



Published in final edited form as:

*Diabetes Care*. 2025 November 01; 48(11): 1960–1970. doi:10.2337/dc25-1087.

## Differences in Prevalence and Incidence of Electrocardiogram Abnormalities and Cardiovascular Autonomic Neuropathy Among Randomized Glucose-lowering Treatments in Early Type 2 Diabetes: The Glycemia Reduction Approaches in Diabetes (GRADE) Cohort

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**ICMJE Statement & Author Contributions:** All authors affirm that authorship is merited based on the ICMJE authorship criteria. RPB, HKS, RKG, SEI, AK, SM, and JBG contributed to the conception and/or design of the research. RPB, HAA, RKG, SEI, SM, ERS, MT, EZS, and JBG contributed to the acquisition of data. SPR and NMB contributed to the statistical analysis of data. RPB, SPR, NMB, HKS, HAA, RKG, SEI, JBM, SM, DSS, ERS, EZS, and JBG contributed to the interpretation of data and results. HKS contributed to obtaining funding. HKS, RKG, SM, ERS, MT, EZS, and JBG contributed to supervision and management of the research. RPB, SPR, NMB, EZS, and JBP contributed to drafting of the manuscript. All authors contributed to critical revision of the manuscript for important intellectual content. All authors approved the final version of the manuscript.

\*See Supplementary Item 1

Trial Registration:

[Clinicaltrials.gov](https://clinicaltrials.gov/ct2/show/study/NCT01794143) identifier: NCT01794143

Date of registration: 2/18/2013

**Prior Presentation:** The abstract for this manuscript was presented as an e-poster and poster at the American Diabetes Association's 85<sup>th</sup> Scientific Sessions in Chicago, Illinois, June 20–23, 2025.

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## Abstract

**Objective:** To describe prevalence and incidence of electrocardiogram (ECG) abnormalities and ECG-derived cardiovascular autonomic neuropathy (CAN) in the GRADE cohort of adults with type 2 diabetes (T2D) <10 years.

**Research Design and Methods:** Individuals with T2D taking metformin alone were randomly assigned to add insulin glargine, glimepiride, liraglutide, or sitagliptin. Resting ECGs were completed at baseline, 2-year, and 4-year study visits and analyzed for minor/major abnormalities and CAN assessed with heart rate variability (HRV) in 4769 participants. Incidence of new major/minor/any ECG abnormalities and CAN by treatment group was analyzed using logistic repeated measures models at years 2 and 4 adjusted for baseline risk factors.

**Results:** At baseline, participants were a mean age of  $57.2 \pm 10.0$  years, 36.3% were women, mean diabetes duration  $4.3 \pm 2.8$  years, and HbA1c  $7.5 \pm 0.5\%$ . Participants with ECG abnormalities at baseline (57.1%) and ECG-derived CAN (52.8%) were older and had more severe cardiovascular risk factors. The incidence of minor and major ECG abnormalities was similar among all treatment groups. However, at year 4, major ECG abnormalities were fewer in the liraglutide vs. non-liraglutide groups (9% vs. 13%;  $p=0.03$ ). The incidence of CAN did not differ between liraglutide vs. non-liraglutide groups across visits ( $p=0.42$ ); however, one measure of HRV (SD of normal-to-normal R-R intervals) was higher at year 2 in liraglutide vs. non-liraglutide groups ( $p=0.02$ ).

**Conclusions:** ECG abnormalities, including those reflecting CAN, are common in individuals with T2D <10 years, more so in those with certain cardiovascular risk factors. The development of major ECG abnormalities may be lower with liraglutide.

## Twitter Summary:

Resting ECG abnormalities predictive of increased cardiovascular risk and mortality are prevalent in contemporary adults with type 2 diabetes of short duration enrolled in GRADE.

## Keywords

type 2 diabetes; electrocardiograms; heart rate variability; insulin; sulfonylurea; GLP-1 receptor agonist; DPP-4 inhibitor

People with type 2 diabetes (T2D) are at increased risk of cardiovascular disease (CVD) and cardiovascular autonomic neuropathy (CAN); however, screening tests are not routinely recommended for asymptomatic individuals. Resting electrocardiogram (ECG) abnormalities, which are associated with an increased risk of morbidity and mortality in the general population, are common in individuals with T2D regardless of CVD status (1). A prospective longitudinal study in the Netherlands of nearly 12,000 people with T2D but without known CVD found that major ECG abnormalities were associated with a twofold to threefold increase in the risk of major adverse cardiovascular (CV) events (2). Importantly, all types of ECG abnormalities – including major abnormalities such as Q-wave patterns, ventricular conduction delays, and left ventricular hypertrophy, as well as minor abnormalities such as isolated ST-T changes, minor Q-waves, and axis deviations—were associated with an increased risk of heart failure (HF) (2). Furthermore, strong evidence from large cohorts of people with diabetes in the U.S. and worldwide demonstrate that HF is now the most prevalent CV complication in diabetes (3). Similar data demonstrating that even minor ECG abnormalities predict severe CV outcomes in T2D were reported by the Detection of Ischemia in Asymptomatic Diabetes (DIAD) study (4).

CAN, often asymptomatic initially, is a common complication of diabetes (5–7) and an independent predictor of serious outcomes such as CVD, HF and CV death (7–9). The earliest indicator of CAN is usually a decrease in heart rate variability (HRV) (7).

The extent of ECG abnormalities in large contemporary cohorts of people with T2D is not clear, as is the differential impact of different classes of glucose-lowering medications on these abnormalities over time. To our knowledge, no prior large U.S.-based trial has reported longitudinal changes in ECG abnormalities and HRV-based CAN metrics across multiple glucose-lowering agents in T2D. Thus, the main objectives of the current analyses were to describe the prevalence and incidence of minor and major ECG abnormalities and of ECG-derived CAN over a 4-year period in the Glycemic Reduction Approaches in Diabetes: A Comparative Effectiveness Study (GRADE) participants, and to determine whether the incidence of these abnormalities differs by treatment group. Additionally, given that prior evidence from other clinical trials and from GRADE reported a reduced CVD risk for liraglutide (10), we also compared the liraglutide group to the other treatment groups evaluated in GRADE.

## METHODS

### GRADE Study Design

The GRADE study was a prospective, multicenter, randomized trial conducted from 2013 to 2021 in the U.S. that compared the glycemic impact of the addition of one of four glucose-lowering medications to metformin: glargine U-100 (a long-acting insulin), glimepiride (a sulfonylurea), liraglutide (a glucagon-like peptide 1 (GLP-1) receptor agonist), and sitagliptin (a dipeptidyl peptidase 4 (DPP-4) inhibitor) in individuals with T2D (11). The protocol, statistical analysis plan, and full list of inclusion/exclusion criteria are available at [ClinicalTrials.gov](https://clinicaltrials.gov/ct2/show/study/NCT01794143) (NCT01794143). Briefly, GRADE enrolled 5047 participants with diagnosed T2D <10 years, HbA1c of 6.8–8.5% (50.8–69.4 mmol/mol), age ≥30 years (≥20 years for Native Americans), and treated with metformin alone for glucose-lowering

treatment (minimum 1000 mg/day and up to 2000 mg/day). At the time of first occurrence of HbA1c >7.5% (confirmed, based on HbA1c measured quarterly) after the addition of randomized glucose-lowering medication, insulin glargine was added to the medication regimen for the non-glargine treatment groups and insulin aspart was added for the glargine group. At the next occurrence of HbA1c >7.5% (confirmed), insulin aspart was added and the randomized medication was discontinued for the non-glargine treatment groups. All clinical centers obtained institutional review board approval for the protocol and informed consent, and all study participants provided written informed consent.

### ECG Procedures and Measures

Participants' resting 12-lead ECG was obtained at baseline, year 2, year 4, and year 6 study visits using a GE/Marquette MAC1200 ECG machine (GE Healthcare, Milwaukee, WI). All ECGs were centrally read and coded at the Epidemiological Cardiology Research Center at the Wake Forest School of Medicine (Winston-Salem, NC). After visual inspection for technical errors and inadequate quality, ECGs were automatically processed using Marquette 12-SL software running under GE MUSE (GE Healthcare). The Minnesota Coding system was applied to classify ECG tracings as either major or minor abnormalities (12). Mappings between Minnesota Codes and major or minor abnormalities are given in Supplementary Table 1.

Major ECG abnormalities included: major ventricular conduction defects, definite myocardial infarction (indicated by major Q wave abnormalities), possible myocardial infarction (indicated by minor Q/-QS wave plus major ST/-T abnormalities), major isolated ST/-T abnormalities, left ventricular hypertrophy, major atrioventricular conduction abnormalities, major QT prolongation, pacemaker use, and others.

Minor ECG abnormalities included: minor isolated Q/-QS waves, minor isolated ST/-T abnormalities, high R waves, ST-segment elevation, incomplete (left or right) bundle branch block, minor QT prolongation, short PR interval, left axis deviation, right axis deviation, frequent ventricular premature beats, and others.

In addition to the above ECG abnormalities, two time-domain HRV measures of ECG-derived CAN were calculated based on the average of three 10-second digital rhythm strips: the SD of all normal-to-normal R-R intervals (SDNN) (milliseconds), and the root mean square of successive differences between all normal-to-normal R-R intervals (RMSD) (milliseconds). The definitions of abnormal HRV were SDNN <17.13 ms and RMSD <24.94 ms, based on our previously validated ECG-derived CAN measure using the gold-standard cardiovascular reflex tests (13).

### Other Baseline Measures

The following baseline factors were analyzed: diabetes duration (tertiles), HbA1c (tertiles), weight (tertiles), height (tertiles), waist circumference (tertiles), history of hyperlipidemia, medication use (statin, angiotensin-converting-enzyme (ACE) inhibitor, angiotensin receptor blocker (ARB), beta blocker, lipid-lowering medication), systolic (<120, 120–<140, or 140+ mmHg) and diastolic (<80, 80–<90, or 90+ mmHg) blood pressure, alcohol use (never, occasionally, or at least weekly), age (<45, 45–<60, or 60+ years), sex, race (Black, White,

or all others), ethnicity (Hispanic vs. non-Hispanic), income (<\$20k, \$20k-<\$35k, \$35k-<\$50k, \$50k-<\$75k, \$75k+), and education (high school/GED or less, some college, college, or graduate school). Body mass index was calculated as weight (kg) divided by height (m) squared (BMI, tertiles). Diabetic peripheral neuropathy was assessed using the Michigan Neuropathy Screening Instrument (MNSI) (14–15); an abnormal score was defined as an MNSI clinical score of 2.5 or more or an MNSI instrument score of 7 or more (15). Estimated glomerular filtration rate (eGFR) was calculated using the CKD-EPI Creatinine Equation (16) and categorized as <90 or 90+ mL/min/1.73m<sup>2</sup>.

## Participants

The GRADE study randomized 5047 participants who underwent quarterly assessments for an average follow-up of 5.0 years (17). This analysis included 4769 participants (94%) with an ECG available at baseline, and participants were included for each follow-up study visit at which an ECG was performed. Among the 4769 participants with baseline ECGs, there were 4437 participants (93%) with ECGs at year 2 and 3519 participants (74%) with ECGs at year 4. A CONSORT flow diagram is shown in Supplementary Figure 1. Most of the analysis sample had less than 6 years of follow-up (3647 participants, 76%), and the COVID-19 pandemic caused logistical challenges at year 6. There was thus a relatively low number of participants with an ECG at year 6 (992 participants, 21%) and year 6 data were excluded from the analyses.

## Statistical Analysis

Descriptive analyses summarized baseline characteristics of participants with versus without any ECG abnormalities and with versus without ECG evidence of CAN across the four treatment groups (these p-values were unadjusted for multiplicity).

Prevalence of ECG abnormalities (minor, major, and any) and ECG-derived CAN at baseline were summarized by treatment group.

Incidence of new ECG abnormalities by treatment group was analyzed using separate generalized estimating equation (GEE) logistic regression models for minor, major, and any ECG abnormalities. The models included treatment group, time-point (i.e., year 2 or 4), and the interaction between treatment and time. Because participants with more baseline ECG abnormalities were ‘eligible’ for fewer new ECG abnormalities at future time points, the models adjusted for the number of relevant baseline ECG abnormalities. The models also adjusted for baseline heart rate, blood pressure (systolic and diastolic), lipids (total cholesterol, HDL, triglycerides), and use of beta blockers. Dependence of the outcomes over time was incorporated into each model by modeling the log odds ratio between year 2 and 4 outcome variables (18). For each model, a Wald test assessed an overall treatment effect over time. Analyses compared the liraglutide treatment group to the three non-liraglutide groups combined, and compared all four treatment groups. The liraglutide vs. three non-liraglutide groups comparison was a prespecified analysis included in the Statistical Analysis Plan. For analyses comparing all four groups, a gatekeeping strategy was used. Separately for each outcome of minor, major, and any ECG abnormalities, first an overall Wald test of treatment group differences was conducted. If the overall test was significant, then Wald tests for all

6 pairwise treatment comparisons were conducted, with these 6 tests adjusted for multiple comparisons using the Holm procedure (19).

Incidence of ECG-derived CAN by treatment group was analyzed using a similar procedure to that used for incidence of new ECG abnormalities, restricted to participants who did not have ECG-derived CAN at baseline. Models were adjusted for baseline heart rate, blood pressure (systolic and diastolic), lipids (total cholesterol, HDL, triglycerides), and use of beta blockers. Indices of HRV indicative of CAN (SDNN and RMSD) were analyzed over time using GEE linear regression models. The distributions of SDNN and RMSD were right-skewed, hence the indices were log-transformed. The models included treatment group, time-point, and the interaction between treatment and time, and were adjusted for the baseline HRV index, heart rate, blood pressure, lipids (total cholesterol, HDL, triglycerides), and use of beta blockers. An autoregressive AR(1) working correlation matrix was used. A procedure similar to the one used to test for treatment differences in incidence of new ECG abnormalities was used to test for treatment differences in the log-transformed HRV indices over time.

Heterogeneity in the treatment effect on ECG abnormalities (minor, major, and any) and ECG-derived CAN was assessed based on the baseline factors listed in the Other Baseline Measures section above. For each baseline factor, separate GEE logistic regression models were fit for minor, major, and any ECG abnormalities and for ECG-derived CAN, with covariates for treatment group, the baseline factor, time-point. Models for and all interactions between treatment, the baseline factor, and time-point. Models for new ECG abnormalities (minor, major, and any) also adjusted for the number of baseline ECG abnormalities as a covariate. These models were unadjusted for other baseline adjustment factors due to concerns about collinearity with the baseline stratification factor. Treatment effect heterogeneity by the baseline factor with respect to the longitudinal progression of ECG abnormalities or ECG-derived CAN was tested with a Wald test of the interaction of treatment with the baseline factor for all time-points, separately for each outcome of minor/major/overall ECG abnormalities and CAN. Separately for each outcome, the 24 tests (corresponding to 24 baseline factors) were adjusted for multiplicity using a 1% false discovery rate (FDR) (20). For each baseline factor for which treatment effect heterogeneity was not significant at the 1% level, the marginal association with longitudinal progression of ECG abnormalities or ECG-derived CAN was tested by fitting a model without treatment and performing a Wald test of the effect of the baseline factor across all time-points, adjusted using a 1% FDR among baseline factors for which the test of treatment effect heterogeneity was not significant (separately for each outcome).

All statistical models used available case analysis (i.e., all available ECGs from baseline, year 2, and year 4 were included in each model, even for participants who were missing a year 2 and/or year 4 ECG).

### Data and Resource Availability

The Glycemia Reduction Approaches in Diabetes: A Comparative Effectiveness Study (GRADE) is funded by the National Institute of Diabetes and Digestive and Kidney Diseases (NIDDK). This manuscript is based on follow-up data and outcome assessments from the

5047 participants enrolled into the study. This database is available in the NIDDK Central Repository at <https://repository.niddk.nih.gov/study/151>.

## RESULTS

Among the GRADE cohort, 4769 participants (94% of overall cohort) with at least a baseline ECG were included in the analyses (12,725 total ECGs from baseline, year 2, and year 4).

### Baseline data on ECG abnormalities and ECG-derived CAN

There were no differences in baseline characteristics across treatment groups (Supplementary Table 2). Table 1 presents baseline characteristics of participants with versus without any ECG abnormalities, and with versus without ECG-derived CAN at baseline. Participants with any ECG abnormality were older; more likely to have longer duration of diabetes, be male, and White; have higher height, weight, BMI, and waist circumference; have higher systolic blood pressure; and more likely to have a history of myocardial infarction or stroke, be a current or past smoker, and use guidelines-directed therapies (lipid-lowering medications, ACE inhibitor or ARB, beta-blockers) (all nominally  $p < 0.05$ ).

Participants with ECG-derived CAN were also older, with longer diabetes duration and higher HbA1c, more likely to be male and White, have higher waist circumference, higher systolic blood pressure, a lower eGFR, and were more likely to have a history of myocardial infarction, diabetic peripheral neuropathy (defined by an abnormal MNSI), history of hyperlipidemia, statin and/or other lipid-lowering medication use, ACE inhibitor or ARB use, and beta blocker use, compared to participants who did not have ECG-derived CAN at baseline.

Due to attrition at year 4, baseline characteristics were compared between participants with and without an ECG at year 4 (Supplementary Table 3). Although there were a few statistically significant differences at baseline between those with and without a year 4 ECG for sex, education, diabetes duration, weight, and LDL, these differences were small and not clinically meaningful. In addition, there were no differences found between those with and without a year 4 ECG in terms of treatment group assignment or cardiovascular disease history (i.e., history of myocardial infarction and stroke) at baseline, and so attrition bias due to missing year 4 ECGs is expected to be minimal.

Table 2 displays descriptive prevalence statistics of ECG abnormalities and of the HRV indices used to define CAN at baseline, overall and across treatment groups. The baseline prevalence of ECG abnormalities and of ECG-derived CAN was high, at 57% for any abnormality and 53% for CAN. The baseline distributions of ECG abnormalities, CAN, and HRV indices were similar across the four treatment groups, as expected. Baseline prevalence statistics for specific ECG abnormalities are presented in Supplementary Table 4.

### **Incidence of minor, major, and overall ECG abnormalities by treatment group**

The incidence analyses are restricted to participants with non-missing data, including for all of the baseline adjustment factors (7956 post-baseline ECGs, 99.2% of sample).

### **Incidence of minor, major, and overall ECG abnormalities across all four treatment groups**

Figure 1 displays the percentages of all participants with minor, major, and overall ECG abnormalities at the baseline, year 2, and year 4 visits. At baseline, 51% and 15% of participants had a minor or a major abnormality, respectively (57% for any abnormality). These percentages are relatively similar at year 2 and rise to 53% and 18% of participants at the year 4 visit (61% have any abnormality).

Figures 2a–2c (Supplementary Table 5) display the estimated incidence of ECG abnormalities at years 2 and 4 post-baseline by the 4 treatment groups, for any minor abnormality, any major abnormality, and any minor or major abnormality, after adjustment for baseline risk factors. There is no significant evidence that the incidence of ECG abnormalities varies across the four treatment groups ( $p=0.90$  for any minor abnormality,  $p=0.13$  for any major abnormality,  $p=0.81$  for any minor or major abnormality).

### **Incidence of minor, major, and overall ECG abnormalities comparing liraglutide and non-liraglutide groups**

Figures 2d–2f (Supplementary Table 5) display the model-estimated probabilities of ECG abnormalities by the liraglutide and non-liraglutide treatment groups, respectively for any minor abnormality, any major abnormality, and any minor or major abnormality, after adjustment for baseline risk factors. There is evidence that incidence of major ECG abnormalities varied between the liraglutide and non-liraglutide groups ( $p=0.03$ ), with higher incidence for the non-liraglutide groups over time. In particular, the model-estimated probability of any new major ECG abnormality was 6.9% (95% CI: 5.4%, 8.6%) for the liraglutide group vs. 8.0% (7.0%, 9.2%) for the non-liraglutide groups at year 2, and 9.3% (7.5%, 11.5%) for the liraglutide group vs. 12.7% (11.3%, 14.3%) for the non-liraglutide groups at year 4.

### **Incidence of ECG-derived CAN by treatment group and comparing liraglutide and non-liraglutide groups**

For CAN incidence, the analyses include only the participants who did not have ECG-derived CAN at baseline and were not missing any data, including all the baseline adjustment factors (1909 participants, 78.6% of sample with no ECG-derived CAN at baseline; 3230 ECGs, 91.9% of post-baseline ECGs among those with no ECG-derived CAN at baseline).

Figure 2g (Supplementary Table 5) displays the estimated incidence of ECG-derived CAN at years 2 and 4 for the liraglutide and non-liraglutide groups, after adjustment for baseline risk factors. There was no evidence that the incidence of ECG-derived CAN was different when comparing the liraglutide and non-liraglutide groups overall across the year 2 and 4 visits ( $p=0.42$ ).

Figures 2h and 2i (Supplementary Table 5) display the model-estimated HRV indices used to define CAN analyzed as continuous variables for the liraglutide and non-liraglutide groups at years 2 and 4. SDNN was higher at year 2 in the liraglutide group compared with the non-liraglutide group ( $p=0.02$  for overall test across years 2 and 4); in particular, the model-estimated mean logarithm of SDNN was 2.77 (95% CI: 2.74, 2.81) for the liraglutide group vs. 2.69 (2.66, 2.72) for the non-liraglutide groups at year 2, and 2.69 (2.63, 2.75) for the liraglutide group vs. 2.67 (2.64, 2.71) for the non-liraglutide groups at year 4. However, no significant treatment group differences were observed for RMSD ( $p=0.48$ ).

### **Association of baseline variables, including social determinants of health, with the incidence and progression of ECG abnormalities and ECG-derived CAN comparing liraglutide and non-liraglutide groups**

We assessed whether the effect of liraglutide vs. non-liraglutide treatment on ECG abnormalities (any minor, any major, and any minor or major) and CAN differed by categories of the baseline variables described in the Other Baseline Measures section above. There were no significant treatment effect interactions between baseline factors and rates of ECG abnormalities or ECG-derived CAN during follow-up (see Supplementary Table 6).

Based on separate regression models, each of the following baseline factors were significantly associated with higher incidence of minor, major, and overall (i.e., any minor or major) ECG abnormalities at years 2 and 4 (see Supplementary Table 6): higher systolic blood pressure, higher weight (minor and overall ECG abnormalities only), higher height (minor ECG abnormalities only), higher waist circumference (minor and overall ECG abnormalities only), diabetic peripheral neuropathy (defined as an abnormal MNSI), lower eGFR, use of beta blockers, use of lipid-lowering medications (overall ECG abnormalities only), older age, and non-Hispanic ethnicity compared to Hispanic ethnicity (minor and overall ECG abnormalities only) (all  $p<0.01$ ). Participant age at study entry was significantly associated with rates of ECG-derived CAN during follow-up ( $p<0.0001$ ), with older age being associated with higher incidence of ECG-derived CAN at years 2 and 4.

### **Sensitivity analysis of missing ECGs during the COVID-19 pandemic**

We conducted a sensitivity analysis where the dataset was truncated to study visits prior to the COVID-19 pandemic, i.e., all visits up to and including March 15, 2020. All analyses were repeated and the overall results, shown in Supplementary Figure 2, mirrored those from the main analyses (shown in Figure 2).

### **Sensitivity analysis using covariate-unadjusted models**

To address concerns that some models may suffer from multicollinearity, and consequently a potential loss in precision in the treatment effect estimates, we repeated all analyses without adjusting for any covariates in regression models. The results are shown in Supplementary Figure 3 and were similar to those from the main analyses (shown in Figure 2). As expected, the significant treatment effects between liraglutide and the non-liraglutide groups for any new major ECG abnormality and for the log of SDNN were also found in the unadjusted analysis (Supplementary Figure 3e and 3i), but with slightly larger  $p$ -values than in the covariate-adjusted analysis, likely due to increased precision from covariate adjustment.

## CONCLUSIONS

In this large contemporary cohort of adults with T2D of relatively short duration (~ 4 years on average) who were enrolled and followed in GRADE, only 6.4% of whom had known prior MI or stroke (10), the prevalence of any ECG abnormalities and ECG-derived CAN at baseline were high, 57% and 53% respectively. As expected and illustrated in Table 1, participants with both ECG abnormalities and with ECG-derived CAN had evidence of higher disease burden and more severe risk factors that were similar among these participants, with a couple of notable differences that included more likely to have higher weight and BMI, and a prior history of stroke in those with ECG abnormalities, and more likely to have higher HbA1c and diabetic peripheral neuropathy as defined by an abnormal MNSI in those with ECG-derived CAN.

To place these data in perspective, the prevalence of any ECG abnormalities in GRADE is somewhat higher than rates reported in prior cohorts, such as Hoorn Diabetes Care System that reported ECG abnormalities in approximately 25% of the T2D population without prior CVD (1) and in approximately 30% overall. Of note, the Hoorn cohort was older than the GRADE participants, and more than 90% were of White of Northern European ancestry. On the other hand, the prevalence of ECG abnormalities in GRADE was comparable to the prevalence reported in a cohort of African American participants with high CVD risk who enrolled in the African American Diabetes Heart Study (AADHS). In AADHS, 60% of the participants had at least one minor or major ECG abnormality; 23% had at least 1 major, and 37% had at least one 1 minor (with no major) (21). In the EDIC study in younger, predominantly white people with type 1 diabetes, about 27.1% of participants had at least 1 ECG abnormality (major or minor) at baseline, whereas 2% had at least one major abnormality (22). This is lower than GRADE likely due to the younger age and a leaner cohort with type 1 diabetes.

Likewise, the prevalence of CAN in GRADE was higher than in other cohorts, such as the ADDITION cohort; however, ADDITION included individuals with screen-detected diabetes, all also of Northern European ancestry (23). Furthermore, the recently validated HRV cut-off thresholds used for the assessment of ECG-derived CAN in these analyses (13) were not available in prior cohorts.

ECG abnormalities in individuals with T2D may serve as early indicators of the presence or risk of developing CVD (1). Prospective studies, particularly those in the general population, have consistently demonstrated links between baseline resting ECG abnormalities and poor outcomes such as CVD and all-cause mortality (24–27). Research examining the progression or incidence of ECG abnormalities reveals even stronger associations with these outcomes, suggesting that changes in ECG patterns provide additional prognostic value (28–29).

Individuals with diabetes have substantially higher risks of atherosclerotic CVD and heart failure compared to those without diabetes (4, 30–31). However, the actual benefits of screening asymptomatic individuals with diabetes for CV disease has been inconsistent across various cohorts over time. While most recently, conclusive evidence demonstrates the benefits of biomarker screening for asymptomatic heart failure in diabetes (4, 31), the

benefit of cardiac screening using ECG has been questioned, and neither the American Diabetes Association nor the most recent U.S. Preventive Services Task Force recommends screening with resting or exercise ECG to predict or prevent CVD events in asymptomatic adults (31–32).

Despite strong evidence demonstrating that CAN is an independent predictor of CVD risk, HF, and mortality (4, 7, 8, 33–35), there are no clear recommendations for CAN screening in asymptomatic individuals with diabetes (36). As a result, the presence or absence of CAN is largely overlooked in daily clinical practice. This was likely due to limited feasibility of assessing CAN in clinical settings until recently, given the burden associated with gold-standard cardiovascular reflex testing. Over the past decades, indices of HRV derived from standard 10-second, 12-lead ECG recordings have emerged as more feasible alternative measures for assessing dysautonomia and/or CAN in population studies such as the Multi-Ethnic Study of Atherosclerosis (MESA) (37) as well as in large diabetes cohort studies such as the MONICA/KORA Augsburg Cohort Study (38) or the Action to Control Cardiovascular Risk in Diabetes (ACCORD) trial (8) and shown to independently predict CVD morbidity and mortality in people with T2D in these cohorts (8). Furthermore, it has recently been demonstrated in the large, well-characterized DCCT/EDIC study cohort that indices of HRV obtained from standard ECGs are valid surrogate measures of CAN (13). Consequently, implementation of ECG-derived CAN testing is becoming feasible at the point of care for risk stratification to guide personalized care for people with diabetes. While current guidelines do not recommend ECG and CAN screening in asymptomatic patients, these data suggest that some patients with a more advanced risk phenotype (multiple CVD risk factors, presence of peripheral neuropathy) may benefit from targeted ECG/HRV screening. However, further research is needed to examine whether actions taken as a result of finding ECG abnormalities would be clinically meaningful or cost effective.

During GRADE follow-up, the incidence of ECG abnormalities or CAN was high. After adjusting for baseline risk factors, the incidence of any minor ECG abnormalities or ECG-derived CAN (among those with no ECG-derived CAN at baseline) did not differ when comparing the liraglutide to non-liraglutide groups across the year 2 and 4 visits. However, the incidence of major ECG abnormalities was lower in the liraglutide group when compared with all other assigned treatment groups. This finding is consistent with previously published analyses of clinical CV outcomes in GRADE (39), which found that the liraglutide-treated group, compared to all other treatment groups combined, had a significantly lower risk of hospitalization for heart failure and composite CV outcomes comprised of CV death, myocardial infarction, stroke, unstable angina requiring hospitalization or revascularization, coronary revascularization, +/- hospitalization for heart failure.

Strengths of this study include a large, well-phenotyped, contemporary cohort of individuals with T2D followed for 4+ years with standardized and comprehensive measurements of risk factors, the centrally-read digital ECGs allowing for standardized outcome assessments, and the use of the validated HRV cut-points. The diversity of the enrolled patient population, of which only a few had a history of established clinical ASCVD at baseline, the minimal dropout rates and small drop-in of non-study medication are additional strengths (17).

Limitations of this study were the exclusion of patients with heart failure and NYHA class III or IV functional status and those with any major cardiovascular events or vascular procedures in the past year, and the limited data collected on cardiovascular status (including heart failure NYHA class 1-II) at baseline. The study did not collect specific details on previous events occurring beyond the exclusion criteria and did not include all relevant contemporary diabetes medication classes (e.g., SGLT2i). Although baseline ECGs were collected in the fasting state, fasting was not required for ECGs conducted at follow-up visits. Baseline ECGs may therefore have been collected under different participant conditions than ECGs collected during follow-up which could impact the ECG results. Additionally, as reported in other cohorts, duration of diabetes and age are established risk factors for both CVD, hence ECG abnormalities, and CAN. Therefore, it is possible that the results from this analysis might not hold in other populations with a longer duration of diabetes. In addition, the observed incidence rates of ECG abnormalities in GRADE may have limited our ability to detect differences in these outcomes between all treatment groups. Another limitation is that although ECG-derived HRV indices have been validated for diagnosing CAN, these indices may have lower sensitivity and specificity compared to the gold-standard of cardiovascular reflex testing. Lastly, although randomized treatment assignment enabled unbiased estimation of treatment effects, GRADE did not consistently collect data from all participants on certain factors, including use of medications such as calcium channel blockers and lifestyle factors such as diet and physical activity. Adjustment for these covariates could have potentially improved precision of the estimated treatment effects.

In summary, the prevalence and incidence of ECG abnormalities and ECG-derived CAN were high in GRADE participants, despite their relatively recent diagnosis of type 2 diabetes. While there were no differences in the incidence of minor ECG abnormalities and ECG-derived CAN among the GRADE interventions, the incidence of major ECG abnormalities was lower with liraglutide treatment compared to all other treatment groups. This finding is consistent with and supports the prior published analysis of clinical CV outcomes in GRADE, which suggests that GLP-1RA treatment may reduce the risk of CV outcomes in patients with T2D at relatively low risk for CV events.

## Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

## ACKNOWLEDGEMENTS

### Funding and Assistance:

The GRADE Study was supported by a grant from the National Institute of Diabetes and Digestive and Kidney Diseases (NIDDK) of the National Institutes of Health under Award Number U01DK098246. The planning of GRADE was supported by a U34 planning grant from the NIDDK (U34-DK-088043). The American Diabetes Association supported the initial planning meeting for the U34 proposal. The National Heart, Lung, and Blood Institute and the Centers for Disease Control and Prevention also provided funding support. The Department of Veterans Affairs provided resources and facilities. Additional support was provided by grant numbers P30 DK017047, P30 DK020541-44, P30 DK020572, P30 DK072476, P30 DK079626, P30 DK092926, U54 GM104940, UL1 TR000439, UL1 TR000445, UL1 TR001108, UL1 TR001409, UL1 TR001449, UL1 TR002243, UL1 TR002345, UL1 TR002378, UL1 TR002489, UL1 TR002529, UL1 TR002535, UL1 TR002537, and UL1 TR002548. Educational materials were provided by the National Diabetes Education Program. Material support in

the form of donated medications and supplies were provided by Becton, Dickinson and Company, Bristol-Myers Squibb, Merck, NovoNordisk, Roche Diagnostics, and Sanofi. The content of this manuscript is solely the responsibility of the authors and does not necessarily represent the official views of the National Institutes of Health.

#### Conflict of Interest Disclosures:

RPB reports grant support paid to her institution from NIDDK, Novo Nordisk, Bayer, and Lexicon Pharmaceuticals; consulting fees from Bayer, Lexicon Pharmaceuticals, Merck, Novo Nordisk, Roche, and Viatrix; support for attending meetings or travel from Roche Diagnostics; participation on a Data Safety Monitoring Board or Advisory Board for Biogen; and a leadership role for the American Diabetes Association (Member of the Board of Directors) outside the submitted work. HAA reports grant support paid to her institution from Novo Nordisk; a lecture payment from HCPLive for the Institutional Perspectives in Type 2 Diabetes symposium on June 22, 2022; and spouse owning stock in Eli Lilly, Medtronic, Merck, Pfizer, and Regeneron outside the submitted work. SEI reports royalties or license from Up-To-Date and McGraw Hill; personal consulting fees from AstraZeneca, Boehringer Ingelheim, Corcept, Merck, Novo Nordisk, and Pfizer; personal payment for lectures, presentations, speakers bureau, manuscript writing or educational events from AstraZeneca and Boehringer Ingelheim; support for attending meetings and/or travel from AstraZeneca, Bayer, Boehringer Ingelheim, and Novo Nordisk; and participation on a Data Safety Monitoring or Advisory Board for Bayer outside the submitted work. SEI reports royalties or license from Up-To-Date and McGraw Hill; personal consulting fees from AstraZeneca, Boehringer Ingelheim, Corcept, Merck, Novo Nordisk, and Pfizer; personal payment for lectures, presentations, speakers bureau, manuscript writing or educational events from AstraZeneca and Boehringer Ingelheim; support for attending meetings and/or travel from AstraZeneca, Bayer, Boehringer Ingelheim, and Novo Nordisk; and participation on a Data Safety Monitoring or Advisory Board for Bayer outside the submitted work. JBM reports grant support paid to her institution from NIH, Biomea, Breakthrough T1D, Diamyd, Lexicon, Novo Nordisk; royalties or licenses from Washington University; personal consulting fees from Bayer, Lilly, Mannkind, Novo Nordisk, and Thermo Fisher; support for attending meetings and/or travel from Bayer; participation on a Data Safety and Monitoring Board or Advisory Board from NIH, Jaeb Center, Pfizer, and Washington University; a leadership role in Diabetes Access to Technology Coalition; and receipt of equipment, materials, drugs, medical writing, gifts or other services from Bayer and Mannkind outside the submitted work. SM reports grant support paid to his institution from NIH/NIDDK outside the submitted work. ERS reports grant support paid to her institution from NIH; personal payment for lectures, presentations, speakers bureaus, manuscript writing or education events from University of Kentucky, University of Pittsburgh, and Endocrine Society; and personal payment for participation on a Data Safety Monitoring Board or Advisory Board from Eli Lilly Advisory Board, University of Michigan Diabetes Center, and NIDDK Advisory Council outside the submitted work. JBG reports grant support paid to her institution from Bluebird, Boehringer Ingelheim, Lilly, Merck, and Roche; and personal consulting fees from Anji, AstraZeneca, Bayer, Boehringer Ingelheim, Corcept, Lilly, Mineralys, Novo Nordisk, Valo, and Vertex outside the submitted work. SPR, NMB, HKS, RKG, AK, DSS, MT, and EZS have nothing to disclose.

#### Guarantor Statement:

RPB and SR are the guarantors of this work and as such, had full access to all the data in the study and take responsibility for the integrity of the data and the accuracy of the data analysis.

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**ARTICLE HIGHLIGHTS**

- Why did we undertake this study?
  - Resting electrocardiogram (ECG) abnormalities predict increased morbidity and mortality.
- What are the specific questions we wanted to answer?
  - What are the prevalence and incidence of ECG abnormalities and ECG-derived cardiovascular autonomic neuropathy (CAN) in adults with type 2 diabetes (T2D) <10 years enrolled in GRADE?
- What did we find?
  - ECG abnormalities and CAN were prevalent in this cohort, particularly in older participants with more severe cardiovascular risk factors. During GRADE, the incidence of major ECG abnormalities was lower in the liraglutide vs. non-liraglutide groups.
- What are the implications of our findings?
  - ECG abnormalities and CAN are common in T2D <10 years, especially with more cardiovascular risk factors. The development of major ECG abnormalities may be lowered by liraglutide.

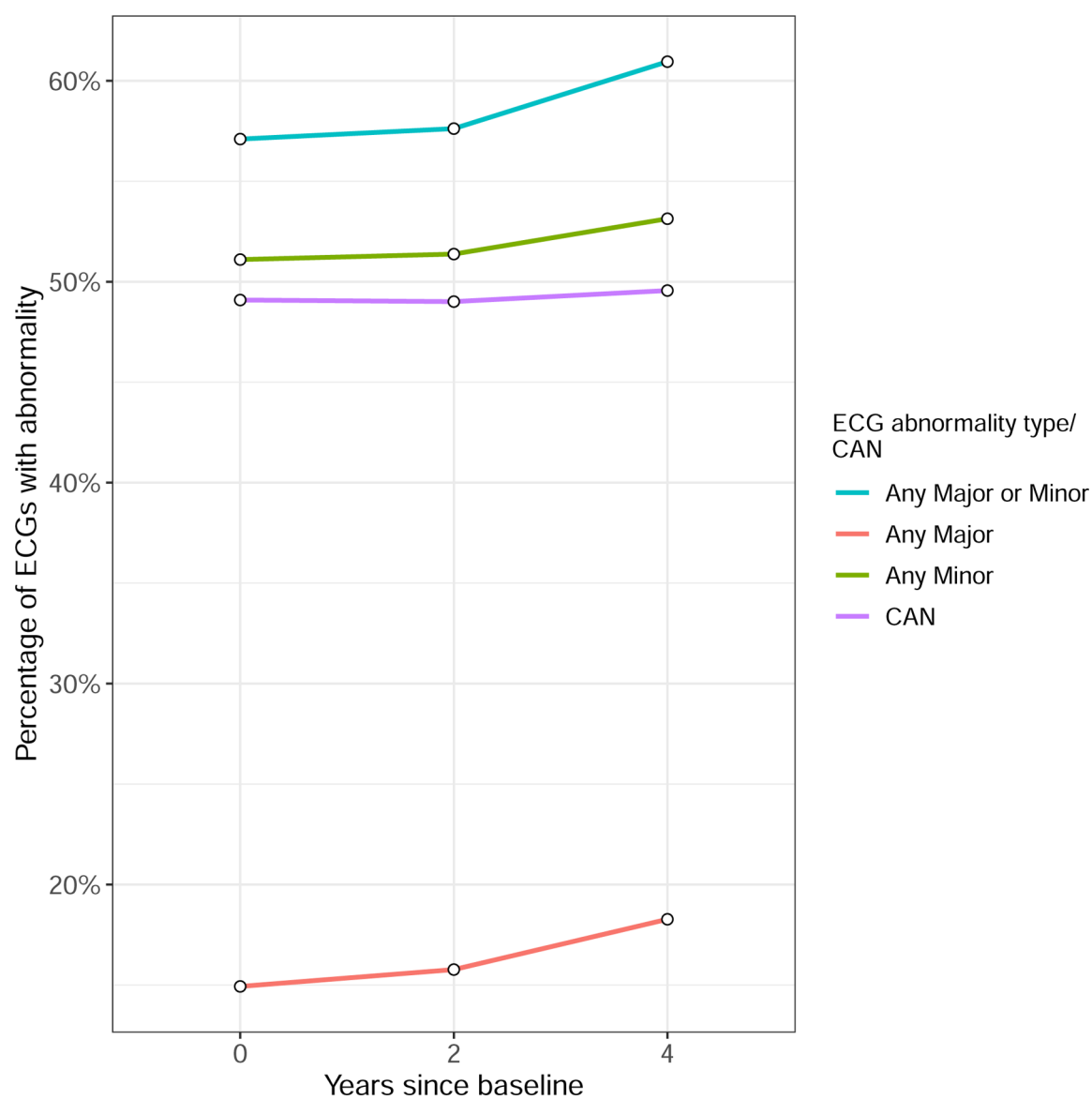
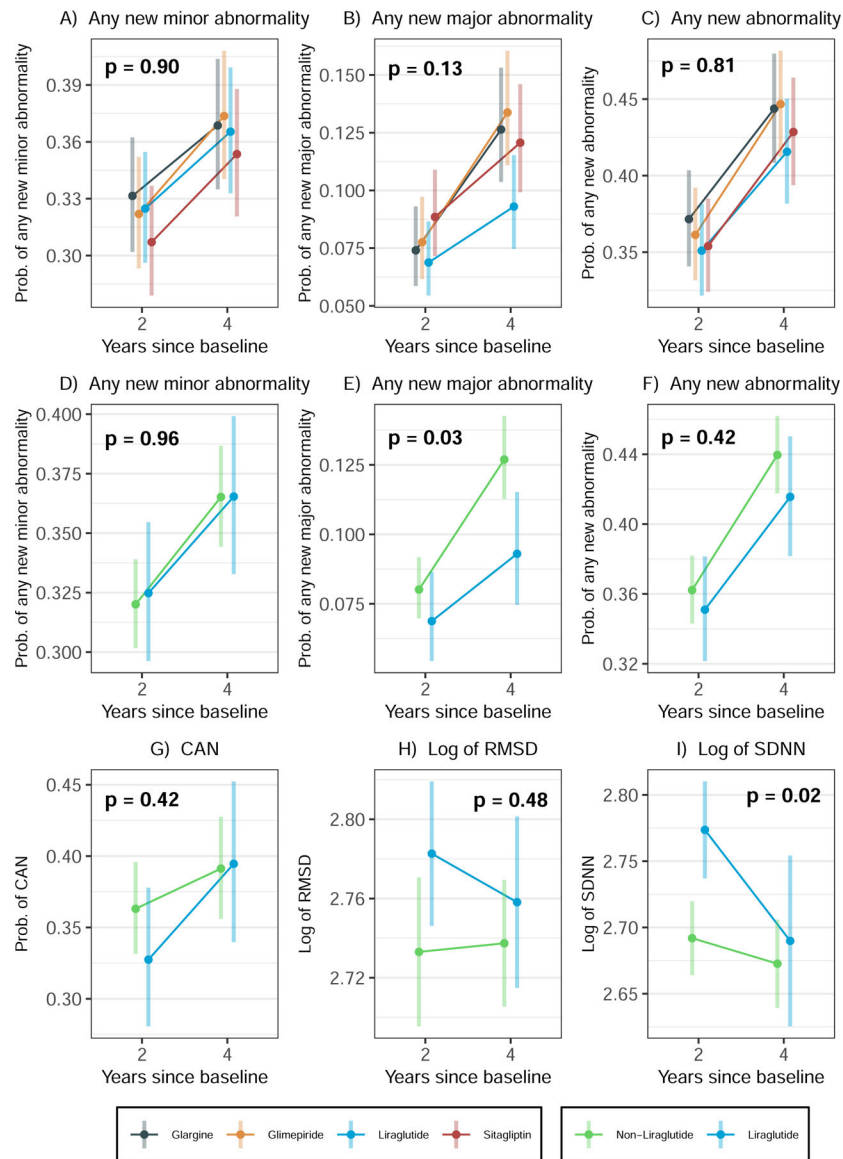


**Figure 1.**  
Prevalence of minor, major, and overall ECG abnormalities, and CAN, at baseline, year 2, and year 4 of follow-up

**Figure 2.**

Model-estimated probabilities (“Prob.”) of the incidence of minor ECG abnormalities (A), major ECG abnormalities (B), and any ECG abnormalities (C) over time, stratified by the 4 GRADE treatment groups. Model-estimated probabilities (“Prob.”) of the incidence of minor ECG abnormalities (D), major ECG abnormalities (E), any ECG abnormalities (F), and ECG-derived CAN (G) over time, stratified by the liraglutide vs. non-liraglutide treatment groups. Model-estimated logarithms of HRV measures (log-RMSD (H) and log-SDNN (I)) over time, stratified by the liraglutide vs. non-liraglutide treatment groups. All model-estimated values are based on GEE models adjusted for baseline factors. All p-values are from a Wald test assessing an overall treatment effect over time. Shaded areas are 95-percent confidence intervals. Points and lines are dodged horizontally away from the 2-year and 4-year points to increase legibility.

**Table 1.** Baseline characteristics comparing GRADE participants with vs. without ECG abnormalities and with vs. without CAN at baseline.

|                                                       | All <sup>a</sup> | Any ECG abnormality at baseline |                 | CAN presence at baseline |                 | p-value <sup>c</sup> |
|-------------------------------------------------------|------------------|---------------------------------|-----------------|--------------------------|-----------------|----------------------|
|                                                       |                  | Yes <sup>b</sup>                | No <sup>b</sup> | Yes <sup>b</sup>         | No <sup>b</sup> |                      |
| Number of participants                                | 4769             | 2723                            | 2046            | 2341                     | 2096            |                      |
| <i>Demographics and Social Determinants of Health</i> |                  |                                 |                 |                          |                 |                      |
| Age (years)                                           | 57.2 ± 10.0      | 58.6 ± 9.8                      | 55.3 ± 9.9      | 58.8 ± 9.2               | 55.0 ± 10.2     | <0.001               |
| Female                                                | 1731 (36.3%)     | 907 (33.3%)                     | 824 (40.3%)     | 833 (35.6%)              | 812 (38.7%)     | 0.032                |
| Race                                                  |                  |                                 |                 |                          |                 | <0.001               |
| All others                                            | 691 (14.5%)      | 356 (13.1%)                     | 335 (16.4%)     | 292 (12.5%)              | 343 (16.4%)     |                      |
| Black                                                 | 923 (19.4%)      | 527 (19.4%)                     | 396 (19.4%)     | 376 (16.1%)              | 486 (23.2%)     |                      |
| White                                                 | 3155 (66.2%)     | 1840 (67.6%)                    | 1315 (64.3%)    | 1673 (71.5%)             | 1267 (60.4%)    |                      |
| Ethnicity                                             |                  |                                 |                 |                          |                 | 0.158                |
| Non-Hispanic                                          | 3856 (81.5%)     | 2271 (84.1%)                    | 1585 (78.0%)    | 1907 (82.1%)             | 1672 (80.4%)    |                      |
| Hispanic                                              | 874 (18.5%)      | 428 (15.9%)                     | 446 (22.0%)     | 416 (17.9%)              | 408 (19.6%)     |                      |
| Income (USD)                                          |                  |                                 |                 |                          |                 | 0.904                |
| <20k                                                  | 802 (19.2%)      | 441 (18.5%)                     | 361 (20.1%)     | 386 (18.8%)              | 355 (19.3%)     |                      |
| 20k – <35k                                            | 630 (14.3%)      | 352 (14.8%)                     | 245 (13.6%)     | 303 (14.7%)              | 256 (14.0%)     |                      |
| 35k – <50k                                            | 743 (16.5%)      | 392 (16.4%)                     | 300 (16.7%)     | 337 (16.4%)              | 300 (16.3%)     |                      |
| 50k – <75k                                            | 783 (17.9%)      | 442 (18.5%)                     | 307 (17.1%)     | 352 (17.1%)              | 329 (17.9%)     |                      |
| 75k+                                                  | 1399 (32.1%)     | 759 (31.8%)                     | 585 (32.5%)     | 678 (33.0%)              | 595 (32.4%)     |                      |
| Education                                             |                  |                                 |                 |                          |                 | 0.398                |
| Less than high school                                 | 347 (7.3%)       | 171 (6.3%)                      | 176 (8.6%)      | 176 (7.5%)               | 156 (7.4%)      |                      |
| High school/GED                                       | 981 (20.6%)      | 565 (20.8%)                     | 416 (20.3%)     | 494 (21.1%)              | 416 (19.8%)     |                      |
| Some college                                          | 1,367 (28.7%)    | 790 (29.0%)                     | 577 (28.2%)     | 640 (27.4%)              | 616 (29.4%)     |                      |
| College                                               | 1265 (26.5%)     | 729 (26.8%)                     | 536 (26.2%)     | 618 (26.4%)              | 569 (27.1%)     |                      |
| Graduate school                                       | 808 (16.9%)      | 467 (17.2%)                     | 341 (16.7%)     | 412 (17.6%)              | 339 (16.2%)     |                      |
| <i>Medical factors</i>                                |                  |                                 |                 |                          |                 |                      |
| Duration of diabetes (years)                          | 4.3 ± 2.8        | 4.4 ± 2.8                       | 4.1 ± 2.8       | 4.4 ± 2.8                | 4.1 ± 2.7       | 0.002                |
| HbA1c (%)                                             | 7.5 ± 0.5        | 7.48 ± 0.47                     | 7.50 ± 0.49     | 7.51 ± 0.49              | 7.47 ± 0.48     | 0.010                |

|                                                       | All <i>a</i>  | Any ECG abnormality at baseline |               |                  | CAN presence at baseline |               |                  |
|-------------------------------------------------------|---------------|---------------------------------|---------------|------------------|--------------------------|---------------|------------------|
|                                                       |               | Yes <i>b</i>                    | No <i>b</i>   | p-value <i>c</i> | Yes <i>b</i>             | No <i>b</i>   | p-value <i>c</i> |
| Weight (kg)                                           | 100.1 ± 22.4  | 101.7 ± 22.6                    | 97.9 ± 22.0   | <0.001           | 100.0 ± 22.8             | 99.6 ± 21.8   | 0.478            |
| Height (cm)                                           | 170.6 ± 9.9   | 171.2 ± 9.7                     | 169.8 ± 10.1  | <0.001           | 170.7 ± 10.0             | 170.2 ± 9.8   | 0.125            |
| BMI (kg/m <sup>2</sup> )                              | 34.3 ± 6.8    | 34.7 ± 6.9                      | 33.9 ± 6.7    | <0.001           | 34.2 ± 6.8               | 34.3 ± 6.8    | 0.718            |
| Waist circumference (cm)                              | 112.5 ± 15.7  | 113.9 ± 15.9                    | 110.7 ± 15.3  | <0.001           | 112.9 ± 15.9             | 111.6 ± 15.5  | 0.005            |
| Diastolic blood pressure (mmHg)                       | 77.3 ± 9.9    | 77.3 ± 10.1                     | 77.3 ± 9.5    | 0.826            | 77.6 ± 10.1              | 77.1 ± 9.6    | 0.105            |
| Systolic blood pressure (mmHg)                        | 128.4 ± 14.8  | 129.4 ± 15.3                    | 127.2 ± 14.0  | <0.001           | 129.2 ± 15.1             | 127.8 ± 14.3  | <0.001           |
| Heart rate (bpm)                                      | 69.9 ± 10.8   | 69.2 ± 11.5                     | 70.8 ± 9.9    | <0.001           | 73.0 ± 11.0              | 66.3 ± 9.2    | <0.001           |
| History of hyperlipidemia                             | 3426 (71.8%)  | 1993 (73.2%)                    | 1433 (70.0%)  | 0.018            | 1758 (75.1%)             | 1404 (67.0%)  | <0.001           |
| HDL                                                   | 43.4 ± 10.6   | 43.4 ± 10.6                     | 43.4 ± 10.5   | 0.831            | 43.1 ± 10.3              | 43.7 ± 10.8   | 0.085            |
| LDL                                                   | 90.5 ± 31.7   | 88.7 ± 31.0                     | 92.8 ± 32.4   | <0.001           | 89.2 ± 31.6              | 92.1 ± 32.0   | 0.003            |
| Triglycerides                                         | 154.8 ± 122.5 | 151.2 ± 114.6                   | 159.7 ± 132.1 | 0.021            | 159.5 ± 118.0            | 150.4 ± 130.7 | 0.016            |
| Creatinine-estimated GFR (mL/min/1.73m <sup>2</sup> ) | 94.8 ± 16.8   | 93.3 ± 16.7                     | 96.8 ± 16.7   | <0.001           | 93.1 ± 16.5              | 97.1 ± 16.7   | <0.001           |
| History of myocardial infarction                      | 242 (5.1%)    | 194 (7.1%)                      | 48 (2.3%)     | <0.001           | 133 (5.7%)               | 83 (4.0%)     | 0.010            |
| History of stroke                                     | 95 (2.0%)     | 69 (2.5%)                       | 26 (1.3%)     | 0.003            | 48 (2.1%)                | 34 (1.6%)     | 0.344            |
| Abnormal MNSI (Neuropathy)                            | 2002 (42.2%)  | 1217 (45.0%)                    | 785 (38.5%)   | <0.001           | 1028 (44.0%)             | 814 (39.1%)   | <0.001           |
| Smoking Status                                        |               |                                 |               | 0.009            |                          |               | <0.001           |
| Never                                                 | 2588 (54.3%)  | 1426 (52.4%)                    | 1162 (56.8%)  |                  | 1220 (52.1%)             | 1220 (58.2%)  |                  |
| Past                                                  | 1524 (32.0%)  | 903 (33.2%)                     | 621 (30.4%)   |                  | 788 (33.7%)              | 615 (29.3%)   |                  |
| Current                                               | 657 (13.8%)   | 394 (14.5%)                     | 263 (12.9%)   |                  | 333 (14.2%)              | 261 (12.5%)   |                  |
| Alcohol use                                           |               |                                 |               | 0.194            |                          |               | 0.167            |
| Never                                                 | 1578 (33.1%)  | 896 (32.9%)                     | 682 (33.3%)   |                  | 772 (33.0%)              | 688 (32.8%)   |                  |
| Occasionally                                          | 2483 (52.1%)  | 1401 (51.5%)                    | 1082 (52.9%)  |                  | 1205 (51.5%)             | 1123 (53.6%)  |                  |
| Weekly                                                | 530 (11.1%)   | 312 (11.5%)                     | 218 (10.7%)   |                  | 267 (11.4%)              | 220 (10.5%)   |                  |
| Daily                                                 | 178 (3.7%)    | 114 (4.2%)                      | 64 (3.1%)     |                  | 97 (4.1%)                | 65 (3.1%)     |                  |
| <i>Medications</i>                                    |               |                                 |               |                  |                          |               |                  |
| Lipid-lowering medication                             | 3149 (66.0%)  | 1874 (68.8%)                    | 1275 (62.3%)  | <0.001           | 1617 (69.1%)             | 1289 (61.5%)  | <0.001           |
| Statin                                                | 3045 (63.8%)  | 1807 (66.4%)                    | 1238 (60.5%)  | <0.001           | 1570 (67.1%)             | 1240 (59.2%)  | <0.001           |
| ACE inhibitor or angiotensin receptor blocker         | 2783 (58.4%)  | 1669 (61.3%)                    | 1114 (54.4%)  | <0.001           | 1418 (60.6%)             | 1173 (56.0%)  | 0.002            |

|              | All <i>a</i> | Any ECG abnormality at baseline |             | CAN presence at baseline |             | p-value <i>c</i> |
|--------------|--------------|---------------------------------|-------------|--------------------------|-------------|------------------|
|              |              | Yes <i>b</i>                    | No <i>b</i> | Yes <i>b</i>             | No <i>b</i> |                  |
| Beta blocker | 873 (18.3%)  | 619 (22.7%)                     | 254 (12.4%) | 462 (19.7%)              | 326 (15.6%) | <0.001           |

<sup>a</sup> Analytic sample included all GRADE participants with an ECG assessment available at baseline.

<sup>b</sup> For categorical variables, counts and percentages are displayed, the latter representing the percent of participants with (without) any ECG abnormalities (with or without CAN) at baseline who have that value of the categorical variable. For continuous variables, means ± standard deviations are displayed.

<sup>c</sup> The p-values are from an ANOVA global F-test for differences in means (continuous variables), or from a chi-square test for differences in proportions (categorical variables) across participants with and without ECG abnormalities (with and without CAN) at baseline. All p-values are nominal, i.e., unadjusted for multiple comparisons.

**Table 2.** Prevalence of ECG abnormalities and ECG-derived cardiac autonomic neuropathy (CAN), and indices of heart rate variability, at baseline by treatment group

|                                                                | All          | Glargine    | Glimepiride | Liraglutide | Sitagliptin |
|----------------------------------------------------------------|--------------|-------------|-------------|-------------|-------------|
| Participants with completed baseline ECGs (%) <sup>*</sup>     | 4769         | 1,184       | 1,189       | 1,200       | 1,196       |
| <i>Overall ECG abnormalities</i>                               |              |             |             |             |             |
| Any minor abnormality                                          | 2437 (51.1%) | 629 (53.1%) | 589 (49.5%) | 620 (51.7%) | 599 (50.1%) |
| Any major abnormality                                          | 712 (14.9%)  | 164 (13.9%) | 172 (14.5%) | 205 (17.1%) | 171 (14.3%) |
| Any minor or major abnormality                                 | 2723 (57.1%) | 680 (57.4%) | 662 (55.7%) | 707 (58.9%) | 674 (56.4%) |
| <i>Heart rate variability (HRV) and CAN assessed by HRV</i>    |              |             |             |             |             |
| Standard deviation of NN intervals (SDNN) <sup>†</sup>         | 19.2 ± 13.9  | 19.4 ± 12.8 | 19.4 ± 14.6 | 18.9 ± 14.5 | 18.9 ± 13.8 |
| Root mean square of successive differences (RMSD) <sup>†</sup> | 21.2 ± 18.9  | 21.1 ± 16.1 | 21.7 ± 20.6 | 21.1 ± 19.8 | 20.8 ± 18.8 |
| CAN                                                            | 2341 (52.8%) | 564 (50.5%) | 551 (50.4%) | 602 (54.2%) | 624 (55.9%) |

<sup>\*</sup> 248 participants did not have a completed baseline ECG.

<sup>†</sup> Numbers displayed are mean ± SD