Applications of Novel HRV Techniques to PGC1a-Deficient and Wild Type Mice

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Abstract

Graphical plots of beat-by-beat heart rate (HR) patterns, e.g., Poincaré plots, provide information beyond traditional HRV, but such plots have not been used in mice. HR patterns from 3 min recording/ hour in 4 wild type and 4 PGC1α-deficient mice were compared. In comparison to normal mice, knockout mice tended to have lower HRs (694 ± 16 vs. 752 ± 51 bpm) consistent with their decreased ability to use glucose in the heart. Poincaré plots from each hour showed that none of the wild-type mice had abnormal, fan-like, complex patterns in any hour of their recording. However, 3 of the 4 knockout mice had a complex pattern in at least 10 hours of the recording. Results suggest that hourly Poincaré plot analyses from multiple short-term recordings may be useful in identifying cardiac autonomic abnormalities in knockout mouse models. Samples from the entire 24-hour period provide more information about autonomic function than a single brief recording.

1. Introduction

Traditional time and frequency domain heart rate variability (HRV) have been used to understand the effect of various genetic knockouts on cardiac autonomic function in different mouse models [1,2,3]. However, the structure of the HR time series can also be characterized by examination of the Poincaré plot of interbeat intervals, *i.e.*, the plot of each normal-to-normal interbeat interval vs. the next [4]. A normal plot, at least in humans, can be described as elliptical or comet-shaped and has a "well-organized" quality [5]. Abnormal Poincaré plots have been described as appearing more disorganized and fan-shaped than normal ones. In humans, the presence of more abnormal Poincaré plots is associated with higher serum norepinephrine levels in heart failure patients, despite similar levels of total HRV. This suggests that abnormal Poincaré plots are a marker for underlying cardiac abnormalities [6]. We have shown that more abnormal Poincaré plots are associated with erratic sinus rhythm, a kind of irregular heart rate pattern that exaggerates HRV, but is actually associated with increased risk of mortality among community-dwelling older adults and post-MI patients [7,8]. The effect of genetic knockouts that induce cardiac dysfunction on Poincaré plot patterns in mice is unknown. We investigated this in the PGC1α knockout mouse. PGC1α is a transcriptional coactivator that regulates energy (glucose and lipid) metabolism [9]. A decrease in PGC1α protein content reduces the amount of mitochondrial DNA and oxidative capacity, reducing mitochondrial enzymatic activities and the ability of the heart to generate ATP efficiently. Thus the ability of the heart to use fatty acids and glucose for energy is limited [10,11]. PGC1α has also been linked with obesity, diabetes, and cardiomyopathy, making it a potential target for drugs that treat obesity and Type 2 diabetes [11]. The purpose of our study was to determine if HR patterns from Poincaré plots would reveal underlying abnormalities in PGC1α knockout mice compared to wild type mice the same age and gender.

2. Methods

N=8 mice, 4 wild-types and 4 PGC1α knockouts, had ECGs collected for 3 minutes per hour for 24 hours with a sampling rate of 1000 Hz. Recordings were down-sampled by a factor of 12 to reduce the mouse heart rate to human range. Thus, 3-minute recordings at mouse heart rates (~700 bpm) were analyzed as 36 minute recordings at human HRs (~58 bpm). Recordings were then loaded onto a MARS 8000 Holter scanner and analyzed using standard research Holter techniques. Figure 1a shows a representative ECG from a wild-type mouse scaled down to human range as seen on the Holter scanner. Figure 1b shows a single-channel human ECG at about the same heart rate.

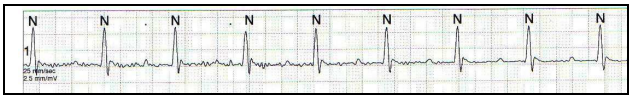

Figure 1a. Example of a wild-type mouse ECG at 68 bpm.

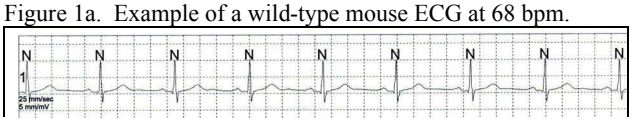

Figure 1b. Example of a human ECG at 67 bpm.

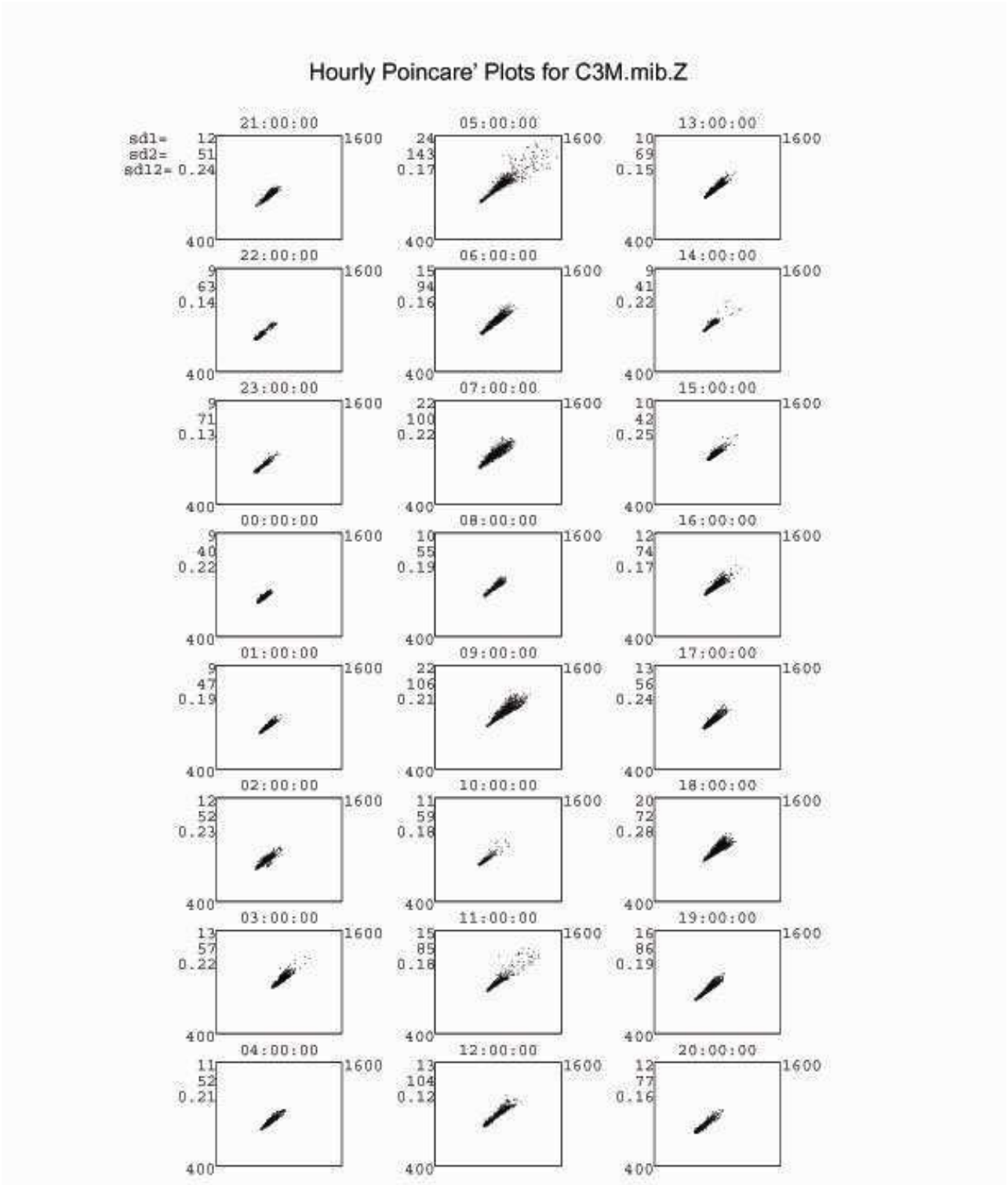

Figure 2. Example of 24-hourly 36-minute Poincaré plots of a normal, wild-type mouse. The beat-to-beat plots were based on a heart rate/12 scale in order to show underlying organization. Plots are well-organized, tightly packed, and either elliptical or cone-shaped.

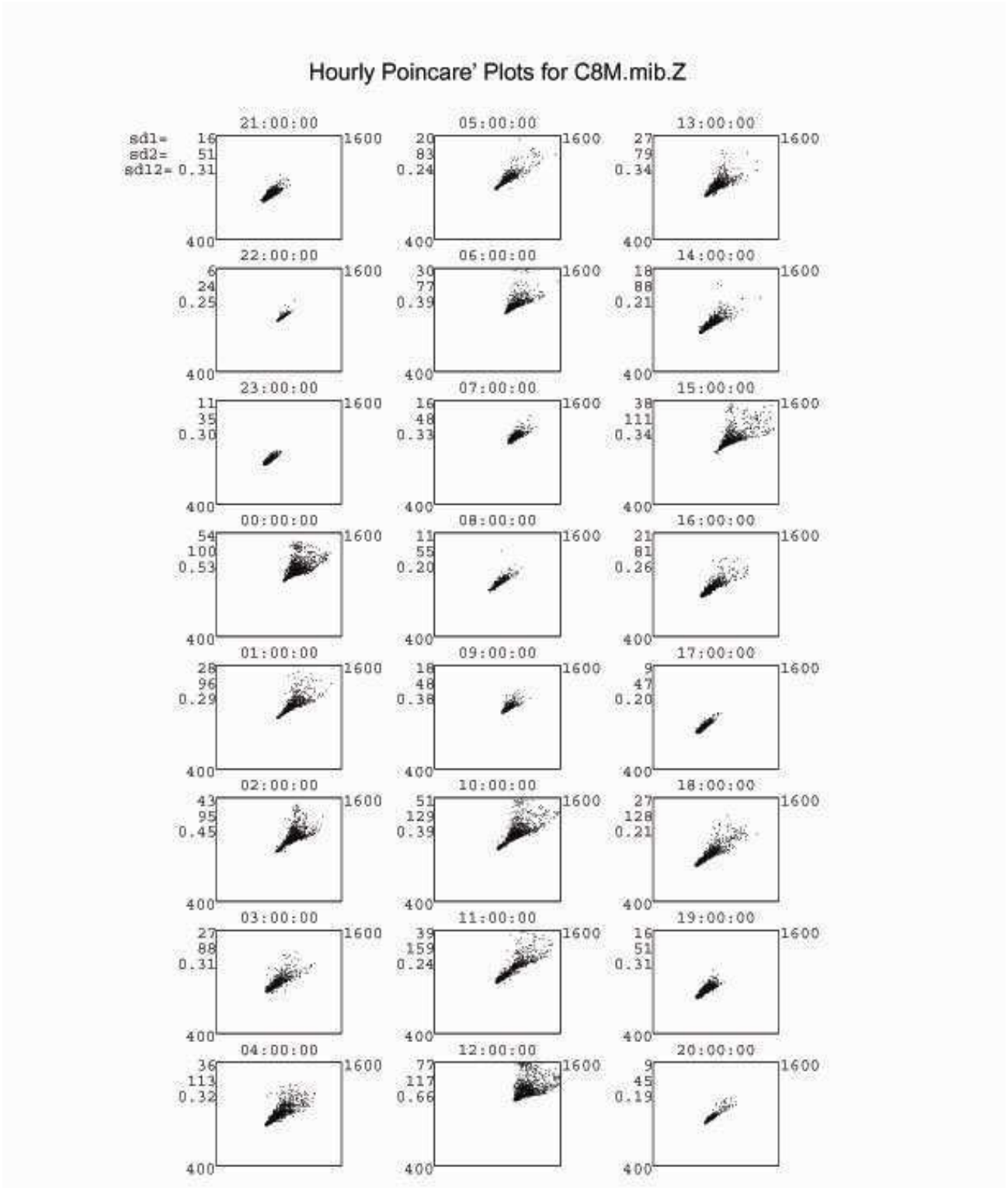

Figure 3. Example of 24-hourly 36-minute Poincaré plots of an abnormal, PGC1α knockout mouse. The beat-to-beat plots were based on a heart rate/12 scale in order to show underlying organization. Many of the plots show fan-shaped, complex heart rate patterns.

Beat-to-beat files were exported, and the interbeat intervals multiplied by 12 to restore the original heart rates. Poincaré plots were generated from the human scale HRs, however. Prevalence of complex patterns was compared between cases and controls.

3. Results

Compared to normal mice, knockout mice tended to have lower average heart rates (695 ± 16 vs. 752 ± 51 bpm, p=0.078) and significantly lower maximum heart rates (778 ± 14 *vs*.844 ± 25 bpm, p=0.002) consistent with their decreased ability to use glucose in the heart.

Examination of the Poincaré plots showed that none of the wild-type mice had an abnormal, fan-like, complex pattern in any hour of their recording, although one mouse had small plots reflecting low HRV. However, 3 of the 4 knockout mice had a complex pattern in at least 10 hours of the recording. Figure 2 shows the hourly Poincaré plots in a normal wild mouse. All of the plots are ellipsoid or comet-shaped. The complex heart rate pattern in a PGC1α knockout mouse is clearly visible at several time points in Figure 3. Notably, not all of the hours are abnormal and a single sample could easily have missed the abnormal periods.

Despite these visible differences in heart rate patterns, standard time and frequency domain HRV, calculated for each 3-minute segment, were averaged for each mouse and were not found to be different between these groups.

4. Discussion and conclusions

Results suggest that analysis of heart rate patterns using Poincaré plots can reveal abnormalities in cardiac function in knockout mice that are not apparent from ordinary HRV analysis. This suggests the importance of including Poincaré plot type analysis in HRV studies.

Results further suggest that a brief sample ECG, as frequently obtained in mouse and other studies may not be adequate to characterize HRV in a meaningful way. Accurate comparisons of HRV between knockout and wild type mice appears to require samples from different time periods (possibly representing periods of activity and rest). Furthermore, rescaling of the ECG signals to human heart rate ranges provides a feasible way to load them onto a commercial Holter scanner and permits accurate characterization of the interbeat intervals.

Finally, it is of interest to note that mouse Poincaré plots using heart rates rescaled to human ranges show patterns that are indistinguishable from those seen in humans verifying a similar structure despite markedly higher actual heart rates.

We conclude that Poincaré plot analysis of heart rate patterns in mice using standard human Holter scanning techniques is feasible and could provide important markers for cardiac abnormalities in various knockout models.

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