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# Cardiovascular autonomic dysfunction in non-obese diabetic mice

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ABSTRACT

It is known that diabetes is associated with autonomic dysfunction; however, data about autonomic function in non-obese diabetic mice (NOD) remain scarce. We evaluated the autonomic profile of NOD mice. Female mice, 24–28 week old, were divided in two groups: NOD (n = 6) and control (n = 6, Swiss mice). NOD mice with glycemia ≥300 mg/dl were used. Heart rate variability (HRV) and arterial pressure variability (APV) in time and frequency domains, symbolic analysis of heart rate (HR) and baroreflex sensitivity were evaluated. HR and arterial pressure (AP) were similar between the groups; however, HRV (total variance of RR interval: NOD = 21.07 ± 3.75 vs. C = 42.02 ± 6.54 $ms^2$) and the vagal modulation index RMSSD were lower in NOD group (4.01 ± 0.32 vs. 8.28 ± 0.97 ms). Moreover, the absolute and normalized low-frequency (LF) components were also enhanced in NOD (normalized = 61.0 ± 4.0%) as compared to control mice (normalized = 20.0 ± 4.0%). Both the absolute and normalized high-frequency (HF) components were lower in NOD (normalized = 39.0 ± 4.0%) when compared to the control group (normalized = 80.0 ± 4.0). In the symbolic analysis the 0V pattern, an indication of sympathetic activity, was higher in NOD and 2LV pattern, an indication of parasympathetic activity, was lower in the NOD than in the control group. Both bradycardic and tachycardic responses were decreased in NOD (3.01 ± 0.72 vs. 4.54 ± 0.36 bpm/mm Hg and 2.49 ± 0.31 vs. C = 3.43 ± 0.33 bpm/mm Hg) when compared to the control group. Correlation analysis showed negative correlations between vagal indexes (RMSSD, %HF and 2LV) and glycemic levels. In conclusion, NOD mice develop severe diabetes correlated with autonomic dysfunction.


## 1. Introduction

The prevalence of diabetes in modern society has been increasing at epidemic rates, largely related to the prevalence of obesity and sedentary lifestyles. Diabetes increases cardiovascular morbidity and mortality related to autonomic dysfunction, nephropathy and neuropathy (Zimmet et al., 1997).

Among these complications, autonomic neuropathy is a common alteration presented in diabetes mellitus which results in autonomic nerve fiber dystrophy in axons and dendrite abnormalities (Schmidt, 2002). In addition, cardiovascular autonomic neuropathy seems to be related to increased mortality in diabetic patients (Vinik and Ziegler, 2007). Moreover, both the parasympathetic and sympathetic neurons can be affected by diabetic neuropathy resulting in alterations of heart rate control and vascular dynamics (Schumer et al., 1998). Our group has showed several autonomic, hemodynamic and functional cardiac alterations promoted by experimental diabetes, resulting in higher mortality (Wichi et al., 2007; Mostarda et al., 2009; Jorge et al., 2012). Additionally, it is widely acknowledged that neuropathy promotes a decrease in heart rate variability (HRV) both in time and frequency domains, as demonstrated by alterations in low frequency and high frequency components. On the other hand, it is well established that HRV is an important prognostic marker of mortality (Malik et al., 1996).

Among several experimental models used to study the pathophysiology of diabetes, non-obese diabetic mice (NOD) have been widely used to evaluate several mechanisms affected by type 1diabetes. These mice exhibit spontaneous autoimmune diabetes by causing the destruction of insulin producing cells, similar to that observed in humans (Kodama et al., 2003). Furthermore, polydipsia, polyuria, glycosuria, hyperglycemia and insulin deficiency are equally observed in these animals, accompanied by a rapid weight loss (Makino et al., 1980).

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Although the association of diabetes and autonomic dysfunction has been widely recognized, data on cardiovascular autonomic function in NOD mice remain scarce. Therefore, the aim of this study was to investigate the cardiovascular autonomic profile of NOD mice.

## 2. Methods

### 2.1. Experimental groups

Groups studied: (1) female hyperglycemic NOD mice (n = 6): blood glucose >300 mg/dl (>17 mmol/l); and (2) Swiss female mice (n = 6): used as control, blood glucose <110 mg/dl (<6 mmol/l).

All surgical procedures and protocols used were approved by the Nove de Julho University Ethical Committee (protocol number AN0029-2012) and strictly followed National Institutes of Health Guide for the Care and Use of Laboratory Animals.

### 2.2. Blood glucose level measurements

Ten-week-old female mice had their blood glucose levels determined weekly using the ACCU-CHECK Sensor® (Roche) until they reached an established level of diabetes. Blood samples were obtained by a vessel puncture in the caudal vein and were used for glucose level determination. Animals with plasma glucose higher than 300 mg/dl were included in this study and Swiss female mice were used as controls (Marco et al., 2008).

### 2.3. Cardiovascular measurements

When the mice were 24–28 weeks old, they were anesthetized (ketamine–xylazine 80:40 mg/kg ip) and polyethylene-tipped Tygon cannulas (4 cm of PE-08 connected to 2 cm of PE-50, Clay Adams) filled with heparinized saline were inserted into the carotid artery and jugular vein for direct measurements of arterial pressure (AP) and drugs administration, respectively. The free ends of the cannulas were tunneled subcutaneously and exteriorized at the top of the skull. After the surgery, the animals received an intramuscular injection of penicillin G (Benzetacil®, Fontoura-Wyeth, 60,000 U) and postoperative care were based on the description of Flecknell (1992). Two days following catheter placement, hemodynamic measurements were made with animals at baseline conditions, namely, conscious, freely moving, in a quiet environment with controlled temperature, deprived of food and water. The arterial cannula was connected to a transducer (Blood Pressure XDCR, Kent Scientific), and AP signals were recorded for a 20-min period using a microcomputer equipped with an analog-to-digital converter (WINDAQ, 4-kHz sampling frequency, Dataq Instruments). The recorded data were analyzed on a beat-to-beat basis to quantify changes in AP and heart rate (HR).

Baroreflex sensitivity (BRS) was evaluated by a mean index relating the tachycardic and the bradycardic responses for mean AP changes (30–40 mm Hg) induced by increasing doses of sodium nitroprusside (100–250 ng/kg body wt. iv) and phenylephrine (80–250 ng/kg body wt. iv) injections, respectively (De Angelis et al., 2004; Wichi et al., 2007; Heeren et al., 2009). Data were expressed as beats per minute (bpm) per mm Hg. Maximal volume per injection was <25 μl.

### 2.4. Heart rate and blood pressure variability

Time-domain analysis consisted in calculating mean pulse interval (PI) variance from its respective time series. For frequency domain analysis, the whole 20-min time series of PI and systolic arterial pressure (SAP), 256 point, overlap 50%, were cubic-spline-interpolated to 27 Hz and decimated to be equally spaced in time. Following linear trend removal, power spectral density was obtained by the fast Fourier transformation as previously described (Soares et al., 2004; Heeren et al., 2009; Zamo et al., 2010). Spectral power for low (LF 0.10–1.0 Hz), and high (HF 1–5 Hz) frequency bands was calculated by means of power spectrum density integration within each frequency bandwidth, using a customized routine (MATLAB 6.0, Mathworks).

Symbolic analysis of 3-beat sequences to distinguish sympathetic and parasympathetic cardiac modulation was used to detect changes in autonomic modulation of cardiovascular variability (Guzzetti et al., 2005; Porta et al., 2007). RR interval sequences of length n = 300 were selected. The full range of the sequences was uniformly spread on 6 levels (from 0 to 5) transforming them into a sequence of integers (i.e., symbols). Patterns (sequences of 3 symbols) were constructed based on the sequence of symbols and all possible patterns were divided into four groups, consisting of patterns with: 1) no variations (0V, three symbols equal that indicate sympathetic modulation); 2) one variation (1V, two consequent symbols were equal and the remaining symbol was different, thus indicating both sympathetic and parasympathetic modulation); 3) two like variations (2LV, with the three symbols forming an ascending or descending ramp, thus indicating parasympathetic modulation); and 4) two unlike variations (2UV, the three symbols forming a peak or a valley, thus indicating parasympathetic modulation (Guzzetti et al., 2005)). The rates of occurrence of these patterns (0V%, 1V%, 2LV%, and 2UV%) were evaluated. All recordings were performed in a sound attenuated room.

### 2.5. Statistical analysis

Data are reported as mean ± SEM and Student's *t* test was used to compare means between the two groups. Pearson correlation was used to study the association between variables. The significance level was established as $P < 0.05$.

## 3. Results

### 3.1. Cardiovascular measurements

AP (systolic, diastolic and mean) and HR were not significantly different between NOD and control groups (Table 1). The results of BRS are shown in Fig. 1. There was significant difference between baroreflex bradycardic responses evoked by phenylephrine in NOD group when compared to the control group ($P = 0.036$). Additionally, baroreflex tachycardic responses were significantly lower in NOD group when compared to the control group ($P = 0.027$).

Time and frequency domains of cardiovascular autonomic modulation in NOD and in control groups are shown in Fig. 2. In time domain analysis, the variance of PI was significantly lower in NOD group ($21.07 \pm 3.75$ ms$^2$) as compared to the control group ($42.02 \pm 6.54$ ms$^2$, $P = 0.02$). Moreover, RMSSD (root mean square of the successive differences in NN intervals, which is a vagal modulation index) showed significantly lower values in NOD group ($4.01 \pm 0.32$ ms) when compared to the control group ($8.28 \pm 0.97$ ms, $P < 0.01$).

The variance of the SAP was not significantly different between NOD (n = 6) and control (n = 6) groups (NOD = $18.60 \pm 0.71$ and C = $20.31 \pm 3.16$ mm Hg$^2$, $P = 0.67$).

**Table 1**
Arterial pressure and heart rate in control and NOD mice.

| Parameters | C (n = 6) | NOD (n = 6) | P value |
|---|---|---|---|
| SAP (mm Hg) | 126 ± 4.7 | 116 ± 6.4 | 0.23 |
| MAP (mm Hg) | 110 ± 4.3 | 103 ± 5.2 | 0.32 |
| DAP (mm Hg) | 93 ± 4.2 | 89 ± 4.0 | 0.5 |
| HR (bpm) | 615 ± 9.0 | 588 ± 10 | 0.07 |

Values are mean ± SEM obtained by direct measurements of arterial pressure. SAP: systolic arterial pressure; MAP: mean arterial pressure; DAP: diastolic arterial pressure; HR: heart rate;