# Effects of Liraglutide on Heart Rate and Heart Rate Variability: A Randomized, Double-Blind, Placebo-Controlled Crossover Study

*Preman Kumarathurai,[1] Christian Anholm,[2] Bjørn S. Larsen,[1] Rasmus Huan Olsen,[1] Sten Madsbad,[3] Ole Kristiansen,[1] Olav W. Nielsen,[1] Steen B. Haugaard,[2,4] and Ahmad Sajadieh[1]*

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## OBJECTIVE

**Reduced heart rate variability (HRV) and increased heart rate (HR) have been associated with cardiovascular mortality. Glucagon-like peptide 1 receptor agonists (GLP-1 RAs) increase HR, and studies have suggested that they may reduce HRV. We examined the effect of the GLP-1 RA liraglutide on HRV and diurnal variation of HR in overweight patients with newly diagnosed type 2 diabetes (T2D) and stable coronary artery disease (CAD).**

## RESEARCH DESIGN AND METHODS

**Liraglutide or placebo was administrated to a backbone therapy of metformin in this double-blind, placebo-controlled 12 + 12–week crossover study. SD of beat-to-beat (NN) intervals (SDNN) was assessed by 24-h Holter monitoring as a measure of HRV. Diurnal HR variation and sympathovagal balance analyzed by root mean square of successive differences (RMSSD) in NN intervals and high-frequency (HF) and low-frequency (LF) power were assessed.**

## RESULTS

**Compared with placebo, liraglutide decreased SDNN in 27 subjects (−33.9 ms; $P < 0.001$, paired analysis); decreased RMSSD (−0.3 log-ms; $P = 0.025$); and increased the mean HR (8.1 beats/min; $P = 0.003$), daytime HR (5.7; $P = 0.083$), and nighttime HR (6.3; $P = 0.026$). In a multivariable regression analysis, the decrease in SDNN remained significant after adjustment for metabolic and HR changes. Liraglutide reduced HF power (−0.7 log-ms$^2$; $P = 0.026$) without any change in LF/HF ratio.**

## CONCLUSIONS

**In overweight patients with CAD and newly diagnosed T2D, liraglutide increased HR and reduced HRV despite significant weight loss and improvement in metabolic parameters. The increase in nightly HR in conjunction with a decrease in parameters of parasympathetic activity suggests that liraglutide may affect sympathovagal balance.**

Autonomic imbalance, characterized by increased sympathetic and decreased parasympathetic activity, is associated with various pathological conditions, such as coronary artery disease (CAD) (1), cardiovascular death (2), and heart failure (3). Heart rate variability (HRV), a measure of beat-to-beat variation in heart rate (HR),

*[1]Department of Cardiology, Copenhagen University Hospital of Bispebjerg, Copenhagen, Denmark*
*[2]Department of Internal Medicine, Copenhagen University Hospital of Amager, Copenhagen, Denmark*
*[3]Department of Endocrinology, Copenhagen University Hospital of Hvidovre, Copenhagen, Denmark*
*[4]Clinical Research Center, Copenhagen University Hospital of Hvidovre, Copenhagen, Denmark*

*Corresponding author: Preman Kumarathurai, preman.kumarathurai@dadlnet.dk.*

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provides information on autonomic balance and is impaired in patients with type 2 diabetes (T2D) (4). The SD of beat-to-beat (NN) intervals (SDNN), derived from 24-h time domain components of Holter monitoring, is a widely used measure of HRV and regarded as a more-sensitive assessment method of autonomic balance than single autonomic reflex tests in subjects with T2D (5). Both reduced SDNN and increased HR are associated with an increased risk of cardiovascular mortality (1,6). High-frequency (HF) and low-frequency (LF) powers are components of the frequency domain parameters of HRV. HF power primarily represents respiratory variation, is mediated by parasympathetic activity, and is highly correlated to the root mean square of successive differences (RMSSD) in R-R variability. LF power reflects primarily the oscillatory rhythm of the baroreceptors and is modulated by both the parasympathetic and the sympathetic nervous systems (7,8). Both time and frequency domain components provide information on the sympathovagal balance and have prognostic value in various populations, such as patients with diabetes (6,9), myocardial infarction (10), and heart failure (11).

Glucagon-like peptide 1 receptor agonists (GLP-1 RAs) have emerged as a promising treatment option in patients with T2D and have been shown to reduce glycated hemoglobin ($HbA_{1c}$) and induce weight loss (12). Although intensified treatment and improvement in glucose metabolic parameters have been associated with HRV improvement (13), some experimental studies have suggested that GLP-1 and GLP-1 RAs may reduce HRV (14–16). Furthermore, some concerns have been raised about the observed increased HR during GLP-1 RA therapy (17). Only a few data exist on how GLP-1 RAs affect long-term HRV in conjunction with changes in glucometabolic parameters. Hence, this study examined the effects of 12 weeks of treatment with the long-lasting GLP-1 RA liraglutide on HR and HRV in overweight patients with newly diagnosed T2D and stable CAD.

## RESEARCH DESIGN AND METHODS

### Study Design and Participants

This is a randomized, double-blind, placebo-controlled 12 + 12–week crossover study with a 2-week washout period. The details of the study protocol have been published previously (18). Briefly, patients with newly diagnosed T2D and stable CAD underwent 12 weeks of liraglutide and placebo treatment added to a backbone therapy of metformin. Patients were recruited through patient files from May 2012 to October 2014 from selected hospitals in Copenhagen, Denmark. Patients who were taking antidiabetic treatment before the study underwent a minimum 2-week washout period before the baseline visit. Subcutaneous injections of liraglutide/placebo and metformin tablets were titrated in an identical manner in both periods. An initial dose of 0.6 mg o.d. liraglutide/placebo + 500 mg b.i.d. metformin was increased after 14 days to 1.2 mg o.d. + (1,000 mg + 500 mg) daily and to 1.8 mg o.d. + 1,000 mg b.i.d. after 28 days. Efforts were made to give subjects the same dosage of study drug in both periods and to keep the background cardiovascular medication unchanged throughout the study. Subjects were instructed not to perform any tasks or physical activity that may influence the recordings. Each subject underwent 48-h Holter monitoring before and after each intervention for a total of four times (weeks 0, 12, 14, and 26). A computer was used to perform 1:1 randomization (placebo first or liraglutide first). Subjects, investigators, and all caregivers were blinded to the treatment sequence, which was concealed until the end of the study.

### Holter Monitoring

Holter monitoring for up to 48 h was performed (Rozinn RZ 153+12; Rozinn Electronics). All recordings were performed with seven leads to create a three-channel electrocardiogram output. The sampling rate for the electrocardiogram recordings was 180/s. The primary editing and analyses of Holter recordings were performed by an experienced technician from the Holter laboratory of the Copenhagen University Hospital of Bispebjerg. If the frequency of ectopic complexes constituted >20% of QRS complexes in a segment, that segment was deleted. If noise implied undetectable QRS complexes in all three leads, the corresponding segment was deleted. All HRV analyses were based on variations in normal complexes. Frequency domain components of HRV were calculated after fast Fourier transformation of the R-R interval data using Cooley-Tukey algorithm with 300-s data segments as input. Time domain components of HRV were analyzed and compared using recordings of equal length (full 24 h) for each subject, and the following parameters were identified: the mean value of all NN intervals and SDNN, RMSSD, SD of the averages of NN intervals in all 5-min segments (SDANN), and SDNN for all 5-min segments of the entire recording (SDNN-index) (7). In frequency domain analysis, the spectral results were obtained from 5-min segments and averaged over 24 h, and the following parameters were assessed: LF power (0.04–0.15 Hz), HF power (0.15–0.4 Hz), total power (TP) (≤0.4 Hz), and LF/HF ratio. Furthermore, normalized units of LF and HF power were calculated to obtain values independent of total variability (LFnorm = LF/TP; HFnorm = HF/TP). Changes in SDNN-index and RMSSD were assessed under more standardized conditions during nighttime (2:00–3:00 A.M.). Mean HR, minimum HR, maximum HR, and diurnal variation in HR were assessed. Diurnal HR variation was analyzed using full recording time, and hourly HR was calculated as the average from 2 consecutive days, when available. In addition, diurnal variations of the following parameters were assessed: SDNN-index, RMSSD, LF, HF, LF/HF ratio, LFnorm, and HFnorm. Daytime was defined as 7:00 A.M.–10:00 P.M. and nighttime as 11:00 P.M.–6:00 A.M.

### Statistical Analysis

Distribution of data (normal or nonnormal) was evaluated by visual assessment of histograms and by Shapiro-Wilk test (19,20). Log-transformation of data was used to normalize variables with skewed distribution (SDNN index, RMSSD, SDANN, LF, HF, LF/HF ratio, LFnorm, and HFnorm) (8,19). Treatment effects of liraglutide and placebo were compared by using the paired *t* test. For nonnormally distributed data, Wilcoxon signed rank test was used. The association between variables of interest was examined by using linear regression. Intention-to-treat (ITT) population was defined as subjects with valid Holter recordings for a minimum of one period. ITT analysis was performed for SDNN by using a linear mixed model with random effects for subjects and fixed effects for

period and treatment. Prespecified power analysis provided a statistical power of 90% for 16 subjects in paired analyses at $P < 0.05$ (18). A two-sided $P < 0.05$ was considered statistically significant. All statistical analyses were performed with Stata 13.1 software (StataCorp, College Station, TX).

### Ethics and Dissemination

This trial was carried out in accordance with the International Conference on Harmonization Good Clinical Practice standards and complies with the Declaration of Helsinki. The Good Clinical Practice Unit for Eastern Denmark performed the monitoring. Written informed consent was obtained from each subject. This trial was approved by the Regional Committee on Biomedical Research Ethics of the Capital Region of Denmark and the Danish Medicines Agency.

## RESULTS

In total, 41 subjects were randomly assigned in this study. Two subjects declined participation before the baseline visit, and nine discontinued the study. Thus, 30 subjects underwent all study visits (Fig. 1). Holter recordings from one or more visits were not readable for three patients due to technical problems.

Holter recordings for a minimum of one period were available for 31 subjects (ITT population). Pre- and post-treatment Holter recordings were available for 27 subjects for both interventions. Holter data were not of sufficient technical quality for frequency domain analysis for one subject, and another did not have data on diurnal HR variation. Thus, data for only 26 subjects were available in these analyses.

The study population comprised mostly men (79%). The mean age was 61.8 ± 7.6 years, and the mean $HbA_{1c}$ was 6.4 ± 0.5% (46.6 ± 5.7 mmol/mol). All subjects were overweight, with a mean BMI of 31.6 ± 4.8 kg/m$^2$, and had newly diagnosed T2D and stable CAD as defined by the inclusion criteria (18). Baseline characteristics of the study population are shown in Table 1.

Liraglutide treatment compared with placebo induced a significant increase in mean HR (6.9 ± 8.6 vs. −1.2 ± 7.1 beats/min [bpm]; $P = 0.003$) and minimum HR (7.0 ± 5.9 vs. −0.7 ± 5.2 bpm; $P < 0.001$). Maximum HR increased during liraglutide treatment but did not reach statistical significance (3.2 ± 13.4 vs. −3.1 ± 11.2 bpm; $P = 0.057$) (Table 2). Figure 2 shows the diurnal variation in post-treatment HR, revealing a persistently elevated HR throughout the 24 h after liraglutide treatment. However, the HR change from baseline was only significantly higher during nighttime compared with changes during the placebo period (Table 2 and Supplementary Fig. 1*A*).

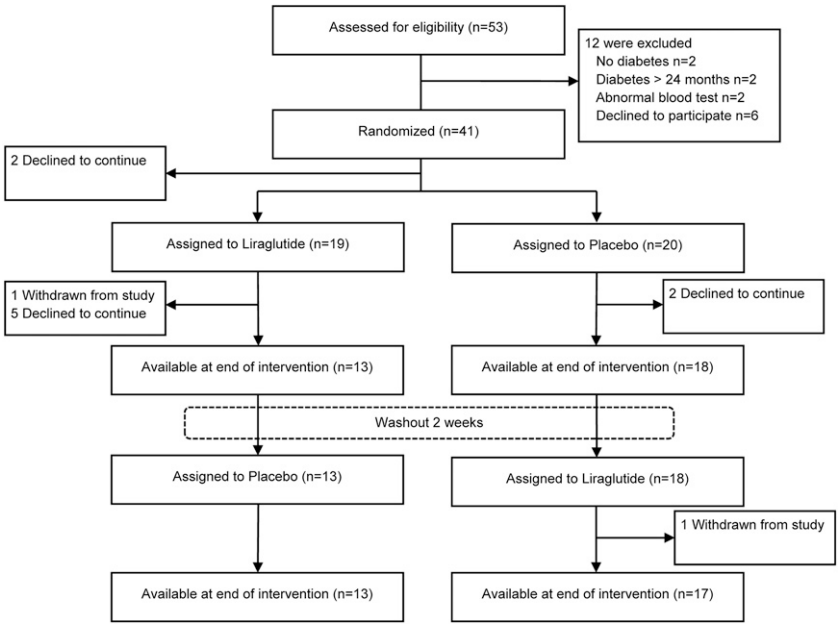


**Figure 1**—Screening, enrollment, and follow-up of the study population.

Analysis of the 24-h time domain parameters of HRV revealed a significant decrease in SDNN after liraglutide treatment compared with placebo (−34.0 ± 19.6 vs. −0.11 ± 28.4 ms; $P < 0.001$) (Table 2). Figure 3 visually represents these changes in SDNN, revealing a general decrease during the liraglutide treatment period for all subjects. ITT analysis confirmed this finding (coefficient −32.5 [95% CI −45.2 to −19.8]; $P < 0.001$). Mean NN was significantly reduced after liraglutide treatment, reflecting the increased HR (−75.5 ± 89.4 vs. 8.5 ± 77.8 bpm; $P = 0.003$) (Table 2). Log-transformed values of RMSSD, SDANN, and SDNN-index were reduced after liraglutide treatment (Table 2). The diurnal variation curves revealed a significant decrease in RMSSD after liraglutide, mainly during nighttime hours (Supplementary Fig. 1*B*). For the SDNN-index, a decrease was observed during both nighttime and morning hours (Supplementary Fig. 1*C*). In sensitivity analyses, SDNN-index and RMSSD were significantly reduced after liraglutide during sleeping hours from 2:00 to 3:00 A.M. (−0.23 ± 0.42 vs. 0.05 ± 0.45 ms [$P = 0.009$] and −0.25 ± 0.53 vs. 0.09 ± 0.61 ms [$P = 0.045$], respectively).

The significant association between liraglutide treatment and changes in SDNN ($\beta = -33.9$; $P < 0.001$) was unchanged after adjustment for mean NN ($\beta = -28.0$; $P < 0.001$) and after further adjustment for other metabolic parameters in a multivariable analysis ($\beta = -32.3$; $P < 0.001$), confirming the independent reduction in SDNN by liraglutide therapy (Table 3).

Analysis of frequency domain parameters showed a significant reduction in log-transformed values of LF and HF power, which was also observed in the diurnal variation curves (Supplementary Fig. 1*D* and *E*). LF/HF ratio, LFnorm, and HFnorm did not change (Table 2); however, the diurnal variation curves revealed reductions in both LFnorm and HFnorm at several time points (Supplementary Fig. 1*F* and *G*). This was not evident in the diurnal curve for the LF/HF ratio (Supplementary Fig. 1*H*).

For the study population, a significant weight loss (−4.2 ± 3.5 vs. −1.0 ± 2.6 kg; $P < 0.001$) and reduction in $HbA_{1c}$ (−0.4 ± 0.3% vs. 0.04 ± 0.4%; $P < 0.001$) was observed after liraglutide treatment compared with placebo. Resting systolic and diastolic blood

**Table 1—Baseline characteristics of the study population ($n$ = 39)**

| Characteristic | Value |
|---|---|
| Clinical characteristics | |
| Age (year) | 61.8 (7.6) |
| Male sex, *n* (%) | 31 (79) |
| Weight (kg) | 96.9 (17.1) |
| BMI (kg/m$^2$) | 31.6 (4.8) |
| Waist (cm) | 110.4 (11.2) |
| Systolic BP (mmHg) | 139.3 (19.4) |
| Diastolic BP (mmHg) | 80.2 (10.1) |
| HR (bpm) | 71.7 (12.1) |
| Risk factors, *n* (%) | |
| Smoker | 14 (36) |
| Hypertension | 29 (74) |
| CAD | |
| Previous MI | 23 (59) |
| Previous CABG | 13 (33) |
| Previous PCI | 25 (64) |
| Coronary stenosis, medical therapy only | 2 (5) |
| Biochemistry | |
| Fasting blood glucose (mmol/L) | 6.5 (1.4) |
| $HbA_{1c}$ (%) | 6.4 (0.5) |
| $HbA_{1c}$ (mmol/mol) | 46.6 (5.7) |
| LDL cholesterol (mmol/L) | 2.3 (0.7) |
| eGFR (mL/min/1.73 m$^2$) | 80.5 (11) |
| HOMA-IR | 4.02 (2.96, 7.49) |
| Fasting insulin (pmol/L) | 93 (64, 155) |
| Medication, *n* (%) | |
| β-Blockers | 24 (62) |
| Calcium antagonists | 21 (54) |
| ACE-I/ARB | 26 (67) |
| Statins | 37 (95) |
| Ivabradine | 1 (3) |
| Diuretics | 11 (28) |
| Nitrate | 11 (28) |
| Aspirin | 37 (95) |
| Prestudy diabetes medication, *n* (%) | |
| Biguanide (metformin) | 15 (38) |
| Sulfonylurea | 1 (3) |
| Diet and lifestyle therapy only | 24 (62) |

Data are mean (SD) or median (interquartile range) unless otherwise indicated. ACE-I, ACE inhibitor; ARB, angiotensin receptor blocker; BP, blood pressure; CABG, coronary artery bypass graft; eGFR, estimated glomerular filtration rate; MI, myocardial infarction; PCI, percutaneous coronary intervention.

pressure, fasting blood glucose, HOMA of insulin resistance (HOMA-IR), plasma insulin, and LDL cholesterol did not change significantly (Supplementary Table 1).

Regression coefficients for the association between changes in HR and SDNN and changes in selected variables of interest for both intervention periods are shown in Supplementary Table 2. No associations among changes in blood pressure, changes in metabolic factors, and changes in SDNN were found during liraglutide therapy. A significant correlation between changes in RMSSD and changes in total HF power and normalized HF power was found ($R^2$ = 0.77 [$P < 0.001$] and 0.62 [$P < 0.001$], respectively), during the liraglutide period. A full list of adverse events are provided in Supplementary Table 3.

## CONCLUSIONS

We demonstrate that 12 weeks of liraglutide treatment induced a significant increase in HR and reduction in long-term HRV in patients with newly diagnosed T2D and stable CAD. We show that the reduction in HRV was not mediated by the increased HR observed after liraglutide therapy. To our knowledge, this randomized placebo-controlled study is the first to assess the effects of a GLP-1 RA on long-term HRV in a clinical setting.

The chronotropic effect of liraglutide may be mediated through the GLP-1 receptor on the sinoatrial node and influence the measures of HRV. However, the multivariable regression analysis revealed no significant association between changes in mean NN and SDNN. Furthermore, the association between liraglutide treatment and SDNN reduction was still significant after adjustment for mean NN. These findings suggest that the impaired HRV may be due to a direct influence on sympathovagal balance.

The time domain parameter RMSSD decreased significantly during liraglutide treatment. Of note, this decrease was mainly during nighttime hours, where vagal activity is considered dominant. The correlation analyses showed a relatively large $R^2$ value for RMSSD and HF power, thus confirming the close association between these two parameters (7). A significant decrease during nighttime hours was also evident for SDNN-index. Although, the normalized values of LF and HF power did not decrease in contrast to the absolute values, the diurnal variation curve revealed a trend for decrease. The less-pronounced reduction in normalized values probably reflects the concomitant reduction in TP. The LF/HF ratio, which is also considered reflective of sympathovagal balance, did not change. However, LF power is subject to both sympathetic and vagal influences, whereas the reduction in both LF and HF power may be attributable to a decrease in mainly parasympathetic activity (7). Taken together, the increase in nighttime HR in conjunction with the significant decrease in SDNN and RMSSD, suggest an impairment of parasympathetic activity. The decrease in HF power together with the trend for decrease in diurnal HFnorm also suggest an impairment of vagal activity after liraglutide therapy.

This hypothesis is supported by findings from previous studies. In a randomized study in 30 patients with T2D, liraglutide induced an increase in HR in conjunction with an increase in the LF/HF ratio during several time points of the day (21). Furthermore, in a small study of seven patients with T2D, 24 weeks of liraglutide treatment increased HR, decreased

SDNN, and decreased LF and HF power, and the authors concluded that this may be due to an inhibiting effect on vagal activity (22). Short-term infusion of exenatide in overweight, healthy participants revealed an increase in HR and a concomitant increase in LF/HF ratio, thus in accordance with a relative increase in sympathetic activity (15). Finally, an experimental study in mice showed an inhibitory effect of GLP-1 on vagal activity because both short- and long-term central administration of the GLP-1 RA exendin-4 increased HR-reduced HF and LF powers and inhibited neurotransmission to vagal neurons (14).

Our findings regarding HR are consistent with previous findings from short-term studies that used 24-h HR monitoring. An HR increase ranging from 6.5 bpm after 7 weeks of liraglutide treatment (23) and up to 9.3 bpm after 8 weeks of treatment with liraglutide as an add-on to insulin glargine therapy has been reported (24). However, data from glucose-lowering trials have consistently reported a lower HR increase. A meta-analysis of six phase 3 LEAD (Liraglutide Effect and Action in Diabetes) studies reported an increase of 3.2 and 3.5 bpm after 26 weeks of treatment with liraglutide 1.2 and 1.8 mg, respectively (25). Recently, the LEADER (Liraglutide Effect and Action in Diabetes: Evaluation of Cardiovascular Outcome Results) study, with a median follow-up of 3.8 years, reported an elevated HR of 3.0 bpm in the liraglutide group compared with the placebo group (26). Longer duration of treatment may attenuate the HR increase, or alternatively, 24-h ambulatory HR monitoring may provide a more accurate assessment of HR because it includes changes in nocturnal HR. HR elevation has been observed for other GLP-1 RAs, and the increase in HR appears to be greater with long-acting than with short-acting agents (27). We did not find an association between changes in resting blood pressure and changes in HR during liraglutide treatment, confirming previous findings that HR elevation is not compensatory to a decrease in blood pressure or systemic vasodilatation (25,28). Whether the HR increase is due to augmented sympathetic activity or a direct effect on GLP-1 receptors in the sinus atrial node remains controversial (29). The nocturnal increase in HR observed in the current study does not appear to be related to the maximum concentration of

**Table 2—Effect of liraglutide versus placebo on HR and HRV parameters**

| | Before liraglutide (baseline) | After liraglutide (12 weeks) | Before placebo (baseline) | After placebo (12 weeks) | *n* | Treatment effect | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | | | | | Liraglutide | Placebo | Difference (95% CI) | *P* value |
| HR parameter (bpm) | | | | | | | | | |
| Mean HR | 74.03 (12.09) | 80.93 (10.61) | 75.93 (11.10) | 74.71 (9.52) | 27 | 6.90 (8.64) | −1.22 (7.15) | 8.11 (3.05 to 13.18) | 0.003 |
| Minimum HR | 48.48 (8.05) | 55.52 (8.55) | 50.81 (7.85) | 50.11 (7.92) | 27 | 7.04 (5.89) | −0.70 (5.17) | 7.74 (4.28 to 11.20) | <0.001 |
| Maximum HR | 126.00 (19.40) | 129.19 (17.91) | 129.56 (18.91) | 126.41 (20.38) | 27 | 3.19 (13.38) | −3.15 (11.22) | 6.33 (−0.22 to 12.88) | 0.058 |
| Daytime HR | 77.08 (12.80) | 83.34 (11.29) | 78.02 (12.04) | 78.61 (10.65) | 26 | 6.26 (9.76) | 0.60 (10.96) | 5.66 (−0.79 to 12.11) | 0.083 |
| Nighttime HR | 70.19 (11.70) | 77.45 (10.21) | 70.11 (10.30) | 71.04 (9.09) | 26 | 7.26 (8.58) | 0.93 (7.61) | 6.34 (0.81 to 11.86) | 0.026 |
| 24-h time domain | | | | | | | | | |
| Mean NN (ms) | 837.34 (143.64) | 761.81 (107.17) | 805.88 (123.80) | 814.35 (109.85) | 27 | −75.52 (89.36) | 8.47 (77.83) | −83.99 (−137.01 to −30.98) | 0.003 |
| SDNN (ms) | 129.63 (30.54) | 95.59 (27.04) | 125.48 (35.50) | 125.37 (32.00) | 27 | −34.04 (19.61) | −0.11 (28.38) | −33.93 (−47.76 to −20.09) | <0.001 |
| SDNN-index (log-ms) | 3.84 (0.35) | 3.57 (0.40) | 3.76 (0.39) | 3.74 (0.36) | 27 | −0.27 (0.28) | −0.02 (0.24) | −0.25 (−0.41 to −0.09) | 0.003 |
| SDANN (log-ms) | 3.18 (0.35) | 2.85 (0.38) | 3.09 (0.39) | 3.06 (0.34) | 27 | −0.33 (0.27) | −0.04 (0.25) | −0.29 (−0.46 to −0.12) | 0.002 |
| RMSSD (log-ms) | 3.52 (0.62) | 3.26 (0.69) | 3.40 (0.63) | 3.46 (0.68) | 27 | −0.26 (0.49) | 0.06 (0.40) | −0.32 (−0.60 to −0.04) | 0.025 |
| Frequency domain | | | | | | | | | |
| Log LF (log-ms$^2$) | 5.33 (0.65) | 4.81 (0.77) | 5.17 (0.81) | 5.13 (0.74) | 26 | −0.52 (0.57) | −0.04 (0.64) | −0.47 (−0.90 to −0.05) | 0.029 |
| Log HF (log-ms$^2$) | 4.46 (0.85) | 3.93 (1.06) | 4.21 (0.92) | 4.36 (1.02) | 26 | −0.53 (0.87) | 0.15 (0.89) | −0.67 (−1.26 to −0.09) | 0.026 |
| Log TP (log-ms$^2$) | 7.61 (0.40) | 7.30 (0.37) | 7.51 (0.38) | 7.51 (0.35) | 26 | −0.31 (0.31) | −0.001 (0.272) | −0.31 (−0.49 to −0.12) | 0.002 |
| Log LF/HF | 0.87 (0.59) | 0.88 (0.70) | 0.96 (0.56) | 0.77 (0.64) | 26 | 0.01 (0.55) | −0.19 (0.48) | 0.20 (−0.11 to 0.51) | 0.201 |
| Log LFnorm | −2.28 (0.43) | −2.49 (0.52) | −2.34 (0.56) | −2.38 (0.49) | 26 | −0.21 (0.33) | −0.04 (0.43) | −0.17 (−0.43 to 0.10) | 0.200 |
| Log HFnorm | −3.15 (0.73) | −3.37 (0.81) | −3.30 (0.72) | −3.15 (0.76) | 26 | −0.22 (0.70) | 0.15 (0.72) | −0.37 (−0.84 to 0.10) | 0.122 |

Data are mean (SD) unless otherwise indicated. *n* is the number of subjects for each treatment phase with complete measurements for all visits. Daytime, 7:00 A.M.–10:00 P.M.; nighttime, 11:00 P.M.–6:00 A.M.

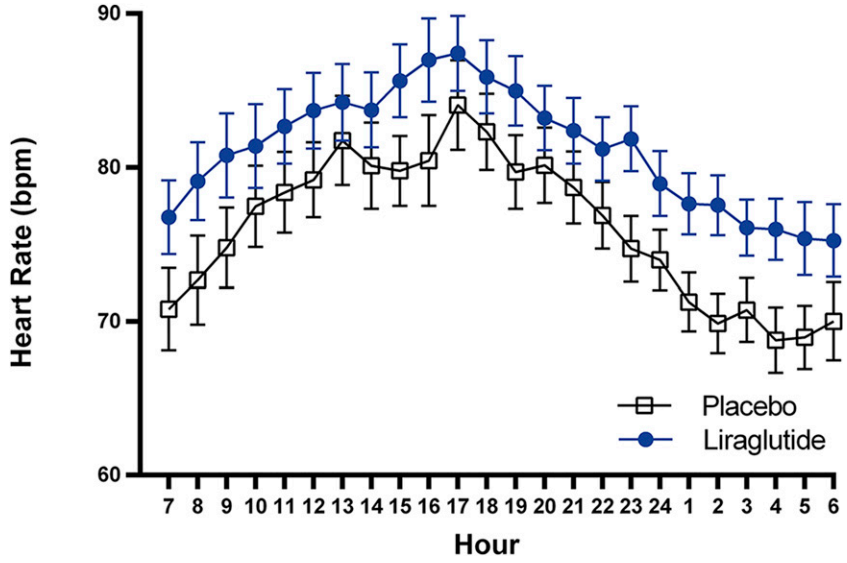


**Figure 2**—Diurnal variation in hourly mean HR. Error bars indicate mean ± SE.

liraglutide reported to be reached after 8–12 h (30). In the studies by Meier et al. (24) and Nakatani et al. (21), the injection of liraglutide was in the morning before breakfast. In both studies, a persistent increase in HR during the nighttime beyond the peak concentration for liraglutide was observed.

In previous studies, weight loss and improvement in metabolic parameters have been associated with an improvement in HRV, such as in obese patients after weight-reducing gastric surgery (31), patients with and without diabetes after an energy-restricted diet (32,33), and patients with T2D after intensive multifactorial intervention (13). In contrast, we observed a deterioration in HRV measures despite significant weight loss and reduction in $HbA_{1c}$ after liraglutide treatment. Hyperglycemia and hyperinsulinemia have been suggested as aggravating factors for HRV (34,35), but we did not see significant changes in these metabolic parameters during liraglutide treatment. Our linear regression analysis revealed that the reduction in SDNN was unaffected by changes in weight, $HbA_{1c}$, HOMA-IR, insulin, and fasting glucose. These findings suggest that liraglutide may have an effect on the autonomic nervous system that is not mediated by an improvement in metabolic parameters.

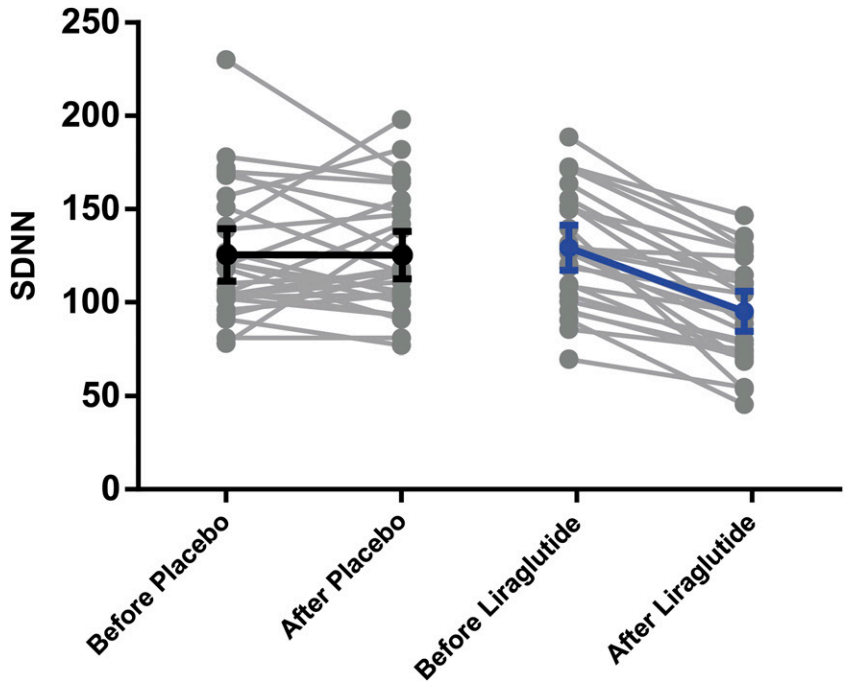


**Figure 3**—SDNN changes during treatment periods. Gray lines represent SDNN values for each patient. Bold lines represent change in mean SDNN in placebo and liraglutide period. Error bars indicate mean ± SE.

Although increased HR and decreased HRV have been associated with poor prognosis in terms of cardiovascular mortality in several studies (5,6,36), beneficial effects of GLP-1 RA therapy in terms of weight loss, improvement in metabolic parameters, and other positive effects on the cardiovascular system are important to note (29). Accordingly, the LEADER study demonstrated a significant reduction in cardiovascular mortality after liraglutide therapy (26,37). However, an unexpected trend toward adverse outcomes after liraglutide therapy was reported in a subgroup of subjects with heart failure and diabetes in a recent randomized study (38). Because both diabetes and heart failure are associated with reduced HRV (5), whether liraglutide induces an additional aggravating effect on autonomic function in patients with both of these conditions and whether it affects cardiovascular outcome need to be explored.

Potential limitations in this study should be considered. The Holter monitoring was not performed under controlled conditions in a laboratory; thus, environmental and behavioral factors may have influenced the results. However, because of the randomized crossover design, such factors would be expected to affect the recordings in both periods to a similar degree. Furthermore, we performed sensitivity analyses during the nighttime, where influences of external factors are limited. Frequency domain analyses should be interpreted with caution when obtained from long-term recordings due to lower stability of HR. Thus, time domain measures are considered ideal under these conditions (7). Nevertheless, the reporting of both these measures is a major strength of this study and

**Table 3—Linear regression models showing the association between liraglutide treatment and changes in SDNN**

| Liraglutide treatment | β-Coefficient | 95% CI | *P* value |
|---|---|---|---|
| Univariable analysis | −33.9 | −47.2 to −20.6 | <0.001 |
| Adjusted for changes in mean NN | −28.0 | −42.9 to −13.1 | <0.001 |
| Multivariable analysis† | −32.3 | −49.5 to −15.0 | <0.001 |

†Multivariable linear regression analysis with adjustment for changes in mean NN, weight, $HbA_{1c}$, systolic blood pressure, HOMA-IR, and insulin; dependent variable, changes in SDNN; independent variable, treatment (liraglutide).

provides a more thorough assessment of the influence of GLP-1 RA treatment on sympathovagal balance. The confounding effects of background medication are considered minimal because an effort was made to not change the medication during the study. However, the use of HR-limiting medications, such as β-blockers and ivabradine, may have attenuated the chronotropic effect of liraglutide and influenced the HRV measurements. Sensitivity analyses based on HR-limiting medications were not performed because of a small and unequal distribution of patients in these subgroups. The gastrointestinal adverse effects or hypoglycemia due to liraglutide therapy may have influenced the HRV measurements. However, the study drug was uptitrated to a maximum dose after 4 weeks, and because Holter monitoring was performed at 12 weeks, the majority of adverse effects are expected to be reduced. Furthermore, no severe hypoglycemic events were observed during the study. Nevertheless, any treatment-related temporary or persistent adverse effects that may influence HRV may be clinically important. Because of the homogeneity of the cohort, we cannot extrapolate the results to other patient groups, such as those with long-standing and poorly controlled diabetes or those with severe CAD or heart failure. The effect on HRV beyond 12 weeks of treatment was not assessed, although we cannot exclude the possibility that HRV changes may attenuate over time. Thus, the effect of GLP-1 RA treatment on HRV needs to be explored in other patient groups and with a longer treatment duration.

In conclusion, liraglutide increased HR and reduced HRV in overweight patients with newly diagnosed T2D and stable CAD despite significant weight loss and improvement in glucometabolic parameters. Liraglutide induced changes in time and frequency domain parameters of HRV, which suggests an effect on sympathovagal balance.

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**Duality of Interest.** During the past 3 years, S.M. has participated on advisory boards for Novartis, Novo Nordisk, Merck Sharp & Dohme, Sanofi, AstraZeneca, Johnson & Johnson, Roche, HumanKind, Boehringer Ingelheim, Zealand Pharma, Eli Lilly, and Intarcia Therapeutics and has received honoraria for lectures from Novo Nordisk, Merck Sharp & Dohme, AstraZeneca, Johnson & Johnson, Roche, Schering-Plough, Sanofi, Novartis, Eli Lilly, and Bristol-Myers Squibb. O.W.N. has received funding of educational and research tasks from ResMed and participated on advisory boards for Novartis. S.B.H. has received funding of educational and research tasks from Novo Nordisk, Abbott, Eli Lilly, Pfizer, Boehringer Ingelheim, Bristol-Myers Squibb, and Merck Sharp & Dohme. No other potential conflicts of interest relevant to this article were reported.

**Author Contributions.** P.K. acquired data, performed the statistical analyses, interpreted data, and drafted and revised the manuscript for important intellectual content and approved the final version. C.A. and B.S.L. acquired data, interpreted data, and revised the manuscript for important intellectual content and approved the final version. R.H.O., S.M., O.K., and O.W.N. interpreted data and revised the manuscript for important intellectual content and approved the final version. S.B.H. conceived and designed the study, interpreted data, and revised the manuscript for important intellectual content and approved the final version. A.S. conceived and designed the study, interpreted data, contributed to the statistical analyses, and revised the manuscript for important intellectual content and approved the final version. P.K. is the guarantor of this work and, as such, had full access to all the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis.

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