film on children learning to self inject insulin. *Behav Ther* 13:186–93, 1982
13. Johnson SB, Pollak T, Silverstein JP, Rosenbloom A, Spillar R, McCallum M, Harkavy J: Cognitive and behavioral knowledge about insulin-dependent diabetes among children and parents. *Pediatrics* 69:708–24, 1983
14. Harkavy J, Johnson SB, Silverstein JP, Spillar R, McCallum M, Rosenbloom A: Who learns what at diabetes summer camp? *J Pediatr Psychol* 8:143–53, 1983
15. Sullivan B: Adjustment in diabetic adolescent girls. I. Development of the diabetic adjustment scale. *Psychosom Med* 41:119–26, 1979
16. LaGreca AM, Follansbee D, Skyler JS: *Behavioral Aspects of Diabetes Management in Children and Adolescents*. Washington, DC, Am. Psychol. Assoc., 1982
17. Allen DA, Tennen H, McGrade BJ, Affleck G, Ratzman S: Parent and child perceptions of the management of juvenile diabetes. *J Pediatr Psychol* 8:129–41, 1983
18. Ingersoll GM, Orr DP, Herrold AJ, Golden MP: Cognitive maturity and self-management among adolescents with insulin-dependent diabetes mellitus. *J Pediatr* 108:620–23, 1986

# Corrected Q-T Interval Prolongation as Diagnostic Tool for Assessment of Cardiac Autonomic Neuropathy in Diabetes Mellitus

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**A simple method for evaluating alterations in cardiac sympathetic innervation may be measurement of the Q-T interval. Seventy-three diabetic patients (46 insulin dependent and 27 non–insulin dependent) were separated into four groups based on the presence and degree of cardiac autonomic neuropathy (CAN) with noninvasive cardiovascular reflexes and blood pressure responses. None of the patients had evidence of ischemic heart disease, kidney disease, or the idiopathic long Q-T–interval syndrome. The corrected Q-T interval (Q-Tc) was determined at rest with Bazett's formula. As a group, diabetic patients with ≥2 abnormalities of cardiac autonomic function had a longer Q-Tc interval than those with no evidence of CAN. Diabetic patients with ≥1 abnormality had a prolonged Q-Tc interval compared with a control group of 96 healthy nondiabetic subjects (mean ± SD 397 ± 18). The frequency of prolonged (>433 ms, normal mean + 2SD) resting Q-Tc intervals increased with the increasing number of abnormalities (0, 1, 2, ≥3): 11, 25, 41, and 75%, respectively. Twenty-three of 25 (92%) patients with a Q-Tc >433 ms had evidence of CAN. However, 57% (31 of 54) of the patients with CAN had a normal Q-Tc interval. These data provide further evidence of a relationship between the presence and severity of CAN and degree of Q-Tc interval prolongation. Compared with cardiovascular reflexes and blood pressure tests for CAN, the Q-Tc interval in the group of diabetic patients studied without evidence of heart or kidney disease was an insensitive but specific marker. An abnormal Q-Tc interval may be an additional diagnostic tool for evaluating CAN in patients with diabetes mellitus.**
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Abnormal autonomic nervous system function is a common complication of diabetes mellitus (1). Congenital or pharmacological perturbation of the autonomic nervous system may result in prolongation of the Q-T interval (2,3). Flugelman et al. (4) and Merdler et al. (5) suggested that Q-T interval prolongation in diabetic patients was a consequence of autonomic neuropathy. We previously demonstrated the existence of a relationship between severity of cardiac autonomic neuropathy (CAN) and degree of Q-T interval prolongation in a small group of diabetic patients without ischemic heart disease (6). Bellavere et al. (7,8) found similar results.

The long-term prognosis is poor for diabetic patients with autonomic neuropathy, with mortality rates estimated to approach 45% within 2.5 yr (9). To objectively diagnose CAN a battery of tests, including postural blood pressure responses and noninvasive cardiovascular reflex testing, were often used (10). These tests are sensitive and reproducible but are labor intensive. We examined the possibility that analysis of a routine electrocardiogram may also provide a noninvasive measurement of CAN. In this study, we examined the value of the resting Q-T interval as a potential marker for the presence and degree of CAN in a large cohort of patients with diabetes mellitus.

## RESEARCH DESIGN AND METHODS

Seventy-three patients with either insulin-dependent ($n$ = 46) or non-insulin-dependent ($n$ = 27) diabetes mellitus were studied. These patients were divided into four groups based on their autonomic function score (CAN score), as described below. None of the diabetic patients were on β-blockers or other medications known to affect cardiac repolarization. All had normal serum creatinine levels. Ninety-six age-matched healthy nondiabetic subjects were used as study controls. All of the healthy control subjects had normal resting electrocardiograms, as determined by an independent cardiologist. All had normal resting systolic and diastolic blood pressures and none took any medications. Clinical fea-

tures of the diabetic and control groups are summarized in Table 1. None of the study subjects (both healthy control subjects and diabetic patients) had evidence of ischemic heart disease on history or on resting electrocardiogram and none had prolonged QRS complexes. All subjects had normal serum electrolytes, none had a history of syncope or a family history of sudden death, and none were deaf. Informed written consent was obtained from each study subject, and the study was approved by the Institutional Review Board Human Use Committee at the University of Michigan Medical Center.

**Measurement of Q-T interval.** After the study subject had rested in the supine position for 15 min, the Q-T intervals of five nonconsecutive sinus beats were measured to the end of the T wave on electrocardiogram tracings taken at a paper speed of 50 mm/s. The Q-T interval corrected (Q-Tc) for the cardiac cycle length was calculated according to Bazett's formula $Q\text{-}Tc = Q\text{-}T/(R\text{-}R)^{1/2}$ (11). The Q-Tc interval for each patient was taken as the mean value of the five measurements made and was reported in milliseconds.

**CAN evaluation.** Diabetic patients were tested for CAN with noninvasive cardiovascular reflex tests, resting heart rate, and postural blood pressure responses, as described previously (12). Modification of a previously described scoring system was used to estimate the extent of autonomic nervous system dysfunction (13). Briefly, each patient was assigned an autonomic function score (CAN score) based on the results of the following tests: *1*) heart-rate variation (HRV) with deep prolonged breathing, *2*) heart-rate response to Valsalva maneuver, *3*) resting heart rate, and *4*) systolic blood pressure response to standing. Each test was graded as normal or abnormal and patients were assigned a CAN score from 0 to 4 based on the number of abnormal tests.

**Statistical methods and analyses.** Differences between groups and variables were tested by one-factor analysis of variance, Student's unpaired *t* test, $\chi^2$-test, and Fisher's exact probability test where appropriate. Correlations among variables were tested by the method of least mean squares. Significant differences were accepted at the 95% confidence interval level ($P < 0.05$).

## RESULTS

**Incidence of CAN.** Of the 73 patients with diabetes, 54 had at least one abnormal test for CAN and 19 had no evidence of autonomic dysfunction (group 0, CAN score 0). Group 1 (CAN score 1) was comprised of 15 patients with an abnormal HRV with deep breathing, 2 patients with an abnormal Valsalva ratio, 2 patients with an abnormal blood pressure response, and 1 patient with an abnormal resting heart rate. Group 2 (CAN score 2) was comprised of 17 patients with abnormalities in both the HRV with deep breathing and Valsalva ratio, 3 patients with abnormalities in both the HRV and resting heart rate, 1 patient with abnormalities in the Valsalva ratio and resting heart rate, and 1 patient with abnormalities in both the HRV and blood pressure response. Nine patients had three abnormal CAN tests, and 3 patients had four abnormal CAN tests. These 12 patients comprised group 3 (CAN score 3 or 4). There were no significant clinical or demographic differences between the four groups (Table 1). The incidence of autonomic neuropathy in insulin-dependent diabetes mellitus (32 of 46, 70%) was not different from non-insulin-dependent diabetes mellitus (22 of 27, 81%), and it was not different between men (28 of 35, 80%) and women (26 of 38, 68%).

**Distribution of resting Q-Tc intervals.** The resting Q-Tc interval ranged from 355 to 436 ms (median 398) for healthy control subjects and from 354 to 490 ms (median 412) for patients with diabetes. The Q-Tc values (mean ± SE) for male and female healthy control subjects were not different (395 ± 8 and 399 ± 8 ms, respectively). Similarly, mean ± SE Q-Tc values for male (423 ± 5 ms) and female (419 ± 3 ms) diabetic patients were not different. The overall mean resting Q-Tc interval for healthy control subjects was 397 ms with an SD of 18. Therefore, a Q-Tc interval >433 ms (nor-

**TABLE 1**
**Clinical characteristics of study subjects**

| Subjects | *n* | Age (yr) | Sex (M/F) | BMI (kg/m²) | Type of diabetes (IDDM/NIDDM) | Diabetes duration (yr) | $HbA_{1c}$ (%)* |
|---|---|---|---|---|---|---|---|
| Control | 96 | 42 ± 1 (18–73) | 49/47 | 24.5 ± 0.5 (17.3–35.5) | | | |
| Diabetes (CAN score) | | | | | | | |
| 0 | 19 | 38 ± 3 (19–57) | 7/12 | 23.7 ± 1.0 (18.1–38.6) | 14/5 | 16 ± 2 (3–32) | 8.9 ± 0.6 (5.7–14.8) |
| 1 | 20 | 44 ± 3 (20–64) | 11/9 | 24.9 ± 1.0 (18.7–38.6) | 11/9 | 15 ± 2 (2–40) | 9.4 ± 0.6 (5.3–15.2) |
| 2 | 22 | 45 ± 3 (23–63) | 10/12 | 27.0 ± 1.1 (16.8–37.6) | 12/10 | 14 ± 2 (1–41) | 9.7 ± 0.5 (6.6–13.8) |
| 3 or 4 | 12 | 39 ± 4 (20–67) | 7/5 | 23.2 ± 1.5 (17.1–33.9) | 9/3 | 16 ± 2 (8–27) | 10.2 ± 1.0 (5.6–14.3) |

Results are expressed as means ± SE with ranges in parentheses. BMI, body mass index; IDDM, insulin-dependent diabetes mellitus; NIDDM, non-insulin-dependent diabetes mellitus; $HbA_{1c}$, glycosylated hemoglobin; CAN, cardiac autonomic neuropathy.
*Normal laboratory range 4–8%.

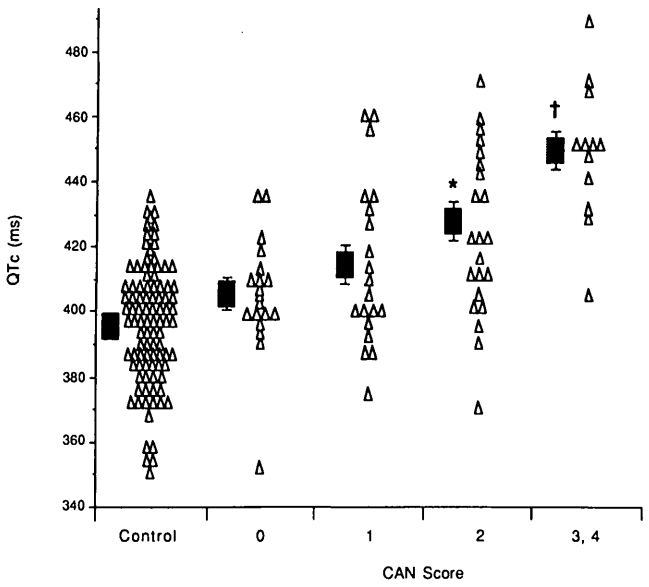


**FIG. 1. Resting corrected Q-T (Q-Tc) intervals versus cardiac autonomic neuropathy (CAN) score. Resting Q-Tc intervals for healthy control subjects are shown. ■, Means ± SE; *shaded area* represents range of Q-Tc values considered abnormal (>433 ms); △, control subjects. *$P < 0.01$ vs. 0. †$P < 0.0001$ vs. 0 and 1, $P < 0.001$ vs. 2. Q-Tc in diabetic patients with CAN ≥1 were significantly greater than control subjects.**

mal mean + 2SD) was considered prolonged. Twenty-five diabetic patients had a prolonged (>433 ms) Q-Tc interval. There were no differences in age, sex, body mass index, type of diabetes, duration of diabetes, and glycosylated hemoglobin between diabetic patients with a Q-Tc interval >433 ms and patients with a Q-Tc interval ≤433 ms.

**Q-Tc interval prolongation and CAN.** Resting Q-Tc intervals are shown for healthy control subjects and for each group of diabetic patients (Fig. 1). As a group, diabetic patients with ≥1 CAN abnormality had a significantly longer Q-Tc interval compared with healthy control subjects. Twenty-three of 25 (92%) patients with a Q-Tc interval >433 ms had evidence of CAN. Compared with the diabetic group without evidence of CAN (group 0), there was a greater frequency of prolonged (>433 ms) Q-Tc intervals in group 3 (CAN score = 3 or 4) (75%; $P < 0.0005$) and in group 2 (41%; $P < 0.05$). The increased frequency of prolonged Q-TC intervals in group 1 (25%) approached but did not achieve statistical significance. Four of 5 group 1 patients (CAN score 1) with a prolonged Q-Tc interval had an abnormal HRV with deep breathing, and 1 patient had an abnormal resting heart rate. Every group 2 patient (CAN score 2) with a prolonged Q-Tc interval ($n = 9$) had an abnormal HRV with deep breathing. Of these 9 patients, 8 also had an abnormal Valsalva ratio, and 1 patient had an abnormal resting heart rate. Six of 9 patients with three CAN abnormalities had a prolonged Q-Tc interval. Of these 6 patients, 3 had combined abnormalites in HRV, Valsalva ratio, and resting heart rate, and 3 patients had combined abnormalities in HRV, Valsalva ratio, and blood pressure response. Each patient with four CAN abnormalities had a resting Q-Tc interval of 450 ms.

## DISCUSSION

In this study, we investigated the relationship between Q-Tc interval prolongation and presence and extent of CAN in a large cohort of patients with diabetes mellitus. The results confirmed and extended our previous observations (6) and those of others (7,8) of a relationship between resting Q-Tc interval prolongation and presence and degree of autonomic dysfunction in diabetes. Twenty-three of 25 (92%) diabetic patients with a prolonged (>433 ms) resting Q-Tc interval had evidence of CAN. However, the Q-Tc interval cannot be used alone to prospectively assess CAN because 57% (31 of 54) of patients with CAN had a normal Q-Tc interval. The resting Q-Tc interval is an insensitive but specific predictor of CAN in diabetic patients who do not have clinical kidney or cardiac disease.

The pathophysiological mechanism for Q-Tc interval prolongation in diabetic patients with CAN is unknown. Scintigraphic mapping with the norepinephrine analogue [$^{123}$I]MIBG has shown altered cardiac adrenergic balance in diabetic patients with CAN (14). Therefore, Q-Tc interval prolongation in diabetic patients with CAN may result from an imbalance in cardiac sympathetic innervation. Altered cardiac sympathetic balance may predispose diabetic patients with CAN to cardiac arrhythmias and sudden death. We found nonuniform loss of adrenergic innervation of the heart associated with severe CAN and Q-T interval prolongation in one patient who subsequently died unexpectedly (15). An imbalance in cardiac sympathetic innervation may also provide a pathophysiological basis for our previous findings of left ventricular dysfunction (16) and abnormal cardiac responses to exercise in diabetic patients with CAN (17).

In conclusion, the Q-Tc interval is an additional noninvasive diagnostic tool in the assessment of CAN in diabetic patients. An abnormal resting Q-Tc interval may be objective evidence for CAN, and the longer the resting Q-Tc interval, the more severe the CAN. However, a normal resting Q-Tc interval does not exclude the presence of CAN. We believe longitudinal studies are necessary to determine the prognostic value of the Q-Tc interval in the prediction of sudden cardiac death in patients with diabetes.

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REFERENCES

1. Ewing DJ, Clarke BF: Diabetic autonomic neuropathy: present insights and future prospects. *Diabetes Care* 9:648–65, 1986
2. Schwartz PJ, Periti M, Malliani A: The long Q-T syndrome. *Am Heart J* 89:378–90, 1975
3. Ahnve S, Vallin H: Influence of heart rate and inhibition of autonomic tone on the QT interval. *Circulation* 65:435–39, 1982
4. Flugelman MY, Kanter Y, Abinader EG, Barzilai D: Rest electrocardiographic patterns (REP) in diabetics without evidence of ischemic heart disease (IHD) (Abstract). *Diabetes* 29 (Suppl. 2):76A, 1980
5. Merdler A, Abinader EG, Flugelman MY, Kantner Y: Beta blockade in asymptomatic diabetics with abnormal rest electrocardiograms. *J Electrocardiol* 16:87–90, 1983
6. Kahn JK, Sisson JC, Vinik AI: QT interval prolongation and sudden cardiac death in autonomic neuropathy. *J Clin Endocrinol Metab* 64:751–54, 1987
7. Bellavere F, Ferri M, Cardone C, Guarini L, Bosello G, Fedele D: Analysis of QT versus RR ECG interval variations in diabetic patients shows a longer QT period in subjects with autonomic neuropathy (Abstract). *Diabetologia* 27:255A, 1984
8. Bellavere F, Ferri M, Guarini L, Bax G, Piccoli A, Cardone C, Fedele D: Prolonged QT period in diabetic autonomic neuropathy: a possible role in sudden cardiac death? *Br Heart J* 59:379–83, 1988
9. Ewing DJ, Campbell IW, Clarke BF: The natural history of diabetic autonomic neuropathy. *Q J Med* 193:95–108, 1980
10. Ewing DJ, Martyn CN, Young RJ, Clarke BF: The value of cardiovascular autonomic function tests: 10 years experience in diabetes. *Diabetes Care* 8:491–98, 1985
11. Bazett HC: An analysis of the time-relations of electrocardiograms. *Heart* 7:353–70, 1920
12. Vinik AI, Glowniak JV: Hormonal secretion in diabetic autonomic neuropathy. *NY State J Med* 82:871–86, 1982
13. Zola B, Kahn JK, Juni JE, Vinik AI: Abnormal cardiac function in diabetics with autonomic neuropathy in the absence of ischemic heart disease. *J Clin Endocrinol Metab* 63:208–14, 1986
14. Sisson JC, Shapiro B, Meyers L, Mallette S, Mangner TJ, Wieland DM, Glowniak JV, Sherman P, Beierwaltes WH: Metaiodobenzylguanidine to map scintigraphically the adrenergic nervous system in man. *J Nucl Med* 28:1625–36, 1987
15. Kahn JK, Sisson JC, Vinik AI: Prediction of sudden cardiac death in diabetic autonomic neuropathy. *J Nucl Med* 29:1605–606, 1988
16. Kahn JK, Zola B, Juni JE, Vinik AI: Radionuclide assessment of left ventricular diastolic filling in diabetes mellitus. *J Am Coll Cardiol* 7:1303–309, 1986
17. Kahn JK, Zola B, Juni JE, Vinik AI: Decreased exercise heart rate and blood pressure response in diabetic subjects with cardiac autonomic neuropathy. *Diabetes Care* 9:389–94, 1986

# Preservatives in Insulin Preparations Impair Leukocyte Function

## In Vitro Study

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***m*-Cresol and methyl *p*-hydroxybenzoate are preservatives in insulin preparations. As previously reported, in diabetic patients on continuous subcutaneous insulin infusion, users of insulin-containing *m*-cresol had significantly more inflamed infusion sites than users of insulin with methyl *p*-hydroxybenzoate. This study assessed the influence of insulin with and without these preservatives on leukocyte function. Leukocyte function was investigated in a killing experiment, expressed as the percentage of bacteria killed after 60 min incubation of bacteria (*Staphylococcus aureus*), polymorphonuclear leukocytes, serum, and insulin preparations. Because preservative is retained by the infusion device, insulin with preservative was tested before and after 1 and 4 days perfusion with a PVC pump catheter. After perfusion, the amount of preservative was reduced (percentage of original concentration after 1 and 4 days 8 and 30% *m*-cresol and 42 and 72% methyl *p*-hydroxybenzoate, respectively). The killing percentage in insulin with *m*-cresol reduced compared with insulin without preservative (mean ± SE 95.4 ± 0.8%) and the control without insulin (95.8 ± 0.8%), both before and after 1 and 4 days perfusion (74.8 ± 0.7, 80.2 ± 2.8, and 80.6 ± 1.6%, respectively; $P < 0.01$). The same occurred in insulin with methyl *p*-hydroxybenzoate (85.0 ± 0.9% before and 88.4 ± 0.9 and 86.2 ± 0.8% after 1 and 4 days perfusion; $P < 0.05$). All insulin preparations with *m*-cresol caused lower killing percentages than corresponding insulin preparations with methyl *p*-hydroxybenzoate ($P < 0.05$). These results demonstrate that both preservatives impaired leukocyte function, but *m*-cresol was the most noxious in this respect. This indicates that preservatives in insulin preparations are possibly implicated in the pathogenesis of local infections in continuous subcutaneous insulin infusion. *Diabetes Care* 13: 71–74, 1990**