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Differential impact of type-1 and type-2 diabetes on control of heart rate in mice

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ABSTRACT

Aims: Cardiac autonomic dysfunction is a serious complication of diabetes. One consequence is disruption of the normal beat-to-beat regulation of heart rate (HR), i.e. HR variability (HRV). However, our understanding of the disease process has been limited by inconsistent HR/HRV data from previous animal studies. We hypothesized that differences in the method of measurement, time of day, and level of stress account for the differing results across studies. Thus, our aim was to systematically assess HR and HRV in two common diabetic mouse models.
Methods: ECG radiotelemetry devices were implanted into *db*/*db* (type-2 diabetic), STZ-treated *db*/+ (type-1 diabetic), and control *db*/+ mice (n = 4 per group). HR and HRV were analyzed over 24 h and during treadmill testing.
Results: 24 h analysis revealed that *db*/*db* mice had an altered pattern of circadian HR changes, and STZ-treated mice had reduced HR throughout. HRV measures linked to sympathetic control were reduced in *db*/*db* mice in the early morning and early afternoon, and partially reduced in STZ-treated mice. HR response to treadmill testing was blunted in both models.
Conclusions: It is important to consider both time of day and level of stress when assessing HR and HRV in diabetic mice. *db*/*db* mice may have altered circadian rhythm of sympathetic control of HR, whereas STZ-treated mice have a relative reduction. This study provides baseline data and a framework for HR analysis that may guide future investigations.

1. Introduction

Diabetes affects almost 400 million people worldwide (Nathan, 2015) and is associated with increased risk of both cardiovascular disease (>2-fold increased risk (Kannel and McGee, 1979)) and autonomic dysfunction (approximately 20% incidence of abnormal cardiovascular autonomic function in diabetic patients (Vinik et al., 2003)). Cardiovascular autonomic dysfunction is linked to increased mortality: a meta-analysis in 2900 patients revealed a relative risk of 2.14, with a 95% confidence interval of 1.83–2.15 ($p < 0.0001$) (Vinik et al., 2003). Various aspects of cardiovascular physiology are under autonomic control, including heart rate (HR). Large changes in HR occur as part of the normal physiological response to stress and exercise, but even during periods of rest HR is tightly controlled; this control is evident as HR variability (HRV). Diabetes-related cardiac autonomic dysfunction increases resting HR (by an average of 6–10% (Ewing et al., 1981)) and decreases HRV (Pop-Busui, 2010). For example, a study by Spallone et al. (1993) showed an 8% increase in HR and a 10–20% decrease in HRV measures in diabetic patients vs. control subjects (n = 25 per group). These changes are associated with worse prognosis in both type-1 and type-2 diabetic populations (Hillis et al., 2012a,b; Singh, 2002; Vinik et al., 2003). Dysfunctional HR regulation also contributes to exercise intolerance, which is common in these patients (Pop-Busui, 2010; Vinik and Ziegler, 2007). In a study of 170 type-2 diabetic patients vs. 56 control subjects, exercise capacity (measured in metabolic equivalents) was reduced by 28% (Fang et al., 2005). In later stages of disease, severe cardiac autonomic neuropathy results in almost no HRV and a fixed HR unresponsive to sleep, stress, or moderate exercise (Pop-Busui, 2010). Thus, cardiac autonomic dysfunction is a serious and potentially devastating complication of diabetes; however, there is no effective treatment and it is currently understudied (Vinik and Ziegler, 2007).

Better understanding of diabetes-induced autonomic dysfunction requires further research that will rely on well-characterized animal models. The most commonly-used mouse models are *db*/*db* mutant

Abbreviations: BP, blood pressure; CO, cardiac output; FFT, fast fourier transform; FS, fractional shortening; HF, high frequency; HR, heart rate; HRV, heart rate variability; IVS, inter-ventricular septum; LF, low frequency; LV, left ventricle; LVIDd, LV internal diameter in diastole; LVIDs, LV internal diameter in systole; LVPW, LV posterior wall; MV A, mitral valve A velocity; MV E, mitral valve E velocity; pNN_6, percentage of RR intervals differing by more than 6 ms; PW, pulse wave; RMSSD, root mean square of successive differences; SDRR, standard deviation of RR intervals; STZ, streptozotocin; SV, stroke volume.

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mice (type-2 diabetes; secondary to hyperphagia and obesity caused by a mutation in the leptin receptor gene) and streptozotocin (STZ)-treated mice (type-1 diabetes; destruction of pancreatic islets by STZ). Published studies using these models, however, contain variable and sometimes conflicting results (Stables et al., 2013). Specifically, HR has been reported to be increased (Goncalves et al., 2009), decreased (Park et al., 2008; Semeniuk et al., 2002; Senador et al., 2009), or unaltered (Su et al., 2008) in *db*/*db* mice, and increased (Kellogg et al., 2009) or decreased (Lin et al., 2010; Mabe and Hoover, 2011) in STZ-treated mice. Papers reporting HRV also show mixed results both in *db*/*db* (Goncalves et al., 2009; Senador et al., 2009) and STZ-treated (Mabe and Hoover, 2011) mice. Our hypothesis was that differences in method of measurement, time of day, and level of stress account for these inconsistent results.

The aim of this study was to assess changes in HR and HRV in *db*/*db* and STZ-treated mice in a more systematic and comprehensive manner than previously published studies. We used both echocardiography and the gold-standard method of implantable ECG radiotelemetry in conscious, unrestrained mice. This enabled analysis of both circadian changes and progression of disease over several weeks. To study response to both stress and exercise, we subjected the telemetered mice to treadmill testing.

2. Methods

2.1. Mice

Male BKS *db*/*db* (type-2 diabetic) and *db*/+ (control) mice (BKS.Cg-Dock7m +/+ Leprdb/J; stock number 000,642) were obtained from Jackson Labs (Bar Harbor, Maine, USA). Principles of laboratory animal care (NIH publication no. 85–23, revised 1985; http://grants1.nih.gov/grants/olaw/references/phspol.htm) were followed. Mice were cared for following the University of Michigan Committee on the Care and Use of Animals guidelines.

2.2. Echocardiography

For the echocardiography (echo) study, 24-week-old *db*/*db* and *db*/+ mice (n = 6 per group) were lightly anesthetized using isoflurane and cardiac echo was conducted as previously described (Zolov et al., 2012). Left ventricular (LV) ejection fraction was measured from the two-dimensional long axis view. Systolic and diastolic dimensions and wall thickness were measured by M-mode in the parasternal short axis view at the level of the papillary muscles. Diastolic function was assessed by conventional pulsed-wave spectral Doppler analysis of mitral valve inflow patterns (early [E] and late [A] filling waves). For further details, see Supplemental methods.

2.3. ECG telemetry

For the ECG telemetry study, three groups of mice were telemetered (n = 4 per group): STZ-treated *db*/+ (type-1 diabetes); vehicle-treated *db*/*db* (type-2 diabetes); and vehicle-treated *db*/+ as a control. An overview of the experimental protocol is shown in Fig. 1. STZ or vehicle was administered at 5 weeks of age, and ECG devices (Data Sciences International (DSI), St. Paul, MN, USA; ETA-F-20) were implanted at 7 weeks of age in approximately lead II configuration. Animals were housed in individual cages with a receiver below each cage. ECG, temperature, and activity were recorded. For clarity, most results are displayed with *db*/*db* vs. control and STZ-treated vs. control on separate graphs. STZ was administered as a single i.p. injection (150 mg/kg in sodium citrate solution); mice were given access to 10% sucrose water for the first 3 days post-STZ and DietGel (ClearH$_2$O, Maine, USA) in their cages thereafter, with cage bedding changed regularly due to increased urination. The room had a 12-h light/dark cycle, with lights on/off at 6 am/6 pm. Animals were fed on normal chow ad libitum, plus DietGel for the STZ-treated mice.

For weekly measurements, recordings were made for 5 min every 10 min (i.e. 5 min on, 5 min off) for 72 h each week for 16 weeks (age 8 to 24 weeks). Average values for HR, temperature, and activity were calculated using DSI's Analysis software. To reduce variability due to noise or human presence we averaged the hourly values over 3 days to obtain an average 24-h dataset for each mouse each week.

HRV was analyzed at 10 weeks of age. To calculate HRV, we first used DSI's Ponemah software to generate RR interval data for one 5-min ECG segment per hour, over 3 days. Next, using the R programming language and environment, we split this data into separate files (one per 5-min interval), and for each file we then excluded RR intervals greater than 3 standard deviations from the mean to remove artifacts. Files for which >5% of RR intervals were excluded in this way were removed

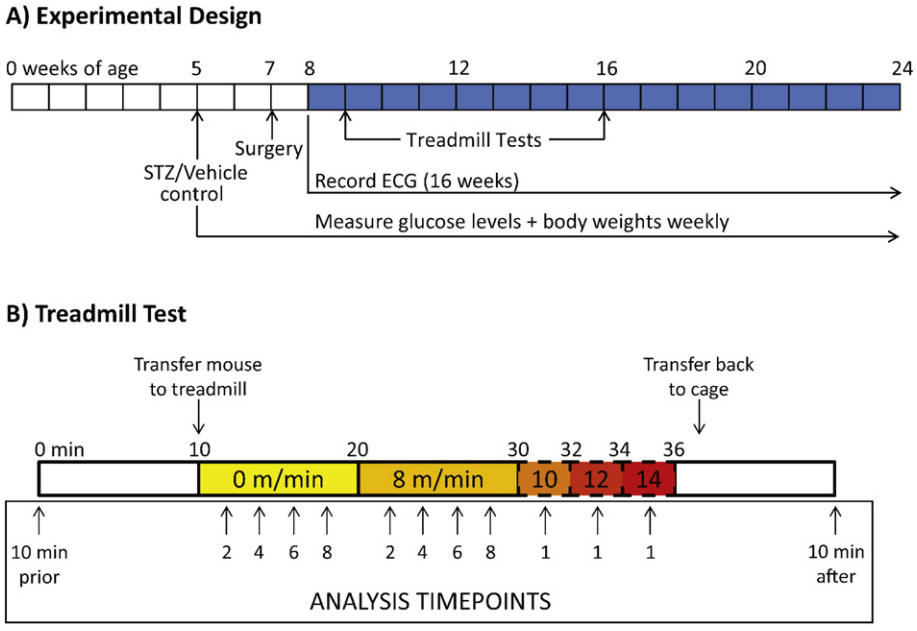

Fig. 1. ECG telemetry experiment design. A) Experiment overview. Streptozotocin (STZ) or vehicle were injected at 5 weeks. Telemetry devices were implanted surgically at 7 weeks. ECG, temperature, and activity were recorded for three days each week. B) Treadmill test protocol. ECG traces (30 s duration) were analyzed at the time points indicated.