Influence of Autonomic Neuropathy on QTc Interval Lengthening During Hypoglycemia in Type 1 Diabetes

Stuart P. Lee,[1] Lishan Yeoh,[1] Nigel D. Harris,[2] Catherine M. Davies,[1] Robert T. Robinson,[1] Andrew Leathard,[3] Christopher Newman,[4] Ian A. Macdonald,[5] and Simon R. Heller[1]

Hypoglycemia produces electrocardiographic QTc lengthening, a predictor of arrhythmia risk and sudden death. This results from both sympatho-adrenal activation and a lowered serum potassium. It has been suggested that cardiac autonomic neuropathy (CAN) might indicate those who are at particular risk. We tested this hypothesis in 28 adults with type 1 diabetes and 8 nondiabetic control subjects. After standard tests of autonomic function and baroreflex sensitivity (BRS) measurement, diabetic participants were divided into three groups: *1*) CAN− with normal BRS (BRS+; n = 10), *2*) CAN− with impaired BRS (BRS−; n = 9), and *3*) CAN+ (n = 9). QTc was then measured during controlled hypoglycemia (2.5 mmol/l) using a hyperinsulinemic clamp. Mean (±SE) QTc lengthened from 377 ± 9 ms (baseline) to a maximum during hypoglycemia of 439 ± 13 ms in BRS+ subjects and from 378 ± 5 to 439 ± 10 ms in control subjects. Peak QTc tended to be lower in CAN+ (baseline, 383 ± 6; maximum, 408 ± 10) and BRS− groups (baseline, 380 ± 8; maximum, 421 ± 11; F = 1.7, P = 0.18). Peak epinephrine concentrations (nmol/l) were 3.1 ± 0.8 (BRS+), 2.6 ± 0.5 (BRS−), 1.4 ± 0.3 (CAN+), and 5.7 ± 0.8 (control subjects). These data do not indicate that those with CAN are at particular risk for abnormal cardiac repolarization during hypoglycemia. Indeed, they suggest that such patients may be relatively protected, perhaps as a result of attenuated sympatho-adrenal responses. *Diabetes* 53: 1535–1542, 2004

In a detailed survey of all deaths that occurred among individuals who had type 1 diabetes and were younger than 50 years during 1989 in the U.K. (a total of 50), Tattersall and Gill (1) highlighted a distinct subgroup of 22 who were found dead in an undisturbed bed, having seemed to be well the previous day. Since the term the "dead in bed syndrome" (2) was used in an accompanying editorial, three separate reports from Scandinavia have subsequently confirmed the existence of this mode of death among young people with type 1 diabetes (3–5). There was circumstantial evidence linking these deaths to hypoglycemia, and the possibility that hypoglycemia might cause cardiac arrhythmias was raised (1,6).

We and others have since demonstrated abnormal cardiac repolarization, manifest by increases in QTc interval and QT dispersion, during experimental (7,8) and clinical hypoglycemia (9). We have proposed that hypoglycemia should be added to the acquired causes of the long QT syndrome (LQTS) and suggested that sudden death in young people with diabetes might be due to ventricular arrhythmias initiated during hypoglycemia (10). However, because such deaths are rare, whereas hypoglycemia is common, it seems likely that such events are manifest only in predisposed individuals under certain circumstances. Factors that might increase susceptibility include mutations in genes encoding cardiac ion channels (as in congenital LQTS), certain medication, and autonomic dysfunction.

Type 1 diabetic patients with cardiac autonomic neuropathy (CAN) have a greater general risk of sudden death (11), which has been linked to QTc prolongation (12). Advanced CAN is relatively rare in young people with type 1 diabetes, but more subtle autonomic abnormalities such as impaired baroreflex sensitivity (BRS) and reduced heart rate variability are more common (13,14). It has been suggested that "subclinical" CAN might contribute to the "dead in bed syndrome," with depressed vagal tone and a relative sympathetic predominance increasing the likelihood of cardiac arrhythmia during severe hypoglycemia (6). A similar pattern of autonomic dysfunction is associated with a high risk of arrhythmia (15) and sudden death (16) after myocardial infarction.

It seems important to establish which of these factors is most relevant, particularly as some, such as sympathetic predominance, might be amenable to medication. This might be exploited to protect patients if those who are at greatest risk could be identified. We therefore have used an experimental model of hypoglycemia to test the hypothesis that those who are at greatest risk of abnormal repolarization during hypoglycemia would be those with established or subclinical autonomic neuropathy. Our aim was to investigate the influence of autonomic dysfunction on QTc lengthening during experimental hypoglycemia. In particular, we wished to determine whether patients with overt or subclinical autonomic dysfunction (measured

From the [1]Department of Medicine, Division of Clinical Sciences, Northern General Hospital, University of Sheffield, U.K.; the [2]Department of Medical Sciences, University of Bath, Bath, U.K.; the [3]Department of Medical Physics, Royal Hallamshire Hospital, Sheffield, U.K.; the [4]Department of Cardiology, Division of Clinical Sciences, Northern General Hospital, University of Sheffield, U.K.; and the [5]School of Biomedical Sciences and Institute of Clinical Research, University of Nottingham Medical School, Nottingham, U.K.

Address correspondence and reprint requests to Simon R. Heller, MD, FRCP, Reader in Medicine, Clinical Sciences Centre, Northern General Hospital, Herries Road, Sheffield S5 7AU, U.K. E-mail: s.heller@sheffield.ac.uk.

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BRS, baroreflex sensitivity; CAN, cardiac autonomic neuropathy; DBP, diastolic blood pressure; ECG, electrocardiogram; LQTS, long QT syndrome; SBP, systolic blood pressure.

using standard criteria or reduced BRS, respectively) exhibit additional QTc lengthening compared with those with normal autonomic function.

RESEARCH DESIGN AND METHODS

A total of 109 adults who have type 1 diabetes and attend our outpatient clinics initially underwent screening for autonomic neuropathy, excluding those with hypertension, ischemic heart disease, peripheral vascular disease, nephropathy (urine albumin-to-creatinine ratio <2.5 mg/mmol in all subjects), and active proliferative retinopathy. Sixty subsequently attended for BRS measurement, 24 of whom agreed to have their QTc interval measured during experimental hypoglycemia. We approached an additional nine patients who had known CAN; four agreed to participate. We also studied eight individuals without diabetes (fasting blood glucose <6 mmol/l) matched with the diabetic groups for age, sex, and BMI. No participant was taking medication that is known to influence the autonomic nervous system or QT interval, and all had a normal resting electrocardiogram (ECG). Any therapy other than insulin was withdrawn 48 h before study. The North Sheffield Research and Ethics committee approved the protocol, and all participants gave written informed consent.

Assessment of autonomic function. Participants were asked to avoid stimulants and exercise for 12 h beforehand, attended at least 2 h after eating, and rested for 10 min before recordings were undertaken. Capillary glucose was measured on arrival. We rescheduled one participant, who experienced biochemical hypoglycemia the day before his study.

Traditional cardiac autonomic function tests. Traditional cardiac autonomic function tests were performed according to a protocol reported by O'Brien et al. (17): *1*) resting heart rate, *2*) R-R interval response to the Valsalva maneuver, *3*) R-R interval response to deep breathing (expiration [E]-to-inspiration [I] ratio; E:I difference), *4*) R-R interval response to standing (30:15 ratio), and *5*) postural blood pressure response. Diabetic participants were designated CAN+ when two or more tests were abnormal (17).

Measurement of BRS. BRS can be considered as the change in heart rate per unit change in blood pressure. For BRS measurement, we used the "sequence" method, based on the automated identification of spontaneous sequences of three or more consecutive beats characterized by either a progressive rise or decline in systolic blood pressure (SBP) and associated changes in R-R interval (18). The sequence technique correlates strongly with BRS measurements obtained using traditional invasive methods (19). Blood pressure was tracked using a Portapres (TNO-TMP Biomedical Instrumentation, Amsterdam, Netherlands), a device measuring beat-to-beat changes in finger arterial pressure using a volume-clamp method. There is a strong correlation between blood pressure measurements obtained using this technique and intra-arterial recordings (20). Before and after BRS measurement, blood pressure was taken manually with a conventional sphygmomanometer to verify Portapres accuracy. For each participant, a minimum of three supine datasets were collected, each consisting of a 3-min recording period.

Analog blood pressure and ECG data were digitized using a PCM-DAS08 card (Amplicon) attached to an on-line personal computer (Toshiba 4090CDS) sampling at 200 Hz. A QRS detection algorithm was used to measure R-R intervals obtained from the three-lead surface ECG. Computer software was developed using Matlab (Mathworks, Nantucket, MA) to identify SBP changes with corresponding increases and decreases in R-R interval length. An "up sequence" (+RR/+SBP) was identified when three or more consecutive SBP readings increased by at least 0.5 mmHg, with a corresponding increase in R-R interval. A "down sequence" (−RR/−SBP) was highlighted when three or more consecutive SBP readings fell, with a reduction in R-R interval. The regression coefficient of these sequences (the slope of the regression line between SBP and pulse interval) was taken as an overall measure of BRS (ms/mmHg) (21). Total BRS was obtained by averaging the +RR/+SBP and −RR/−SBP regression coefficients. SDs of the recordings were computed for up, down, and total BRS values.

BRS was measured in 69 diabetic participants (51 CAN−, 18 CAN+). We classified participants as having normal or impaired BRS according to published reference values (22). Those with a mean supine BRS value below the 10th centile relative to age-matched healthy control subjects were designated BRS−; BRS+ participants had a mean BRS above the 25th centile. CAN− participants whose BRS fell between the 10th and 25th centiles were excluded from further study.

Diabetic participants were assigned to one of three groups: *1*) "normal" autonomic function (CAN− with normal BRS [BRS+]), *2*) "mild" autonomic neuropathy (CAN− with impaired BRS [BRS−]), or *3*) advanced autonomic neuropathy (CAN+).

Hypoglycemic clamp studies. Of the 69 subjects with type 1 diabetes, 28 (10 BRS+, 9 BRS−, 9 CAN+) underwent sequential euglycemic-hypoglycemic clamps, along with 8 nondiabetic participants. All refrained from stimulants and strenuous exercise for 12 h beforehand. To reduce the risk of antecedent hypoglycemia (diabetic participants), evening insulin dose was reduced by 20–25% the day before study and participants were instructed to check capillary glucose at bedtime, at 0300, and before breakfast. Symptomatic hypoglycemia during the preceding 3 days or a blood glucose <4 mmol/l precluded individuals from participating on that occasion.

Diabetic participants were admitted at 0800 on the study day, having consumed a light breakfast but omitted their morning insulin dose. On arrival, soluble insulin (Human Actrapid; Novo Nordisk Laboratories, Copenhagen, Denmark) was infused via an 18-g cannula inserted into an antecubital vein to maintain capillary blood glucose between 5 and 10 mmol/l.

Between 1100 and 1130, a retrograde cannula for sampling was inserted into a dorsal hand vein and kept patent with a slow 0.9% saline infusion; this hand rested in a heated chamber at 55°C to arterialize venous blood. Baseline data were collected at least 30 min later. When blood glucose was stable at ~5 mmol/l (around 1230), insulin infusion rate was increased to 60 mU · min^{-1} · m^{-2} and a 20% glucose solution was administered. Glucose infusion rate was adjusted according to blood glucose measurements obtained every 3–5 min. After a 60-min euglycemic period (5 mmol/l), glucose was allowed to fall over 30 min to a target of 2.5 mmol/l and held at 2.5 mmol/l for an additional 60 min.

Physiological and ECG data were collected at baseline and every 30 min during each glycemic plateau (expressed as 5 mmol/l: E0, E30, and E60; 2.5 mmol/l: H0, H30, and H60). Plasma catecholamines were measured at baseline, E60, H30, and H60, and free insulin was measured at E60 and H60.

Biochemical analyses. Blood glucose was measured by a glucose oxidase method (YSI 2300 Stat Plus analyzer; Yellow Springs Instruments, Yellow Springs, OH). HbA_{1c} was measured using Primus 385 and 330 instruments (Primus, Kansas City, MO), using a boronate affinity and high-performance liquid chromatography method (23). Plasma potassium, magnesium, calcium, and albumin were measured using a dry slide automated analyser (Vitros 250; Ortho Clinical Diagnostics, Rochester, NY). Samples for catecholamine analysis were taken into chilled tubes that contained 75 μl of EGTA-glutathione, centrifuged promptly, and stored at −80°C until assayed by high-performance liquid chromatography and electrochemical detection (24). Free-insulin samples were centrifuged promptly, and 0.5 ml of the supernatant was added to chilled tubes that contained 0.5 ml of polyethylene glycol buffer. After centrifugation, the resulting supernatant was stored at −80°C until analyzed by radioimmunoassay (Pharmacia & Upjohn Diagnostics AB, Uppsala, Sweden). Inter- and intra-assay coefficients of variation were 9.9 and 8.5%, respectively (epinephrine), 3.9 and 1.2% (norepinephrine), and 5.8 and <5% (insulin).

QTc interval. A custom-built, high-resolution ECG analysis system, acquiring three bipolar orthogonal X, Y, and Z leads, was used for QTc measurement (25). For each lead, signals from sinus beats were amplified, digitized, filtered, and averaged online over a 1-min recording period.

A semiautomated gradient method (25,26) was used, with a tangent automatically applied to the downward slope of the T wave at the point of maximum gradient, and the end of the T wave was taken as the intersection of this tangent with the baseline. The tangent position was verified and adjusted if necessary by a single observer who was blinded to all other data. The values for QT interval and mean associated heart rate were then displayed on the computer screen, and QTc was derived from QT using the modified Bazett formula (27). Finally, the root mean square QTc across the three leads was calculated.

Physiological measurements. Heart rate, blood pressure, sweating, and tremor responses were determined using previously described methods (28). Briefly, heart rate and blood pressure recordings were made using an automatic oscillometric sphygmomanometer (Accutorr 4; Datascope, Huntingdon, U.K.). Finger tremor was measured using a ring accelerometer placed on the index finger of the outstretched hand over a 1-min period, and sweating was determined using a ventilated chamber evaporimeter placed on the sternum, which records the difference in humidity between inflowing and outflowing air.

Statistics. Comparisons between variables measured at several time points were made using repeated-measures ANOVA to evaluate between-group differences and within-subject changes over time. In the event of a significant group by time interaction, summary measures were compared by one-way ANOVA followed by Scheffe post hoc test for multiple comparisons. Group comparisons not involving time were also made by one-way ANOVA with Scheffe subanalysis. The relationship between plasma epinephrine and QTc length was explored using Spearman rank correlation. Data are presented as mean ± SE unless otherwise stated, and $P < 0.05$ was judged significant. Analyses were performed using the statistical package SPSS version 10.0 (SPSS, Chicago, IL).

TABLE 1
Cardiac autonomic function

	Normal (n = 8)	BRS+ (n = 10)	BRS− (n = 9)	CAN+ (n = 9)
Heart rate (bpm)	67 ± 2	68 ± 2	73 ± 2	86 ± 2
Valsalva ratio	1.95 ± 0.14	1.93 ± 0.13	1.62 ± 0.09	1.24 ± 0.12
E:I ratio	1.45 ± 0.05	1.41 ± 0.07	1.34 ± 0.05	1.07 ± 0.01
Beat-to-beat variation (bts)	25 ± 3	23 ± 3	22 ± 3	6 ± 1
SBP fall on standing (mmHg)	5 ± 2	6 ± 2	6 ± 3	13 ± 4
30:15 ratio	1.36 ± 0.05	1.38 ± 0.05	1.24 ± 0.02	1.06 ± 0.02
BRS (ms/mmHg)	16.8 ± 2.0	16.4 ± 1.4	7.3 ± 0.7	4.8 ± 0.6

Data are means ± SE. E:I ratio, expiration-to-inspiration ratio.

RESULTS

Autonomic function. Of 109 type 1 diabetic subjects initially screened, 9 were classified as CAN+. The diagnosis of CAN was confirmed in all of the nine additional individuals who had known CAN and were also studied. BRS was unequivocally low (below the 10th centile) in 15 of 51 CAN− participants (29%) and fell between the 10th and 25th centiles in 5 CAN− participants, leaving 31 (61%) with "normal" BRS. In contrast, 14 of 18 CAN+ participants (78%) exhibited low BRS, with 3 showing intermediate (10th to 25th centiles) results.

Cardiac autonomic function data relating to the hypoglycemic clamp participants are summarized in Table 1. BRS was above the 25th centile relative to age-matched control subjects in only one CAN+ participant.

Hypoglycemic clamp participant characteristics. CAN+ participants tended to be older than the nondiabetic and CAN− participants. The groups were otherwise well matched (Table 2).

Biochemical analyses. Blood glucose was similar among the groups (Fig. 1). Within each group, plasma free-insulin concentrations were similar at steady-state euglycemia (E60) and hypoglycemia (H60; BRS+, 82.1 ± 5.7 vs. 76.8 ± 5.3 mU/l, P = 0.26; BRS−, 76.7 ± 2.8 vs. 70.7 ± 5.1 mU/l, P = 0.17; CAN+, 71.7 ± 5.6 vs. 77.8 ± 6.2 mU/l, P = 0.48; N, 82.2 ± 5.5 vs. 80.4 ± 7.3 mU/l, P = 0.76). There were no group differences in insulin concentrations at euglycemia (F = 0.96, P = 0.42) or hypoglycemia (F = 0.466, P = 0.71).

Plasma epinephrine concentrations (Fig. 2) were similar at baseline among the groups (F = 0.88, P = 0.46). There was a significant difference between the groups with respect to hypoglycemic epinephrine responses (F = 6.21, P < 0.001). Nondiabetic participants exhibited greater epinephrine responses (peak increase from baseline 5.50 ± 0.82 nmol/l) than the diabetic groups (BRS+, 2.93 ± 0.81 nmol/l, P = 0.065 vs. control subjects; BRS−, 2.37 ± 0.43 nmol/l, P = 0.02 vs. control subjects; CAN+, 1.31 ± 0.32 nmol/l, P = 0.001 vs. control subjects). Epinephrine responses tended to be blunted in CAN+ participants compared with the other diabetic groups, but this was not statistically significant (vs. BRS+, P = 0.35; vs. BRS−, P = 0.71).

Basal plasma norepinephrine concentrations were similar between the groups (F = 0.32, P = 0.81). The groups differed with respect to hypoglycemic norepinephrine responses (F = 2.48, P = 0.038), with nondiabetic participants tending to show greater norepinephrine increases (peak rise above baseline 1.42 ± 0.36 nmol/l) than the diabetic groups. However, there were no significant differences between individual groups (BRS+, 0.67 ± 0.19, P = 0.3 vs. control subjects; BRS−, 0.68 ± 0.23, P = 0.32 vs. control subjects; CAN+, 0.80 ± 0.30, P = 0.49 vs. control subjects).

Plasma potassium (Fig. 3) was similar at baseline between the groups (F = 1.612, P = 0.21). By the end of euglycemia, potassium was significantly lower in all groups (P < 0.001); the euglycemic decrement in potassium was similar between the groups (F = 0.806, P = 0.59).

During hypoglycemia, the decrement in potassium differed significantly between the groups (F = 2.543, P = 0.029). This was largely accounted for by a twofold greater potassium fall in the nondiabetic participants (0.59 ± 0.06 mmol/l) when compared with the diabetic groups (BRS+, 0.29 ± 0.07 mmol/l, P = 0.047 vs. control subjects; BRS−, 0.30 ± 0.09 mmol/l, P = 0.08 vs. control subjects; CAN+, 0.33 ± 0.06 mmol/l, P = 0.12 vs. control subjects). The diabetic groups exhibited similar potassium falls (BRS+ vs. BRS−, P = 0.998; BRS+ vs. CAN+, P = 0.98; BRS− vs. CAN+, P = 0.998). Plasma calcium (corrected for albumin) and magnesium concentrations were similar among the groups and remained unchanged during the studies (data not shown).

TABLE 2
Participant characteristics

	BRS+ (n = 10)	BRS− (n = 9)	CAN+ (n = 9)	Normal (n = 8)	P value
Male:female ratio	9:1	9:0	8:1	7:1	
Age (years)	33.8 ± 2.8	33.4 ± 1.9	40.6 ± 1.7	33.8 ± 2.3	0.1
Diabetes duration (years)	14.6 ± 2.2	16.8 ± 2.5	18.6 ± 2.9	—	0.51
HbA_{1c} (%)	9.1 ± 0.5	8.8 ± 0.3	9.6 ± 0.4	5.0 ± 0.07	0.44*
BMI (kg/m^2)	23.7 ± 0.9	25.8 ± 1.1	23.6 ± 1.1	24.3 ± 1.2	0.49
BDR	4	4	3	—	
Laser-treated DR	0	2	2	—	
PN	1	2	8	—	

Data are means ± SE. BDR, background diabetic retinopathy; DR, diabetic retinopathy; PN, peripheral neuropathy. *Excluding normal subjects.

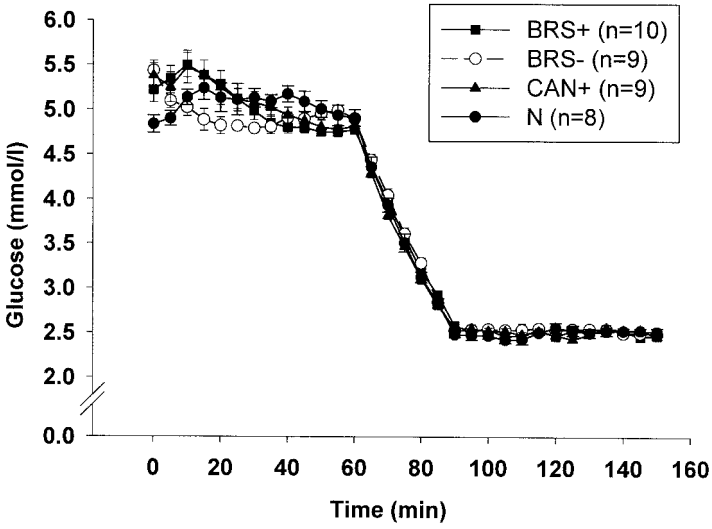

FIG. 1. Arterialized venous blood glucose concentrations. Error bars denote SE. N, normal.

Heart rate, blood pressure, sweat, and tremor responses to hypoglycemia. At baseline, heart rate differed between the groups ($F = 7.42$, $P = 0.001$), being greater in CAN+ participants than in nondiabetic ($P = 0.015$) and BRS+ ($P = 0.001$) participants; there were otherwise no significant group differences (Table 3).

During hypoglycemia, heart rate increased in the control (peak increase from baseline 12 ± 4.3 bpm; $P = 0.02$), BRS+ (12 ± 2.3 bpm; $P < 0.001$), and BRS− (10 ± 1.8 bpm, $P < 0.001$) groups; by contrast, CAN+ participants did not show a significant rise in heart rate (peak increase 5 ± 3.3 bpm; $P = 0.36$). However, hypoglycemic heart rate changes did not differ significantly between the groups ($F = 0.85$, $P = 0.56$).

SBP was similar at baseline among the groups ($F = 1.16$, $P = 0.34$). SBP tended to increase during hypoglycemia in all groups, although this was significant only for the nondiabetic (peak increase 13 ± 2.2 mmHg; $P = 0.001$) and BRS+ (10 ± 1.9 mmHg; $P = 0.02$) groups. Overall, there was no significant difference between the groups with respect to change in SBP during hypoglycemia ($F = 0.637$, $P = 0.82$).

Baseline diastolic blood pressure (DBP) was similar among the groups (F = 0.79, $P = 0.51$). Although DBP tended to fall during hypoglycemia in the BRS+ and control groups, the change in DBP was not significantly different between the groups ($F = 1.069$, $P = 0.39$).

Mean arterial pressure was similar at baseline and remained unchanged during hypoglycemia across all groups. At baseline, finger tremor was similar among all groups ($F = 1.80$, $P = 0.17$). There was a significant difference in hypoglycemic tremor responses between the groups ($F = 2.016$, $P = 0.047$). Although tremor increased during hypoglycemia in all groups, this tended to be blunted in CAN+ participants (peak incremental tremor 0.049 ± 0.010 $g \cdot m^{-2} \cdot min^{-1}$), particularly in comparison with the control (0.163 ± 0.044, $P = 0.06$ vs. CAN+) and BRS+ groups (0.142 ± 0.023, $P = 0.12$ vs. CAN+).

Sweating increased across all groups during hypoglycemia. The magnitude of the sweating response did not differ between the groups ($F = 0.59$, $P = 0.70$).

QTc interval. Baseline QTc was similar between the

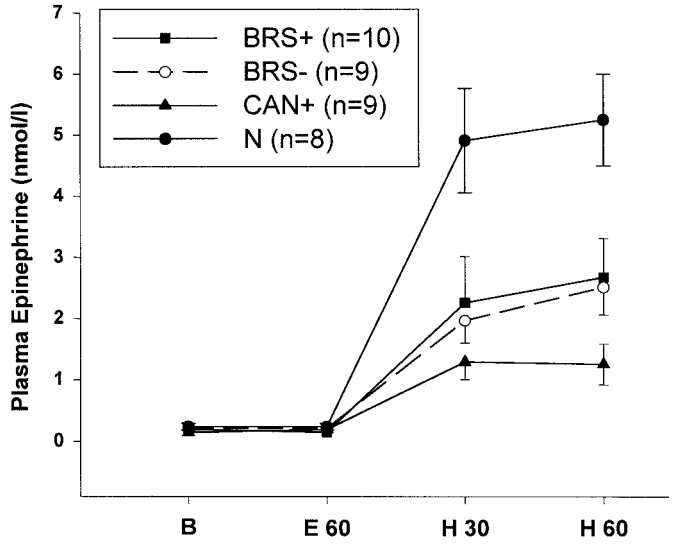

FIG. 2. Mean ± SE circulating epinephrine concentrations at baseline (B), at the end of euglycemia (E60), and after 30 (H30) and 60 (H60) min of hypoglycemia. N, normal.

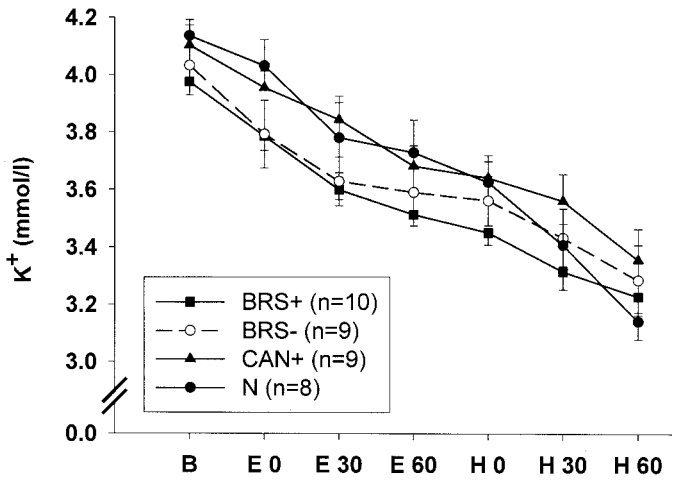

FIG. 3. Mean ± SE plasma potassium at baseline (B) and after 0, 30, and 60 min of euglycemia (E0, E30, and E60, respectively) and hypoglycemia (H0, H30, and H60, respectively). N, normal.

TABLE 3
Physiological responses

	B	E0	E30	E60	H0	H30	H60
Heart rate (bpm)							
BRS+	62 ± 2	62 ± 2	64 ± 2	62 ± 2	71 ± 3	72 ± 3	71 ± 2
BRS−	69 ± 3	72 ± 3	70 ± 2	71 ± 3	74 ± 3	77 ± 2	76 ± 4
CAN+	79 ± 3	80 ± 3	81 ± 4	80 ± 3	83 ± 4	83 ± 3	83 ± 3
Normal	65 ± 2	64 ± 2	67 ± 2	66 ± 2	70 ± 3	76 ± 5	71 ± 4
SBP (mmHg)							
BRS+	117 ± 2	118 ± 2	119 ± 2	118 ± 2	118 ± 2	124 ± 3	122 ± 3
BRS−	123 ± 4	123 ± 3	124 ± 3	125 ± 3	125 ± 2	127 ± 3	132 ± 5
CAN+	125 ± 5	122 ± 4	121 ± 3	124 ± 6	122 ± 7	128 ± 6	129 ± 4
Normal	121 ± 3	124 ± 2	122 ± 2	121 ± 3	123 ± 3	132 ± 5	126 ± 4
DBP (mmHg)							
BRS+	72 ± 2	71 ± 2	71 ± 2	72 ± 2	70 ± 2	68 ± 2	67 ± 2
BRS−	77 ± 2	78 ± 2	74 ± 2	77 ± 2	75 ± 2	74 ± 2	77 ± 2
CAN+	76 ± 3	75 ± 2	76 ± 3	78 ± 3	72 ± 3	74 ± 2	75 ± 2
Normal	74 ± 3	73 ± 4	75 ± 3	77 ± 3	77 ± 3	75 ± 4	71 ± 4
Sweat ($g \cdot m^{-2} \cdot min^{-1}$)							
BRS+	28.9 ± 3.1	22.6 ± 3.2	21.6 ± 3.2	26.8 ± 4.6	45.6 ± 21.4	110.0 ± 54.3	103.9 ± 51.5
BRS−	29.3 ± 5.9	38.4 ± 14.8	28.9 ± 6.2	32.5 ± 5.2	36.2 ± 5.9	167.2 ± 55.4	119.9 ± 49.0
CAN+	47.2 ± 8.5	44.7 ± 9.0	34.7 ± 6.9	36.1 ± 7.6	126.7 ± 61.4	205.2 ± 67.1	178.2 ± 60.1
Normal	35.5 ± 4.7	30.3 ± 4.2	32.4 ± 4.8	25.8 ± 3.6	98.4 ± 38.1	236.5 ± 72.8	221.6 ± 67.0
Tremor (RMS V/s)							
BRS+	0.106 ± 0.006	0.128 ± 0.007	0.129 ± 0.005	0.126 ± 0.012	0.143 ± 0.013	0.214 ± 0.015	0.170 ± 0.021
BRS−	0.099 ± 0.012	0.113 ± 0.010	0.120 ± 0.010	0.117 ± 0.014	0.159 ± 0.018	0.180 ± 0.036	0.149 ± 0.020
CAN+	0.108 ± 0.008	0.145 ± 0.010	0.143 ± 0.010	0.139 ± 0.011	0.168 ± 0.022	0.140 ± 0.011	0.136 ± 0.008
Normal	0.082 ± 0.006	0.112 ± 0.014	0.120 ± 0.010	0.123 ± 0.015	0.135 ± 0.016	0.232 ± 0.046	0.192 ± 0.040

Data are means ± SE. RMS, root mean square.

groups (control, 378 ± 4 ms; BRS+, 377 ± 9 ms; BRS−, 380 ± 8 ms; CAN+, 383 ± 6 ms; $F = 0.124$, $P = 0.95$; Fig. 4). During euglycemia, there was a small (10–20 ms) but significant increase in QTc across all groups; these increases were similar between the groups ($F = 0.94$, $P = 0.49$).

During hypoglycemia, QTc lengthened significantly among all four groups ($P < 0.001$). However, there was considerable interindividual variability. BRS+ participants exhibited peak QTc increases above baseline between 13 and 90 ms (mean, 61 ms). QTc lengthened by 4 to 84 ms (mean, 41 ms) in BRS− participants, by −9 to 49 ms (mean, 25 ms) in CAN+ participants, and by 28 to 106 ms (mean, 61 ms) in the nondiabetic participants.

The groups differed with respect to the magnitude of hypoglycemic QTc lengthening ($F = 3.442$, $P = 0.007$). When expressed as peak increase above baseline, CAN+ participants showed blunted QTc increases in comparison with both the control ($P = 0.038$) and BRS+ ($P = 0.025$) groups. There were no significant differences among the control, BRS+, and BRS− groups.

Maximum QTc interval during hypoglycemia did not differ significantly between the groups ($F = 1.731$, $P = 0.18$), although there was a trend toward lower peak QTc in CAN (408 ± 10 ms) and BRS− (421 ± 11 ms) participants when compared with BRS+ (439 ± 13 ms) and control (439 ± 10 ms) groups.

There was a significant positive correlation (Fig. 5) between peak incremental plasma epinephrine response and the magnitude of QTc lengthening ($r_s = 0.43$, $P = 0.009$).

DISCUSSION

Our data show that type 1 diabetic patients with CAN (as evidenced by either abnormal conventional cardiovascular tests or impaired BRS) do not exhibit greater hypoglycemic QTc lengthening than those without CAN. Indeed, CAN+ participants tended to show the smallest QTc increases. Thus, our findings refute the hypothesis that autonomic neuropathy is an important factor increasing the risk of QTc lengthening during hypoglycemia in young adults with type 1 diabetes. This suggests that autonomic neuropathy is not an important contributory factor to sudden death from hypoglycemia in young people with diabetes.

Although the mechanisms underlying these deaths are not fully understood, there is strong circumstantial evidence implicating hypoglycemia and associated cardiac arrhythmia (1,6). In Tattersall and Gill's series, most deaths occurred at night, and 14 patients had experienced significant nocturnal hypoglycemia during the preceding months. Generally, the bedclothes were not disturbed, and no anatomical lesion (e.g., neuropathological evidence of hypoglycemia) was found at autopsy. Therefore, it seems unlikely that the deaths were related to hypoglycemic brain damage. The evidence points to a sudden cardiac or respiratory arrest as the terminal event. It is noteworthy that, although apparently uncommon, both supraventricular (29,30) and ventricular (31–33) arrhythmias have been reported during hypoglycemia.

Epinephrine infusion lengthens QTc interval in normal participants (34), predominantly through a β-adrenoceptor–mediated process, whereby the cardiac action potential is prolonged by delayed inactivation of inward calcium currents (35). In this study, we observed a relationship, albeit weak, between the magnitude of the hypoglycemic epinephrine response and the degree of QTc lengthening. These observations suggest that hypoglycemic QTc length-

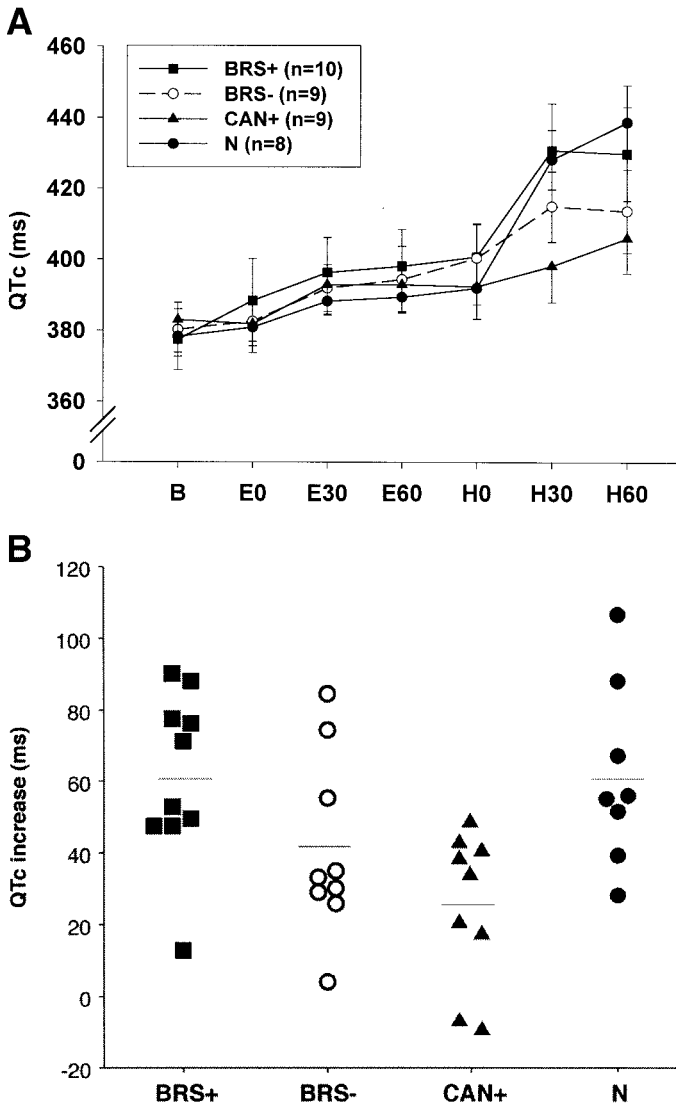

FIG. 4. Effect of hypoglycemia on QTc interval. *A*: Mean ± SE QTc at baseline (B) and after 0, 30, and 60 min of euglycemia (E0, E30, and E60, respectively) and hypoglycemia (H0, H30, and H60, respectively). *B*: Individual (and mean) hypoglycemic QTc responses expressed as peak increase above baseline. N, normal.

ening is predominantly mediated through the sympatho-adrenal response, although there seems to be wide interindividual variability in myocardial epinephrine sensitivity (7,36). Type 1 diabetic patients with autonomic neuropathy tend to exhibit impaired plasma epinephrine responses to hypoglycemia in comparison with their non-neuropathic counterparts (37,38), and this seems the most likely explanation for our findings.

We did not restrict recruitment according to HbA_{1c} or match it over the three diabetic groups because of the limited number of participants. However, we made some attempt to ensure that HbA_{1c} in the three diabetic groups was similar because glycemic control modulates the sympatho-adrenal response and hence could influence the degree of QT lengthening. Glycemic control tended to be poor in the diabetic participants, perhaps not surprising in those with diabetic neuropathy. It is possible that both the hypoglycemic sympatho-adrenal response and the magnitude of QT lengthening would have been smaller if we had studied participants with better glycemic control. However, because HbA_{1c} was similar across the groups (with a trend to higher values in CAN+ participants), we do not believe that the level of glycemic control influenced our findings.

Our laboratory-based study inevitably has limitations when considering its relevance to the clinical situation. Because the depth of experimental hypoglycemia must be limited for ethical reasons, we have used QT interval as a surrogate marker of abnormal cardiac repolarization. Thus, the influence of autonomic dysfunction on cardiac electrical pathophysiology during clinical hypoglycemic episodes must remain somewhat uncertain, as is the influence of QT duration on arrhythmia risk. However, there is good evidence in families with congenital LQTS that the risk of ventricular arrhythmia is related to QT interval (39), and this is also the case in acquired QT lengthening caused by medication (40) or after myocardial infarction (41).

Experimental hypoglycemia is induced using greater circulating insulin concentrations than those generally observed clinically, and this tends to produce greater potassium falls than those that occur during clinical episodes. This might be expected to produce greater QTc lengthening. However, we recently showed that, in comparison with the rise in plasma epinephrine, an associated fall in potassium has a relatively minor effect on hypoglycemic QTc prolongation (8). Similarly, preventing a fall in plasma potassium does not prevent QTc lengthening induced by epinephrine infusion (36).

The clinical relevance of our data in relation to the risks of intensive insulin therapy is also uncertain. Nocturnal

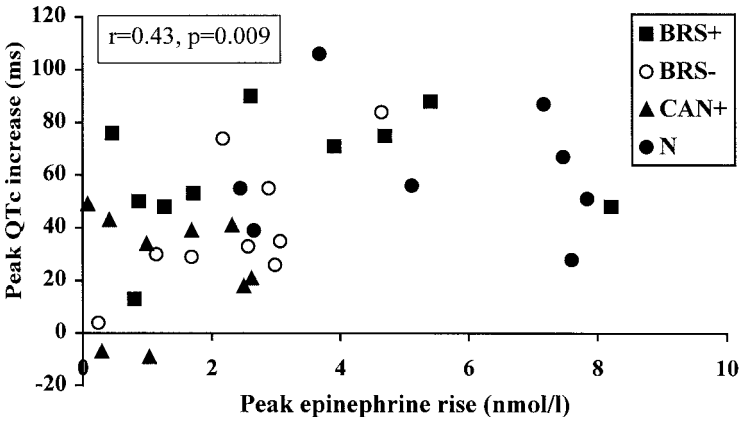

FIG. 5. Relationship between plasma epinephrine response and magnitude of hypoglycemic QTc response. N, normal.

hypoglycemia is common in both adults and children with type 1 diabetes whether they are undertaking intensive insulin therapy or not, although it is likely to occur more frequently in those who aim for tight glucose targets. Thordarson and Sovik (4) noted a rising incidence of hypoglycemic death during the 1980s and attributed this in part to the increasing use of intensive insulin therapy. Conversely, tight glycemic control is associated with diminished sympatho-adrenal responses, which, although increasing the risk of severe hypoglycemia, might actually reduce the degree of abnormal cardiac repolarization and any associated arrhythmic risk. Nevertheless, we advocate the use of approaches to reduce nocturnal hypoglycemia, such as insulin analogs or insulin pump therapy, in those who are at particular risk during intensive insulin therapy.

In conclusion, we have demonstrated that the presence of autonomic neuropathy does not result in greater QTc lengthening during moderate experimental hypoglycemia in type 1 diabetic participants. Rather, our data support the view that patients with the strongest hypoglycemic sympatho-adrenal responses, typically those with relatively short disease duration and poor glycemic control, may be most at risk for significant QTc lengthening and perhaps sudden death. Other factors, including mutations or polymorphisms in candidate genes, also need to be explored in future work because this might identify those who are at particular risk of this rare but devastating condition.

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REFERENCES

1. Tattersall RB, Gill GV: Unexplained deaths of type 1 diabetic patients. *Diabet Med* 8:49–58, 1991
2. Campbell IW: Dead in bed syndrome: a new manifestation of nocturnal hypoglycaemia? (Editorial). *Diabet Med* 8:3–4, 1991
3. Borch-Johnsen K, Helweg-Larsen K: Sudden death and human insulin: is there a link? *Diabet Med* 10:255–259, 1993
4. Thordarson H, Sovik O: Dead in bed syndrome in young diabetic patients in Norway. *Diabet Med* 12:782–787, 1995
5. Sartor G, Dahlquist G: Short-term mortality in childhood onset insulin-dependent diabetes mellitus: a high frequency of unexpected deaths in bed. *Diabet Med* 12:607–611, 1995
6. 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* 16:626–631, 1999
7. 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* 14:648–654, 1997
8. Robinson RTCE, Harris ND, Ireland RH, Lee S, Newman C, Heller SR: Mechanisms of abnormal cardiac repolarization during insulin-induced hypoglycemia. *Diabetes* 52:1469–1474, 2003
9. Robinson RTCE, 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* 47:312–315, 2004
10. Harris ND, Heller SR: Sudden death in young patients with type 1 diabetes: a consequence of disease, treatment or both? *Diabet Med* 16:1–3, 1999
11. Ziegler D: Diabetic cardiovascular autonomic neuropathy: prognosis, diagnosis and treatment. *Diabetes Metab Rev* 10:339–383, 1994
12. Sivieri R, Veglio M, Chinaglia A, Scaglione P, Cavallo-Perin P: Prevalence of QT prolongation in a type 1 diabetic population and its association with autonomic neuropathy: the Neuropathy Study Group of the Italian Society for the Study of Diabetes. *Diabet Med* 10:920–924, 1993
13. Malpas SC, Maling TJ: Heart-rate variability and cardiac autonomic function in diabetes. *Diabetes* 39:1177–1181, 1990
14. Weston PJ, James MA, Panerai R, McNally PG, Potter JF, Thurston H, Swales JD: Abnormal baroreceptor-cardiac reflex sensitivity is not detected by conventional tests of autonomic function in patients with insulin-dependent diabetes mellitus. *Clin Sci (Lond)* 91:59–64, 1996
15. Farrell TG, Odemuyiwa O, Bashir Y, Cripps TR, Malik M, Ward DE, Camm AJ: Prognostic value of baroreflex sensitivity testing after acute myocardial infarction. *Br Heart J* 67:129–137, 1992
16. La Rovere MT, Bigger JT Jr, Marcus FI, Mortara A, Schwartz PJ: Baroreflex sensitivity and heart-rate variability in prediction of total cardiac mortality after myocardial infarction. *Lancet* 351:478–484, 1998
17. O'Brien IA, O'Hare P, Corrall RJM: Heart rate variability in healthy subjects: effect of age and the derivation of normal ranges for tests of autonomic function. *Br Heart J* 55:348–354, 1986
18. Di Rienzo M, Bertinieri G, Mancia G, Pedotti A: A new method for evaluating the baroreflex role by a joint pattern analysis of pulse interval and systolic blood pressure series. *Med Biol Eng Comput* 23 (Suppl. 1):313–314, 1985
19. Pitzalis MV, Mastropasqua F, Passantino A, Massari F, Ligurgo L, Forleo C, Balducci C, Lombardi F, Rizzon P: Comparison between noninvasive indices of baroreceptor sensitivity and the phenylephrine method in post-myocardial infarction patients. *Circulation* 97:1362–1367, 1998
20. Parati G, Casadei R, Groppelli A, Di Rienzo M, Mancia G: Comparison of finger and intra-arterial blood pressure monitoring at rest and during laboratory testing. *Hypertension* 13:647–655, 1989
21. Parati G, Di Rienzo M, Bertinieri G, Pomidossi G, Casadei R, Groppelli A, Pedotti A, Zanchetti A, Mancia G: Evaluation of the baroreceptor-heart rate reflex by 24-hour intra-arterial blood pressure monitoring in humans. *Hypertension* 12:214–222, 1988
22. Tank J, Baevski RM, Fender A, Baevski AR, Graves KF, Ploewka K, Weck M: Reference values of indices of spontaneous baroreceptor reflex sensitivity. *Am J Hypertens* 13:268–275, 2000
23. Fairbanks VF, Zimmerman BR: Measurement of glycosylated hemoglobin by affinity chromatography. *Mayo Clin Proc* 58:770–773, 1983
24. Forster CD, Macdonald IA: The assay of the catecholamine content of small volumes of human plasma. *Biomed Chromatogr* 13:209–215, 1999
25. Ireland RH, Robinson RTCE, Heller SR, Marques JLB, Harris ND: Measurement of high resolution ECG QT interval during controlled euglycaemia and hypoglycaemia. *Physiol Meas* 21:295–303, 2000
26. Lepeschkin E, Surawicz B: The measurement of the QT interval of the electrocardiogram. *Circulation* 6:378–388, 1952
27. Bazett HC: An analysis of the time relations of electrocardiograms. *Heart* 7:353–370, 1920
28. George E, Harris N, Bedford C, Macdonald IA, Hardisty CA, Heller SR: Prolonged but partial impairment of the hypoglycaemic physiological response following short-term hypoglycaemia in normal subjects. *Diabetologia* 38:1183–1190, 1995
29. Collier A, Matthews DM, Young RJ, Clarke BF: Transient atrial fibrillation precipitated by hypoglycaemia: 2 case reports. *Postgrad Med J* 63:895–897, 1987
30. Fisher BM, Marshall DA, MacCuish AC: Hypoglycaemia and atrial flutter. *Postgrad Med J* 67:1083, 1991
31. Judson WE, Hollander W: The effects of insulin-induced hypoglycaemia in patients with angina pectoris: before and after intravenous hexamethonium. *Am Heart J* 52:198–209, 1956
32. Miley GB, Binnick SA, Block PJ: Hypoglycemia and ventricular tachycardia due to adenocarcinoma of the stomach. *Am J Med Sci* 269:403–408, 1975
33. Chelliah YR: Ventricular arrhythmias associated with hypoglycaemia. *Anaesth Intensive Care* 28:698–700, 2000
34. Struthers AD, Reid JL, Whitesmith R, Rodger JC: The effects of cardioselective and non-selective beta-adrenoceptor blockade on the hypokalaemic and cardiovascular responses to adrenomedullary hormones in man. *Clin Sci* 65:143–147, 1983
35. Yue DT, Herzig S, Marban E: Beta-adrenergic stimulation of calcium channels occurs by potentiation of high-activity gating models. *Proc Natl Acad Sci U S A* 87:742–747, 1990
36. Lee S, Harris ND, Robinson RT, Yeoh L, Macdonald IA, Heller SR: Effects of adrenaline and potassium on QTc interval and QT dispersion in man. *Eur J Clin Invest* 33:93–98, 2003
37. Hilsted J, Madsbad S, Krarup T, Sestoft L, Christensen NJ, Tronier B, Galbo

H: Hormonal, metabolic, and cardiovascular responses to hypoglycemia in diabetic autonomic neuropathy. *Diabetes* 30:626–633, 1981
38. Bottini P, Boschetti E, Pampanelli S, Ciofetta M, Del Sindaco P, Scionti L, Brunetti P, Bolli GB: Contribution of autonomic neuropathy to reduced plasma adrenaline responses to hypoglycemia in IDDM: evidence for a nonselective defect. *Diabetes* 46:814–823, 1997
39. Moss AJ, Schwartz PJ, Crampton RS, Tzivoni D, Locati EH, MacCluer J, Hall WJ, Weitkamp L, Vincent GM, Garson A: The long QT syndrome: prospective longitudinal study of 328 families. *Circulation* 84:1136–1144, 1991
40. Shah RR: The significance of QT interval in drug development. *Br J Clin Pharmacol* 54:188–202, 2002
41. Dabrowski A, Kramarz E, Kubik L: Prolongation of QT interval corrected for heart rate by Bazett's equation and linear formula as predictor of arrhythmic events after myocardial infarction. *Am J Cardiol* 86:469–472, 2000