Diurnal Differences in Risk of Cardiac Arrhythmias During Spontaneous Hypoglycemia in Young People With Type 1 Diabetes

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OBJECTIVE

Hypoglycemia may exert proarrhythmogenic effects on the heart via sympathoadrenal stimulation and hypokalemia. Hypoglycemia-induced cardiac dysrhythmias are linked to the "dead-in-bed syndrome," a rare, but devastating, condition. We examined the effect of nocturnal and daytime clinical hypoglycemia on electrocardiogram (ECG) in young people with type 1 diabetes.

RESEARCH DESIGN AND METHODS

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

RESULTS

A total of 2,395 h of simultaneous ECG and CGM recordings were obtained; 159 h were designated hypoglycemia and 1,355 h euglycemia. A median duration of nocturnal hypoglycemia 60 min (interquartile range 40–135) was longer than daytime hypoglycemia 44 min (30–70) (P = 0.020). Only 24.1% of nocturnal and 51.0% of daytime episodes were symptomatic. Bradycardia was more frequent during nocturnal hypoglycemia compared with 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). Prolonged QTc, T-peak to T-end interval duration, and decreased T-wave symmetry were detected during nocturnal and daytime hypoglycemia.

CONCLUSIONS

Asymptomatic hypoglycemia was common. We identified differences in arrhythmic risk and cardiac repolarization during nocturnal versus daytime hypoglycemia in young adults with type 1 diabetes. Our data provide further evidence that hypoglycemia is proarrhythmogenic.

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

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analogs 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 Tattersall 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 experimental hypoglycemia in both 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 is likely to contribute (14). There is, however, considerable uncertainty in the way and extent to which these factors contribute to the development of the presumed malignant disturbances of cardiac rhythm (6,17,18). Presumably only under certain circumstances does a combination of these and perhaps other factors 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 with 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 that of 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 explain the pathophysiology of the dead-in-bed syndrome.

The aim of this study was to examine the effect of clinical hypoglycemia in young people ($\leq$50 years of age) with type 1 diabetes—compared with 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 β-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 an estimated glomerular filtration rate (eGFR) $<$30 mL/min/1.73 m^2. Data on presence and severity of diabetic microvascular complications were obtained from a local electronic patient database. Written informed consent was obtained from all participants, and the study received local ethics committee approval (National Research Ethics Service, NRES Committee, East Midlands, Derby, U.K.).

Baseline Assessment

Urea, electrolytes, and glycated hemoglobin 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, MA). Participants were instructed to avoid vigorous exercise, caffeine, and smoking 12 h prior to morning testing. Participants were classified with 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 analog Likert-type scale of 1 to 7 as previously described (24).

Monitoring

All participants underwent 96 h of simultaneous 12-lead Holter ECG and CGM monitoring while continuing their usual daily activities and diabetes management. Twelve-lead high-frequency (HF) ambulatory ECGs (H12+; Mortara Instrument, Milwaukee, WI) were recorded at a 1,000-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, U.K.). 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 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 below,

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 (FreeStyle CoPilot Health Management System; 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 threshold for $\geq$20 min (27). The first reading of IG $\leq$3.5 mmol/L marked the start of 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 HScribe software, version 4.34 (Mortara Instrument). 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 s), 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 HScribe 4.34 software and were paired with hourly mean IG, which was categorized into hypoglycemia (mean hourly IG value $\leq$3.5 mmol/L) and euglycemia (mean hourly IG value between 5 and 10 mmol/L). Analyses were separated into day and night (2300–0700 h) to take into account diurnal variation.

HRV Analysis

R-R intervals were calculated from annotated normal beats (NN intervals), which were identified by the HScribe 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 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 had previously been suggested to indicate the level of sympathetic modulation in HRV (29,30).

Repolarization Analysis

Repolarization analysis and detection of QT interval duration were performed using custom-built, semi-automatic software based on a selective beat averaging approach (31). Cubic spline interpolation and 40-Hz 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 μV. 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 the Bazett 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 versus matching euglycemia as described below.

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 $\pm$ SD, while skewed data were summarized using the median (interquartile range [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 correlated measurements from individuals who experienced more than one episode of hypoglycemia or hyperglycemia were taken into account. Data were fitted with a negative binomial model with the assumption that rates for individuals came from a distribution with a mixed but nonzero variance. This allows modeling of variables that are overdispersed (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 with the period spent in hypoglycemia. HRV, 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, IL). A *P* value $\leq$0.05 was deemed statistically significant.

RESULTS

Participant Characteristics

A total of 3,165 h of IG recordings and 2,395 h of valid simultaneous ECG and IG recordings were obtained from 37 participants. A total of 159 h of IG data were recorded in the hypoglycemic range, out of which 88 h were recorded during the night and 71 h during the day. Out of 1,355 h of euglycemia, 506 h were recorded during the night and 849 h during daytime (Supplementary Fig. 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

Table 1—Baseline participant characteristics

Number of participants	37
Age (years)	34 (25.5–40.5)
Male	19 (51.4)
Duration of diabetes (years)	19.3 ± 9.6
Insulin regimen	
Basal, prandial (MDI)	26 (70.3)
Twice daily biphasic	4 (10.8)
Insulin pump (CSII)	7 (18.9)
Insulin type	
Human	4 (10.8)
Analog	31 (83.8)
Human and analog combined	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 (μmol/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	5 (13.5)
Definitive CAN	3 (8.1)
No CAN	29 (78.4)
Diabetic retinopathy	
No retinopathy	9/37 (24.3)
Background retinopathy	23/37 (62.2)
Treated proliferative retinopathy	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 (mL/min/1.73 m^2)	
>90 (CKD1)	24/37 (64.9)
60–89 (CKD2)	12/37 (32.4)
30–59 (CKD3)	1/37 (2.7)

Data are mean ± SD, median (IQR), *n* (%), or *n*/total (%). eGFR calculated with the Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI) formula. CKD1–3, chronic kidney disease stages 1–3; CSII, continuous subcutaneous insulin infusion; DBP, diastolic blood pressure; DSPN, chronic sensorimotor distal symmetrical polyneuropathy; MDI, multiple daily injections of insulin; SBP, systolic blood pressure. *Microalbuminuria was defined as albumin-to-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-to-creatinine ratio >30 mg/mmol.

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 duration of nocturnal hypoglycemic episodes was 60 min (IQR 40–135), which is significantly longer than the duration of daytime hypoglycemic episodes: 44 min (30–70) (P = 0.020). There was a trend toward lower glucose at nadir of nocturnal hypoglycemia (median 2.66 mmol/L [IQR 1.89–3.14]) in comparison with nadir of daytime hypoglycemia (3.00 mmol/L [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 a record of any symptomatic hypoglycemia for the duration of the monitoring period. The return rate of the diaries was 26 of 37 (70.3%). Of 29 nocturnal hypoglycemia episodes detected by CGM in these participants, only 7 were reported to be symptomatic (24.1%), and of 49 daytime hypoglycemia episodes 25 were reported to be 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 duration of nocturnal symptomatic hypoglycemic episodes was 60 min (IQR 50–90) with a nadir glucose 2.77 mmol/L (2.55–3.27). The median duration of nocturnal asymptomatic hypoglycemic episodes was 45 min (20–113) with a nadir value 2.77 mmol/L (1.79–3.20). During the day, the median duration of symptomatic hypoglycemic episodes was 50 min (30–70) and of asymptomatic episodes 40 min (23–70). With daytime and nighttime hypoglycemic episodes combined, there was a trend toward lower nadir values during symptomatic (median 2.83 mmol/L [IQR 2.28–3.13]) versus asymptomatic (3.13 mmol/L [2.62–3.33]) episodes (P = 0.055).

Arrhythmias During Nocturnal and Daytime Hypoglycemia

We compared total and relative frequencies of distinct types of arrhythmias during hypoglycemia versus euglycemia 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 (Fig. 1). Bradycardia was more than sixfold higher during nocturnal hypoglycemia compared with nocturnal euglycemia (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 (0.023 [0.002, 0.26], P = 0.002). The median duration of nocturnal bradycardic episodes was 5 s (minimum–maximum

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, min, median (IQR)	60 (40–135)	44 (30–70)	0.020
Nadir, mmol/L, median (IQR)	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.

5–23). The median duration of daytime bradycardic episodes was 5 s (5–786). During daytime hypoglycemia, frequency of atrial ectopics was more than twofold higher in comparison with 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 with euglycemia (Fig. 1).

HRV

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 for 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. Cardioacceleration was detected during daytime hypoglycemia (mean heart rate 80 ± 14 bpm) compared with matched euglycemia (mean heart rate 77 ± 12 bpm), with mean paired difference 3.5 (95% CI 0.5, 6.4), $P = 0.022$, which was accompanied by a trend toward lower HF power ($P = 0.106$) and root mean square of successive differences between adjacent NN intervals (RMSSD) ($P = 0.078$). LF power (log LF) was significantly lower during daytime hypoglycemia (2.82 ± 0.31) in comparison with euglycemia (2.90 ± 0.27), with mean paired difference −0.077 (95% CI −0.151, –0.004), $P = 0.04$. No differences in heart rate or 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), with mean paired difference 5.4 ms (95% CI 0.5, 10.3), $P = 0.031$, as well as during daytime hypoglycemia (413 ± 30 ms) compared with euglycemia (401 ± 29 ms), with mean paired difference 11.7 ms (5.7, 17.6), $P < 0.001$ (Table 3). TpTend was prolonged during nocturnal hypoglycemia (71 ± 9 ms) compared with matched euglycemia (67 ± 7 ms), with mean paired difference 4.95 ms (2.76, 7.14), $P < 0.001$, and with corresponding decrease in Tsym: 1.64 ± 0.41 vs. 1.77 ± 0.27, with mean paired difference −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), with mean paired difference 5.41 ms (2.53, 8.30), $P < 0.001$, and with corresponding decrease in Tsym (1.42 ± 0.35 vs. 1.54 ± 0.34), with mean paired difference −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 Fig. 2). 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 from 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 one nocturnal and two daytime hypoglycemia episodes in three 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, 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 an increased risk of bradycardia during nocturnal hypoglycemia, whereas during daytime hypoglycemia, bradycardia was significantly lower and frequency of atrial ectopics significantly higher in comparison with euglycemia. Cardioacceleration was detected during daytime hypoglycemia but not during nocturnal hypoglycemia. Lastly, we confirmed a proarrhythmogenic effect of hypoglycemia by showing significant

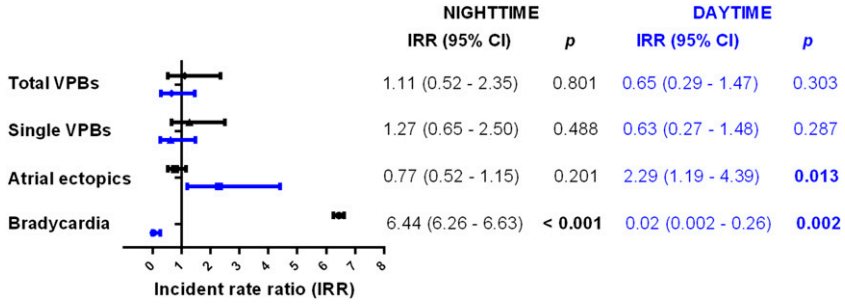

Figure 1—IRRs of distinct types of arrhythmias during hypoglycemia vs. euglycemia. Comparison between nocturnal (2300–0700 h) and daytime episodes. No complex ventricular paroxysmal beats (VPBs) were detected during nocturnal hypoglycemia (see also Table 3) and therefore no IRRs could be calculated for this type of arrhythmia. *P* values indicate significance of difference in arrhythmia rates during hypoglycemia vs. euglycemia.

Table 3—HRV 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 normalized	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 normalized	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 mean ± SD unless otherwise indicated. P values indicate comparison of nocturnal and daytime hypoglycemic data via paired t test. Eu, euglycemia; Hypo, hypoglycemia; Mean diff., mean paired difference; SDNN, SD of all NN intervals; TpTend_cB, TpTend interval corrected for heart rate (Bazett formula).

prolongation of QTc interval and TpTend interval together with a change toward abnormal, symmetric shape of the T wave during both night and day.

One of the main objectives of this study was to explore the different effects of nocturnal and daytime hypoglycemia on the frequency of cardiac arrhythmias, HRV, and cardiac repolarization. Factors that might affect these responses include diurnal variability in autonomic tone (19), different sympathoadrenal responses 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 with 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 bias from a variable length of episodes (the range of the duration ranged from 20 to 460 min) as well as from 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, predefining a hypoglycemic episode precisely, imposes its own limitation. By only counting hypoglycemic episodes that lasted at least 20 min, we probably underestimated the amount of hypoglycemia. Nevertheless, since we defined euglycemia as a glucose concentration between 5 and 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 the duration of symptomatic and asymptomatic hypoglycemia 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 subgroups, such as those with CAN. The majority of cardiac arrhythmias occurred during euglycemia, since periods of euglycemia were more frequent and long-lasting compared with hypoglycemia. Relative risks for distinct types of arrhythmias during hypoglycemia versus euglycemia are reflected by presented IRRs. The applied statistical model was used, as it adjusts for uneven distribution of hypoglycemic episodes and cardiac arrhythmias among studied individuals and adjusts for individuals with no episodes and those who experienced multiple episodes of hypoglycemia. Nevertheless, arrhythmia IRRs 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 the Bazett correction is the most commonly used. The Bazett formula corrects intervals to a rate of 60 bpm and tends to result in overcorrection at high heart rates and undercorrection 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 with matched euglycemia. These changes are smaller than the differences in QTc duration usually observed during experimental hypoglycemia and are probably due to both lower insulin levels and sympathoadrenal responses during spontaneous compared with experimental episodes (12,18). However, the difference compared with mean QTc duration during matched euglycemia was statistically significant ($P < 0.001$). This degree of QTc prolongation is 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: TpTend and 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 reentrant ventricular dysrhythmias and is associated with increased risk of sudden cardiac death (38). We detected significant prolongation of TpTend during 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 observed a consistent change toward more symmetrical shape during hypoglycemia during both day and night.

Cardioacceleration and a trend toward vagal withdrawal were observed during daytime hypoglycemia together with greater increases in QTc. In contrast, during nocturnal hypoglycemia no changes in HRV were detected and QTc increases were smaller. We have 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 with the night, since sympathoadrenal activation is suppressed both 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 current study suggest that this hypoglycemia-induced mechanism is independent of the type of diabetes, age, or cardiovascular risk profile. Importantly, our observation that certain types of arrhythmia (for example, bradycardia) were confined to just a few subjects suggests that susceptibility to arrhythmias during hypoglycemia 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 with daytime hypoglycemia. We confirm an HF 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 proarrhythmogenic.

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