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Does empagliflozin modulate the autonomic nervous system among individuals with type 2 diabetes and coronary artery disease? The EMPA-HEART CardioLink-6 Holter analysis

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ABSTRACT

Context: We examined if empagliflozin was associated with modulation of cardiac autonomic tone among subjects with type 2 diabetes and stable coronary artery disease (CAD) relative to placebo.
Methods: Using ambulatory 24-h Holter electrocardiographic data prospectively collected from a randomized trial, we compared changes in heart rate variability (HRV) parameters between empagliflozin- and placebo-assigned subjects over a follow-up period of 6 months. Measured HRV domains included: standard deviation (SD) of NN intervals (SDNN), SD of average NN intervals per 5-min (SDANN), root mean square of successive RR interval differences (RMSSD), % successive NN intervals differing >50 ms (ms) (pNN50), low frequency (LF), high frequency (HF) and the LF/HF ratio (LF:HF). Differences in HRV parameters between the 2 groups were compared with analysis of covariance (ANCOVA). Statistical measures of significance were reported as adjusted differences between the 2 groups and their corresponding 95% confidence intervals.
Results: Sixty-six subjects completed 24-h Holter monitoring at baseline and 6-months. Over 6 months, the change in HRV was similar between subjects treated with empagliflozin vs. placebo for the following parameters: RMSSD -1.2 ms (-6.0 to 3.6 ms); pNN50 0.5% (-2.6 to 3.6%); VLF -907.8 ms^2 (-2388.8 to 573.1 ms^2); LF -341 ms^2 (-878.7 to 196.7 ms^2); HF -33.8 ms^2 (-111.1 to 43.5 ms^2); LF:HF -0.1 (-0.4 to 0.2). Subjects who received placebo experienced an increase in SDNN 18.6 ms (2.8–34.3 ms) and SDANN 20.2 ms (3.2–37.3 ms) relative to those treated with empagliflozin.
Conclusion: Compared to placebo, empagliflozin did not result in changes in autonomic tone among individuals with type 2 diabetes and stable coronary artery disease.

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Abbreviations

ANCOVA	analysis of covariance
bpm	beats per minute
HRV	heart rate variability
LV	left ventricle
LVMi	left ventricular mass indexed to body surface area
pNN50	percentage of successive NN intervals that differ by > 50 ms
RMSSD	root mean square of successive RR interval differences
SDNN	standard deviation of normal to normal intervals
SDANN	standard deviation of the average NN intervals for each 5-min segment of the 24-h recording
SGLT2	sodium-glucose cotransporter 2

1. Introduction

Sodium-glucose cotransporter 2 (SGLT2) inhibitors improve cardiovascular outcomes among individuals with type 2 diabetes [1–4] and heart failure [4]. In the EMPAgliflozin Removal of Excess Glucose (EMPA-REG OUTCOME) trial, empagliflozin reduced cardiovascular mortality and heart failure hospitalization by 38% and 35% relative to placebo [1]. Despite these proven benefits, the mechanisms by which these agents improve outcomes remain elusive.

Autonomic dysfunction is prevalent among individuals with diabetes, heart failure, or both and is associated with increased mortality [5,6]. Pathologic imbalance between the parasympathetic and sympathetic nervous systems reflects increased β1 adrenergic receptor activity and/or reduced parasympathetic activity. This, in turn, is associated with adverse cardiac effects on a clinical and cellular level [5,6]. Heart rate variability (HRV), which measures variations in time intervals between adjacent consecutive heart beats, is a well-established metric to characterize the sympathetic and parasympathetic aspects of the autonomic nervous system. Many studies have shown that lower HRV is associated with increased risks of myocardial infarction, heart failure, sudden cardiac death, and cardiovascular mortality [7,8]. The effect of empagliflozin on HRV has not been studied.

We recently completed the EMPA-HEART CardioLink-6 trial which compared empagliflozin versus placebo on cardiac remodeling among subjects with type 2 diabetes and established coronary artery disease (CAD) and reported that empagliflozin use for 6 months reduced left ventricular (LV) mass (indexed to body surface area, LVMi) [9]. Using data collected from Holter monitoring, we conducted an exploratory analysis comparing the effect of empagliflozin vs. placebo on HRV parameters in order to gather further insight into how this agent may exert its cardiovascular benefits.

2. Methods

2.1. Cohort

The primary findings and design of the EMPA-HEART CardioLink-6 study have been published elsewhere [9]. In brief, it was a double-blind, placebo-controlled, randomized clinical trial that enrolled subjects with type 2 diabetes and established CAD (defined as a history of myocardial infarction or coronary revascularization) who were stably treated with antihyperglycemic therapy for ≥2 months prior to enrollment [9]. These subjects were randomized to empagliflozin 10 mg daily or placebo for 6 months. The primary endpoint was the change in LVMi from baseline to 6 months. The protocol mandated the following investigations for all subjects before treatment and at 6 months: cardiac magnetic resonance imaging (MRI), transthoracic echo, 24-h Holter monitoring, 24-h continuous blood pressure monitoring, 12-lead electrocardiogram and bloodwork (hematocrit and N-terminal pro B-type natriuretic peptide). All subjects provided informed written consent prior to randomization and were enrolled at St. Michael's Hospital (Toronto, Canada).

2.2. Holter monitoring

All 24-h Holter recordings were overread by cardiac technologists at St. Michael's Hospital using an ambulatory analysis system (MUSE, General Electric Healthcare). Readers were blinded to treatment allocation and study timing. Using the software's automated features, time and frequency domain measures were collected to inform HRV analysis. Frequency domain measures included: very low frequency (VLF), low frequency (LF), high frequency (HF) and low/high frequency ratio (LF:HF). Time domain measures included: standard deviation of NN (normal to normal) intervals (SDNN), standard deviation of the average NN intervals for each 5-min segment of the 24-h recording (SDANN), root mean square of successive RR interval differences (RMSSD), and the percentage of successive NN intervals that differ by > 50 ms (pNN50). Subjects whose Holter recordings demonstrated atrial fibrillation (AF) were excluded from analysis.

2.3. Statistical analysis

Subject data were analyzed according to the randomization status. Categorical data are presented as number and percentages; continuous variables are reported as means and standard deviation. The exposure was empagliflozin or placebo. Differences in HRV parameters between the 2 groups were compared with analysis of covariance (ANCOVA). Statistical measures of significance were reported as adjusted differences with corresponding 95% confidence intervals (CI). Since LV mass regression with empagliflozin use was most pronounced among subjects with a baseline indexed LVMi of ≥60 g/m^2 in the main EMPA-HEART trial, we conducted a stratified sub-analysis using this cut-off. The primary analysis consisted of subjects who completed a Holter study at baseline and 6 months. For each comparison, a 2-sided p-value of <0.05 was considered to be statistically significant. We did not perform a formal power calculation for this sub-study given the post hoc and exploratory nature of this analysis. Data were analyzed with SAS version 9.4 (SAS Institute Inc, Cary, NC, USA) and R version 3.5.1 (R Foundation for Statistical Computing, Vienna, Austria).

3. Results

3.1. Baseline characteristics

Of the 97 individuals randomized in the EMPA-HEART CardioLink-6 trial, 66 (68%) completed 24-h Holter monitoring tests at both baseline and 6-month visits. Five individuals withdrew from the study, 3 declined Holter monitoring, 5 were lost to follow up, 2 did not complete Holter monitoring for other reasons, 1 did not return their Holter monitor, while 15 individuals' Holter monitors

could not be downloaded for analysis due to technical reasons. The key baseline characteristics of the 66 subjects (n = 33 in each study arm) were similar (Table 1). In this cohort, the average duration of type 2 diabetes was over 10 years. Most (94%) were treated with metformin while 21% were treated with insulin. Most were treated with beta-blockers (85%) and the proportion was similar between those randomized to empagliflozin and placebo (82% vs. 88%). The proportion of subjects treated with guideline-directed secondary prevention medications such as angiotensin-converting enzyme inhibitors/angiotensin receptor blockers (ACEi/ARB) and lipid-lowering therapies was high (>80%). There were 9 (14%) patients (n = 4 in the empagliflozin group and n = 5 in the placebo group) who experienced a change in their beta-blocker or ACEi/ARB therapy over the 6-month follow-up. In the empagliflozin group, 2 patients were started on ACEi/ARB and the ACEi/ARB dose for 1 was increased. Beta-blocker therapy was changed for 3 patients: initiation (n = 1), substitution (n = 1), and dose decrease (n = 1). Two patients experienced a change in both their beta-blocker and ACEi/ARB therapies. In the placebo group, 1 patient was started on an ACEi/ARB, 2 patients had ACEi/ARB substitutions, and 2 patients had their ACEi/ARB dose increased. No patient in the placebo group experienced a change in beta-blocker therapy over 6 months.

3.2. Heart rate and HRV

The average HR at baseline and at 6 months for the empagliflozin group was 75.9 ± 10.3 beats per minute (bpm) and 76.6 ± 10.4 bpm, respectively. In the placebo group, the average HR was 69.9 ± 9.5 bpm at baseline and 72.6 ± 8.1 bpm at 6 months. No significant intra-group difference was observed for the empagliflozin (0.7 ± 8.7 bpm) and placebo group (2.6 ± 7.2 bpm) between baseline and 6 month. Adjusting for baseline, there was no difference in the change of the average HR between the 2 treatment arms at 6 months (adjusted difference between empagliflozin and placebo: 0.32 bpm, 95% CI -3.34 to 3.97, p = 0.86) (Table 2). Similarly, no intra- or inter-group differences were observed between the 2 groups in terms of the minimum and maximum HR (Table 2).

Time domain and frequency domain measures for HRV are summarized in Table 2. Baseline SDNN and SDANN values were similar between subjects in the empagliflozin and placebo groups (100.2 ± 45.8 ms vs. 108.3 ± 29.9 ms, p = 0.40 and 87.0 ± 41.0 vs. 93.9 ± 28.2 ms, p = 0.42). At 6 months, SDNN and SDANN increased (i.e. in a favorable direction) among subjects in the placebo group (ΔSDNN 11.9 ± 44.4 ms and ΔSDANN 12.9 ± 47.1 ms, p < 0.01 for both comparisons) while no change was observed among subjects in the empagliflozin group (ΔSDNN -3.3 ± 23.4 ms and ΔSDANN -4.6 ± 23.0 ms, p = NS for both comparisons). For all other HRV parameters, no difference was observed between the 2 treatment arms over 6 months (Table 2).

Over the 6-month follow-up, no difference in frequency domain outcomes was observed between the empagliflozin and placebo groups. Amongst time domain measures, RMMSD and pNN50 were similar between the 2 groups. On the other hand, SDNN and SDANN were lower amongst subjects in the empagliflozin group compared to the placebo group (adjusted difference -18.6 ms, 95% CI -34.3 to -2.8 ms, p = 0.02 and -20.2 ms, 95% CI -37.3 to -3.2 ms, p = 0.02, respectively).

3.2.1. Subgroup analysis according to LVMi

There were 38 and 28 subjects whose baseline LVMI was <60 g/m^2 and ≥60 g/m^2, respectively. Within these 2 subgroups, the changes in HRV parameters over 6 months were numerically and directionally similar between the subjects randomized to receive empagliflozin or placebo (Supplementary Table 1).

3.2.2. Subgroup analysis according to baseline beta-blocker use

No difference was observed in baseline HRV parameters and changes of these parameters over 6 months in relation to subjects' use (vs. non-use) of beta-blocker at baseline. Given the small number of subjects who were not treated with a beta-blocker at baseline, inferential statistical comparison was not performed (Supplementary Table 2).

3.2.3. Sensitivity analysis

There were 91 subjects who completed at least 1 Holter at baseline or 6 months (n = 46 (empagliflozin) and n = 45 (placebo)). This constituted 94% of the overall sample size. No systematic differences in HRV values were observed between the sensitivity cohort and the primary analysis cohort (Supplementary Table 3).

4. Discussion

Clinical trials examining the cardiovascular and renal effects of SGLT2 inhibitors have shown that this class of antihyperglycemic

Table 1
Baseline characteristics.

	Empagliflozin 10 mg daily (n = 33)	Placebo (n = 33)
Age (years)	61.6 (8.7)	64.6 (9.5)
Male	30 (90.9)	33 (100)
Duration of type 2 diabetes (years)	11.9 (9.4)	10.1 (7.2)
HbA1c (%)	7.9 (0.9)	7.9 (0.9)
BMI (kg/m^2)	28.2 (4.9)	26.8 (4.6)
Office-based systolic BP (mmHg)	139.2 (23.0)	136.4 (21.4)
Office-based diastolic BP (mmHg)	79.6 (12.4)	75.1 (11.1)
eGFR (mL/min/1.73m^2)	90.2 (16.3)	86.6 (17.4)
Hypertension	29 (87.9)	29 (87.9)
Hypercholesterolemia	24 (72.7)	26 (78.8)
Stroke or TIA	7 (21.2)	4 (12.1)
Peripheral artery disease	2 (6.1)	2 (6.1)
History of heart failure	1 (3.0)	4 (12.1)
History of smoking	13 (39.4)	15 (45.5)
ACEi/ARB	29 (87.9)	29 (87.9)
Beta-blocker	27 (81.8)	29 (87.9)
Calcium channel blocker	5 (15.2)	8 (24.2)

Categorical data are presented as n (%); continuous variables are reported as mean (SD).
ACEi, angiotensin-converting enzyme inhibitor; ARB, angiotensin receptor blocker; BMI, body mass index; BP, blood pressure; eGFR, estimated glomerular filtration rate; HbA1c, glycated hemoglobin; TIA, transient ischemic attack.

Table 2
Baseline HRV parameters and changes over 6 months.

	Empagliflozin 10 mg daily (n = 33)		Placebo (n = 33)		Adjusted difference between empagliflozin and placebo	
	Baseline	Change from baseline at 6 months	Baseline	Change from baseline at 6 months	Mean (95% CI)	p
SDNN (ms)	100.2 (45.8)	-3.3 (23.4)	108.3 (29.9)	11.9 (44.4)	-18.6 (-34.3, -2.8)	0.02
SDANN (ms)	87.0 (41)	-4.6 (23)	93.9 (28.2)	12.9 (47.1)	-20.2 (-37.3, -3.2)	0.02
RMSSD (ms)	26.4 (12.2)	0.4 (7.9)	25.6 (9.9)	2.0 (12.5)	-1.2 (-6.0, 3.6)	0.61
pNN50 (%)	7.6 (7.8)	0.7 (5.0)	6.7 (6.7)	0.5 (8.0)	0.5 (-2.6, 3.6)	0.75
LF:HF	1.7 (0.6)	0.04 (0.6)	1.6 (0.4)	0.2 (0.8)	-0.1 (-0.4, 0.2)	0.60
VLF (ms^2)	656.6 (512.9)	118.3 (1106.2)	684.1 (437.4)	984.8 (4198.1)	-907.8 (-2388.8, 573.1)	0.23
LF (ms^2)	317.4 (304.5)	34.2 (376.0)	285.6 (432.6)	408.4 (1584.5)	-341.0 (-878.7, 196.7)	0.21
HF (ms^2)	134.6 (143.5)	5.9 (85.7)	111.0 (92.6)	50.1 (215.2)	-33.8 (-111.1, 43.5)	0.39
Min HR (bpm)	54.1 (7.6)	0.6 (5.6)	51.1 (7.0)	0.1 (5.3)	1.6 (-0.8, 4.0)	0.20
Mean HR (bpm)	75.9 (10.4)	0.7 (8.7)	70 (9.5)	2.6 (7.2)	0.3 (-3.3, 4.0)	0.86
Max HR (bpm)	111.9 (15.0)	1.8 (13.8)	107.6 (12.6)	3.4 (13.3)	0.3 (-5.8, 6.3)	0.93

Data are presented as mean (SD).
bpm, beats per minute; HF, high frequency; HR, heart rate; LF, low frequency; pNN50, percentage of successive NN intervals that differ by > 50 ms; RMSSD, root mean square of successive RR interval differences; standard deviation; SDNN, standard deviation of normal to normal intervals; SDANN, standard deviation of the average normal to normal intervals for each 5-min segment of the 24-h recording; VLF, very low frequency.

agents is beneficial for patients with type 2 diabetes and/or heart failure [1–4]. Accordingly, there is tremendous interest to elucidate the mechanisms responsible for these benefits [10–18]. In the context of a randomized trial, the present analysis explores the impact of empagliflozin (vs. placebo) on cardiac autonomic activity. Collectively, our results suggest that cardiac autonomic activity as assessed by HRV was similar between subjects treated with empagliflozin or placebo over 6 months. Our study is one of the first to address the relationship between SGLT2 inhibition and cardiac autonomic tone using HRV parameters which were prospectively collected from non-invasive ambulatory Holter ECG monitoring.

Human studies involving empagliflozin and other SGLT2 inhibitors have consistently demonstrated that blood pressure is reduced without an accompanying increase in heart rate [1–4]. This suggests that sympathetic activity is attenuated by this agent class [19]. The modulatory effect of SGLT2 inhibitors upon the autonomic nervous system, particularly the sympathetic arm, is supported by emerging data from animal models [20]. In a carefully designed study of neurogenic hypertensive mice, Herat et al. showed that dapagliflozin, another SGLT2 inhibitor, lowered blood pressure, renal norepinephrine levels, and renal tyrosine hydroxylase levels [20]. These findings suggested that dapagliflozin was associated with renal sympathoinhibition in the hypertensive mouse model. Of note, the relationship between SGLT2 inhibition and cardiac sympathetic activity was not specifically examined in this study. Accordingly, it has been postulated that reduction in renal afferent tone may lower central sympathetic activity, which in turn will attenuate sympathetic outflow to the heart, allowing for positive ventricular remodeling (e.g. decreased volume overload, wall stress, fibrosis, and/or hypertrophy) to occur [21].

In the EMPA-HEART CardioLink-6 trial, subjects treated with empagliflozin experienced reduction in their LVMi and blood pressure (systolic and diastolic) relative to those who received placebo. In keeping with these changes, subjects in the empagliflozin group would have been expected to have lower cardiac sympathetic tone. Interestingly, our results are counter to prevailing concepts on the positive effect of SGLT2 inhibition on cardiac autonomic activity. There are several reasons to account for our findings. Unlike parameters such as the high frequency component in which there is general agreement that it is largely influenced by vagal tone (i.e. parasympathetic activity), no HRV parameter is felt to be primarily reflective of sympathetic activity [22]. Parameters such as SDNN and LF have been proposed as candidate metrics to reflect cardiac sympathetic tone, but disagreement exists in the literature [22]. Rather, these parameters are felt to represent the interplay between the vagal and sympathetic arms of the cardiac autonomic system [22]. Thus, it can be argued that HRV parameters may not be an optimal method to assess for changes in cardiac autonomic tone related to SGLT2 inhibitors, particularly if it is the sympathetic arm that is primarily affected by these agents.

Furthermore, it should be highlighted that our cohort consisted of subjects with stable CAD. More pronounced changes in HRV parameters with empagliflozin might have been demonstrated if we enrolled subjects with more significant cardiovascular comorbidities with greater perturbation of their autonomic tone. Aggregate findings from the published literature showed that SDNN <100 ms, SDANN <50 ms, pNN50 < 3%, and RMSSD <25 ms were prognostic for increased mortality risk among patients with acute MI or heart failure [23,24]. For instance, studies of patients with acute MI demonstrated a 4-fold increased risk of death with a SDNN <50 ms relative to a SDNN >100 ms [23]. Conversely, the average baseline HRV parameters in our cohort reflected a healthier group of subjects without these high-risk features. For example, the mean baseline SDNN was >100 ms in our study cohort, indicative of a less deranged autonomic state. Therefore, it would not be surprising that no major changes in these HRV parameters were detected in our subjects with more normalized autonomic states. This however is speculative and requires prospective confirmation.

In a study of 16 subjects with diabetic nephropathy and LV hypertrophy who received weekly evaluation for glycemic and blood pressure control over 1 year, Weinrauch et al. showed that regression in LV mass was associated with improvement in the entire panel of HRV parameters (SDNN, SDANN, LF, HF) [25]. On the other hand, our study, despite having a larger sample size, did not demonstrate such an association even though LVMi improved for subjects in the empagliflozin arm. One plausible explanation relates to the differences in LV mass between the 2 studies. The baseline LV mass and its subsequent reduction were considerably higher in Weinrauch's study when compared to ours (206 vs. 119 g and 19 vs. 5 g, respectively). If indeed LV mass regression is correlated with improvement in HRV parameters, it is conceivable that such an association would be more likely observed in Weinrauch's study since it included subjects with considerably greater degree of LV hypertrophy. These contrasting results highlight that small sample sizes do not allow for definitive comparisons of HRV parameters unless large differences exist.

Alternative modalities may provide further insight on the relationship between SGLT2 inhibition and modulation of cardiac

autonomic tone. In a case report by Kiuchi et al. use of ipragliflozin (a SGLT2 inhibitor prescribed in Japan) over 1 year resulted in significant improvement in symptoms, weight, and BNP levels of an 83-year old man with heart failure with preserved LV ejection fraction [26]. In addition, imaging with ^{123}I-meta-iodobenzylguanidine cardiac-scintigraphy (^{123}I-MIBG) demonstrated reduction in cardiac sympathetic activity. An ongoing randomized, double-blind clinical trial in Japan will compare empagliflozin to placebo on cardiac sympathetic activity among subjects with acute MI. The investigators will employ an array of ECG and imaging modalities to assess cardiac autonomic tone, including HRV, ^{123}I-MIBG, T-wave alternans, late potentials, and heart rate turbulence [27]. Coupled with ongoing animal studies, results from clinical trials will provide valuable insight on the interplay between SGLT2 inhibition and cardiac autonomic tone.

In our study, subjects who received placebo experienced an increase (i.e. directionally more favorable) in SDNN and SDANN over 6 months while no change was noted for those treated with empagliflozin. On the other hand, no change in all other HRV parameters was observed between the 2 treatment arms over 6 months. Several aspects should be highlighted to contextualize our study findings. First, previous studies which examined the effect of therapies (e.g. beta-blocker, ivabradine, cardiac rehabilitation) on HRV reported improvement across all of its component parameters (e.g. SDNN, SDANN, RMSSD, LF, HF). In contrast, in our study, changes in most of the HRV parameters were similar between the empagliflozin and placebo arms, except for SDNN and SDANN. Since HRV parameters are correlated and complement each other in describing cardiac autonomic activity, no one single HRV parameter is more dominant or informative than another in terms of its characterization of cardiac autonomic tone. Accordingly, on the basis of the totality of our data, we conclude that cardiac autonomic activity as assessed by HRV markers was similar between subjects treated with empagliflozin relative to placebo. Finally, given that multiple endpoints were examined in this analysis, a case could be made that the p-value cut-off chosen to denote statistical significance should be considerably lower than 0.05. When we planned our analysis, we elected to not employ a lower p-value cut-off. As such, the difference noted in the change of SDNN and SDANN between the 2 groups would not have been considered statistically significant if a lower p-value cut-off was selected.

There are a number of important limitations in this study. First, this was a post hoc, exploratory analysis examining the potential relationship between empagliflozin and cardiac autonomic tone. The EMPA-HEART trial was not powered to assess for prespecified differences in HRV parameters. As such, our results and conclusions are only hypothesis-generating and must be interpreted with caution. Second, the number of subjects included in this analysis was small ($n = 66$), precluding association between changes in HRV metrics and clinical outcomes. Third, Holter monitoring was performed on a cohort of subjects with stable CAD. It is possible that different results may occur if a more medically vulnerable population is examined, such as those with acute MI or those with significant LV dysfunction. Fourth, since we did not collect the doses of beta-blocker nor ACEi/ARB prescribed for subjects in this study, we were not able to examine whether dosing could have influenced HRV parameters in this cohort. Finally, we only employed HRV to assess cardiac autonomic tone. Use of other modalities such as heart rate turbulence, heart rate recovery after exercise, or imaging of the autonomic nervous system with radiotracers would have provided a more comprehensive assessment of cardiac sympathetic activity, but this was beyond the scope of our study.

In conclusion, our results did not support the hypothesis that empagliflozin use among individuals with type 2 diabetes and stable CAD was associated with alteration in cardiac autonomic tone, as assessed by HRV parameters. This underscores the need for additional research to confirm or refute our findings. Furthermore, whether SGLT2 inhibitors may impact autonomic function in different cardiovascular patient populations requires future study.

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Declaration of competing interest

SV: Research support and/or speaking/advisory board honoraria from AstraZeneca, Boehringer Ingelheim, Eli Lilly, EOCI Pharmacomm Ltd, Janssen, Merck, Novartis, Novo Nordisk, Sanofi, Sun Pharmaceuticals, and the Toronto Knowledge Translation Working Group.

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All other authors have no relevant conflicts of interest to declare.

CRediT authorship contribution statement

Vinay Garg: Conceptualization, Methodology, Data curation, Writing - original draft, Writing - review & editing. **Subodh Verma:** Conceptualization, Methodology, Writing - review & editing, Supervision, Funding acquisition. **Kim A. Connelly:** Conceptualization, Methodology, Writing - review & editing. **Andrew T. Yan:** Conceptualization, Methodology, Writing - review & editing. **Aditya Sikand:** Data curation, Writing - review & editing. **Ankit Garg:** Data curation, Writing - review & editing. **Paul Dorian:** Writing - review & editing. **Fei Zuo:** Formal analysis, Writing - original draft, Writing - review & editing. **Lawrence A. Leiter:** Writing - review & editing. **Bernard Zinman:** Writing - review & editing, Funding acquisition. **Peter Jüni:** Formal analysis, Writing - review & editing. **Atul Verma:** Writing - review & editing. **Hwee Teoh:** Writing - review & editing. **Adrian Quan:** Writing - review & editing, Project administration. **C. David Mazer:** Conceptualization, Methodology, Formal analysis, Writing - review & editing, Supervision. **Andrew C.T. Ha:** Conceptualization, Methodology, Writing - original draft, Writing - review & editing, Supervision.

Appendix A. Supplementary data

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References

[1] Zinman B, Wanner C, Lachin JM, Fitchett D, Bluhmki E, Hantel S, et al. Empagliflozin, cardiovascular outcomes, and mortality in type 2 diabetes. N Engl J Med 2015;373:2117–28.

[2] Neal B, Perkovic V, Mahaffey KW, de Zeeuw D, Fulcher G, Erondu N, et al. Canagliflozin and cardiovascular and renal events in type 2 diabetes. N Engl J Med 2017;377:644–57.

[3] Wiviott SD, Raz I, Bonaca MP, Mosenzon O, Kato ET, Cahn A, et al. Dapagliflozin and cardiovascular outcomes in type 2 diabetes. N Engl J Med 2019;380:347–57.

[4] McMurray JJV, Solomon SD, Inzucchi SE, Kober L, Kosiborod MN, Martinez FA, et al. Dapagliflozin in patients with heart failure and reduced ejection fraction. N Engl J Med 2019;381:1995–2008.

[5] Floras JS. Sympathetic nervous system activation in human heart failure: clinical implications of an updated model. J Am Coll Cardiol 2009;54:375–85.

[6] Jia G, Hill MA, Sowers JR. Diabetic cardiomyopathy: an update of mechanisms contributing to this clinical entity. Circ Res 2018;122:624–38.

[7] Vaseghi M, Shivkumar K. The role of the autonomic nervous system in sudden cardiac death. Prog Cardiovasc Dis 2008;50:404–19.

[8] Maheshwari A, Norby FL, Soliman EZ, Adabag S, Whitsel EA, Alonso A, et al. Low heart rate variability in a 2-minute electrocardiogram recording is associated with an increased risk of sudden cardiac death in the general population: the atherosclerosis risk in communities study. PLoS One 2016;11:e0161648.

[9] Verma S, Mazer CD, Yan AT, Mason T, Garg V, Teoh H, et al. Effect of empagliflozin on left ventricular mass in patients with type 2 diabetes mellitus and coronary artery disease: the EMPA-HEART CardioLink-6 randomized clinical trial. Circulation 2019;140:1693–702.

[10] Byrne NJ, Parajuli N, Levasseur JL, Boisvenue J, Beker DL, Masson G, et al. Empagliflozin prevents worsening of cardiac function in an experimental model of pressure overload-induced heart failure. JACC Basic Transl Sci 2017;2:347–54.

[11] Chiba Y, Yamada T, Tsukita S, Takahashi K, Munakata Y, Shirai Y, et al. Dapagliflozin, a sodium-glucose Co-transporter 2 inhibitor, acutely reduces energy expenditure in BAT via neural signals in mice. PLoS One 2016;11:e0150756.

[12] Connelly KA, Zhang Y, Visram A, Advani A, Batchu SN, Desjardins JF, et al. Empagliflozin improves diastolic function in a nondiabetic rodent model of heart failure with preserved ejection fraction. JACC Basic Transl Sci 2019;4:27–37.

[13] Diaz-Rodriguez E, Agra RM, Fernandez AL, Adrio B, Garcia-Caballero T, Gonzalez-Juanatey JR, et al. Effects of dapagliflozin on human epicardial adipose tissue: modulation of insulin resistance, inflammatory chemokine production, and differentiation ability. Cardiovasc Res 2018;114:336–46.

[14] Hess DA, Terenzi DC, Trac JZ, Quan A, Mason T, Al-Omran M, et al. SGLT2 inhibition with empagliflozin increases circulating provascular progenitor cells in people with type 2 diabetes mellitus. Cell Metabol 2019;30:609–13.

[15] Iborra-Egea O, Santiago-Vacas E, Yurista SR, Lupón L, Packer M, Heymans S, et al. Unraveling the molecular mechanism of action of empagliflozin in heart failure with reduced ejection fraction with or without diabetes. JACC Basic Transl Sci 2019;4:831–40.

[16] Mazer CD, Hare GMT, Connelly PW, Gilbert RE, Shehata N, Quan A, et al. Effect of empagliflozin on erythropoietin levels, iron stores and red blood cell morphology in patients with type 2 diabetes and coronary artery disease. Circulation 2019;141(8):704–7.

[17] Verma S, McMurray JJV. SGLT2 inhibitors and mechanisms of cardiovascular benefit: a state-of-the-art review. Diabetologia 2018;61:2108–17.

[18] Verma S, Rawat S, Ho KL, Wagg CS, Zhang L, Teoh H, et al. Empagliflozin increases cardiac energy production in diabetes: novel translational insights into the heart failure benefits of SGLT2 inhibitors. JACC Basic Transl Sci 2018;3:575–87.

[19] Verma S. Are the cardiorenal benefits of SGLT2 inhibitors due to inhibition of the sympathetic nervous system? JACC Basic Transl Sci 2020 Feb 24;5(2):180–2.

[20] Herat LY, Magno AL, Rudnicka C, Hricova J, Carnagarin R, Ward NC, et al. SGLT2 inhibitor-induced sympathoinhibition: a novel mechanism for cardiorenal protection. JACC Basic Transl Sci 2020 Jan 29;5(2):169–79.

[21] Sano M. A new class of drugs for heart failure: SGLT2 inhibitors reduce sympathetic overactivity. J Cardiol 2018 May;71(5):471–6.

[22] Heart rate variability: standards of measurement, physiological interpretation and clinical use. Task force of the European society of cardiology and the north American society of pacing and electrophysiology. Circulation 1996 Mar 1;93(5):1043–65.

[23] Kleiger RE, Miller JP, Bigger Jr JT, Moss AJ. Decreased heart rate variability and its association with increased mortality after acute myocardial infarction. Am J Cardiol 1987;59:256–62.

[24] Manfrini O, Pizzi C, Trerè D, Fontana F, Bugiardini R. Parasympathetic failure and risk of subsequent coronary events in unstable angina and non-ST-segment elevation myocardial infarction. Eur Heart J 2003 Sep;24(17):1560–6.

[25] Weinrauch LA, Berger AJ, Aronson D, Gleason RE, Lee AT, D'Elia JA. Regression of left ventricular hypertrophy in diabetic nephropathy: loss of parasympathetic function predicts response to treatment. J Clin Hypertens (Greenwich). 2006;8:330–5.

[26] Kiuchi S, Hisatake S, Kabuki T, Fujii T, Oka T, Dobashi S, et al. Long-term use of ipragliflozin improved cardiac sympathetic nerve activity in a patient with heart failure: a case report. Drug Discov Ther 2018 Mar 19;12(1):51–4.

[27] Kubota Y, Yamamoto T, Tara S, Tokita Y, Yodogawa K, Iwasaki Y, et al. Effect of empagliflozin versus placebo on cardiac sympathetic activity in acute myocardial infarction patients with type 2 diabetes mellitus: rationale. Diabetes Ther 2018 Oct;9(5):2107–16.