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Diurnal Differences in Risk of Cardiac Arrhythmias during Spontaneous Hypoglycemia in Young People with Type 1 Diabetes

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ABSTRACT

Objective

Hypoglycemia exerts proarrhythmogenic effect on the heart via sympathoadrenal stimulation and hypokalemia. Hypoglycemia-induced cardiac dysrhythmias are linked to the 'Dead in bed' syndrome, a rare, but devastating event. We examined the effect of nocturnal and daytime clinical hypoglycemia on the electrocardiogram in young people with type 1 diabetes.

Research Design and Methods

Thirty-seven individuals with type 1 diabetes underwent 96 hours of simultaneous ambulatory electrocardiogram (ECG) and blinded continuous interstitial glucose monitoring (CGM) while recording symptomatic hypoglycemia. Frequency of arrhythmias, heart rate variability and cardiac repolarization were measured during hypoglycemia and compared to time-matched euglycemia separately at night and day.

Results

A total of 2395 hours of simultaneous ECG and CGM recordings were obtained; 159 hours were designated hypoglycemia and 1355 hours euglycemia. Median (interquartile range) duration of nocturnal hypoglycemia 60 (40 – 135) min was longer than daytime hypoglycemia 44 (30 – 70) min ($p = 0.020$). Only 24.1% of nocturnal and 51.0% of daytime episodes were symptomatic. Bradycardia was more frequent during nocturnal hypoglycemia compared to matched euglycemia (Incident rate ratio (IRR) 6.44 [95% CI 6.26 – 6.66], $p < 0.001$). During daytime hypoglycemia, bradycardia was less frequent (IRR 0.023 [95% CI 0.002 – 0.26], $p = 0.002$) and atrial ectopics more frequent (IRR 2.29 [95% CI 1.19 – 4.39], $p = 0.013$) compared to euglycemia. Prolonged rate-corrected QT (QTc), TpTend interval and decreased T-wave symmetry were detected during nocturnal and daytime hypoglycemia.

Conclusions

We have identified differences in arrhythmic risk and cardiac repolarization during nocturnal versus daytime hypoglycemia in young adults with type 1 diabetes. Asymptomatic hypoglycemia was common and our data support the concept of hypoglycemia as a proarrhytmogenic state.

Hypoglycemia is an inevitable consequence of the current management of type 1 diabetes (1). Improved glycemic control is frequently accompanied by an increased risk of inducing iatrogenic hypoglycemia (2). Observational studies indicate that rates of severe hypoglycemia have generally not fallen despite the introduction of insulin analogues and advanced methods of glucose monitoring (3, 4). Hypoglycemia thus continues to be a major limiting factor in the management of type 1 diabetes.

It has been nearly three decades since Tatersall and Gill published their original report describing 22 nocturnal deaths of young people with type 1 diabetes (5). The 'dead in bed syndrome' was characterized by young and otherwise healthy individuals with type 1 diabetes who had retired to bed in good health but were found dead in undisturbed beds on the following day. Subsequent autopsies could not establish a cause of death. Similar reports from other parts of the world have continued to appear in the literature (6-8).

Nocturnal hypoglycemia has been consistently implicated, yet the precise mechanism remains unclear. A case report involving continuous interstitial glucose monitoring (CGM) has confirmed that hypoglycemia was present at the time of death (9). Our group and others have reported QT interval prolongation during both experimental hypoglycemia in healthy individuals (10) and people with diabetes (11) as well as during clinical episodes (12). Abnormal cardiac repolarization during hypoglycemia appears to be mediated by both direct effect of sympathoadrenal stimulation and catecholamine and insulin-induced hypokalemia on cardiac ion channels (13). Hypoglycemia is increasingly recognized as a potential proarrhythmic event (14), but direct evidence linking electrocardiographic changes and the 'dead in bed' syndrome is missing (15).

Given the fact that nocturnal hypoglycemia is very common and sudden deaths in type 1 diabetes are rare (6, 16) an interplay of several factors such as overt or undetected autonomic neuropathy, genetic contribution or abnormally intensive sympathoadrenal response are likely to contribute (14). There is, however, considerable uncertainty in the way and extent in which

these factors contribute to the development of the presumed malignant disturbances of cardiac rhythm (6, 17, 18). Presumably only under certain circumstances a combination of these and perhaps other factors can lead to an unfavorable course of events resulting in a fatal outcome.

One of the defining characteristics of the 'dead in bed' syndrome is that it seems to occur only at night. A physiological diurnal variability in the autonomic tone with reduction in sympathetic tone and consequent relative increase in parasympathetic activity during sleep has been well documented (19). Diminished epinephrine responses to experimental hypoglycemia during sleep in comparison to daytime hypoglycemia have been reported in people with type 1 diabetes and healthy individuals (20). Additionally, we have recently reported differences in increased susceptibility to cardiac arrhythmias, heart rate variability (HRV) and cardiac repolarization during nocturnal and daytime hypoglycemia in people with type 2 diabetes with increased cardiovascular risk in a study design similar to the currently presented report (21). We have hypothesized that similar diurnal differences in cardiac electrophysiological responses to hypoglycemia may exist in young people with type 1 diabetes and if so, could help to elucidate the pathophysiology of the 'dead in bed' syndrome.

The aim of this study was to examine the effect of clinical hypoglycemia in young people (≤ 50 years of age) with type 1 diabetes – as compared to matched euglycemia - on the frequency of cardiac arrhythmias, HRV and cardiac repolarization. We particularly sought to compare differences between nocturnal and daytime hypoglycemia given the above described diurnal differences in sympathetic adrenomedullary responses to hypoglycemia.

RESEARCH DESIGN AND METHODS

Thirty–seven individuals with type 1 diabetes below the age of 50 years and with duration of disease for at least 4 years were recruited from Sheffield Teaching Hospitals outpatient clinics. Participants taking beta-blocking and QT-interval prolonging agents were excluded.

Baseline 12-lead electrocardiogram (ECG) was performed prior to further testing and participants with bundle branch block or atrial fibrillation were excluded. We also excluded participants with diabetic maculopathy and severe visual impairment as well as participants with estimated glomerular filtration rate (eGFR) < 30 ml/min/1.73m^2. Data on presence and severity of diabetic microvascular complications were obtained from local electronic patient database. Written informed consent was obtained from all participants and the study received local ethics approval.

Baseline Assessment

Urea, electrolytes and glycated haemoglobin A_{1c} (HBA_{1c}) were measured at the onset of the monitoring period. HBA_{1c} was established using ion-exchange high-performance liquid chromatography. Cardiovascular autonomic tests were performed at the onset of the monitoring period in accordance with a recently published consensus statement (22). ECG signals were acquired using a g.USBamp biosignal amplifier unit together with the g.Recorder software (g.tec Medical Engineering GMBH, Schiedlberg, Austria) and analysis was undertaken using custom software written in MATLAB (MathWorks, Natick, Massachusetts, USA). Participants were instructed to avoid vigorous exercise, caffeine and smoking 12 hours prior to morning testing. Participants were classified as possible cardiac autonomic neuropathy (CAN) when one cardioreflex test was below the age-adjusted reference range and definite CAN when two or more cardioreflex tests were below the age-adjusted reference range (22, 23). Hypoglycemia awareness was assessed using a visual analogue Likert-type scale of 1 to 7, as previously described (24).

Monitoring

All participants underwent 96 hours of simultaneous 12-lead Holter ECG and CGM monitoring. Participants carried on with their usual daily activities and diabetes management. Twelve-lead high-frequency ambulatory ECGs (Mortara H12+; Mortara Instrument Inc.

Milwaukee, Wisconsin, USA) were recorded at a 1000 Hz sampling rate with electrodes in a Mason-Likar configuration. Participants also had a time-synchronized CGM monitoring system attached (FreeStyle Navigator II, Abbott Diabetes Care, Maidenhead, UK). Calibrations were performed at least five times during the study period according to the manufacturer`s instructions. Mindful of the potential limitations of the CGM, we selected a system that with the lowest detection limit of 1.1 mmol/L (20 mg/dL) and with acceptable mean absolute differences between the interstitial glucose (IG) values and actual blood glucose levels (25). Predictive alarms were switched off and participants were blinded to the real-time IG levels by disabling the display of IG values on the device. Participants were asked to keep a record of any symptomatic hypoglycemia in provided diaries. Any hypoglycemia episode, as defined in the next paragraph, without simultaneous self-report of symptoms in the diary was regarded as asymptomatic.

CGM Analysis

The IG was measured every minute by the CGM, and 10-min averages were reported (CoPilot Health Management; Abbott Diabetes Care). Hypoglycemia was defined as IG $\leq$ 3.5 mmol/L in accordance with previously published studies (21, 26). Euglycemia was defined as an IG value between 5 and 10 mmol/L. We defined a valid hypoglycemic episode as a period of IG below the threshold for $\geq$ 20 min (27). The first reading of IG $\leq$ 3.5 mmol/L marked the start of the hypoglycemia, and the first reading of IG $\geq$ 3.5 mmol/L signified the end of the episode. The lowest IG within the hypoglycemic episode was designated the hypoglycemia nadir and was matched with a euglycemic time point from the same individual at the same time (within 20 min) on a different day.

Arrhythmia Analysis

The 12-lead Holter ECG data were reviewed using H-Scribe 4.34 software (Mortara Instrument Inc.). The software automatically separated normal ECG traces from artifacts and

detected arrhythmic events according to predetermined event definitions. These included atrial ectopic beats (prematurity threshold 30%), bradycardia (consecutive beats at rate <45 bpm for >5 seconds), single ventricular premature beats (VPBs), complex VPBs (VPB couplets and runs) and total VPBs (sum of all VPBs). All identified arrhythmic events were manually verified for accuracy and modified if needed. Investigators were blinded to glucose values during arrhythmia identification. Hourly counts for each type of arrhythmia were provided by H-Scribe 4.34 software and were paired with hourly mean IG, which was categorized into hypoglycemia (mean hourly IG value ≤ 3.5 mmol/L) and euglycemia (mean hourly IG value between 5 and 10 mmol/L). Analyses were separated into day and night (23.00 – 07.00 h) to take into account diurnal variation.

Heart Rate Variability Analysis

R-R intervals were calculated from annotated normal beats (NN intervals), which were identified by the H-Scribe 4.34 software. A 5-min segment of successive NN intervals, centered on the nadir IG value was selected and spectral analysis was performed using Fourier transformation. Spectral analysis was performed in accordance with recommendations of the Taskforce on Heart Rate Variability (28). The low-frequency (LF) band was defined as 0.04 –0.15 Hertz (Hz) and high-frequency (HF) band as 0.15 – 0.4 Hz. The ratio between the LF power and total power (LF + HF power) was calculated (LFnorm). HF power reflects parasympathetic activity. LFnorm was previously suggested to indicate the level of sympathetic modulation in HRV (29, 30).

Repolarization Analysis

Repolarization analysis and detection of QT interval duration was performed using custom-built, semiautomatic software based on a selective beat averaging approach (31). Cubic spline interpolation and 40Hz low-pass filtering were applied to ECG leads. Average beats were calculated from stable normal beats within 5-min segments, centered on each IG value.

Analysis was performed on a composite wave, which was calculated by combining averaged beats derived from leads I, II, and V5 (31). The onset of the Q wave was marked as the first positive deflection from the isoelectric line > 10 microvolts. The end of the T wave was determined using the tangent method, where the tangent to the steepest downslope of the T wave crosses the isoelectric line. All fiducial points were reviewed and adjusted if necessary by an observer blinded to glucose values. QT intervals were corrected for heart rate (QTc) using Bazett's formula (32). Cardiac repolarization was characterized by calculating rate-independent parameters, including T-peak to T-end interval duration (TpTend) and symmetry of the T wave (Tsym) (33). All parameters, including HRV indices were compared at hypoglycemia nadir vs matching euglycemia as described in the next paragraph.

Statistical Analysis

This was an observational study, and thus no power calculations were performed. The numbers chosen were based upon an assessment of the number of patients it was possible to examine given the constraints on recruitment and projected hypoglycemia rates. Data were inspected for normality. Data that followed an approximate normal distribution were summarized using mean ± SD, while skewed data were summarized using the median (IQR). Median duration and median nadir values of nocturnal and daytime hypoglycemic episodes were compared using the Mann-Whitney *U* test. The generalized estimated equations approach was used to investigate the effect of glycemic status on arrhythmia counts while taking into account correlated measurements from individuals who experienced more than one episode of hypoglycemia or hyperglycemia. Data were fitted with a negative binomial model with the assumption that rates for individuals came from a distribution with a mixed but non-zero variance. This allows modelling of variables that are over-dispersed (i.e. where the sample variance exceeds the sample mean) relative to a Poisson model which is usually used in analyzing count data. A first-order autoregressive correlation structure was applied to adjust for within-individual correlation. Exponentiated regression coefficients represent

incident rate ratios (IRRs). The IRRs of arrhythmias during hypoglycemia compared with euglycemia were calculated adjusting for the longer period participants were at euglycemic levels compared to the period spent in hypoglycemia . HRV, corrected QT intervals (QTc) and repolarization parameters were compared at the hypoglycemia nadir against an equivalent euglycemic time point on a different day. Where there was more than one matching hypoglycemic-euglycemic episode in an individual participant over the course of the recording period, the mean values from all daytime and nocturnal episodes from that individual were reported. Data were compared using a paired *t*-test or the Mann-Whitney *U* test. Statistical analysis was performed with SPSS (version 22; IBM, Chicago, Illinois, USA). A p value ≤ 0.05 was deemed statistically significant.

RESULTS

Participant Characteristics

A total of 3165 hours of interstitial glucose (IG) recordings and 2395 hours of valid simultaneous ECG and IG recordings were obtained from 37 participants. 159 hours of IG data were recorded in the hypoglycemic range out of which 88 hours were recorded during the night and 71 hours during the day. Out of 1355 hours of euglycemia, 506 hours were recorded during the night and 849 hours during daytime (Supplementary Figure 1). Baseline participant characteristics are shown in Table 1. Of 37 participants, 32 (86.5%) experienced at least one episode of hypoglycemia; 23 (62.2%) experienced at least one episode of nocturnal hypoglycemia and 28 (75.7%) experienced at least one episode of daytime hypoglycemia.

Nocturnal and Daytime Hypoglycemia Characteristics

Altogether 44 nocturnal and 69 daytime hypoglycemic episodes were analyzed. There were diurnal differences in the duration of hypoglycemic episodes (Table 2). The median (IQR)

duration of nocturnal hypoglycemic episodes was 60 (40 – 135) min significantly longer than the duration of daytime hypoglycemic episodes 44 (30 – 70) min (p = 0.020). There was a trend towards lower glucose at nadir of nocturnal hypoglycemia, median (IQR) 2.66 (1.89 – 3.14) mmol/L in comparison to nadir of daytime hypoglycemia 3.00 (2.44 – 3.22) mmol/L, but the difference did not reach statistical significance (p = 0.116).

Symptomatic versus Asymptomatic Hypoglycemia Episodes

Participants were provided with diaries to keep record of any symptomatic hypoglycemia for the duration of the monitoring period. The return rate of the diaries was 26/37 (70.3%). Out of 29 nocturnal hypoglycemia episodes detected by CGM in these participants, only 7 were reported symptomatic (24.1%), and out of 49 daytime hypoglycemia episodes 25 were reported symptomatic (51.0%). We did not detect any significant differences in relation to duration or nadir values between symptomatic and asymptomatic hypoglycemia episodes. The median (IQR) duration of nocturnal symptomatic hypoglycemic episodes was 60 (50 – 90) min with a nadir glucose 2.77 (2.55 – 3.27) mmol/L. The median (IQR) duration of nocturnal asymptomatic hypoglycemic episodes was 45 (20 – 113) min with a nadir value 2.77 (1.79 – 3.20) mmol/L. During the day, the median (IQR) duration of symptomatic hypoglycemic episodes was 50 (30 – 70) min and of asymptomatic episodes 40 (23 – 70) min. Combining daytime and nightime hypoglycemic episodes, there was a trend towards lower nadir values during symptomatic 2.83 (2.28 – 3.13) mmol/L versus asymptomatic episodes 3.13 (2.62 – 3.33) mmol/L (p = 0.055).

Arrhythmias during Nocturnal and Daytime Hypoglycemia

We compared total and relative frequencies of distinct types of arrhythmias during hypoglycemia versus euglycemia during night and day. Total frequencies of arrhythmias were low (Supplementary Table 1). However, comparison of relative frequencies showed several differences between hypoglycemia and euglycemia and between nocturnal and

daytime hypoglycemia, respectively (Figure 1). Bradycardia was more than six-fold higher during nocturnal hypoglycemia compared with nocturnal euglycemia (Incident rate ratio IRR 6.44 [95% CI 6.26 – 6.66], $p < 0.001$). On the contrary, bradycardia was significantly less frequent during daytime hypoglycemia compared with daytime euglycemia (IRR 0.023 [95% CI 0.002 – 0.26], $p = 0.002$). The median duration (min-max) of nocturnal bradycardic episodes was 5 (5-23) seconds. The median duration (min-max) of daytime bradycardic episodes was 5 (5-786) seconds. During daytime hypoglycemia frequency of atrial ectopics was more than two-fold higher in comparison to euglycemia (IRR 2.29 [95% CI 1.19 – 4.39], $p = 0.013$). We did not detect any significant differences in frequencies of ventricular arrhythmias during nocturnal or daytime hypoglycemia in comparison to euglycemia (Figure 1).

Heart Rate Variability

In relation to HRV and cardiac repolarization characteristics we analyzed 45 nocturnal hypoglycemic episodes in 21 participants and 59 daytime hypoglycemic episodes in 24 participants and compared them with time-matched euglycemia. Episodes to which at least one time-matched euglycemic episode could not be found were not included.

HRV parameters at nadir of nocturnal and daytime hypoglycemia were compared with matched euglycemic episodes. Cardio-acceleration was detected during daytime hypoglycemia (mean heart rate (HR) 80 ± 14 bpm) compared with matched euglycemia (mean HR 77 ± 12 bpm); mean paired difference (95%CI) 3.5 (0.5 – 6.4), $p = 0.022$, which was accompanied by a trend towards lower HF power (p = 0.106) and RMSSD (p = 0.078). Low frequency power (logLF) was significantly lower during daytime hypoglycemia (2.82 ± 0.31) in comparison to euglycemia (2.90 ± 0.27); mean paired difference (95%CI) -0.077 (-0.151 – [-0.004]), p = 0.04. No differences in heart rate and parameters of HRV were detected during nocturnal hypoglycemia compared with matched euglycemia (Table 3).

Cardiac Repolarization

Mean QTc was prolonged during nocturnal hypoglycemia (405 ± 27 ms) compared with euglycemia (400 ± 22 ms); mean paired difference (95% CI) 5.4 (0.5 – 10.3) ms, $p = 0.031$ as well as during daytime hypoglycemia (413 ± 30 ms) compared with euglycemia (401 ± 29 ms); mean paired difference (95% CI) 11.7 (5.7 – 17.6) ms, $p < 0.001$ (Table 3). TpTend was prolonged during nocturnal hypoglycemia (71 ± 9 ms) compared with matched euglycemia (67 ± 7 ms); mean paired difference (95% CI) 4.95 (2.76 – 7.14) ms, $p < 0.001$, with corresponding decrease in T wave symmetry: Tsym (1.64 ± 0.41 vs. 1.77 ± 0.27); mean paired difference (95% CI) -0.13 (-2.23 – [-0.04]), $p = 0.007$. TpTend was also prolonged during daytime hypoglycemia (74 ± 12 ms) compared with matched euglycemia (69 ± 12 ms), mean paired difference (95% CI) 5.41 (2.53 – 8.30), $p < 0.001$, with corresponding decrease in Tsym (1.42 ± 0.35 vs. 1.54 ± 0.34), mean paired difference (95% CI) -0.12 (-0.20 – [-0.04]), $p = 0.002$) (Table 3).

We examined changes in HRV and cardiac repolarization parameters during a 440 min long asymptomatic nocturnal hypoglycemic episode in a 29-year old male participant with CAN (Supplementary Figure 2, panels A-G). This was the only nocturnal hypoglycemic episode recorded in a participant with CAN and also one of the longest of all episodes. Interestingly, cardiac repolarization parameters during this episode show opposite trends than the mean values across all episodes as described in the previous paragraph. QTc and TpTend tend to decrease over the duration of the hypoglycemia (panels E and F), along with gradually increasing Tsym (panel G). Only 1 nocturnal and 2 daytime hypoglycemia episodes in 3 participants with confirmed CAN were identified, which precludes any further statistical analysis of the above parameters.

CONCLUSIONS

To the best of our knowledge this is the largest observational study to date examining the effect of spontaneous hypoglycemia in young people with type 1 diabetes on frequency of

cardiac arrhythmias, heart rate variability (HRV) and cardiac repolarization and the first study that directly compares diurnal differences in these characteristics. We confirmed that hypoglycemia and particularly asymptomatic hypoglycemia continues to occur frequently in young people with type 1 diabetes. We also observed several differences in cardiac electrophysiological responses to hypoglycemia between day and night. We saw increased risk of bradycardia during nocturnal hypoglycemia whereas during daytime hypoglycemia, bradycardia was significantly lower and frequency of atrial ectopics significantly higher in comparison to euglycemia. Cardio-acceleration was detected during daytime hypoglycemia but not during nocturnal hypoglycemia. Lastly, we confirmed a pro-arrhythmogenic effect of hypoglycemia by showing significant prolongation of QTc interval and TpTend interval together with a change towards abnormal, symmetric shape of the T wave both during night and day.

One of the main objectives of this study was to explore the different effects of nocturnal and daytime hypoglycemia on frequency of cardiac arrhythmias, HRV and cardiac repolarization. Factors which might affect these responses include diurnal variability in autonomic tone (19), different sympathoadrenal response to hypoglycemia when awake or asleep (20) and the effect of body position (34). Previous studies in this field have either compared measurements of cardiac repolarization during hypoglycemia to those at euglycemia immediately before hypoglycemia (26) or averaged HRV and repolarization across the total duration of episodes (35). We chose to compare measurements at glucose nadir. In this way we hoped to avoid a bias due to a variable length of episodes (the range of the duration ranged from 20 to 460 minutes) as well as averaging out the changes during phasic responses in episodes of long duration. We also controlled robustly for diurnal increases in arrhythmic risk by comparing changes during a matched period of euglycemia at the same time on a different day.

However pre-defining a hypoglycaemic episode precisely imposes its own limitation. By only counting hypoglycemic episodes that lasted at least 20 min, we probably underestimated the amount of hypoglycaemia. Nevertheless since we defined euglycemia as a glucose concentration between 5-10 mmol/l, we are confident that periods defined as euglycemia (to control for circadian influences on arrhythmias) were calculated accurately. A further limitation was the 70% return rate of questionnaires used to identify symptomatic responses. While this probably represents a reasonably good return in an observational clinical study our estimates of symptomatic and asymptomatic hypoglycaemia must be regarded with some uncertainty.

Absolute numbers of arrhythmias in the studied population were low meaning that we were unable to measure the changes in sub-groups, such as those with CAN. The majority of cardiac arrhythmias occurred during euglycemia, since periods of euglycemia were more frequent and long-lasting compared to hypoglycemia. Relative risks for distinct types of arrhythmias during hypoglycemia versus euglycemia are reflected by presented incident rate ratios (IRRs). The applied statistical model was used to adjust for uneven distribution of hypoglycemic episodes and cardiac arrhythmias among studied individuals as well as to adjust for individuals with no episodes and those who experienced multiple episodes of hypoglycemia. However, arrhythmia incident rate ratios are weighted by data from individuals who are more prone to arrhythmias and the findings need to be confirmed by studies in different populations.

The QT interval duration varies according to heart rate and different formulas are used to correct for heart rate, of which Bazett`s correction is the most commonly used. The Bazett`s formula corrects intervals to a rate of 60 bpm and tends to result in overcorrection at high heart rates and under-correction at lower heart rates. Nevertheless, it is generally considered a reasonably accurate correction in the setting of hypoglycemia (36). In our study we detected a mean QTc prolongation of 5 ms during nocturnal hypoglycemia and 12 ms during

daytime hypoglycemia in comparison to matched euglycemia. Despite being smaller than the differences in QTc duration measured during experimental hypoglycemia (attributed to lower insulin levels and sympathoadrenal responses during spontaneous compared to experimental episodes) (12, 18), the comparison with mean QTc duration during matched euglycemia reached statistical significance ($p < 0.001$). QTc prolongation of this scale are probably clinically relevant, given that the current FDA recommendations for testing the effects of new agents on QT/QTc interval prolongation indicate a difference in QT/QTc of 5 ms or larger as a reason for regulatory concern (37).

In the light of the potential limitations of heart rate correction, we also included measurements of rate-independent indicators of cardiac repolarization: T-peak to T-end interval duration (TpTend) and symmetry of the T wave (Tsym) (33). TpTend is a measure of dispersion of repolarization in the left ventricle; its prolongation is considered to represent a period of potential vulnerability to re-entrant ventricular dysrhythmias and is associated with increased risk of sudden cardiac death (38). We detected significant prolongation of TpTend in both nocturnal and daytime hypoglycemia. Symmetrical T waves can be found in various pathological states and computational models show that T waves become more symmetrical with an increase in the dispersion of repolarization (39). In our study T waves did not become totally symmetric (Tsym = 1) but we found a consistent change towards more symmetrical shape during hypoglycemia both during day and night.

Cardio-acceleration and a trend towards vagal withdrawal were observed during daytime hypoglycemia together with greater increase in QTc. In contrast, during nocturnal hypoglycemia no changes in HRV were detected and QTc increases were smaller. We reported similar findings in an ambulatory study in type 2 diabetes (21). One potential mechanism is a predominance of sympathoadrenal response during the day compared to the night where sympathoadrenal activation may be suppressed by prone posture and during sleep (21).

In contrast to differences in the magnitude of QTc prolongation, the observed changes in rate-independent characteristics of cardiac repolarization (TpTend, Tsym) during daytime and nighttime hypoglycemia are rather similar. This suggests that during hypoglycemia, cardiac repolarization is influenced by factors additional to cardiac autonomic responses, perhaps hypokalemia or hypoglycemia *per se*. In this observational study no direct measurements of catecholamine or potassium levels were possible.

Increased frequency of bradycardias during nocturnal hypoglycemia in young people with type 1 diabetes might be clinically relevant given the fact that bradycardia may lead to early afterdepolarizations (EADs) via increased intracellular Ca^{2+} concentration in cardiomyocytes (40). EADs represent one of the most important arrhythmogenic mechanisms promoting arrhythmias in acquired and congenital long QT syndromes including torsade des pointes, polymorphic ventricular tachycardia and ventricular fibrillation (40). Additionally, diabetes and hypoglycemia *per se* are linked with increased incidence of EADs independent of heart rate (14). Nocturnal hypoglycemia with concomitant bradycardia in people with diabetes thus represents an event with increased proarrhythmogenic potential. We have previously reported an increased risk of bradycardias during spontaneous nocturnal hypoglycemia in people with type 2 diabetes at increased cardiovascular risk (21). The findings in the present study suggest that this hypoglycemia-induced mechanism is independent of the type of diabetes, age or cardiovascular risk profile. Importantly, our observations that certain types of arrhythmia (for example, bradycardia) were confined to just a few subjects suggest that susceptibility to arrhythmias during hypoglycaemia is highly individual. The increasing use of CGM as a clinical rather than a research tool might permit screening of individuals with type 1 diabetes to identify those at high arrhythmic risk.

In summary, we have shown a contrast in the frequencies of arrhythmias and cardiac electrophysiological responses during nocturnal compared to daytime hypoglycemia. We confirm a high frequency of hypoglycemia, particularly of nocturnal asymptomatic episodes

among young people with type 1 diabetes and our data add to the body of evidence suggesting that hypoglycemia is pro-arrhythmogenic.

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AUTHOR CONTRIBUTIONS

*PN and AB contributed equally to the present study. P.N. collected and analyzed the data and wrote the manuscript. A.B. developed the methodology and software, analyzed the data and reviewed the manuscript. E.C. collected the data and reviewed the manuscript. A.I. contributed to the discussion and reviewed the manuscript. L.S., S.W and R.A.F. collected the data. B.P contributed to the discussion. R.J provided statistical support and reviewed the manuscript. J.L.B.M. reviewed the manuscript and contributed to the discussion. P.J.S. and S.R.H. designed the study, reviewed the data and reviewed/edited the manuscript. P.N. is the guarantor of this work and, as such, had full access to all the data in the study and takes responsibility for the integrity of the data and accuracy of the data analysis.

CONFLICT OF INTEREST STATEMENT

S.R.H. received research grants from Medtronic UK Ltd. He has served on speaker panels for Sanofi Aventis, Eli Lilly, Takeda, NovoNordisk and Astra Zeneca for which he has received remuneration. He has served on advisory panels or as a consultant for Boeringher Ingelheim, NovoNordisk, Eli Lilly and Takeda for which his institution has received remuneration. All other authors of this work have no relevant conflict of interest to disclose.

PRIOR PRESENTATION

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REFERENCES

1. 5. Glycemic Targets. Diabetes Care. 2016 Jan;39 Suppl 1:S39-46.
2. Cryer PE. Glycemic goals in diabetes: trade-off between glycemic control and iatrogenic hypoglycemia. Diabetes. 2014 Jul;63(7):2188-95.
3. MacLeod KM, Hepburn DA, Frier BM. Frequency and morbidity of severe hypoglycaemia in insulin-treated diabetic patients. Diabet Med. 1993 Apr;10(3):238-45.
4. Kristensen PL, Hansen LS, Jespersen MJ, Pedersen-Bjergaard U, Beck-Nielsen H, Christiansen JS, Norgaard K, Perrild H, Parving HH, Thorsteinsson B, Tarnow L. Insulin analogues and severe hypoglycaemia in type 1 diabetes. Diabetes Res Clin Pract. 2012 Apr;96(1):17-23.
5. Tattersall RB, Gill GV. Unexplained deaths of type 1 diabetic patients. Diabet Med. 1991 Jan;8(1):49-58.
6. Tu E, Twigg SM, Duflou J, Semsarian C. Causes of death in young Australians with type 1 diabetes: a review of coronial postmortem examinations. Med J Aust. 2008 Jun 16;188(12):699-702.
7. Dahlquist G, Kallen B. Mortality in childhood-onset type 1 diabetes: a population-based study. Diabetes Care. 2005 Oct;28(10):2384-7.
8. Thordarson H, Sovik O. Dead in bed syndrome in young diabetic patients in Norway. Diabet Med. 1995 Sep;12(9):782-7.
9. Tanenberg RJ, Newton CA, Drake AJ. Confirmation of hypoglycemia in the "dead-in-bed" syndrome, as captured by a retrospective continuous glucose monitoring system. Endocr Pract. 2010 Mar-Apr;16(2):244-8.
10. Eckert B, Agardh CD. Hypoglycaemia leads to an increased QT interval in normal men. Clin Physiol. 1998 Nov;18(6):570-5.
11. Marques JL, George E, Peacey SR, Harris ND, Macdonald IA, Cochrane T, Heller SR. Altered ventricular repolarization during hypoglycaemia in patients with diabetes. Diabet Med. 1997 Aug;14(8):648-54.
12. Robinson RT, Harris ND, Ireland RH, Macdonald IA, Heller SR. Changes in cardiac repolarization during clinical episodes of nocturnal hypoglycaemia in adults with Type 1 diabetes. Diabetologia. 2004 Feb;47(2):312-5.
13. Robinson RT, Harris ND, Ireland RH, Lee S, Newman C, Heller SR. Mechanisms of abnormal cardiac repolarization during insulin-induced hypoglycemia. Diabetes. 2003 Jun;52(6):1469-74.
14. Nordin C. The case for hypoglycaemia as a proarrhythmic event: basic and clinical evidence. Diabetologia. 2010 Aug;53(8):1552-61.

15. Heller SR. Abnormalities of the electrocardiogram during hypoglycaemia: the cause of the dead in bed syndrome? Int J Clin Pract Suppl. 2002 Jul(129):27-32.
16. Sovik O, Thordarson H. Dead-in-bed syndrome in young diabetic patients. Diabetes Care. 1999 Mar;22 Suppl 2:B40-2.
17. Weston PJ, Gill GV. Is undetected autonomic dysfunction responsible for sudden death in Type 1 diabetes mellitus? The 'dead in bed' syndrome revisited. Diabet Med. 1999 Aug;16(8):626-31.
18. Lee SP, Yeoh L, Harris ND, Davies CM, Robinson RT, Leathard A, Newman C, Macdonald IA, Heller SR. Influence of autonomic neuropathy on QTc interval lengthening during hypoglycemia in type 1 diabetes. Diabetes. 2004 Jun;53(6):1535-42.
19. Furlan R, Guzzetti S, Crivellaro W, Dassi S, Tinelli M, Baselli G, Cerutti S, Lombardi F, Pagani M, Malliani A. Continuous 24-hour assessment of the neural regulation of systemic arterial pressure and RR variabilities in ambulant subjects. Circulation. 1990 Feb;81(2):537-47.
20. Jones TW, Porter P, Sherwin RS, Davis EA, O'Leary P, Frazer F, Byrne G, Stick S, Tamborlane WV. Decreased epinephrine responses to hypoglycemia during sleep. N Engl J Med. 1998 Jun 4;338(23):1657-62.
21. Chow E, Bernjak A, Williams S, Fawdry RA, Hibbert S, Freeman J, Sheridan PJ, Heller SR. Risk of cardiac arrhythmias during hypoglycemia in patients with type 2 diabetes and cardiovascular risk. Diabetes. 2014 May;63(5):1738-47.
22. Tesfaye S, Boulton AJ, Dyck PJ, Freeman R, Horowitz M, Kempler P, Lauria G, Malik RA, Spallone V, Vinik A, Bernardi L, Valensi P. Diabetic neuropathies: update on definitions, diagnostic criteria, estimation of severity, and treatments. Diabetes Care. 2010 Oct;33(10):2285-93.
23. O'Brien IA, O'Hare P, Corrall RJ. Heart rate variability in healthy subjects: effect of age and the derivation of normal ranges for tests of autonomic function. Br Heart J. 1986 Apr;55(4):348-54.
24. Gold AE, MacLeod KM, Frier BM. Frequency of severe hypoglycemia in patients with type I diabetes with impaired awareness of hypoglycemia. Diabetes Care. 1994 Jul;17(7):697-703.
25. Weinstein RL, Schwartz SL, Brazg RL, Bugler JR, Peyser TA, McGarraugh GV. Accuracy of the 5-day FreeStyle Navigator Continuous Glucose Monitoring System: comparison with frequent laboratory reference measurements. Diabetes Care. 2007 May;30(5):1125-30.
26. Gill GV, Woodward A, Casson IF, Weston PJ. Cardiac arrhythmia and nocturnal hypoglycaemia in type 1 diabetes--the 'dead in bed' syndrome revisited. Diabetologia. 2009 Jan;52(1):42-5.
27. Risk of hypoglycaemia in types 1 and 2 diabetes: effects of treatment modalities and their duration. Diabetologia. 2007 Jun;50(6):1140-7.
28. Heart rate variability: standards of measurement, physiological interpretation and clinical use. Task Force of the European Society of Cardiology and the North American Society of Pacing and Electrophysiology. Circulation. 1996 Mar 1;93(5):1043-65.
29. Bernardi L, Spallone V, Stevens M, Hilsted J, Frontoni S, Pop-Busui R, Ziegler D, Kempler P, Freeman R, Low P, Tesfaye S, Valensi P. Methods of investigation for cardiac autonomic dysfunction in human research studies. Diabetes Metab Res Rev. 2011 Oct;27(7):654-64.
30. Montano N, Ruscone TG, Porta A, Lombardi F, Pagani M, Malliani A. Power spectrum analysis of heart rate variability to assess the changes in sympathovagal balance during graded orthostatic tilt. Circulation. 1994 Oct;90(4):1826-31.
31. Badilini F, Maison-Blanche P, Childers R, Coumel P. QT interval analysis on ambulatory electrocardiogram recordings: a selective beat averaging approach. Med Biol Eng Comput. 1999 Jan;37(1):71-9.
32. Christensen TF, Randlov J, Kristensen LE, Eldrup E, Hejlesen OK, Struijk JJ. QT Measurement and Heart Rate Correction during Hypoglycemia: Is There a Bias? Cardiol Res Pract. 2010;2010:961290.
33. Merri M, Benhorin J, Alberti M, Locati E, Moss AJ. Electrocardiographic quantitation of ventricular repolarization. Circulation. 1989 Nov;80(5):1301-8.
34. Hirsch IB, Heller SR, Cryer PE. Increased symptoms of hypoglycaemia in the standing position in insulin-dependent diabetes mellitus. Clin Sci (Lond). 1991 Jun;80(6):583-6.

35. Koivikko ML, Tulppo MP, Kiviniemi AM, Kallio MA, Perkiomaki JS, Salmela PI, Airaksinen KE, Huikuri HV. Autonomic cardiac regulation during spontaneous nocturnal hypoglycemia in patients with type 1 diabetes. Diabetes Care. 2012 Jul;35(7):1585-90.
36. Christensen TF, Tarnow L, Randlov J, Kristensen LE, Struijk JJ, Eldrup E, Hejlesen OK. QT interval prolongation during spontaneous episodes of hypoglycaemia in type 1 diabetes: the impact of heart rate correction. Diabetologia. 2010 Sep;53(9):2036-41.
37. FDA. E14 Clinical Evaluation of QT/QTc Interval Prolongation and Proarrhythmic Potential for Non-Antiarrhythmic Drugs. 2005; Available from: http://www.fda.gov/downloads/drugs/guidancecomplianceregulatoryinformation/guidances/ucm073153.pdf.
38. Panikkath R, Reinier K, Uy-Evanado A, Teodorescu C, Hattenhauer J, Mariani R, Gunson K, Jui J, Chugh SS. Prolonged Tpeak-to-tend interval on the resting ECG is associated with increased risk of sudden cardiac death. Circ Arrhythm Electrophysiol. 2011 Aug;4(4):441-7.
39. di Bernardo D, Murray A. Explaining the T-wave shape in the ECG. Nature. 2000 Jan 6;403(6765):40.
40. Weiss JN, Garfinkel A, Karagueuzian HS, Chen PS, Qu Z. Early afterdepolarizations and cardiac arrhythmias. Heart Rhythm. 2010 Dec;7(12):1891-9.

Tables

Table 1 – Baseline participant characteristics	
Number of participants , n	37
Age (years)	34 (25.5 – 40.5)
Male, n (%)	19 (51.4%)
Duration of diabetes (years)	19.3 ± 9.6
Insulin regimen	
Basal – prandial (MDI), n (%)	26 (70.3%)
Twice daily biphasic, n (%)	4 (10.8%)
Insulin pump (CSII), n (%)	7 (18.9%)
Insulin type	
Human, n (%)	4 (10.8%)
Analog, n (%)	31 (83.8%)
Human and analog combined, n (%)	2 (5.4%)
HbA_1C	
%	8.1 (7.5 – 8.8)
mmol/mol	65 (58.5 – 73)
BMI (kg/m^2)	25 (22.8 – 27.9)
SBP (mmHg)	123 ± 13
DBP (mmHg)	72 ± 7
Heart rate (bpm)	73 ± 14
Baseline QTc (ms)	417 ± 27
Baseline creatinine (umol/L)	72.5 (55.5 - 89.5)
Baseline potassium (mmol/L)	4.55 (4.05 - 4.95)
GOLD score (1-7) (n=26)	

1	7/26 (26.9%)
2-3	16/26 (61.5%)
≥4	3/26 (11.5%)
CAN status	
possible CAN, n (%)	5 (13.5%)
definitive CAN, n (%)	3 (8.1%)
no CAN, n (%)	29 (78.4%)
Diabetic retinopathy	
R0	9/37 (24.3%)
R1	19/37 (51.4%)
R2	4/37 (10.8%)
R3	5/37 (13.5%)
Diabetic peripheral neuropathy	
No DSPN	36/37 (97.3%)
Possible DSPN	1/37 (2.7%)
Diabetic nephropathy	
Absent	32/37 (86.5%)
Microalbuminuria*	5/37 (13.5%)
Macroalbuminuria†	0/37 (0%)
eGFR	
>90 ml/min/1.73m^2 (CKD1)	24/37 (64.9%)
60-89 ml/min/1.73m^2 (CKD2)	12/37 (32.4%)
30-59 ml/min/1.73m^2 (CKD3)	1/37 (2.7%)

Data are displayed as mean ± SD or median (interquartile range). MDI – multiple daily injections of insulin. CSII – continuous subcutaneous insulin infusion. SBP – systolic blood

pressure, DBP – diastolic blood pressure. CAN – cardiac autonomic neuropathy. DSPN – chronic sensorimotor distal symmetrical polyneuropathy. *Microalbuminuria was defined as albumin/creatinine ratio ≥ 2.5 mg/mmol (men) and ≥ 3.5 mg/mmol (women) on 2 separate measurements at least 6 months apart. †Macroalbuminuria was defined as albumin/creatinine ratio > 30 mg/mmol. eGFR – estimated glomerular filtration rate (CKD-EPI formula). CKD1-3 – chronic kidney disease stages 1-3.

Table 2 - Comparison of nocturnal and daytime hypoglycemic episodes			
	NIGHTTIME	**DAYTIME**	***p***
Episodes, n	44	69	
Participants who experienced at least one episode, n/total (%)	23/37 (62.2%)	28/37 (75.7%)	
Symptomatic episodes, n/total (%)	7/29 (24.1%)	25/49 (51.0%)	
Duration of episodes, median (IQR) (min)	60 (40 – 135)	44 (30 – 70)	0.020
Nadir, median (IQR) (mmol/L)	2.66 (1.89 – 3.14)	3.00 (2.44 – 3.22)	0.116

P values indicate comparison of nocturnal and daytime hypoglycemic episode characteristics via Mann-Whitney *U* test.

Table 3 – Heart rate variability and cardiac repolarization characteristics					
	NIGHTTIME (n=45 episodes)				
	Hypo	**Eu**	**Mean diff.**	**95% CI**	***p***
Heart rate (bpm)	68 ± 12	66 ± 9	1.5	(-1.3, 4.4)	0.285
SD NN (ms)	68.1 ± 42.7	67.8 ± 34.7	0.28	(-12.30, 12.85)	0.965
RMSSD (ms)	33.0 ± 15.1	32.8 ± 14.1	0.21	(-2.70, 3.13)	0.884
log LF	2.74 ± 0.49	2.75 ± 0.44	-0.009	(-0.115, 0.097)	0.871
log HF	2.26 ± 0.48	2.25 ± 0.49	0.006	(-0.082, 0.094)	0.89
LF normalised	0.73 ± 0.14	0.74 ± 0.13	-0.004	(-0.041, 0.033)	0.829
QTc (ms)	405 ± 27	400 ± 22	5.4	(0.5, 10.3)	0.031
TpTend (ms)	71.5 ± 9.4	66.5 ± 7.3	4.95	(2.76, 7.14)	<0.001
TpTend_cB (ms)	75.7 ± 11.9	69.6 ± 7.7	6.06	(3.33, 8.78)	<0.001
Tsym	1.64 ± 0.41	1.77 ± 0.27	-0.132	(-0.226, -0.037)	0.007
	DAYTIME (n=59 episodes)				
	Hypo	**Eu**	**Mean diff.**	**95% CI**	***p***
Heart rate (bpm)	80 ± 14	77 ± 12	3.5	(0.5, 6.4)	0.022
SD NN (ms)	61.1 ± 28.9	61.5 ± 20.8	-0.35	(-7.67, 6.96)	0.923
RMSSD (ms)	27.5 ± 12.8	29.6 ± 12.7	-2.16	(-4.57, 0.25)	0.078
log LF	2.82 ± 0.31	2.90 ± 0.27	-0.077	(-0.151, -0.004)	0.040
log HF	2.18 ± 0.46	2.25 ± 0.42	-0.075	(-0.166, 0.016)	0.106
LF normalised	0.79 ± 0.12	0.80 ± 0.08	-0.009	(-0.034, 0.017)	0.506
QTc (ms)	413 ± 30	401 ± 29	11.7	(5.7, 17.6)	<0.001
TpTend (ms)	74.2 ± 12.3	68.8 ± 12.3	5.41	(2.53, 8.30)	<0.001
TpTend_cB (ms)	84.2 ± 14.9	76.6 ± 15.8	7.61	(3.61, 11.62)	<0.001
Tsym	1.42 ± 0.35	1.54 ± 0.34	-0.121	(-0.197, -0.045)	0.002

Data are displayed as mean ± SD. RMSSD – root mean square of successive differences between adjacent NN intervals. TpTend – T-peak to T-end interval duration. TpTend_cB - TpTend interval corrected for heart rate (Bazett`s formula). Mean diff. – mean paired difference. *P* values indicate comparison of nocturnal and daytime hypoglycemic data via paired *t*-test.

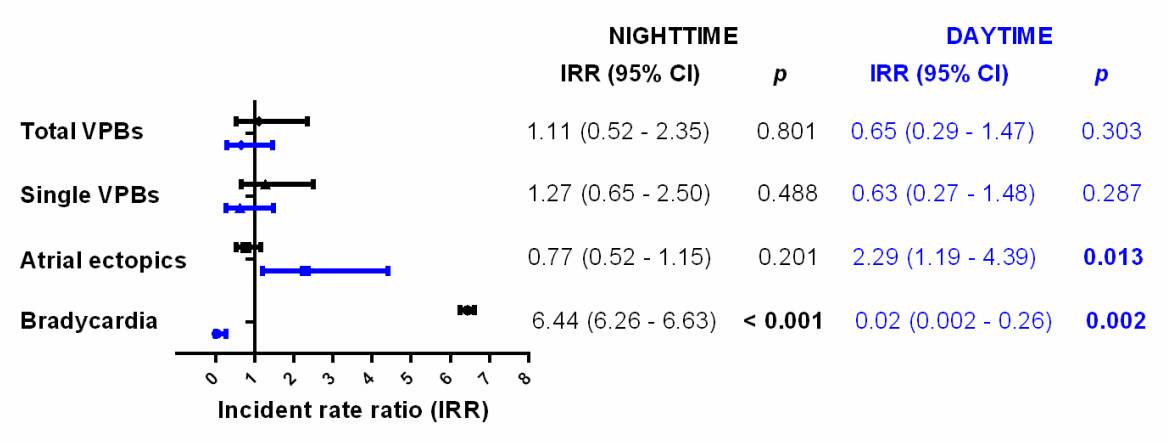

Figure 1 – Incident rate ratios (IRRs) of distinct types of arrhythmias during hypoglycemia vs. euglycemia. Comparison between nocturnal (23.00 – 07.00h) and daytime episodes. No complex ventricular paroxysmal beats (VPBs) were detected during nocturnal hypoglycemia (see also Table 3) and therefore no IRR could be calculated for this type of arrhythmia. *P* – significance of difference in arrhythmia rates during hypoglycemia vs. euglycemia.