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Cardiac arrhythmias and electrophysiologic responses during spontaneous hyperglycaemia in adults with type 1 diabetes mellitus

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ABSTRACT

Aim

We examined the effect of spontaneous hyperglycaemia in adults with type 1 diabetes mellitus (T1DM) and without history of cardiovascular disease on heart rate variability (HRV), cardiac repolarisation and incidence of cardiac arrhythmias.

Methods

Thirty-seven individuals with T1DM (age 17-50 years, 19 males, mean duration of diabetes 19.3 SD(9.6) years) underwent 96 hours of simultaneous ambulatory 12-lead Holter ECG and blinded continuous interstitial glucose (IG) monitoring (CGM). HRV, QT interval and cardiac repolarisation were assessed during hyperglycaemia (IG ≥15mmol/l) and compared with matched euglycaemia (IG 5-10mmol/l) on a different day, separately during the day and night. Rates of arrhythmias were assessed by calculating incidence rate differences.

Results

Simultaneous ECG and CGM data were recorded for 2395 hours. During daytime hyperglycaemia vs euglycaemia the mean QT_c interval duration was 404 SD(21)ms vs 407 SD(20)ms, p=0.263. T-peak to T-end interval duration corrected for heart rate ($T_pT_{end}c$) shortened (74.8 SD(16.1)ms vs 79.0 SD(14.8)ms, p=0.033) and T-wave symmetry increased (1.62 SD(0.33) vs 1.50 SD(0.39), p=0.02). During nighttime hyperglycaemia vs euglycaemia, the mean QT_c interval duration was 401 SD(26)ms vs 404 SD(27)ms, p=0.13 and T_pT_{end} shortened (62.4 SD(12.0)ms vs 67.1 SD(11.8)ms, p=0.003). The number of cardiac arrhythmias was low and confined to bradycardia and isolated ectopic beats. A considerable inter-subject and diurnal variability was observed.

Conclusions

Hyperglycaemia in individuals with T1DM without known cardiovascular disease was not associated with clinically important cardiac arrhythmias.

KEYWORDS

hyperglycemia; type 1 diabetes mellitus (T1DM); QT_c interval; cardiac electrophysiology; cardiac arrhythmias; heart rate variability (HRV).

INTRODUCTION

Hyperglycaemia represents the main risk factor for long-term micro- and macrovascular diabetic complications [1]. Little is known, however, about the direct effect of acute hyperglycaemia on cardiac electrophysiology and risk of cardiac arrhythmias in people with type 1 diabetes mellitus (T1DM) and in diabetes in general. To the best of our knowledge, the risk of cardiac arrhythmias during spontaneous hyperglycaemia in adults with diabetes or in healthy individuals has not been investigated. Existing data on the effect of hyperglycaemia on QT interval duration provide conflicting results and there is a marked discrepancy between the results obtained from observational studies on adolescents with T1DM and the data from experimental clamp studies on adults with and without diabetes. A small observational study using continuous glucose monitoring (CGM) and Holter ECG in 8 adolescents with T1DM showed a significant negative correlation between QT interval duration and interstitial glucose (IG) levels in a proportion (5/8) of participants – QT interval duration was shortest during hyperglycaemia and longest during hypoglycaemia [2]. Another observational study involving individuals with T1DM confirmed significant QT_c interval and $T_pT_{end}c$ shortening during hyperglycaemia [3]. By contrast an experimental study in 20 healthy adults showed that after a 2-hour hyperglycaemic clamp (15 mmol/l), catecholamine levels, blood pressure and heart rate increased and QT_c interval prolonged significantly versus baseline [4]. Similarly, QT_c interval prolongation was reported in another hyperglycaemia clamp study in both healthy adult males and males with T1DM [5].

We have previously reported diurnal differences in relative risks of cardiac arrhythmias and pro-arrhythmic electrophysiological changes during spontaneous hypoglycaemia in a group of 37 young individuals with T1DM [6]. In the presented study we examined the effect of spontaneous hyperglycaemia (IG ≥15mmol/l) in the same group of individuals on heart rate

variability (HRV), cardiac electrophysiological characteristics and individual`s relative risks of cardiac arrhythmias in comparison to matched euglycaemia (IG 5–10 mmol/l).

MATERIALS AND METHODS

Thirty-seven individuals with T1DM (history of presentation with acute symptoms of hyperglycaemia, weight loss, polyuria, polydipisa and/or DKA and later confirmed by low c-peptide levels and presence of specific antibodies) and without history of cardiovascular disease were recruited from Sheffield Teaching Hospitals outpatient clinics. Participants taking beta-blocking and QT interval prolonging agents (interference with QT interval duration), those with diabetic maculopathy and severe visual impairment (inability to handle the CGM and Holter ECG equipment) and with estimated glomerular filtration rate (eGFR) <30 ml min^{-1} $1.73m^{-2}$ (high risk of electrolyte abnormalities affecting cardiac repolarisation) were excluded. Exclusion criteria also included bundle branch block and atrial fibrillation. Written informed consent was obtained from all participants and the study received local ethics approval (National Research Ethics Service, NRES Committee, East Midlands, Derby, UK).

Baseline assessment

Urea, electrolytes, HbA_{1c}, body weight and height, BMI, blood pressure and heart rate were recorded at the onset of the monitoring period. Early morning spot urine sample was tested for ketonuria. Those with significant ketonuria were excluded from the study and were further treated as appropriate. Baseline QTc was established on a resting 12-lead ECG. Participants were instructed to avoid vigorous exercise, caffeine and smoking 12 hours prior to morning testing. Cardiovascular autonomic tests were performed at the onset of the monitoring period in accordance with the latest consensus statement [6, 7] and included heart rate responses to deep breathing, Valsalva maneuver and postural change and blood pressure responses to

postural change. Participants were classified as having possible cardiac autonomic neuropathy (CAN) when at least one cardioreflex test was outside the normal age-adjusted range [8] or definite CAN when two or more cardioreflex tests were outside the age-adjusted normal range [7].

Monitoring

All participants underwent 96 hours of time-synchronised 12-lead Holter ECG (Mortara H12+, Mortara Instrument Inc., Milwaukee, Wisconsin, USA) and CGM recordings (Freestyle Navigator II, Abbott Diabetes Care, Maidenhead, UK). Participants continued their usual daily activities and diabetes management including blood glucose monitoring (using their own blood glucose meters) and appropriate insulin dose adjustments. CGM calibrations were performed at least five times during the study period. The CGM has a lowest detection limit of 1.1 mmol/l. Participants were blinded to the IG levels by disabling the alarms and display on the device.

CGM analysis

The CGM system reported IG data in 10 min intervals. Hyperglycaemia was defined as IG ≥15 mmol/l and a hyperglycaemic episode as IG above this threshold for ≥20 min. Euglycaemia was defined as IG between 5–10 mmol/l. The hyperglycaemia – euglycaemia matching for heart rate variability and cardiac electrophysiological parameters was performed by identifying the highest (peak) IG within the hyperglycaemic episode and pairing it with a euglycaemic time point from the same individual at the same time (within 20 min either way) on a different day.

Arrhythmia analysis

The 12-lead Holter ECG data were reviewed using H-Scribe 4.34 software (Mortara Instrument Inc., Milwaukee, Wisconsin, USA). The software automatically separated normal ECG traces from artefacts and detected arrhythmic events: atrial ectopic beats (prematurity threshold 30%), bradycardia (consecutive beats at rate <45 bpm for >5 seconds) and ventricular premature beats

(VPBs). Hourly counts for each type of arrhythmia were provided by H-Scribe software and the mean IG was calculated for each corresponding hour. Hyperglycaemic hours (mean hourly IG ≥15 mmol/l) and euglycaemic hours (mean hourly IG 5–10 mmol/l) were identified for further statistical analysis of arrhythmias, separated into day and night (23:00–07:00) to take into account diurnal variation.

Heart rate variability analysis

R-R intervals were identified by the H-Scribe software and spectral analysis was performed on 5-minute segments using the Fourier transformation in accordance with the recommendations of the Task Force on Heart Rate Variability [9]. Heart rate variability (HRV) parameters included the low-frequency power (LF) of the variability, calculated within the 0.04–0.15 Hertz (Hz) interval and the high-frequency (HF) power within 0.15–0.4 Hz, which reflects parasympathetic activity [9, 10].

Cardiac repolarisation analysis

Repolarisation analysis and detection of QT-interval duration was performed using custom-built, semiautomatic software [11]. In brief, 5-minute ECG segments, centered on hyperglycaemia peak time points were identified and the analyses were performed on a composite wave, derived from combined ECG leads I, II, and V5. QT-intervals were corrected for heart rate (QT_c) using the Bazett formula [12]. The onset and end of T-wave were defined by tangents to both sides of the T-wave [13]. The isoelectric line and marker time points were reviewed and adjusted if necessary by an observer blinded to glucose values. The following parameters of cardiac repolarisation were further calculated: T-peak to T-end interval duration (T_pT_{end}), T_pT_{end} corrected for heart rate using the Bazett formula ($T_pT_{end}c$) and symmetry of the T-wave area (T_{sym}), which was calculated as the ratio of the area under the T-wave before and after the peak [6, 14]. Cardiac repolarisation and HRV parameters were calculated at matched hyperglycaemia and euglycaemia time points (see section CGM analysis) and compared using

paired statistical analysis. Where there were multiple euglycaemia time points identified for a single hyperglycaemia time point, average values of parameters at all euglycaemia time points were calculated.

Statistical analysis

Heart rate variability and cardiac electrophysiology parameters were compared between matched hyperglycaemic and euglycaemic time points using linear mixed effects models for continuous data. Individual variability was modelled using a random effect and the difference between hyperglycaemia and euglycaemia was estimated using a fixed effect, separately during day time and night time. For the arrhythmia analysis, hourly counts of cardiac arrhythmias were compared between hours of hyperglycaemia and euglycaemia (hourly IG averages) within individuals by calculating incidence rate differences (IRD). For an individual, the IRD is the mean number of arrhythmias per hyperglycemic hour minus the mean number of arrhythmias per euglycaemic hour. IRD was chosen over incident rate ratio (IRR) as a summary measure because it can be calculated even when there are zero arrhythmia counts in an individual. See discussion for further details. Forest plots were used to display the IRD calculated for each individual. Statistical analysis was performed with R (https://cran.r-project.org/). Linear mixed effects models were fitted using the NLME package [15] and Forest Plots drawn using the Metafor package [16]. $P < 0.05$ was deemed statistically significant.

RESULTS

Participant and hyperglycaemic episode characteristics

Baseline participant characteristics are shown in Table 1. A total of 3165 hours (h) of IG data were recorded, of which 416h were in the hyperglycaemic range (IG ≥15 mmol/l): 287h during the day and 129h during the night (23.00 – 07.00). Out of 1355h of euglycaemia, 849h were

recorded during the day and 506h at night. In total, 113 hyperglycaemic episodes were identified during the day and 53 during the night. Of 37 participants, 34 (91.9%) experienced at least one daytime hyperglycaemic episode and 22 (59.5%) experienced at least one nocturnal hyperglycaemic episode. The median and interquartile range (IQR) duration of daytime hyperglycaemic episodes was 107 min IQR(50 – 190 min) compared to euglycaemic episodes at 70 min IQR(38 – 200 min) (p=0.13, Mann-Whitney U Test). The median peak IG value during daytime hyperglycaemic episodes was 18.3 mmol/l IQR(16.5 – 21.4 mmol/l) and during nocturnal episodes it was 17.1 mmol/l IQR(15.9 – 21.4 mmol/l) (p=0.11, Mann-Whitney U Test).

Heart rate variability (HRV) and cardiac electrophysiology analysis

A total of 2395h of simultaneous CGM and ECG data were recorded, comprising 54 daytime and 26 nocturnal hyperglycaemic episodes that could thus be included in the HRV and cardiac electrophysiological analysis.

No significant differences between daytime hyperglycaemic episodes vs matched euglycaemia were detected in any of the examined HRV parameters (Table 2). The mean heart rate during daytime hyperglycaemia was 82 SD(13) bpm and during matched euglycaemia it was 85 SD(14) bpm (p=0.14). The QT_c interval duration during hyperglycaemia vs matched euglycaemia was 404 SD(21) ms vs 407 SD(20) ms (p=0.263). The mean T_pT_{end} interval duration was shorter during daytime hyperglycaemia in comparison to euglycaemia (64.4 SD(12.3) ms vs 67.1 SD(11.7) ms (p=0.057). $T_pT_{end}c$ was significantly shorter during hyperglycaemia vs euglycaemia: 74.8 SD(16.1) ms vs 79.0 SD(14.8) ms, mean paired difference -4.2 ms (95%CI: -8.1 to -0.4 ms), p=0.033. T-wave symmetry (T_{sym}) was significantly higher during hyperglycaemia at 1.62 SD(0.33) vs euglycaemia 1.50 SD(0.39), mean paired difference 0.12 (95%CI: 0.02 to 0.21), p = 0.015.

A higher heart rate was noted during nocturnal hyperglycaemic episodes vs matched euglycaemia (75 SD(15) vs 70 SD(10) bpm), but this was not statistically significant (p=0.072) (Table 2). The mean QT_c during hyperglycaemia vs matched euglycaemia was 401 SD(26) ms vs 404 SD(27) ms (p=0.128). The mean T_pT_{end} interval was significantly shorter during hyperglycaemia: 62.4 SD(12.0) ms vs 67.1 SD(11.8) ms, mean paired difference -4.7 ms (95%CI: -7.6 to -1.8 ms), p=0.003. $T_pT_{end}c$ and T_{sym} were not significantly different during nocturnal hyperglycaemia compared to euglycaemia (Table 2).

We repeated the above paired comparisons by excluding the data from individuals with possible and definite CAN and the results were consistent with those obtained from all participants (data not shown).

Cardiac arrhythmias

The incidence rate differences (IRD) between hyperglycaemic and euglycaemic hours for distinct types of arrhythmias were calculated for 26 out of 37 individuals. IRDs could not be calculated for 11 participants because they did not have at least one hyperglycaemic and one euglycaemic hour recorded either during daytime or nighttime.The data for bradycardia, atrial ectopic beats and ventricular premature beats are presented in Supplementary Figs 1-3.In the 26 individuals a total of 2094 hours of valid simultaneous CGM and Holter ECG data were recorded.

Analysis of cardiac arrhythmias revealed that during daytime, IRD for bradycardia was significantly positive in one individual (IRD 1.52, 95%CI: 0.58 to 2.45), indicating a greater incidence of bradycardia during hyperglycaemia vs euglycaemia. IRD was significantly negative in one individual (-0.22, 95%CI: -0.44 to -0.004). At night, IRD for bradycardia was significantly negative in 4 individuals and the highest negative IRD was -99.08 (95%CI: -104.67 to -93.49) (Supplementary Fig. 1).

The IRD for atrial ectopic beats during daytime was significantly negative in 4 individuals and significantly positive in 2 individuals. At night, the IRD for atrial ectopic beats was significantly negative in 2 individuals and significantly positive in one individual (Supplementary Fig. 2).

The incidence of VPBs during daytime hyperglycaemia was significantly lower compared to euglycaemia in 7 individuals (negative IRDs) and significantly higher in one individual (positive IRDs). At night, the incidence of VPBs during hyperglycaemia vs euglycaemia was significantly higher in one individual, in whom the highest positive IRD was calculated (48.84, 95%CI: 45.14 to 52.54) and significantly lower in 2 individuals (Supplementary Fig. 3). In all figures, participants with possible CAN can be identified by IDs 1, 2, 7 and 9 and participants with definite CAN by IDs 6, 15 and 21. IRDs could not be calculated for one individual with possible CAN.

Table 3 complements the above IRD data with a breakdown of number of hours in which arrhythmias occurred, the respective number of individuals and the total individual arrhythmia beat counts, listed separately for day and night.

DISCUSSION

In the present study we report for the first time the effect of spontaneous hyperglycaemia on HRV, cardiac electrophysiology, and individual incidences of cardiac arrhythmias in a group of adults with T1DM without known cardiovascular disease. Our main findings include non-significant shortenings of the QT_c interval duration during hyperglycaemia at night and day, accompanied with significant shortening of another characteristic of ventricular repolarisation, the T_pT_{end} interval. We observed considerable inter-individual and diurnal variability in incidences of cardiac arrhythmias during hyperglycaemia vs euglycaemia. The number of

detected arrhythmias was, however, low and generally confined to bradycardia and isolated ectopic beats, rather than clinically important arrhythmias.

Conflicting data exist on the effect of hyperglycaemia on cardiac electrophysiology, in particular the effect of hyperglycaemia on QT/QT_c interval duration. Existing experimental hyperglycaemia studies report prolongation of QT_c interval duration [4, 5] and increased catecholamine levels [4]. In our opinion, it is plausible to assume that the QT_c prolongation observed in these studies might have been caused by concomitantly increased catecholamine levels. The QT_c interval prolonging effect of catecholamines has been well documented [17] and represents one of the main mechanisms by which hypoglycaemia causes QT interval prolongation [18]. We are not aware of any data suggesting that frequently encountered clinical hyperglycaemia causes significant increase in plasma catecholamine levels in people with diabetes. Experimentally induced hyperglycaemia, on the other hand, may result in more rapid alterations in glucose levels which could conceivably induce stronger sympathoadrenal activation. Contrary to experimental human studies, data from existing observational studies on adolescents with T1DM report QT/QT_c shortening effect of clinical hyperglycaemia [2, 3]. Our study is in agreement with these findings. We also confirm that other electrophysiological parameters indicate either no changes or shortening of cardiac repolarisation during hyperglycaemia, together with no significant differences in any of the parameters of heart rate variability.

In the presented study we introduced an individualised assessment of the risk of cardiac arrhythmias during hyperglycaemia vs euglycaemia by calculating individual incidence rate differences (IRDs). In our previous observational studies we investigated the incidence of arrhythmias during hypoglycaemia vs euglycaemia using ‘incident rate ratios’ (IRRs) which represented a summary measure of the relative risk of arrhythmia for the whole studied group [6, 19]. IRR as a summary measure however does not sufficiently reflect the inter-individual

differences in the incidence of arrhythmias [20]. Also, given that the absolute numbers of arrhythmias were relatively low and occurred in a small proportion of individuals, the interpretation of the summary IRR data could be misleading [20-22]. In addition, the cohort in our present study was heterogenous in relation to presence of CAN. For these reasons, we decided to introduce individualised assessment of the risk of arrhythmias. In line with our previous studies, the absolute number of arrhythmias encountered during hyperglycaemia was relatively low. We noted, however, individual differences in the incidence of arrhythmias and also a degree of their diurnal variability. Given the relatively small number of participants we were unable to perform any further sub-group analysis to investigate the inter-individual variability further. Our current findings provide reassurance that hyperglycaemia in relatively young individuals with T1DM without known cardiovascular disease is not associated with clinically important cardiac arrhythmias. This statement should not be, however, generalised to all people with diabetes. The prevalence of sub-clinical cardiovascular disease is higher in people with T1DM compared to non-diabetic population [23]. Although we didn't examine for the presence of sub-clinical cardiovascular disease in our study, participants served as their own controls in relation to the risk of cardiac arrhythmias during hyperglycaemia vs euglycaemia. The association of hyperglycaemia with cardiac arrhythmias might be different in those with clinical cardiovascular disease, in particular immediately after acute myocardial infarction (MI). This was shown in a retrospective analysis by Tran et al. who report that patients who were admitted to hospital with acute MI and with hyperglycaemia (>140 mg/dl, equals to >7.8 mmol/l) on admission had higher chance of developing early (within 48 hours) ventricular tachycardia (VT) (multivariable adjusted OR = 1.39, 95% CI = 1.11–1.73). Interestingly, similar associations were found in those with and without diabetes and in those with and without ST-segment elevation MI (STEMI) [24]. In addition, a retrospective study on over 300 implantable cardioverter-defibrillator (ICD) patients with type 2 diabetes mellitus

(T2DM) and patients without diabetes showed that suboptimal metabolic control is associated, independently of QT/QT_c interval duration, with increased risk of ventricular arrhythmias [25].

The strength of our study includes an effort to minimise diurnal and inter-individual variability in HRV and cardiac repolarisation parameters by matching hyperglycaemia and euglycaemia time points. A use of semi-automatic analysis also insures a robust detection of parameters, which is highly relevant in ambulatory recordings. Cardiac repolarisation parameters T_pT_{end} and T-wave area symmetry ratio were chosen because, in contrast to QT interval duration, they have been previously demonstrated to be heart rate independent [14]. There are differing opinions whether T_pT_{end} should be corrected for heart rate [26, 27], and corrected $T_pT_{end}c$ has been demonstrated as a superior predictor of cardiovascular risk [28, 29]. For this reason, we report both T_pT_{end} and $T_pT_{end}c$ in the current study and while the statistical outputs aren`t identical, both parameters demonstrate the same trend.

Our study has several limitations. This is the largest study of its kind accomplished thus far, yet a larger number of participants would have also enabled a sub-group analysis comparing participants with and without CAN. A prolonged resting QT_c interval has been reported in people with T2DM and present autonomic neuropathy in comparison to those without autonomic neuropathy [30]. In addition, an altered relationship between QT interval duration and RR has also been identified in those with T2DM and autonomic neuropathy [31]. Exclusion of subjects with CAN did not affect the findings of the present study (data not shown), but additional studies are needed to investigate the effect of hyperglycaemia in subjects with T1DM and CAN. Interpretation of heart rate variability data and their physiological relevance is a challenging topic [10, 32, 33]. Due to the ambulatory nature of our study we were not able to control for physical activity,frequency and depth of respiration. While we applied a rigorous approach to assess HRV power, we were mindful not to over-interpret these data for the above reasons. We separated the arrhythmia analysis into day and night (23:00–07:00) to take into

account diurnal variation, but other variables including sleep state (asleep/awake), level of physical activity or emotional distress were not recorded to limit complexity of in an already complicated study. Finally, the observational character of the study didn't allow us to measure plasma catecholamine, potassium and magnesium levels, which, given the above mentioned data available from experimental and animal studies, would have helped to explore the contribution of these factors. On the other hand, the 'real-life' nature of our study is relevant in determining whether hyperglycaemia has clinically relevant cardiac electrophysiological consequences.

Conclusions

In conclusion, we report for the first time the effect of spontaneous hyperglycaemia on HRV, cardiac electrophysiology and individual relative incidence of several types of cardiac arrhythmias in a group of individuals with T1DM. Our results show considerable variability in the incidences of cardiac arrhythmias but few of any major clinical importance. Shortening of the duration of ventricular repolarisation ($T_pT_{end}c$/ T_pT_{end}) along with shorter, although not statistically significant, duration of the QT_c interval during hyperglycaemia in our study is in keeping with data from small observational studies in adolescents with T1DM, but contradicts experimental studies in adults with and without diabetes. Further observational studies are required in the future to fully establish the clinical significance of our findings, particularly in those with T2DM at increased cardiovascular risk.

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DECLARATION OF INTEREST

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Table 1. Baseline participant characteristics. Quantitative data are displayed as mean (SD) or median (IQR).

Number of participants, *n*	37
Age (years)	34, range: 17-50
Male, *n* (%)	19 (51.4%)
BMI (kg/m^2)	25 (22.8 – 27.9)
SBP (mmHg)	123 (13)
DBP (mmHg)	72 (7)
Heart rate (bpm)	73 (14)
Baseline QT_c (ms)	417 (27)
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)
Baseline fasting creatinine (umol/l)	72.5 (55.5 - 89.5)
Baseline fasting potassium (mmol/l)	4.55 (4.05 - 4.95)
eGFR (ml/min/1.73m^2), *n* (%)	
≥90 (CKD1)	24 (64.9%)
60-89 (CKD2)	12 (32.4%)
30-59 (CKD3)	1 (2.7%)
Diabetic nephropathy, *n* (%)	
Absent	32 (86.5%)
Microalbuminuria[a]	5 (13.5%)
Macroalbuminuria[b]	0 (0%)
Diabetic retinopathy, *n* (%)	
No retinopathy (R0)	9 (24.3%)
Non-proliferative retinopathy (R1+R2)	23 (62.2%)
Treated proliferative retinopathy (R3)	5 (13.5%)
Diabetic peripheral neuropathy (DSPN), *n* (%)	
Possible DSPN	1 (2.7%)
CAN status, *n* (%)	
possible CAN	5 (13.5%)
definite CAN	3 (8.1%)
no CAN	29 (78.4%)

[a]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. [b]Macroalbuminuria was defined as albumin/creatinine ratio > 30 mg/mmol. eGFR – estimated glomerular filtration rate (CKD-EPI formula). MDI – multiple daily injections of insulin, CSII – continuous subcutaneous insulin infusion, SBP – systolic blood pressure, DBP – diastolic blood pressure, DSPN – chronic sensorimotor distal symmetrical polyneuropathy, CAN – cardiovascular autonomic neuropathy, CKD1-3 – chronic kidney disease stages 1-3.

Table 2. Heart rate variability (HRV) characteristics during peak hyperglycaemia vs matching euglycaemia

DAYTIME (n=54 episodes in 27 subjects)							
	HYPER		**EU**		**HYPER – EU**		
	Mean	**SD**	**Mean**	**SD**	**Mean Diff.**	**95% CI**	***p* value**
HR (bpm)	82.4	12.8	85.2	14.2	-2.8	-6.6 to 1.0	0.143
SDNN (ms)	51.1	22.5	55.1	22.3	-4.0	-10.1 to 2.1	0.193
RMSSD (ms)	25.0	13.7	23.8	13.9	1.2	-2.1 to 4.4	0.476
logHF	2.1	0.5	2.0	0.5	0.1	-0.05 to 0.2	0.275
logLF	2.71	0.41	2.69	0.41	0.03	-0.07 to 0.13	0.604
QT_c (ms)	404.4	20.8	406.8	20.3	-2.4	-6.6 to 1.8	0.263
T_pT_{end} (ms)	64.4	12.3	67.1	11.7	-2.7	-5.6 to 0.1	0.057
$T_pT_{end}c$ (ms)	74.8	16.1	79.0	14.8	-4.2	-8.1 to -0.4	**0.033**
T_{sym}	1.62	0.33	1.50	0.39	0.12	0.02 to 0.21	**0.015**
NIGHTTIME (n=26 episodes in 18 subjects)							
	HYPER		**EU**		**HYPER – EU**		
	Mean	**SD**	**Mean**	**SD**	**Mean Diff.**	**95% CI**	***p* value**
HR (bpm)	75.3	14.8	70.3	9.5	5.0	-0.5 to 10.4	0.072
SDNN (ms)	51.4	27.0	48.9	24.5	2.4	-6.9 to 11.7	0.597
RMSSD (ms)	25.6	17.4	25.5	12.5	0.1	-5.0 to 5.2	0.972
logHF	2.0	0.6	2.0	0.5	-0.1	-0.3 to 0.2	0.580
logLF	2.57	0.48	2.59	0.50	-0.03	-0.24 to 0.19	0.794
QT_c (ms)	401.1	26.1	404.2	27.0	-3.0	-7.0 to 0.9	0.128
T_pT_{end} (ms)	62.4	12.0	67.1	11.8	-4.7	-7.6 to -1.8	**0.003**
$T_pT_{end}c$ (ms)	69.0	15.1	71.7	12.5	-2.8	-6.2 to 0.7	0.111
T_{sym}	1.76	0.33	1.74	0.19	0.02	-0.10 to 0.13	0.782

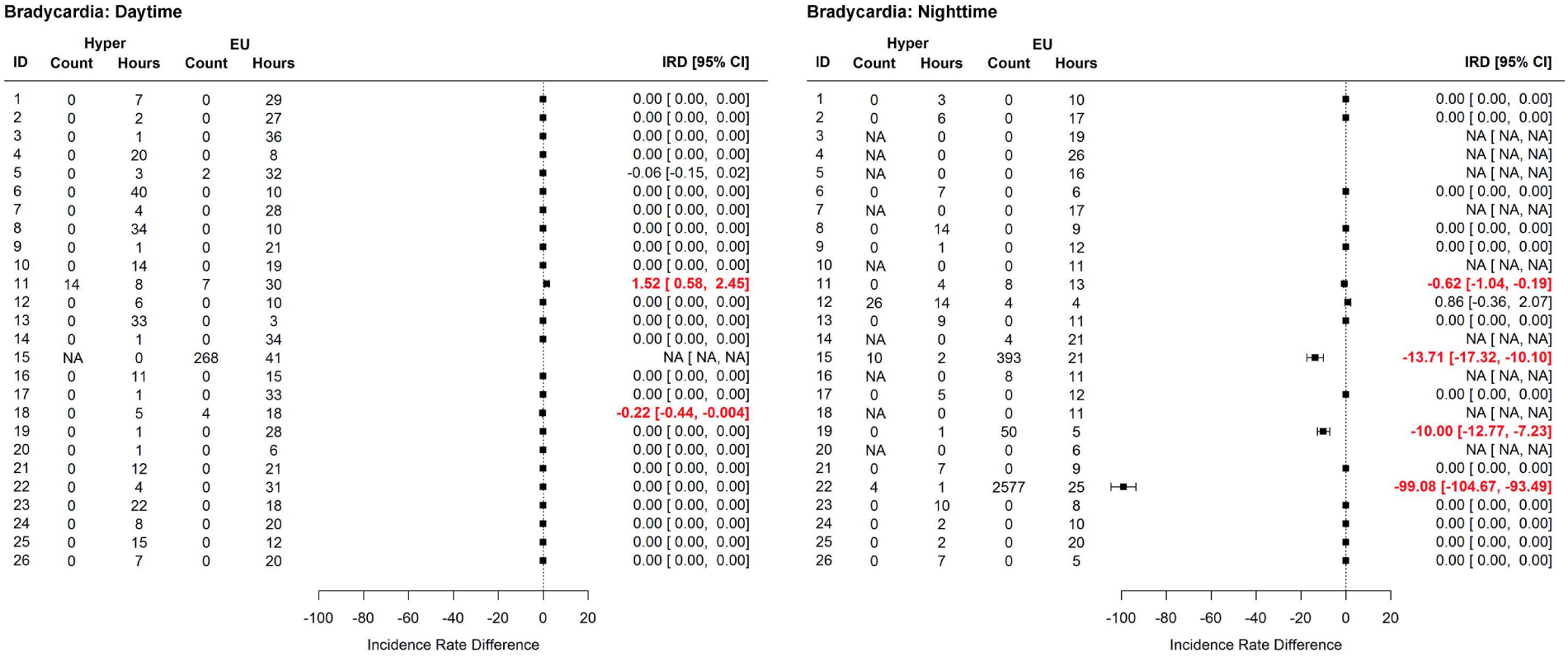
HR – heart rate, SDNN – standard deviation of NN intervals, RMSSD – root mean square of successive differences, logHF – logarithm of HF power, logLF – logarithm of LF power, QT_c – corrected QT interval, T_pT_{end} – T-peak to T-end interval, $T_pT_{end}c$ – corrected T_pT_{end}, T_{sym} – T wave area symmetry ratio.

Table 3. Frequency and numbers of arrhythmic beatsduring night and day time

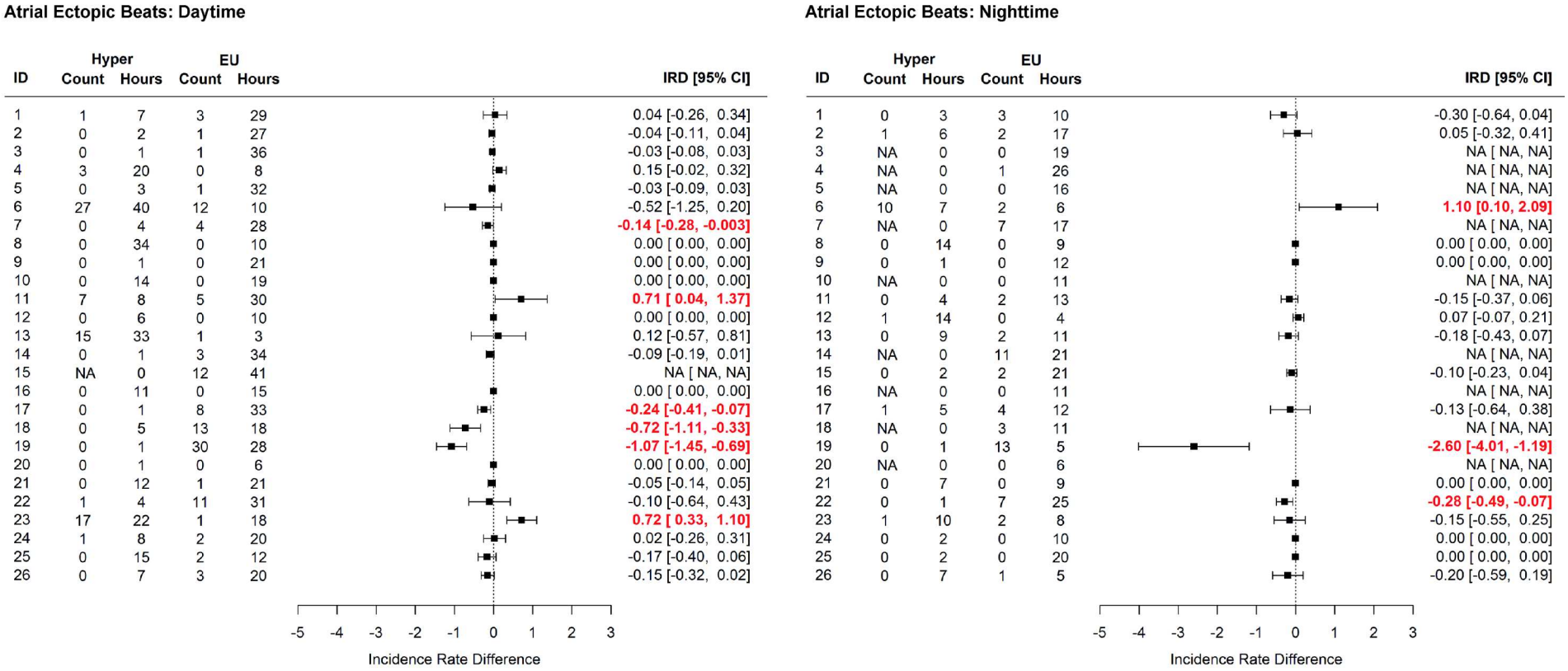
	NIGHTTIME				DAYTIME			
	Hyperglycaemia		**Euglycaemia**		**Hyperglycaemia**		**Euglycaemia**	
Hourly IG and ECG (h)[a]	**95**		**335**		**261**		**560**	
	Hours (subjects)	**Beat count**	**Hours (subjects)**	**Beat count**	**Hours (subjects)**	**Beat count**	**Hours (subjects)**	**Beat count**
Bradycardia	6 (3)	40	31 (7)	3044	2 (1)	14	10 (4)	281
Atrial ectopic beats	9 (5)	14	38 (15)	62	46 (8)	72	65 (19)	114
Total VPBs	19 (5)	696	36 (13)	63	37 (14)	146	82 (19)	903

[a]Length of valid simultaneous CGM and ECG data. Durations of hourly euglycaemia (IG 5-10 mmol/l) and hyperglycaemia (IG ≥ 15) are presented. The total length of simultaneous IG and ECG data was 2094h.

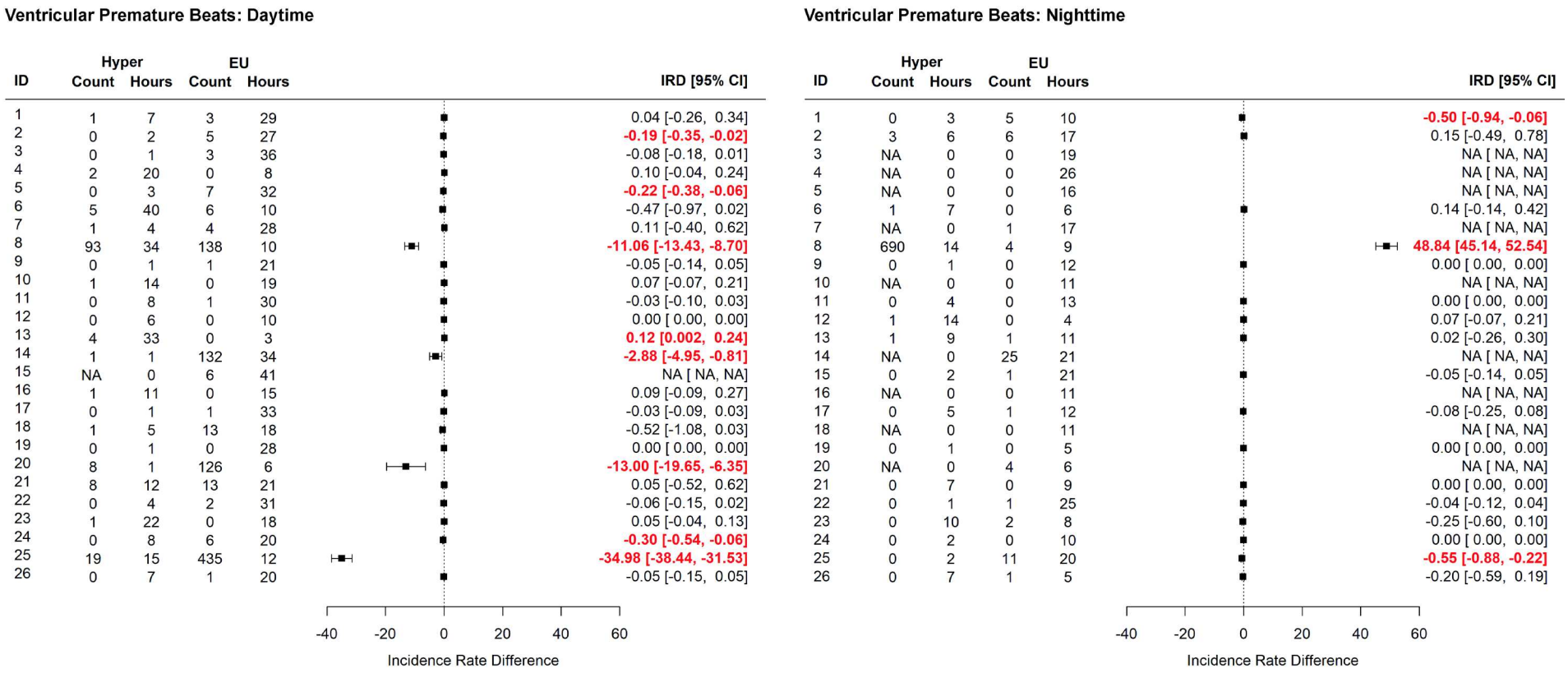
Hours - numbers of hourly segments in which arrhythmic beats were detected, separated for the day and night (23:00 – 7:00). Beat count - total number of arrhythmic beats and the number of corresponding participants (in parenthesis). Only participants who experienced both hyperglycaemia and euglycaemia are included (n = 26). IG – interstitial glucose, ECG – 12-lead Holter ECG, Total VPBs – total number of individual ventricular premature beats.



Supplementary Figure 1. Incidence Rate Differences (IRD) for bradycardia between hyperglycaemic (Hyper) and euglycaemic (EU) hours for daytime and nighttime. Each line in the Forest plot represents an individual participant (IDs 1-26). Numbers of hyperglycaemic and euglycaemic hours and total numbers of individual beats for each participant are recorded. Significant IRDs are highlighted in red. Note that the approximating for the confidence is unlikely to hold for extreme counts, e.g. ID 22 for nighttime. Participants 1, 2, 7 and 9 were classified as having possible CAN and participants 6, 15 and 21 as definite CAN.



Supplementary Figure 2. Incidence Rate Differences (IRD) for atrial ectopic beats between hyperglycaemic (Hyper) and euglycaemic (EU) hours for daytime and nighttime. Each line in the Forrest plot represents an individual participant (IDs 1-26). Numbers of hyperglycaemic and euglycaemic hours and total numbers of arrhythmic beats for each participant are recorded. Significant IRDs are highlighted in red. Participants 1, 2, 7 and 9 were classified as having possible CAN and participants 6, 15 and 21 as definite CAN.



Supplementary Figure 3. Incidence Rate Differences (IRD) for ventricular ectopic beats between hyperglycaemic (Hyper) and euglycaemic EU) hours for daytime and nighttime. Each line in the Forest plot represents an individual participant (IDs 1-26). Numbers of hyperglycaemic and euglycaemic hours and total numbers of arrhythmic beats for each participant are recorded. Significant IRDs are highlighted in red. Participants 1, 2, 7 and 9 were classified as having possible CAN and participants 6, 15 and 21 as definite CAN.