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Title Page

Title: Longer QTc interval associates with higher HbA1c and autonomic nerve dysfunction in adolescents with type 1 diabetes

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Longer QTc associates with higher HbA1c and autonomic nerve dysfunction in adolescents with type 1 diabetes

Abstract

Aims: To examine QT intervals corrected for heart rate (QTc) in adolescents with type 1 diabetes (T1DM) compared to controls, and determine associations with metabolic control and autonomic function.

Methods: Resting electrocardiogram recordings of 142 adolescents with T1DM (mean (SD) age 15.3 (±2.0) years, diabetes duration 9.0 (±3.5) years, HbA1c 8.7 (±1.6)% (71 (17) mmol/mol)) and 125 controls (age 15.7 (±2.5) years) were used to calculate QTc duration and derive mean heart rate (HR) and heart rate variability (HRV) parameters. Linear and logistic regression models were used to examine associations between QTc, metabolic control and autonomic function (HRV and pupillary function).

Results: QTc duration was not significantly different between T1DM and control subjects (mean: 392 vs. 391ms; p =0.65). In T1DM, QTc was positively associated with HbA1c (β 4 (95% CI: 2, 6); p<0.001); and inversely associated with severe hypoglycemia events (-10 (-20,-2); p=0.01), less insulin/kg (-12 (-22, -2); p=0.024) and less HRV. In T1DM, QTc in the highest quintile (≥409ms) vs. quintiles 1-4 had more pupillary abnormalities (83% vs. 56%; p =0.03), lower pupillary maximum constriction velocity (4.8 vs. 5.3mm/s; p =0.04), higher HR (78 vs. 72bpm, p =0.02) and lower HRV (SDNN 4.0 vs. 4.3ms, p =0.004 and RMSSD 3.7 vs. 4.1ms, p =0.004).

Conclusions: Contrary to general hypoglycemia concerns in T1DM, chronic hyperglycemia, rather than intermittent hypoglycemia, appears to be more deleterious to autonomic cardiac function even in adolescence. Longer QTc was associated with higher HbA1c, lower risk of hypoglycemia and autonomic dysfunction. Longitudinal studies are warranted.

Introduction

The incidence of sudden unexplained death in childhood onset type 1 diabetes (T1DM) cohorts has recently been estimated to be 45-48 per 100,000 patient years (1) and 5-6% of all early deaths (<40 years old) due to diabetes (2). The underlying cause is thought to relate to a cardiac arrhythmia triggered by hypoglycemia and QT interval prolongation (3). The QT interval on the electrocardiogram (EKG) measures the total time for ventricular depolarization and repolarization, and prolonged QT interval corrected for heart rate (QTc) may be a trigger for ventricular arrhythmia and, consequently, sudden death in the non-diabetic population (4). The QT interval can be acutely prolonged with hypoglycemia (3).

In people with diabetes, a prolonged QTc interval has been associated with cardiac autonomic neuropathy (5), though the association may be stronger in younger people with diabetes (6). As cardiac pace-making is a neural function, there may be links between QTc length and other measures of autonomic nerve function, such as pupillary function, which we and others have found to be abnormal in adolescents with T1DM (7, 8). A link between HRV and QTc length has been found in adults (9), however, no study to date has identified a link between abnormal pupillometry and QTc length, and studies for both modalities are limited in the adolescent population. We therefore hypothesized that: (1) Type 1 diabetes would cause longer QTc intervals than in control subjects during adolescence; and (2) longer QTc interval would be associated with diabetic autonomic neuropathy (DAN), as measured by adverse changes in HRV and pupillometry.

Methods

Subjects

Adolescents aged 10-18 years with T1DM (n=142) were recruited from the Children's Hospital at Westmead during 2008-2011, and 125 healthy adolescents aged 10-18 years (controls) were recruited from schools and hospital relatives between 2010-2013. No subjects were taking cardiac or anti-hypertensive medications. Ethics approval for the study was obtained from the Children's Hospital at Westmead and the University of Sydney. Written informed consent was obtained from adolescents and their families.

Clinical and biochemical assessment

All participants with T1DM underwent standardized clinical assessment. If experienced diabetes research nurses detected clinical signs of hypoglycemia, which was confirmed on blood glucose testing, it was treated prior to the EKG recording. The number of severe hypoglycemic episodes (defined as unconsciousness or seizures) experienced by T1DM subjects in the previous six months, as well the number experienced during their lifetime, were recorded. Total daily dose of insulin (units/kg) was recorded as reported by the subject or their parent. Height and weight were measured, and body mass index (BMI) calculated. Height, weight and BMI standard deviation scores (SDS) were calculated using the LMS method which summarizes the changing distribution of centiles according to age by three curves representing median (M), coefficient of variation (S) and skewness (L) (10). Blood pressure was measured with the subject supine after five minutes of resting and recorded by auscultation with a standard sphygmomanometer. Age and sex-related

SDS for systolic BP (SBP) and diastolic BP (DBP) were calculated according to published standards (11).

HbA1c was assessed by high performance liquid chromatography (Variant analyzer, Bio-Rad Laboratories, CA, USA until December 2009, then Adams Arkray Inc., Kyoto, Japan from January 2010 onwards; Adams = 1.0566x Variant, where R^2 = 0.98).

Total cholesterol was measured using VITROS CHOl slides and VITROS Chemistry Products Calibrator Kit 2 on VITROS 5,1 FS (Ortho Clinical Diagnostics). HDL-cholesterol was measured using VITROS dHDL slides and the VITROS Chemistry Products Calibrator Kit 25 on VITROS 5,1 FS. Thyroid stimulating hormone (TSH) was measured on an Architect i2000 (Abbott Diagnostics) by a chemiluminescent microparticle immunoassay.

EKG analysis

Using the same machine, 10-minute EKG recordings were performed using LabChart® and LabChart® Pro software, an electronic recording system, using a standardized protocol, measuring the reading of lead I (negative electrode placed on right arm, positive electrode on left arm, earth electrode on right hip) in rested supine subjects.

The first five readable beats for each minute in the first 10 minutes of the EKG recordings (i.e. 50 beats in total) for each subject were analyzed. ‘Readable’ beats refers to those beats for which there was little or no interference or noise and the QT interval and RR interval could be accurately determined. QTc interval was calculated using three formulae: Bazett’s (12), Framingham (13) and Fridericia (14). The

software analysis was manually checked by one EKG reader to confirm that i) the beat analyzed did not have excessive noise or activity and ii) QT and RR intervals were accurately measured. QT intervals were identified from the beginning of the QRS complex to the end of the T wave. The end of the T wave was identified as the return of the T wave to the isoelectric line, and in the presence of a U wave, the end of the T wave was identified as the nadir between the T wave and the U wave. The RR interval was taken from the peak of the R wave of the previous beat to the peak of the R wave of the analyzed beat. To the extent that the software did not produce accurate measurements, for example, misidentification of the end of the T wave or the peak of the R wave, these were manually corrected. Subjects were excluded if there was excessive noise interference in their EKG.

One reviewer (KS) analyzed and edited all EKG data. A second (cardiologist) reviewer analyzed and edited a randomly chosen 10% of the EKG data. Concordance between the two EKG readers was assessed using a two one-sided tests (TOST) equivalence paired t-test with an acceptable equivalence bound of 20ms on each side found ($p < 0.0001$; confidence interval: -0.01, -0.005). For each individual, a mean QTc and maximum QTc were calculated using the three formulae.

The entire 10-minute EKG trace was used for HRV analysis, with exclusion of ectopic beats (<500ms, >1100ms). The trace was visually scanned for R-waves amongst artefacts and ectopic beats by one operator, and R-waves manually inserted for analysis (8). Mean HR and HRV parameters were derived using LabChart Pro. The mean HR was calculated from the NN intervals, defined as the distance between adjacent QRS complexes. Derived time domain measures included: standard deviation of mean NN intervals (SDNN), root mean squared difference of successive NN intervals (RMSDD), and HRV triangular index (TI),

which are estimates of overall HRV. Frequency domain measures included: low frequency (LF) representing the sympathetic component, defined as >0.04Hz and <0.15Hz, and high frequency (HF) representing the parasympathetic component, defined as >0.15Hz and <0.4Hz, and the LF:HF ratio (15), indicating sympathovagal balance.

Autonomic and microvascular assessment

Pupillary autonomic function at baseline and reference ranges were assessed by measuring the dark-adapted pupil size before and 3-seconds after a light stimulus delivered using an infrared pupillometer (Pupilscan; Fairvil Medical Optics). Variables measured as continuous variables included initial pupil diameter, maximum constriction velocity and reflex amplitude. Abnormal was defined as <5th centile for any of the three variables (16).

Seven-field stereoscopic fundal photography was used to detect early retinopathy, defined as the presence of at least one microaneurysm or hemorrhage (≥21) graded according to the modified Airlie House classification. Urinary albumin was measured using an Immulite analyzer (Siemans). Microalbuminuria was defined as AER ≥20μg/min in at least two of three samples from timed overnight urine collections or mean ACR ≥4.1 mg/mmol (17).

Statistical analysis

Statistical tests were performed using SPSS version 21. HRV parameters (SDNN, RMSSD, LF, HF, LF:HF and TI) were log transformed (base e) before analysis.

QTc interval was divided into quintiles based on the QTc distribution in the control group, with quintile 5 being ≥409ms. The lower quintiles 1-4 were combined for analysis, and compared to the highest quintile 5.

Independent sample T-tests were used to examine the differences between the diabetes and control groups, as well as differences in participant characteristics and autonomic function in the two quintile groups (5 vs. 1-4). General linear models were used to calculate estimated marginal mean for QTc for the two groups, each adjusted for BMI, age and gender. Chi-square statistic was used to determine differences in frequency of microvascular complications in the two quintile groups.

Linear regression was used to examine associations between QTc length, as a continuous outcome, and clinical explanatory variables (HbA1c, diabetes duration, BMI SDS, SBP SDS, DBP SDS, total and HDL-cholesterol levels and gender), treatment variables (insulin dose and significant hypoglycemia) and autonomic function (HRV parameters and pupillometry) in the diabetes group.

Multivariate regression models were used to examine highest vs. lower QTc quintiles as a binary outcome with clinical explanatory variables (age, gender, HbA1c, diabetes duration, height SDS, weight SDS, BMI SDS, SBP SDS, DBP SDS, total and HDL-cholesterol and TSH), and autonomic nerve tests.

Statistical significance was taken at $p<0.05$.

Results

EKG traces were analyzed from 142 subjects with T1DM and 123 control subjects. Table 1 summarizes clinical characteristics for both groups. T1DM participants had higher BMI SDS than controls. In the T1DM group, mean (SD) HbA1c was 8.7% (1.6) (71(17) mmol/mol) and diabetes duration 9.0 (3.5) years. Nine (7%) had a severe hypoglycemic episode in the last 6-months, and 42 (33%) had severe hypoglycemia during their lifetime.

There was no significant difference in QTc between T1DM and controls using Bazett's formula for QTc (Table 1), or using Framingham or Fridericia formulae (mean 378 vs. 379; p=0.77 and mean 375 vs. 379; p=0.62 respectively). A measure of long QTc >440ms occurred in 67 controls and 64 T1DM subjects (p=0.14), with an upper range of 831ms and 792ms respectively. Resting HR was faster in T1DM than controls with less HRV (Table 1).

In the T1DM cohort, longer QTc was associated with higher HbA1c, less exogenous insulin, younger age, higher total cholesterol levels and not having severe hypoglycemia in the last 6-months, but not with SBP, DBP, BMI, HDL-cholesterol nor diabetes duration. In multivariate analysis, HbA1c accounted for 10% of the variation, a further 4% was explained by hypoglycemia, 4% by age, 10% by gender and 1% by insulin dose/kg. Total cholesterol level did not remain significant.

Longer QTc interval was significantly associated with HRV measures: lnRMSSD, lnLF:HF ratio, even after adjustment for above variables, adding a further 3% and 2% variation to the model (Table 2).

The threshold for the highest QTc quintile was ≥409ms. Adolescents with T1DM in the highest QTc quintile had significantly higher HbA1c, BMI SDS, total cholesterol and were more likely to be female than those in the lower four quintiles (Table 3). They also had significantly higher HR and lower HRV (as measured by lower lnSDNN and lnRMSSD), and a significantly greater proportion of pupillary abnormalities (8% vs. 56%; p=0.03), but not significantly less retinopathy or microalbuminuria (Table 3). There was no association between QTc interval as a continuous outcome and pupillary variables.

Discussion

In adolescents of mean age 15 years and 9 years T1DM duration, we did not find a longer QTc duration than in their non-diabetic peers. This was consistent regardless of QTc formula used for heart rate (Bazett's, Framingham or Fridericia formulae). Longer QTc interval was associated with the vascular risk factor of higher HbA1c and adverse changes in cardiac and pupillary autonomic nerve function, but not other microvascular complications. This is the first study to identify a link between abnormal pupillometry and longer QTc in T1DM, supporting prolongation of resting QTc interval as part of autonomic nerve dysfunction.

The primary concern of a prolonged QTc interval is the initiation of a potentially fatal arrhythmia and sudden cardiac death (4), the risk of which is increased in the setting of hypoglycemia. QTc intervals over 440ms are generally considered 'prolonged' and predictive of mortality in adults (4). Even if not over 440ms, longer QTc intervals have been positively associated with increased risk of subsequent cardiovascular disease, stroke and heart failure (18). Our quintile analysis (QTc ≥409ms) suggests that a lower threshold for QTc interval differentiates adolescents at risk of other diabetes complications.

Our study showed a positive association between HbA1c and longer QTc interval, suggesting that poorer glycemic control may be associated with increased risk of arrhythmia, and that improvement in glycemic control, without increasing hypoglycemia risk, could be an important preventative measure against prolonged QTc intervals and cardiac arrhythmias. Severe hypoglycemia in the last 6-months was not recalled in the highest QTc quintile group, but was higher over their lifetime. This implies that glycemic control should be the major focus to improve QTc at rest,

but severe hypoglycemia should still be avoided as this could acutely prolong the QTc interval and induce cardiac arrhythmias.

Hypoglycemia has been proposed as a trigger for QTc interval prolongation. Our clinical data did not support hypoglycemia as a risk factor, with an absence of severe hypoglycemia in the previous 6-months associated with QTc length at rest in the daytime. Experimental hypoglycemia has been shown to induce prolonged QTc intervals (19), and other studies have confirmed this clinically, showing that prolonged QTc interval occurred frequently with spontaneous overnight hypoglycemia in diabetes subjects (20). Unfortunately, in our study, blood glucose testing was only performed if clinical hypoglycemia was detected by experienced nurses. In fact, this clinical exclusion of hypoglycemia prior to testing could be a possible reason that we found less prolongation than other younger adolescent studies which may not have excluded clinical hypoglycemia.

Our study design did not allow for testing overnight because testing was performed in the morning. It has been shown that nocturnal hypoglycemia is associated with QTc lengthening (20) and that there are diurnal variations in QTc, with longer QTc overnight than during daytime (21). All but two EKG recordings were taken in the morning, and so the true effect of hypoglycemia on QTc intervals may not have been evident.

The similarity of QTc intervals in those with and without diabetes is consistent with a previous report in adults (21), but contrary to adolescent studies (22, 23). Our T1DM group had higher BMI, higher HR and less HRV than controls, all of which are consistent with other literature (8), but not with studies which found differences in QTc measurements. Indeed the most recent adolescent study found longer QTc did

not remain significant after adjustment for BMI (23); the BMI was not stated in the earlier adolescent study (22). Conversely, we were unable to demonstrate an association of BMI with QTc, but we found longer QTc with younger age. The previous adolescent studies were younger than in our study, so this could explain the difference. The earlier adolescent study surprisingly did not find differences in HR or HRV; nor any relationship of QTc to HbA1c in the diabetic group. Our study was conducted in a larger and older study population than previous pediatric studies, and also reflects the contemporary increase in BMI in adolescents with T1DM. A longer EKG recording was used, allowing 50 beats for each subject to be analyzed, and with HRV and dynamic pupillary studies, giving a more comprehensive and larger data base than previous studies.

Whilst cardiac autonomic neuropathy is often measured with the Ewing battery of cardiovascular reflex tests (24), HRV is more specific and a better discriminator for early subclinical autonomic neuropathy (25). Furthermore, it is an easier test to perform in adolescents, and is more widely available and low cost. The different measures of HRV give information on both parasympathetic and sympathetic function (15). As such, our findings of lower HRV parameters associated with QTc length suggest a predominant parasympathetic defect. Our study results are consistent with associations with cardiovascular reflex testing (5) and HRV (9).

Pupillometry is also a measure of autonomic function. The dynamic pupillary response to light is an early predictor of microvascular disease, such as retinopathy and microalbuminuria (16), though is not yet used in routine clinical practice. It has also been shown to be an early predictor of, and diagnostic tool for, DAN (26). Patients with T1DM have been shown to have smaller and less reactive pupils compared to control subjects (26). Our pupillary testing measured both sympathetic

(pupil size at rest) and parasympathetic (maximal constriction velocity) function, and found that having any pupillary abnormality, and specifically a lower maximum constriction velocity, a marker of parasympathetic damage, is associated with highest QTc quintile. There is a paucity of literature on direct associations between QTc and pupillometry, with only one study identified, which found no association (27). That study was smaller (60 diabetic patients) and in an older age group, and only examined a continuous association, not looking for threshold effects for QTc length. We found the association in quintile analysis and not continuous regression analysis.

DAN itself is associated with an increased risk of mortality and sudden death in diabetic patients (28). Whether this association is independent or linked to QTc lengthening is debated (28), however a combined abnormality of QTc length and HRV has been shown to result in a worsening of prognosis (29). More extensive longitudinal studies will need to be undertaken to confirm whether this is indeed the case. Interestingly, our study found a female predominance in the highest QTc quintile. This is consistent with previous studies of adolescents having more females in the higher risk groups (23). Our female adolescents also had significantly higher HbA1c, weight and BMI; and in females QTc was significantly associated with lower HRV. Female adolescents have been found to be at greater risk of diabetes-related deaths (30). Our findings might indicate a causative link between longer QTc intervals and sudden death, and could indicate that females are at greater risk due to a combination of inherent longer QTc interval, higher HbA1c or higher BMI during adolescence. Long-term studies will help to determine whether QTc intervals may be useful in identifying adolescents with T1DM with or at higher risk of vascular and neurologic damage and at increased risk of sudden death. While QTc intervals are

not generally increased in T1DM subjects compared to non-diabetic subjects, longer QTc duration in youth with T1DM is associated with autonomic dysfunction measured by HRV and pupillometry and as such further dialogue around routine EKG testing is warranted.

Acknowledgements

KS, KCD, PB and YHC were involved in study design, data analysis, researching data and writing the manuscript. AJJ and ACK were involved in study design and manuscript review. MM was involved in manuscript review. PM was involved in recruitment of controls.

The authors would like to thank RuDee Ting for reviewing an EKG sample, and Albert Chan for data retrieval. The authors would also like to thank the participants for their involvement in the study.

The authors declare they have no competing interests to disclose.

Table 1 – Characteristics and EKGs of type 1 diabetic subjects and control subjects

Patient characteristics	**Diabetes**	**Control**	**P value**
Age (years)	15.3 (2.0)	15.7 (2.5)	0.13
BMI SDS	0.79 (0.76)	0.09 (0.92)	**<0.001**
Mean QTc interval (ms)	392 (21)	391 (22)	0.65
Adjusted mean QTc interval (ms)*	391 (388-394)	394 (390-401)	0.25
Number with QTc >440ms	64	67	0.14
Mean maximum QTc interval (ms)	433 (24)	435 (26)	0.63
HR (bpm)	73 (10)	67 (11)	**<0.001**
lnSDNN (ms)	4.21 (0.43)	4.39 (0.43)	**0.001**
lnRMSSD (ms)	4.03 (0.65)	4.33 (0.61)	**<0.001**
lnLF:HF ratio	-0.06 (0.74)	-0.21 (0.64)	0.08
lnTI	2.75 (0.39)	2.87 (0.39)	**0.007**

HR - heart rate; SDNN - standard deviation of mean NN intervals; RMSSD - root mean square difference of successive NN intervals; LF - low frequency; HF - high frequency; TI – triangular index; QTc - QT interval corrected for heart rate.

All data are presented as mean (SD).

* Adjusted mean QTc is presented with 95% confidence intervals adjusted for BMI, age and gender

Table 2 – Predictors of mean QTc interval (as continuous variable): demographic, treatment and HRV measures

QTc outcome	Explanatory variables	β (95%CI)	P value
Model 1	HbA1c	3 (1, 5)	**0.005**
R^2 33%	Severe hypoglycemia	-13 (-21, -5)	**0.002**
	Age	-2 (-4, -1)	**0.006**
	Gender	-12 (-19, -6)	**<0.001**
	Insulin/kg/day	-12 (-22, -2)	**0.024**
	lnRMSSD	-6 (-11, -2)	**0.011**
Model 2	HbA1c	3 (1, 5)	**0.001**
R^2 32%	Severe hypoglycemia	-13 (-21, -5)	**0.001**
	Age	-2 (-4, -1)	**0.008**
	Gender	-13 (-19, -6)	**<0.001**
	Insulin/kg/day	-11 (-22, -2)	**0.035**
	lnLF:HF ratio	5 (-11, -2)	**0.022**

Severe hypoglycemia – unconsciousness or seizure in last 6-months; RMSSD - root mean square difference of successive NN intervals; LF- low frequency; HF - high frequency.

Table 3 – Characteristics of T1DM subjects in QTc quintiles 1-4 vs. quintile 5

	Quintiles 1-4	**Quintile 5 (≥409ms)**	**P value**
Age (years)	15.3 (2.1)	14.9 (1.8)	0.37
Gender	F:51 M:64	F:23 M:4	**<0.001**
Diabetes duration (years)	9.2 (3.6)	8.4 (3.1)	0.30
HbA1c (%), (mmol/mol)	8.4 (1.4), 68 (15)	9.8 (2.0), 83 (22)	**<0.001**
BMI SDS	0.71 (0.74)	1.15 (0.75)	**0.006**
Total cholesterol (mmol/L)	4.5 (0.9)	5.0 (1.0)	**0.02**
Proportion on CSII	23%	40%	0.13
Insulin dose (units/day)	65 (24)	67 (29)	0.71
Severe hypoglycemia last 6-mths	9/106 (9%)	0/20 (0%)	0.35
Severe hypoglycemia ever	35/109 (32%)	7/20 (35%)	0.80
Autonomic function			
Heart rate (bpm)	72 (10.01)	78 (10.38)	**0.008**
lnSDNN (ms)	4.26 (0.43)	4.00 (0.36)	**0.004**
lnRMSSD (ms)	4.10 (0.65)	3.71 (0.55)	**0.004**
lnLF:HF ratio	-0.10 (0.75)	0.12 (0.62)	0.15
lnTI	2.78 (0.39)	2.60 (0.36)	**0.03**
Pupillary abnormalities	42/75 (56%)	15/18 (83%)	**0.03**
Initial pupillary diameter (mm)	6.04 (0.57)	5.85 (0.56)	0.21
Reflex amplitude (mm)	1.64 (0.32)	1.48 (0.30)	0.07
Max. constriction velocity (mm/s)	5.28 (0.95)	4.79 (0.72)	**0.04**
Microvascular assessment			

Retinopathy	19/110 (17%)	6/25 (24%)	0.43
Peripheral nerve abnormalities	33/113 (29%)	7/25 (28%)	0.90
Microalbuminuria	4/101 (4%)	2/24 (8%)	0.37

BMI - body mass index; SDS - standard deviation score; CSII – continuous subcutaneous insulin infusion; HR - heart rate; SDNN - standard deviation of mean NN intervals; RMSSD - root mean square difference of successive NN intervals; LF - low frequency; HF - high frequency; TI – triangular index.

All data are presented as mean (SD) and frequency (percentage).

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