 1997; de Moura-Tonello et al., 2016; Holwerda et al., 2016; Kück et al., 2020; Maeda et al., 1995; Ruiz et al., 2005). The mechanism underlying the autonomic impairment that manifests as baroreflex dysfunction in T2DM remains poorly understood. Previous studies have suggested that attenuated baroreflex sensitivity (BRS) in T2DM is associated with both hyperglycemia (Kück et al., 2020; Matsutani et al., 2018; Wu et al., 2014) and insulin resistance (Al-Assi et al., 2018; Anan et al., 2005; Kück et al., 2020), as well as the co-existence of obesity (Holwerda et al., 2016). Indeed, obesity can markedly decrease BRS in nondiabetic individuals (Indumathy et al., 2015) and it has been suggested that the specific location of fat accumulation (i.e., visceral fat) can be even more detrimental to BRS (Del Colle et al., 2007; Li et al., 2020). However, to what extent the metabolic impairments associated with obesity in T2DM patients affect baroreflex function needs to be further elucidated.

As an endocrine organ, adipose tissue is responsible for the synthesis and secretion of a variety of metabolites, hormones, and cytokines (Coelho et al., 2013). Adiponectin is the most abundant adipocytokine secreted by adipose tissue and has been shown to have anti-inflammatory and protective effects on the cardiovascular system (Hui et al., 2012; Swarbrick and Havel, 2008). Previous studies have shown that individuals with T2DM often present with a lower level of adiponectin and this is inversely correlated with insulin and glucose concentrations (Cnop et al., 2003; Hotta et al., 2000; Takahashi et al., 2007; Weyer et al., 2001). Hypoadiponectinemia has also been shown to be associated with sympathetic activation, resulting in a shift in the sympathovagal balance in T2DM patients (Hansen et al.,

2017; Takahashi et al., 2007; Wakabayashi and Aso, 2004). However, whether adiponectin contributes to the autonomic dysfunction reflected by an attenuated baroreflex in the context of T2DM is unknown. Therefore, the purpose of this study was to investigate the role of obesity and adiponectin on baroreflex dysfunction in a validated rat model of T2DM. We hypothesized that BRS would be attenuated in T2DM rats compared with healthy age-matched controls due to increased visceral fat, which is well known to be associated with lower circulating concentrations of adiponectin.

2. Methods

All procedures were reviewed and approved by the Institutional Animal Care and Use Committee of The University of Texas at Austin (Protocol AUP-2022-0049) and were conducted following the National Institutes of Health Guide for the Care and Use of Laboratory Animals (2011). Young (<6 months of age) adult, male University of California Davis (UCD)-T2DM rats (n=24) were provided from the breeding colony of UCD-T2DM rats in the Department of Nutrition at UCD. The UCD-T2DM rat model presents with polygenic adult-onset obesity and insulin resistance with inadequate β-cell compensation, which leads to the development of hyperglycemia and diabetes over time (Cummings et al., 2008). As a result, this rat model exhibits a T2DM etiology more similar to the development of T2DM in humans when compared with other rodent models (Kleinert et al., 2018). Since female UCD-T2DM rats develop diabetes much later than male rats (Cummings et al., 2008), we were only able to use young T2DM males in the current study. Experiments were also performed on age-matched healthy Sprague-Dawley rats (n=24; Charles River Laboratories, Wilmington, MA, USA) as controls. Rats were housed two per cage in a temperature (24 ± 1°C) and light-dark cycle (12:12h) controlled room. Rats were fed a standard diet and tap water *ad libitum*.

2.1. Grouping of animals

Each UCD-T2DM rat was fasted for 12 hours before weekly measures of glucose (Stat Strip Xpress®, Nova Biomedical, Waltham, MA, USA) and hemoglobin A1c (HbA1c, A1CNow®+, PTS Diagnostics, Indianapolis, IN, USA), which were obtained by pricking the tail. These measurements started at 8 weeks of age and continued until diabetes onset. The criteria used for T2DM diagnosis was HbA1c ≥5.6%. We found fasting HbA1c to be a more stable indicator for determining diabetes onset in the UCD-T2DM rat model than fasted or non-fasted glucose measurements (Supplemental data available in DOI: 10.6084/m9.figshare.22307449). T2DM rats were then assigned to one of four experimental groups according to diabetes onset (n=6 in each group): prediabetic (Pre), diabetes-onset (T0), 4 weeks after onset (T4), and12 weeks after onset (T12). Four groups of healthy, age-matched Sprague-Dawley rats served as controls (n=6 in each group). The experimental protocols included in this study are terminal experiments, which precluded the use of the same groups of rats across different time points. Thus, different rats from the same colony were assigned to the groups according to the diabetes onset.

2.2. Dual-energy X-ray absorptiometry

On the day of the experiments, rats were fasted overnight and blood glucose and HbA1c were assessed prior to experimental protocols. Total and regional body composition were measured using dual-energy x-ray absorptiometry (DEXA, iDXA/Lunar Prodigy; GE Medical Systems, Madison, WI, USA) with software specific for rats (enCORE, version 15.0; GE Medical Systems). This non-invasive technique has been found to be reliable and accurate for assessments of rat body composition (Bertin et al., 1998; Stevenson and van Tets, 2008). A specially designed small animal spine phantom made of calcium hydroxyapatite embedded in a lucite block was scanned daily to calibrate the instrument and adjust the scanning field accordingly. Prior to each scan, rats were anesthetized by intraperitoneal injection of a combination of dexmedetomidine hydrochloride (0.25 mg/kg, Pivetal®, Liberty, MO, USA) and ketamine hydrochloride (50 mg/kg, Dechra Veterinary Products, Overland Park, KS, USA). Once the rat was fully anesthetized, it was placed in a prone position on the platform and a whole-body scan was performed which lasted for ~180 s. All rats were placed on the same area of the instrument platform and in the same geometry (i.e., straight and flat on their ventral side, limbs spread, and tail curled on the side). The body was scanned starting from the nose and extending to the end of the tail. Scans were performed in triplicate in a sub-group of rats [T2DM (n=3) and age-matched healthy (n=3)] to assess the reliability of the DEXA measurements in assessing the body composition of the rats. Three consecutive scans were performed as described above but the animals were removed from the platform and repositioned each time between scans. After the DEXA scan, rats were injected with atipamezole hydrochloride (5 mg/mL, i.p.; Antisedan®, Orion Pharma, Kalamazoo, MI, USA), and vital signs were closely monitored until they fully recovered.

2.3. Hemodynamic evaluation

The animals were anesthetized with isoflurane gas (2-3%) in 100% oxygen and the right common carotid artery was cannulated (polyethylene-50). The catheter was connected to a pressure transducer (Deltran®, Utah Medical Products, Inc., Midvale, UT, USA) for recording pulsatile BP waveforms using Spike2 software (CED, v.8.24, Cambridge, UK). Rats were placed in a prone position and resting BP waveforms were recorded for 5 min. After baseline measurements, blood was drawn from the arterial catheter and rats were euthanized with an overdose of isoflurane followed by an intravenous injection of saturated potassium chloride (>200 mg/kg).

2.4. Body composition

Offline analysis was conducted for global and regional body composition using the same software as above (enCORE, version 15.0; GE Medical Systems). Whole-body measurements were automatically provided and included body weight, fat mass, and lean mass. To assess visceral fat, a region of interest (ROI) from the whole-body scan was defined by enlarging the total spine and extending a rectangular box vertically from L3/L2 vertebral space to L5/L6 vertebral space and laterally to the edge of the abdominal soft tissue. This visceral fat region has been shown to have the strongest correlation ($r=0.94$, $p<0.001$) with *ex-vivo* fat mass, assessed by weighting the total perirenal and

peri-epididymal adipose tissue (Gerbaix et al., 2010). All analyses were performed by the same researcher (M.S.) to avoid any bias linked to inter-operator error.

2.5. Spontaneous baroreflex sensitivity and heart rate variability

Spontaneous BRS was assessed in a beat-to-beat time series using the sequence technique (CardioSeries, v2.4, Brazil) (Bertinieri et al., 1985; Parati et al., 2000; Samora et al., 2018; Teixeira et al., 2018a, 2018b). Briefly, this approach consisted of the identification of 3 or more consecutive cardiac cycles (no delay) in which progressive increases in systolic BP are followed by RR interval lengthening ($Ramps_{up}$), or decreases in systolic BP are followed by RR interval shortening ($Ramps_{down}$). A linear regression was applied to each sequence, and only those with a correlation coefficient >0.80 were accepted. The BRS was determined for all combined sequences ($Gain_{all}$) as well as separately for both upward (increases in systolic BP, $Gain_{up}$) and downward (decreases in systolic BP, $Gain_{down}$) sequences.

Heart rate variability (HRV) in linear methods in the time domain was represented by the root mean square of successive differences (RMSSD) in RR interval, which is a measure of parasympathetic modulation of sinus node function. In addition, power spectral analysis of HRV was performed in frequency domain, where three main spectral components are distinguished in very low frequency (VLF: <0.04 Hz), low frequency (LF: 0.04-0.15 Hz) and high frequency (HF: 0.15-0.4 Hz). Normalized units (nu) of LF and HF represent the relative value of each spectral band in proportion to the total component minus the VLF. The LF/HF ratio was calculated to estimate the sympathovagal balance of the heart (Task, 1996).

2.6. Blood sampling and measurements

Arterial blood was drawn from the carotid artery into a serum separator vacutainer tube. Blood was allowed to clot at room temperature for 30 min, centrifuged for 10 min at 1400 xg, and then the serum was aliquoted into tubes and frozen at −80°C for subsequent analyses. An ultrasensitive rat insulin ELISA kit (ALPCO®, 80-INSRTU-E01, Salem, NH, USA) was used to determine insulin concentration in neat samples and a rat total adiponectin immunoassay ELISA kit (Quantikine®, RRP 300, R&D Systems, Inc., Minneapolis, MN, USA) was used to determine adiponectin concentration in samples diluted 1:1000. Standards, controls, and samples were added, in duplicate, to 96-well plates according to instructions provided by the manufacturers and absorbance was measured (Infinite® F200 PRO, TECAN, Männdorf, CH). For both analytes, four-parameter-Marquardt logistic regression was used to construct standard curves, which were then used to calculate unknown concentrations. The coefficient of determination (R^2) for both standard curves was 0.999, the coefficient of variation (CV) between sample duplicates was <6.2% for insulin and <2.7% for adiponectin, and controls were within the range specified by the manufacturers.

2.7. Data analysis

The pulsatile BP waveform was continuously recorded using a Spike2 data acquisition system with a sampling rate of 100 Hz. The signal was exported from Spike2 and a linear interpolation was applied for an output sampling rate of 1000 Hz. The continuous arterial pressure recordings were filtered by a digital low-pass filter with a cutoff frequency

of 20 Hz to reduce signal noise and provide a smoother trace (LabChart Pro, v.8.1,13, ADInstruments, Bella Vista, NSW, Australia). All hemodynamic variables were obtained on a beat-to-beat basis and the baseline was determined as the average of 5 min of data recording. Both relative fat mass (%) and relative lean mass (%) were calculated as the ratio between corresponding absolute values (in g) and body weight (in g). Relative visceral fat mass (%) was calculated by dividing the fat mass (g) by the total tissue mass (g) within the ROI. Statistical comparisons were made using two-way analysis of variance (ANOVA), in which group and time were the main factors. When indicated, Sidak post hoc analysis was performed for multiple comparisons. Reproducibility of the 3 DEXA scans was assessed by calculating both CV and intraclass correlation coefficient (ICC) with the 95% confidence interval based on a mean-rating (k=3), absolute-agreement, and 2-way mixed-effects (Hopkins, 2000; Koo and Li, 2016). Data are presented as mean ± SD, and the criteria for the level of significance was set at $P<0.05$. Statistical analyses were performed, and figures were created using Prism® (GraphPad, v.6.01, San Diego, CA, USA).

3. Results

The descriptive characteristics of T2DM and age-matched healthy rats are presented in Table 1. Rats studied were from 12 to 22 weeks of age and, by design, it was evenly matched between T2DM and healthy rats within each group. As expected, HbA1c was elevated in T2DM compared with age-matched healthy rats. T2DM rats have similar fasting blood glucose until T12 suggesting that worsening insulin resistance is leading to progressive hyperglycemia.

Baseline hemodynamic variables are presented in Table 2. T2DM rats present a higher systolic BP at T4 and lower diastolic BP at T12 compared to healthy rats. For the indices of spontaneous BRS, $Gain_{up}$ is significantly lower in T2DM rats at the onset of the disease compared to age-matched healthy rats. In addition, heart rate is lower, and RR interval is higher in T2DM rats at T12 compared to the pre-diabetic group. This finding aligns with the increased RMSSD and HF (nu) with a significant decrease in LF (nu) at T12 compared with T0.

Spontaneous BRS was attenuated in T2DM rats throughout all time points of the disease, except during T12 (Healthy: 9.2 ± 2.1 ms/mmHg; 16.1 ± 8.4 ms/mmHg; 11.2 ± 8.9 ms/mmHg; 9.2 ± 6.0 ms/mmHg and T2DM: 5.2 ± 2.6 ms/mmHg; 3.7 ± 3.1 ms/mmHg; 6.0 ± 4.4 ms/mmHg; 13.4 ± 8.1 ms/mmHg; Fig. 1). Specifically, BRS was significantly lower in T2DM when compared to age-matched healthy rats at T0 ($P=0.01$). However, T2DM rats had a significantly higher BRS at T12, thereby abolishing the differences between T2DM and healthy rats ($P=0.16$). It is noteworthy to mention that the number of baroreflex sequences were not different between T2DM and healthy rats (data not shown).

DEXA scans were done in triplicate in a subset of rats (n=6) to assess reliability. The primary measures, body weight (g), fat (g), and lean mass (g), showed low CVs between consecutive scans (CV: 0.2%, 3.6%, and 1.0%, respectively) and ICC was 1.00 [1.00-1.00], $P<0.001$ for all measurements, which indicates excellent reliability (Hopkins, 2000; Koo and Li, 2016). Body composition in T2DM and age-matched healthy rats are shown in Figure

2. Although healthy rats increased body weight with age as expected, they remained smaller than T2DM rats (Healthy: 355 ± 13 g; 377 ± 29 g; 411 ± 27 g; 442 ± 26 g and T2DM: 485 ± 14 g; 562 ± 58 g; 636 ± 59 g; 589 ± 42 g; All $P<0.05$; Fig. 2A). Of note, T2DM rats attained their highest body weight at T4 and then started to lose weight thereafter, which is likely due to loss of calories through spillover of glucose in the urine as glycosuria becomes more pronounced (Cummings et al., 2008).

Whole-body fat mass did not change in age-matched healthy rats, and, as expected, T2DM rats had significantly greater fat mass compared with controls (Healthy: 63 ± 6g; 74 ± 15g; 85 ± 20g; 102 ± 7g and T2DM: 136 ± 14g; 175 ± 24g; 207 ± 44g; 163 ± 45g; All $P<0.05$; Fig. 2B). Normalizing both fat and lean mass to body weight revealed a main disease effect (all $P<0.01$), suggesting that the increased body weight in T2DM rats is being driven primarily by increased fat mass (Fig. 2C) since lean mass is lower compared with age-matched healthy rats (Fig. 2E).

Figure 3 shows representative DEXA scan images for healthy (Fig. 3A) and T2DM rats (Figs. 3B-E). Although no interaction was found for relative visceral fat ($P=0.09$), there was a disease effect ($P<0.01$) in which T2DM rats presented with higher visceral fat when compared to age-matched healthy rats (Fig. 3F).

Serum adiponectin and insulin concentrations are shown in Figure 4. No significant interaction between group and time was found for adiponectin concentration ($P=0.06$; Fig. 4A), but there was a disease effect ($P<0.01$). Contrary to our hypothesis, T2DM rats have higher adiponectin levels compared to healthy rats, presenting the peak at T0 and a slight decrease at T12 (Healthy: 4.5 ± 1.1 μg/mL; 4.7 ± 1.7 μg/mL; 5.6 ± 1.6 μg/mL; 4.2 ± 0.5 μg/mL and T2DM: 12.5 ± 2.2 μg/mL; 13.1 ± 4.6 μg/mL; 12.0 ± 2.8 μg/mL; 7.6 ± 2.3 μg/mL). Although no significant interaction was found for insulin ($P=0.24$; Fig. 4B), there was a tendency towards hyperinsulinemia in T2DM rats ($P=0.06$), which also appears to peak at T0 and decrease at T12 (Healthy: 0.3 ± 0.1 ng/mL; 0.4 ± 0.3 ng/mL; 0.4 ± 0.4 ng/mL; 0.5 ± 0.2 ng/mL and T2DM: 0.4 ± 0.3 ng/mL; 0.9 ± 0.6 ng/mL; 0.7 ± 0.3 ng/mL; 0.5 ± 0.1 ng/mL).

4. Discussion

The aim of this study was to investigate the role of obesity and adiponectin, the most abundant cytokine produced by adipocytes, on baroreflex dysfunction in T2DM rats. Interestingly, we found a temporal change in spontaneous BRS early in the disease in T2DM rats. Consistent with our hypothesis, T2DM rats exhibited greater adiposity, including visceral fat, relative to controls, even in the prediabetic state. Contrary to our hypothesis, T2DM rats presented with higher adiponectin concentrations relative to controls, which were mostly present at diabetes onset. Interestingly, changes in adiponectin and insulin concentrations across the measured time points mirrored each other and were opposite those of the spontaneous BRS in T2DM rats. Collectively, these findings demonstrate that obesity-related metabolic impairments may contribute to baroreflex dysfunction in T2DM rats, thereby suggesting a link between metabolic and autonomic dysfunction in diabetes.

The attenuated spontaneous BRS at the onset of the disease in T2DM rats in the present study is supported by previous findings (Cseh et al., 2020; de Moura-Tonello et al., 2016; Holwerda et al., 2016; Kück et al., 2020; Ruiz et al., 2005). Although the underlying mechanisms for an attenuated BRS in T2DM are not fully understood, it is likely that dynamic obesity-related metabolic changes over the course of the disease play a significant role in this baroreflex dysfunction (Al-Assi et al., 2018; Anan et al., 2005; Holwerda et al., 2016; Kück et al., 2020; Matsutani et al., 2018; Wu et al., 2014). We found that T2DM rats had a higher relative fat mass and lower lean mass compared with healthy rats, suggesting an unfavorable body composition profile with regards to metabolic health. This is further supported by the greater visceral fat in the T2DM rats, given that central obesity has an additional negative effect on baroreflex function (Del Colle et al., 2007; Li et al., 2020). Previous studies have shown that reduced arterial baroreflex in obese Zucker rats is caused by an impairment in both the sympathetic (Grassi et al., 2000; Huber and Schreihofer, 2010; Schreihofer et al., 2007) and parasympathetic (Barringer and Buñag, 1989; Buñag and Barringer, 1988) nervous systems. In contrast, Holwerda et al. (2016) demonstrated preserved baroreflex control of sympathetic nerve activity with a selective impairment in baroreflex control of heart rate in both T2DM patients and weight-matched control subjects when compared to lean subjects (Holwerda et al., 2016). Care should be taken comparing results from these previous studies where a different technique (i.e., modified Oxford) was used to evaluate baroreflex function in T2DM. However, spontaneous BRS predominantly reflects changes in vagal tone, and there is a tendency for lower indexes of cardiac parasympathetic nerve activity (i.e., RMSSD and HF) in T2DM rats compared with healthy controls at the onset of the disease. Surprisingly, we found that spontaneous BRS was higher in T2DM rats 12 weeks after the onset of the disease, thereby abolishing the differences between T2DM and healthy rats. Given that T2DM rats still have greater visceral fat mass compared with healthy controls at T12, obesity does not completely explain this temporal change in spontaneous BRS early in the disease. Thus, we speculate that other obesity-associated metabolic changes occurring over time in T2DM rats may also be affecting this dynamic baroreflex dysfunction.

Adiponectin, an important adipocytokine released by adipose tissue, has been shown to have significant protective cardiovascular effects (Havel, 2004; Hui et al., 2012). Accordingly, previous studies have reported lower adiponectin levels in obese (Arita et al., 1999) and T2DM patients (Cnop et al., 2003; Hansen et al., 2017; Takahashi et al., 2007; Wakabayashi and Aso, 2004). However, and contrary to our hypothesis, we found that T2DM rats had a higher serum adiponectin concentration relative to control rats. The reason for this discrepancy is unknown; however, it is possible that we are capturing a very early and transient increase that precedes the hypoadiponectinemia reported in other studies. Corroborating our findings, Hotta et al. (2001) also found similar longitudinal changes in plasma adiponectin levels in T2DM rhesus monkeys, which develop diabetes spontaneously over time similar to the T2DM rats used in the current study (Hotta et al., 2001). An alternative explanation for the hyperadiponectinemia found in T2DM rats compared with controls is the secretory pattern of adiponectin in regard to mean adipocyte size (Bahceci et al., 2007; Swarbrick and Havel, 2008). It is possible that T2DM rats have more small fat cells (i.e., adipocyte hyperplasia) rather than larger fat cells (i.e., adipocyte hypertrophy), the

latter of which are known to secrete less adiponectin per surface area (Skurk et al., 2007). It is important to note that this is only speculation since assessment of adipocyte size was beyond the scope of this study. Yet, the exact mechanism by which hyperadiponectinemia may be affecting baroreflex function early in the disease in T2DM rats is not clear. Adiponectin receptors are expressed in cardiac tissue (Lord et al., 2005), and a previous study has shown that T2DM rats have a decrease in cardiac adiponectin expression that is associated with cardiac dysfunction and is linked to oxidative stress, inflammation, and reactive hypertrophy (Gupta et al., 2020). The authors suggest that these biochemical alterations in the heart might be the precipitating factor for the reduction in BRS (Gupta et al., 2020). Therefore, we speculate that the attenuated BRS in T2DM rats early in the disease could be associated with a downregulation of adiponectin receptor expression in cardiac tissue. However, since adiponectin protein receptor expression and adipocyte size were not quantified in the current study, further investigation is needed.

Changes in serum insulin concentration in T2DM rats followed a similar pattern as adiponectin. We observed a tendency for hyperinsulinemia in T2DM rats at the onset of the disease, which likely represents pancreatic β-cell compensation in response to insulin resistance in an attempt to maintain glucose homeostasis (Cummings et al., 2008). This is consistent with our findings showing a sustained fasting glucose early in the disease but not later. It is possible that this hyperinsulinemia at the onset of the disease might also contribute to the attenuated BRS in T2DM rats. A previous study has shown that insulin-resistant rats without frank diabetes (i.e., hyperinsulinemia with normal glucose levels) have an impaired baroreflex response to increases in BP, whereas the reflex response to decreases in BP remain intact (Miller et al., 1999). These results are in accordance with our findings, where $Gain_{up}$, but not $Gain_{down,}$ was attenuated in T2DM rats at the onset of the disease. Miller et al. (1999) reported that the administration of atropine abolished the differences in baroreflex function between insulin-resistant and control groups, suggesting an insulin-induced reduction in the vagal component of the reflex. Indeed, there is a tendency for lower indexes of cardiac parasympathetic nerve activity (i.e., RMSSD and HF) in T2DM rats compared with healthy controls at the onset of the disease. Thus, we speculate that dynamic changes in insulin over time might partially contribute to attenuating the spontaneous BRS in T2DM rats. With the progression of the disease, however, worsening insulin resistance leads to hyperglycemia with a resultant decrease in insulin production due to a β-cell decompensation (Cummings et al., 2008). A reduction in insulin over time may have resulted in a loss of the inhibitory effect of hyperinsulinemia on parasympathetic control of the heart. This cardiac-vagal reactivation is aligned with the resting bradycardia, higher RMSSD and HF, and restoration of the spontaneous SBR in T2DM rats at T12. However, future studies are needed to investigate the precise role of insulin in baroreflex dysfunction in T2DM.

It is worthwhile to mention that the higher BRS in T2DM at T12, driven primarily by increases in vagal tone, does not rule out the possibility of even worsening baroreflex control. Notably, there are only a few studies that have evaluated baroreflex function during the progression of the disease in T2DM patients and results have been contradictory. For example, early baroreflex dysfunction in individuals with well-controlled recent-onset T2DM diagnosis (~186 days) was found to not progress over 5 years of follow-up in

a prospective cohort (Kück et al., 2020). In contrast, worsening BRS over 2 years was reported in individuals with ~9 years since T2DM diagnosis (Matsutani et al., 2018). It is important to note that the true onset and duration of the disease are rarely, if ever, known in T2DM patients, explaining, at least in part, this discrepancy. Since diabetes onset was accurately diagnosed in the rats used in this study, we propose that temporal changes in BRS are occurring early in the disease and further investigation into underlying mechanisms is warranted. Furthermore, hypoadiponectinemia has been shown to be associated with sympathetic activation (Hansen et al., 2017; Takahashi et al., 2007; Wakabayashi and Aso, 2004), and sympathetic activation has an inhibitory effect on adiponectin production (Delporte et al., 2002). Thus, it is reasonable to suggest a “vicious cycle” between adiponectin production and sympathetic activation. In addition, insulin is also known to activate the sympathetic nervous system centrally (Muntzel et al., 1995; Pricher et al., 2008; Rahmouni et al., 2004), thus it is possible that changes in insulin concentration over time might be affecting the sympathetic outflow to the heart as the disease progresses. Importantly, during this period when both adiponectin and insulin concentrations are changing, it is possible that the return of spontaneous BRS levels toward those of control rats might be a compensatory increase in parasympathetic activity to the heart to counteract increases in sympathetic activity. However, as with other compensatory mechanisms, this increase in parasympathetic activity to the heart would not be sustainable and sympathetic activity would eventually overwhelm the increase in vagal activity. Thus, it is reasonable to speculate that spontaneous BRS would become attenuated again as the disease progresses. Importantly, to what extent the sympathovagal balance drives baroreflex dysfunction in T2DM rats remains to be determined. In addition, given this temporal change in spontaneous BRS in T2DM rats, it is suggested that future studies dive deeper into the effects of T2DM progression on baroreflex function in humans using mechanistic studies while controlling for, or at the very least noting, varying time points of the disease.

4.1. Limitations

Although the current study found significant differences in body composition and baroreflex function in T2DM rats compared to controls, sample sizes were not large. This may have led to an underestimation of significant statistical interactions. However, changes over time in each group still occurred and a larger sample size would not necessarily change the physiological interpretation of the results. One of the main limitations of the present study is the lack of sympathetic nerve activity assessment. Since we could not establish a direct relationship between sympathetic and vagal activity in the current study, further studies are needed to investigate whether changes in the sympathovagal balance are contributing to the baroreflex dysfunction in T2DM over time. Furthermore, the current study included only male rats, and therefore, these findings should not be extrapolated to females. Previous studies have shown sex differences in baroreflex function in healthy humans (Abdel-Rahman et al., 1994; Beske et al., 1985; Fu et al., 2009; Samora et al., 2020, 2019, 2018; Teixeira et al., 2018b), thus future investigation is urgently needed to determine whether sex also plays a role in the baroreflex dysfunction in obese and T2DM patients.

Lastly, it is important to consider the possible effect of a volatile anesthetic on baroreflex function. It is well established that isoflurane disrupts the regulation of circulation and

also depresses baroreflex control of heart rate (Lee et al., 2002; Seagard et al., 1983; Skovsted and Sapthavichaikul, 1977). However, to the best of our knowledge, there is no evidence showing that the effect of anesthesia is different between healthy and T2DM rats. Thus, we can assume that suppression of baroreflex control of heart rate would happen to a similar degree in both groups. Of notable interest, we did not find significant differences in the number of baroreflex ramps between groups (data not shown). This is important to point out since a loss in the number of baroreflex sequences could limit the application of the sequence method, and could lead to misinterpretation of the results (Laude et al., 2009). Finally, a previous study reported that isoflurane depresses baroreflex function predominantly by inhibiting the sympathetic component of the reflex (Lee et al., 2002), which is not the underlying mechanism proposed in the current study to explain the attenuated spontaneous BRS in T2DM rats. Thus, we believe that the effect of isoflurane on hemodynamic variables and baroreflex function did not change the interpretation of the results in the present study.

5. Conclusions

We found a temporal change in spontaneous BRS early in the disease in T2DM rats, and this dynamic baroreflex dysfunction might be partially driven by obesity-associated changes in adiponectin and insulin. Since we did not observe a significant reduction in parasympathetic activity in T2DM rats compared with healthy controls at the onset of diabetes, alterations in vagal tone may not be the only possible explanation for an attenuated spontaneous BRS in T2DM rats. Future studies are warranted to further investigate the effects of T2DM, with the co-existence of obesity, on the afferent (Sheehan et al., 1941) and efferent (Gottsäter et al., 2003) components of the baroreflex arc, connections in the central nervous system (Yan et al., 2009), or receptors in the heart (Gianaros et al., 2002). Our findings demonstrate that obesity-related metabolic impairments may play a role in the attenuated spontaneous BRS in T2DM, suggesting a link between metabolic and autonomic dysfunction in diabetes.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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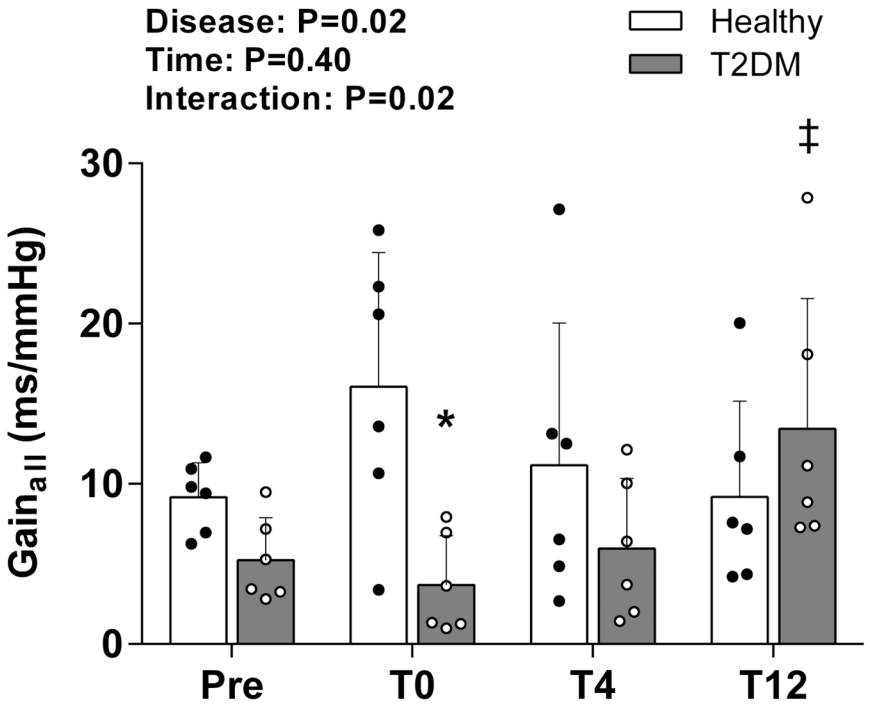

Figure 1. Spontaneous baroreflex sensitivity in T2DM and age-matched healthy rats.

Values are presented as group averaged and individual data points. Data were compared using a two-way ANOVA with *Sidak* post hoc analysis for multiple comparisons and are expressed as means ± SD. Sample size is the same for every group (n=6). Pre, pre diabetic; T0, diabetes-onset; T4, 4 weeks after onset; T12, 12 weeks after onset. *P<0.05 *vs.* healthy within time-point. ‡P<0.05 *vs.* T0 within group.

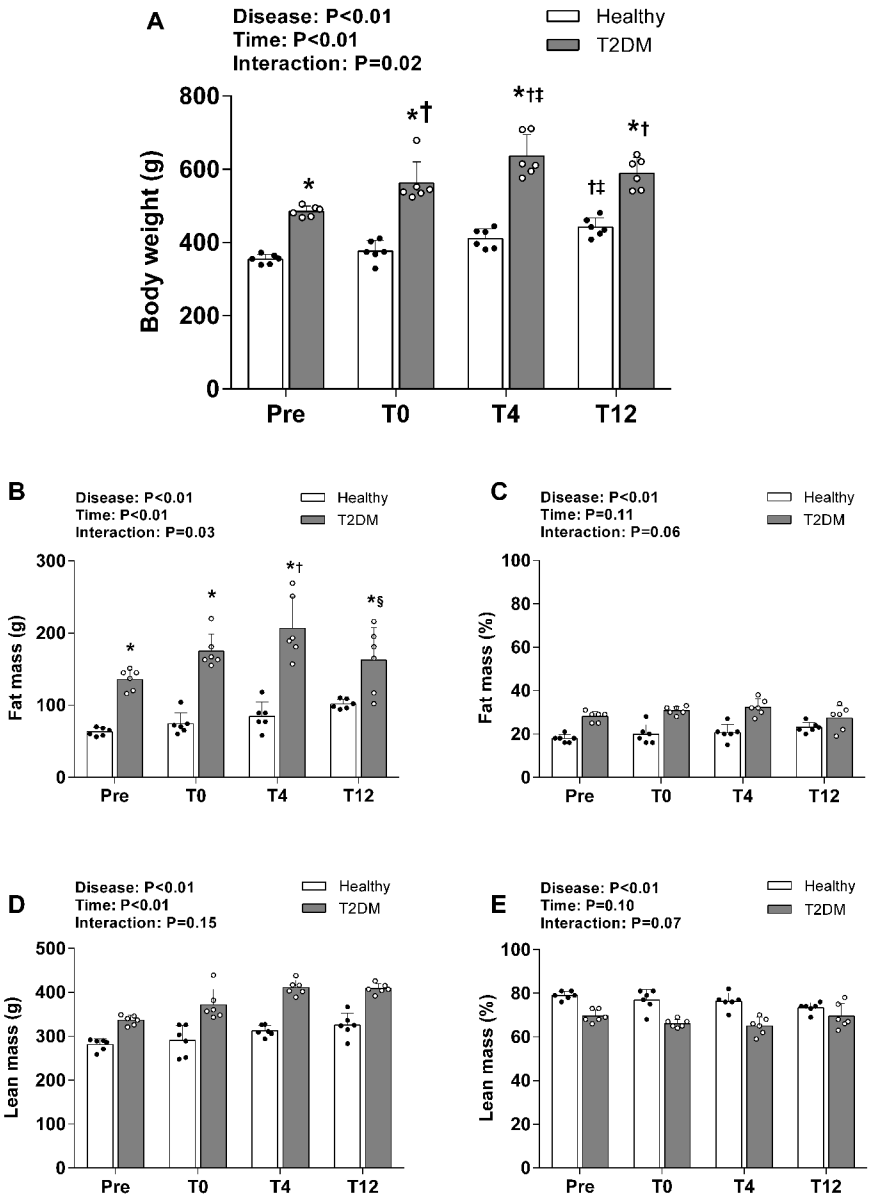

Figure 2. Body composition in T2DM and age-matched healthy rats.

Values are presented as group averaged and individual data points. Both fat and lean mass were normalized to body weight. Data were compared using a two-way ANOVA with *Sidak* post hoc analysis for multiple comparisons when applicable and are expressed as means ± SD. Sample size is the same for every group (n=6). Pre, pre diabetic; T0, diabetes-onset; T4, 4 weeks after onset; T12, 12 weeks after onset. *P<0.05 *vs.* healthy within time-point. †P<0.05 *vs.* Pre within group. ‡P<0.05 *vs.* T0 within group. §P<0.05 *vs.* T4 within group.

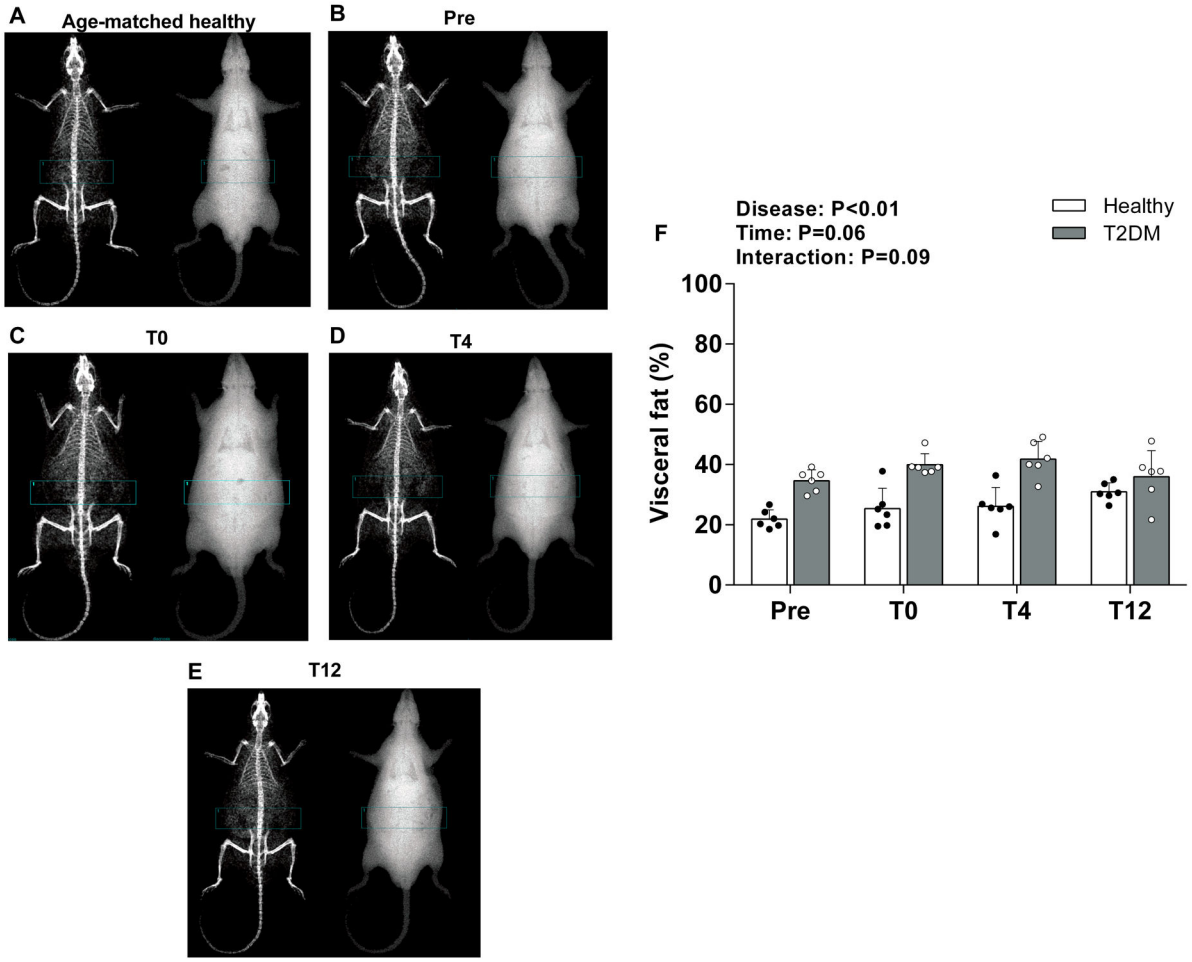

Figure 3. DEXA scan images for a single representative healthy and T2DM rats.

Region of interest (ROI) from the whole-body scan extended from L3/L2 vertebral space to L5/L6 vertebral space with the lateral borders extending to the edge of the abdominal soft tissue (blue rectangle on the scans). Panel F shows the group averaged and individual data points for the percent of visceral fat in T2DM and healthy rats. Percent visceral fat is the ratio between fat mass and total tissue within the ROI. Data were compared using a two-way ANOVA and are expressed as means ± SD. Sample size is the same for every group (n=6). Pre, pre diabetic; T0, diabetes-onset; T4, 4 weeks after onset; T12, 12 weeks after onset.

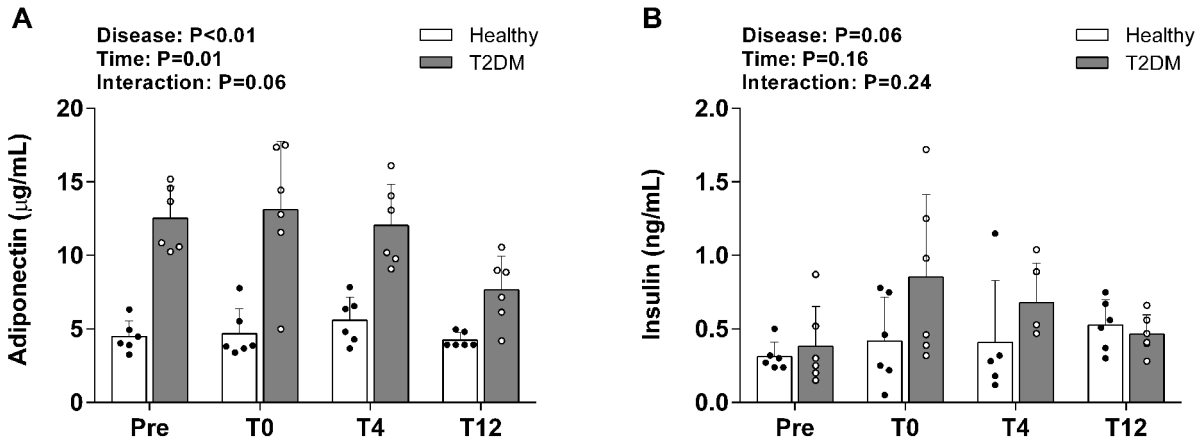

Figure 4. Serum adiponectin (A) and insulin (B) concentrations in T2DM and age-matched healthy rats.

Values are presented as group averaged and individual data points. Data were compared using a two-way ANOVA and are expressed as means ± SD. Sample size is the same for every group (n=6), except one T2DM rat and one healthy rat from the T4 insulin groups that were considered outliers (i.e., values greater than 2.5 standard deviations from the mean) and were excluded. Pre, pre diabetic; T0, diabetes-onset; T4, 4 weeks after onset; T12, 12 weeks after onset.

Table 1.

Characteristics of the experimental groups.

	Pre		T0		T4		T12		*P* Value		
	Healthy	**Diabetic**	**Healthy**	**Diabetic**	**Healthy**	**Diabetic**	**Healthy**	**Diabetic**	**Time**	**Disease**	**Interaction**
Age (weeks)	12 ± 0.5	12 ± 0.2	15 ± 3.0	14 ± 3.0	20 ± 4.8	20 ± 4.9	22 ± 1.0	22 ± 0.7	<0.001	0.921	1.000
Fasting blood glucose (mg/dL)	67 ± 10	108 ± 7	76 ± 7	113 ± 13	71 ± 8	113 ± 9	68 ± 3	244 ± 100 *†‡§	<0.001	<0.001	**<0.001**
HbA1c (%)	4.5 ± 0.1	5.4 ± 0.2 *	4.7 ± 0.3	5.9 ± 0.2 *	4.7 ± 0.2	5.6 ± 0.7 *	4.7 ± 0.2	12.1 ± 11.1 *†‡§	<0.001	<0.001	**<0.001**

Data are means ± SD. Data were analyzed using two-way ANOVA in which group and time were the main factors. *Post hoc* analysis was employed using the *Sidak* test for multiple comparisons when applicable. Sample size is the same for every group (n=6). Pre, pre diabetic; T0, diabetes-onset; T4, 4 weeks after onset; T12, 12 weeks after onset. * P<0.05 *vs.* healthy within time-point. †P<0.05 *vs.* Pre within group. ‡P<0.05 *vs.* T0 within group. §P<0.05 *vs.* T4 within group.

Table 2.

Hemodynamics, indices of spontaneous baroreflex sensitivity and heart rate variability in T2DM and age-matched healthy rats.

	Pre		T0		T4		T12		*P* Value		
	Healthy	**Diabetic**	**Healthy**	**Diabetic**	**Healthy**	**Diabetic**	**Healthy**	**Diabetic**	**Time**	**Disease**	**Interaction**
Hemodynamic variables											
Systolic BP (mmHg)	77 ± 5	86 ± 13	77 ± 5	79 ± 12	72 ± 4	93 ± 11 *	90 ± 14 §	83 ± 9	0.231	0.040	**0.014**
Diastolic BP (mmHg)	48 ± 6	51 ± 12	48 ± 5	43 ± 11	46 ± 6	54 ± 10	63 ± 9 †‡§	49 ± 11 *	0.064	0.416	**0.024**
Mean BP (mmHg)	58 ± 5	62 ± 12	57 ± 5	55 ± 11	55 ± 4	67 ± 10	72 ± 11 §	60 ± 10	0.092	0.839	**0.016**
Heart rate (bpm)	220 ± 32	247 ± 31	205 ± 23	218 ± 10	227 ± 13	222 ± 20	232 ± 19	203 ± 18 †	0.099	0.799	**0.023**
RR interval (ms)	277 ± 34	247 ± 31	297 ± 35	273 ± 16	265 ± 14	272 ± 24	261 ± 22	298 ± 26 †	0.144	0.727	**0.011**
Indices of BRS											
$Gain_{up}$ (ms/mmHg)	11.8 ± 4.6	4.0 ± 3.6	22.3 ± 15.8	3.4 ± 3.1 *	15.3 ± 11.7	6.6 ± 4.7	11.5 ± 8.8	15.9 ± 10.5	0.411	0.005	**0.027**
$Gain_{down}$ (ms/mmHg)	6.9 ± 2.2	5.9 ± 3.3	8.8 ± 5.3	3.5 ± 3.7	7.4 ± 4.5	2.5 ± 1.5	6.4 ± 3.0	7.9 ± 4.0	0.545	0.027	0.095
Heart rate variability											
RMSSD (ms)	7.5 ± 3.2	6.5 ± 2.2	12.1 ± 6.5	6.9 ± 5.1	6.6 ± 2.9	6.8 ± 2.6	7.0 ± 3.7	14.0 ± 4.6 *†‡§	0.084	0.997	**0.012**
VLF (ms^2)	0.33 ± 0.19	0.13 ± 0.12	0.42 ± 0.38	0.51 ± 0.59	1.42 ± 1.86	0.17 ± 0.09	0.36 ± 0.20	0.21 ± 0.14	0.218	0.075	0.118
LF (ms^2)	0.35 ± 0.23	0.26 ± 0.33	0.41 ± 0.30	0.87 ± 1.36	0.51 ± 0.53	0.25 ± 0.25	0.32 ± 0.25	0.34 ± 0.18	0.443	0.852	0.443
HF (ms^2)	1.17 ± 0.87	0.49 ± 0.47	1.66 ± 1.81	0.46 ± 0.55	0.88 ± 0.95	0.74 ± 0.96	0.81 ± 0.71	1.96 ± 1.35	0.519	0.483	0.060
LF (nu)	24 ± 9	28 ± 8	28 ± 10	36 ± 13	37 ± 6	29 ± 10	34 ± 5	21 ± 13 ‡	0.206	0.466	**0.025**
HF (nu)	76 ± 9	72 ± 8	73 ± 10	61 ± 12	63 ± 6	71 ± 10	66 ± 5	80 ± 13 ‡	0.125	0.644	**0.010**
LF/HF	0.38 ± 0.19	0.49 ± 0.25	0.46 ± 0.23	0.87 ± 0.48	0.75 ± 0.23	0.52 ± 0.37	0.67 ± 0.21	0.37 ± 0.35	0.236	0.969	**0.026**

Data are means ± SD. Data were analyzed using two-way ANOVA in which group and time were the main factors. Post hoc analysis was employed using the *Sidak* test for multiple comparisons when applicable. Sample size is the same for every group (n=6). BP, blood pressure; BRS, baroreflex sensitivity; HF, high frequency; LF, low frequency; Pre, pre diabetic; RMSSD, root mean square of successive differences; T0, diabetes-onset; T4, 4 weeks after onset; T12, 12 weeks after onset; VLF, very low frequency * P<0.05 vs. healthy within time-point. †P<0.05 vs. Pre within group. ‡P<0.05 vs. T0 within group. §P<0.05 vs. T4 within group.