DOI: 10.1111/dme.14279

Research: Treatment

Influence of an SGLT2 inhibitor, tofogliflozin, on the resting heart rate in relation to adipose tissue insulin resistance

T. Nojima[1,2], Y. Matsubayashi[1], A. Yoshida[1,3], H. Suganami[2], T. Abe[1], M. Ishizawa[1], K. Fujihara[1], S. Tanaka[4], K. Kaku[5] and H. Sone[1]

[1]Department of Hematology, Endocrinology and Metabolism, Niigata University Faculty of Medicine, Niigata, [2]Clinical Data Science Department, [3]Kowa Co., Ltd., Tokyo, Japan, [4]Department of Clinical Biostatistics, Graduate School of Medicine, Kyoto University, Kyoto and [5]Kawasaki Medical School, Okayama, Japan

Accepted 22 February 2020

Abstract

Aims To examine the effects of a sodium–glucose co-transporter 2 (SGLT2) inhibitor, tofogliflozin, on resting heart rate by exploring baseline factors that independently influenced changes in the resting heart rate.

Methods Data on 419 participants in tofogliflozin phase 2/3 trials were analysed. Changes in resting heart rate from baseline to week 24 were analysed using an analysis of covariance (ANCOVA) model with groups (tofogliflozin/placebo) as a fixed effect and baseline values as covariates. The antilipolytic effect was evaluated as adipose tissue insulin resistance (Adipo-IR) and was calculated as the product of fasting insulin and free fatty acid. Multivariate analysis evaluated independent factors for changes in resting heart rate from baseline to week 24.

Results Of the participants, 58% were men, and mean age, HbA_{1c}, BMI and resting heart rate were 57.6 years, 65 mmol/mol (8.1%), 25.5 kg/m^2 and 66 bpm, respectively. At week 24, adjusted mean difference vs. placebo in the change from baseline was −2.3 bpm [95% confidence interval (CI) −4.6, −0.1] with tofogliflozin. Changes in resting heart rate were positively correlated with changes in Adipo-IR, whereas reductions in HbA_{1c}, body weight and blood pressure were similar independent of changes in resting heart among quartiles of resting heart rate change. On multivariate analysis, higher baseline resting heart rates and Adipo-IR values were significantly associated with greater reductions in resting heart rate.

Conclusions Tofogliflozin corrected resting heart rate levels in accordance with baseline levels. Correction of high resting heart rates may be attributed to improved adipose tissue insulin resistance, leading to correction of hyperinsulinaemia.

Diabet. Med. 37, 1316–1325 (2020)

Introduction

Resting heart rate is determined by the activity of the sinoatrial node. It is considered a marker of autonomic nervous system activity and is largely influenced by the interaction of sympathetic and vagal activities [1]; thus an elevated resting heart rate implies sympathetic hyperactivity and/or reduced parasympathetic activity [2]. Epidemiological evidence from the general population suggests that a high resting heart rate is related to increased cardiovascular morbidity and mortality independent of conventional risk factors [3]. Furthermore, in the ADVANCE study of type 2 diabetes mellitus, a high resting heart rate was reported as a risk factor for not only cardiovascular disease, but also microvascular complications [4,5]. Insulin resistance and elevated sympathetic system activity were reported to be closely related [6,7].

A sodium–glucose co-transporter 2 (SGLT2) inhibitor facilitates urinary glucose excretion by inhibiting SGLT2 in the proximal tubule and produces weight loss, in particular, reduction in fat mass, as well as a reduction in blood glucose [8]. In addition, as it lowers the blood glucose level without promoting insulin secretion, it has been reported to decrease fasting and postprandial insulin levels, and improve insulin

Correspondence to: Hirohito Sone. E-mail: sone@med.niigata-u.ac.jp

What's new?

- A high resting heart rate is related to increased cardiovascular and microvascular risks in persons with type 2 diabetes.
- Tofogliflozin significantly reduced resting heart rate compared with placebo, irrespective of reductions in HbA_{1c}, body weight and blood pressure.
- Reduction in resting heart rate was associated with improvements in adipose tissue insulin resistance.
- Higher baseline adipose tissue insulin resistance levels were independently associated with a greater decline in resting heart rate.
- Correcting the resting heart rate via improved adipose tissue insulin resistance by tofogliflozin treatment might contribute to lowering the risks of cardiovascular diseases.

sensitivity [9]. In recent years, the effect of SGLT2 inhibitors on the onset of cardiovascular events has been reported by the EMPA-REG OUTCOME trial, the CANVAS Program and the DECLARE–TIMI 58 study [10–13]. These trials suggested that SGLT2 inhibitors suppress composite endpoints, such as hospitalization owing to heart failure and the occurrence of cardiovascular death. However, their mode of action is not fully understood, and few reports have addressed their effect on resting heart rate, one of the cardiovascular risk factors in type 2 diabetes, or have focused on their effect on insulin resistance in adipose tissue and resting heart rate. Therefore, we investigated the effect of an SGLT2 inhibitor, tofogliflozin, on resting heart rate using tofogliflozin phase 2/3 trial data. We also explored independent factors that affect the resting heart rate and the relationship between insulin resistance and resting heart rate upon administration of tofogliflozin.

Participants and methods

We conducted a pooled analysis of data from two tofogliflozin phase 2/3 trials that followed participants with type 2 diabetes for at least 24 weeks (Table 1). CSG003JP (placebo; tofogliflozin 10, 20 and 40 mg monotherapy) was a 24-week multicentre, randomized, placebo-controlled, double-blind, phase 2/3 trial [14]. CSG004JP (tofogliflozin 20 and 40 mg monotherapy) was a 52-week, multicentre, randomized, controlled, open-label phase 3 trial [15]. Major inclusion criteria were: (1) participants at least 20 years old (CSG003JP) or 20–74 years old (CSG004JP) with type 2 diabetes; (2) BMI of 18.5–44.9 kg/m^2; and (3) HbA_{1c} at screening of ≥ 51 mmol/mol (6.8%) (CSG003JP) or ≥ 56 mmol/mol (7.3%) (CSG004JP) to < 89 mmol/mol (10.3%). Details of the design and results of the above

Table 1 Integrated analysis of two clinical studies

Study	Design	Treatment	Dosage (*n*)	Periods (weeks)	Baseline						
					Sex (M : F)	Age (years)	BMI (kg/m^2)	HbA_{1c} (mmol/mol)	HbA_{1c} (%)	eGFR (ml min^{-1} 1.73 m^{-2})	Resting heart rate (bpm)
003JP [14]	Randomized, placebo controlled, double-blind, parallel-group comparative study	Monotherapy	Placebo (56) 10 mg (57) 20 mg (58) 40 mg (58)	24	153 : 76	57.3 (9.7)	25.5 (4.1)	68 (8)	8.4 (0.8)	85.9 (19.2)	67 (11)
004JP [15]	Open-label, randomized controlled study	Monotherapy	20 mg (63) 40 mg (127)	52	126 : 64	58.1 (10.8)	25.6 (4.5)	62 (10)	7.8 (0.9)	83.0 (18.2)	65 (10)
Total	Integrated two prospective studies	Monotherapy	Total (419)	24	279 : 140	57.6 (10.2)	25.5 (4.3)	65 (10)	8.1 (0.9)	84.3 (18.6)	66 (11)
			Placebo (56)		37 : 19	56.8 (9.9)	26.0 (4.1)	68 (9)	8.4 (0.8)	83.8 (17.7)	66 (9)
			Tofogliflozin (363)		242 : 121	57.8 (10.3)	25.4 (4.3)	65 (10)	8.1 (0.9)	84.4 (18.7)	66 (11)

Data are expressed as mean (SD).

studies, including inclusion and exclusion criteria, have been reported previously [14,15]. Data acquired from baseline, that is, from week 0 of study entry, to week 24 of each study were included in this pooled analysis. All studies were conducted in accordance with the Declaration of Helsinki and the ICH Good Clinical Practice Guidelines. The protocol was reviewed and approved by the Institutional Review Committee of each participating centre. Before enrolment into the two trials, all participants had provided written informed consent, including permission to use the resultant data. These trials were registered by the Japan Pharmaceutical Information Center clinical trials information (JapicCTI) as 101349 and 101351.

Measurements

The following baseline laboratory values were used: HbA_{1c}, fasting plasma glucose, fasting insulin, fasting C-peptide, fasting free fatty acids, homeostatic model assessment of insulin resistance scores [HOMA-IR: F-IRI (μU/ml) × fasting plasma glucose (mg/dl)/405], 24-variable homeostasis model assessment of β-cell function (iHOMA2%β) and insulin sensitivity (iHOMA2%S) [16], and Adipo-IR determined by fasting insulin × fasting free fatty acid [17], which was reported to be associated with insulin sensitivity in adipose tissue using the clamp method [18]. Values for adiponectin, uric acid, haematocrit, serum creatinine and the eGFR calculated from serum creatinine [19] were determined. Furthermore, assessments using study baseline data included BMI, waist circumference, SBP, DBP, mean average pressure [DBP + (SBP – DBP)/3], pulse pressure (SBP – DBP), double product (SBP × resting heart rate) and pulse rate. A meal tolerance test was performed in the CSG004JP study. For that study, after fasting for at least 10 h, participants came to the medical institution taking part in the clinical trials and underwent a meal tolerance test. The test meal contained 314 kcal (1314 kJ; 5.45 g protein, 73.05 g carbohydrate and 0 g lipids). Urinary glucose excretion during the meal tolerance test was measured.

Statistical analysis

In each group, the demographics were summarized with appropriate descriptive statistics (means and SD for continuous variables, and frequencies and percentages for categorical variables). In addition, differences in baseline assessments across groups were analysed using Student's *t*-test and Fisher's exact test. Assessments of changes in the resting heart rate from baseline to week 24 were analysed using an analysis of covariance (ANCOVA) model with the group as a fixed effect and baseline values as covariates to determine differences across groups. The amount of change was calculated for each participant from the values at two time points, baseline (week 0) and 24 weeks after administration as an endpoint. Also, as an exploratory analysis, the change in resting heart rate from baseline to week 24 was compared between participants who did and did not use a concomitant anti-hypertensive agent (angiotensin II receptor blocker, angiotensin converting enzyme inhibitor, calcium channel blocker, β-blocker and diuretic); for this analysis, an ANCOVA model was used with the anti-hypertensive agent as the fixed effect and baseline values as covariates to examine differences between using and not using such an agent. Analyses of the correlation or relationship between resting heart rate and other variables were performed using Pearson's product-moment correlation coefficients and Spearman rank-order correlation coefficients. Participants receiving tofogliflozin were divided into four groups based on quartiles of change in resting heart rate from baseline to week 24. Laboratory variables were also evaluated from baseline to week 24.

Multivariate general linear models were fitted to the change in resting heart rate from baseline to week 24. Covariates in the models included: sex; age; angiotensin II receptor blocker, angiotensin converting enzyme inhibitor, calcium channel blocker, β-blocker and diuretic dosages; duration of diabetes; DBP; HbA_{1c}; fasting plasma glucose level; BMI; haematocrit; eGFR; uric acid level; Adipo-IR; HOMA-IR; iHOMA2%S; iHOMA2%β; adiponectin level; and resting heart rate at baseline. The variables in the models were selected through stepwise variable selection with $P < 0.05$. Resting heart rate was measured using a 12-lead ECG. Data were analysed using the SAS system, Release 9.3 (SAS Institute, Cary, NC, USA). The significance level for each test was 0.05 (two-sided).

Results

Data collected at baseline and week 24 of tofogliflozin or placebo administration were examined in people with type 2 diabetes who had insufficient glycaemic control using diet and exercise therapy (placebo: $n = 56$; tofogliflozin: $n = 363$) (Table 1). The average age of the target population was 57.6 years and BMI was 25.5 kg/m^2, HbA_{1c} was 65 mmol/mol (8.1%), and resting heart rate was 66 bpm. At week 24, the change in resting heart rate was −1.1 bpm in the tofogliflozin group and +1.2 bpm in the placebo group; the decrease in the tofogliflozin group was significant in comparison with that in the placebo group, as indicated by ANCOVA ($P = 0.041$). In the tofogliflozin group, a negative correlation was observed between the change in resting heart rate and the baseline value, with a greater resting heart rate reduction at week 24 in participants with higher baseline values ($r = -0.37$) (Fig. 1). Baseline resting heart rate values were the same in the placebo group as in the tofogliflozin group.

Associations of changes in resting heart rate with other variables after tofogliflozin administration

Baseline factors were compared among participants divided according to quartiles of resting heart rate change after tofogliflozin administration (Table 2). HOMA-IR and Adipo-

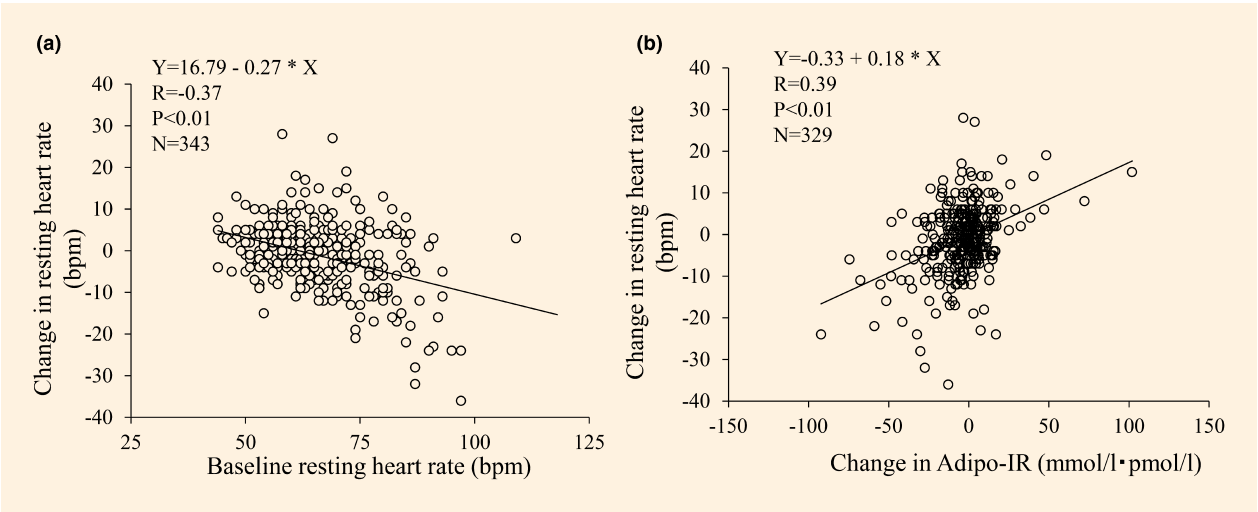

FIGURE 1 Correlation between change in resting heart rate and other parameters in study participants administered tofogliflozin. (a) Correlation between baseline resting heart rate and its change at week 24 in tofogliflozin group. (b) Correlation between change in resting heart rate and change in Adipo-IR at week 24 in tofogliflozin group. Pearson's product-moment correlation coefficient.

IR, which are indicators of insulin resistance, were highest, and iHOMA2S, which is an index of insulin sensitivity, was the lowest in the Quartile 1 (Q1) group. The greatest reduction in resting heart rate (least squares mean −11.2 bpm) among quartiles was observed in Q1 (Fig. 2). Changes in each variable from baseline were examined according to quartiles of resting heart rate change after tofogliflozin administration (Table 3). For each change in SBP, DBP, HbA_{1c} and body weight, the same degree of decrease was observed among quartiles of the resting heart rate change (Fig. 2). Among glucose-related indicators, fasting plasma glucose, C-peptide and postprandial blood glucose area under the curve ($AUC_{0\text{-}120min}$) had the greatest decreases in Q1 as did Adipo-IR and insulin, which are indicators of insulin resistance. A significant positive correlation was also observed between the reduction in resting heart rate and Adipo-IR levels (Fig. 1).

Baseline predictors that influenced changes in resting heart rate

Baseline factors that affected changes in resting heart rate were examined by multivariate analysis (Table 4). Higher baseline resting heart rate and Adipo-IR values were significantly associated with a greater decline in resting heart rate (for each 1 unit increase, declines were 0.24 and 0.07, respectively), whereas higher baseline iHOMA2%β and eGFR were significantly associated with an increase in resting heart rate. Even when these factors were adjusted, tofogliflozin significantly reduced the resting heart rate compared with placebo.

Discussion

This study is the first to evaluate the influence of an SGLT2 inhibitor on resting heart rate and factors related to such an influence. The SGLT2 inhibitor, tofogliflozin, was shown to significantly reduce resting heart rate compared with a placebo. Furthermore, an ~ 11 bpm reduction in resting heart rate was observed in participants with higher baseline resting heart rate and greater insulin resistance. This decrease was also associated with improvements in adipose tissue insulin resistance. Therefore, it was speculated that the correction in resting heart rate was associated with normalization of sympathetic nervous activity by not only correcting hyperglycaemia, but also improving adipose tissue insulin resistance, leading to the correction of hyperinsulinaemia.

Epidemiological studies have shown that a high heart rate is an independent risk factor for cardiovascular disease or death [20]. It was reported that an elevated resting heart rate presents a risk of cardiovascular disease and microvascular disease in type 2 diabetes [4,5]. In the current study, the resting heart rate showed a significant decrease at week 24 in the tofogliflozin group compared with the placebo group. In particular, a negative correlation was found between the change in resting heart rate from baseline to week 24 and the baseline resting heart rate; participants with a high baseline resting heart rate had a greater decrease in resting heart rate. Therefore, we infer that tofogliflozin effectively corrects high resting heart rate values. Interestingly, irrespective of the change in resting heart rate, the same degree of reduction in HbA_{1c}, body weight and blood pressure was observed among the quartiles of resting heart rate change. Previous reports have suggested that SGLT2 inhibitors lead to a reduction in blood pressure without affecting heart rate [21,22]. That the degree of blood pressure reduction was not significantly different in this study among groups according to quartiles of resting heart rate, that is, irrespective of heart rate change, is consistent with these past reports. Taken together, these results suggest that SGLT2 inhibitors affect the resting heart

Table 2 Baseline characteristics according to quartiles of change in resting heart rate at week 24 after administration of tofogliflozin

	Quartile 1 (Δ resting heart rate < −5) (*n* = 76)	Quartile 2 (−5 ≤ Δ resting heart rate < −1) (*n* = 84)	Quartile 3 (−1≤ Δ resting heart rate < 4) (*n* = 92)	Quartile 4 (4 ≤ Δ resting heart rate) (*n* = 91)	*P*-value
Age (years)	58.6 (9.5)	58.2 (10.4)	58.5 (10.7)	55.9 (10.0)	0.255
Sex (M : F)*	46 :30 (60.5 : 39.5)	56 : 28 (66.7 : 33.3)	59 : 33 (64.1 : 35.9)	64 : 27 (70.3 : 29.7)	0.597
Tofogliflozin*					0.035
10 mg	8 (10.5)	19 (22.6)	13 (14.1)	14 (15.4)	
20 mg	25 (32.9)	26 (31.0)	22 (23.9)	39 (42.9)	
40 mg	43 (56.6)	39 (46.4)	57 (62.0)	38 (41.8)	
Anti-hypertensive agents*	37 (48.7)	35 (41.7)	27 (29.4)	29 (31.9)	0.037
Angiotensin II receptor blocker	27 (35.5)	26 (31.0)	17 (18.5)	23 (25.3)	0.069
Angiotensin converting enzyme inhibitor	1 (1.3)	1 (1.2)	1 (1.1)	2 (2.2)	0.937
Calcium channel blocker	22 (28.9)	21 (25.0)	19 (20.7)	15 (16.5)	0.240
β-Blocker	5 (6.6)	4 (4.8)	2 (2.2)	0 (0.0)	0.048
Diuretics	4 (5.3)	5 (6.0)	4 (4.3)	2 (2.2)	0.614
Duration of diabetes (years)	5.5 (4.5)	6.1 (6.8)	6.3 (5.3)	5.7 (5.1)	0.803
eGFR (ml min^{-1} 1.73 m^{-2})	82.3 (18.9)	82.5 (18.5)	86.5 (20.1)	86.2 (17.8)	0.296
Body weight (kg)	69.6 (14.8)	68.6 (15.3)	66.3 (12.8)	68.5 (13.2)	0.480
Waist circumference (cm)	90.7 (11.1)	88.3 (10.2)	87.8 (9.8)	89.6 (11.4)	0.280
BMI (kg/m^2)	25.8 (4.3)	25.5 (4.2)	25.0 (4.1)	25.3 (4.1)	0.651
HbA_{1c} (mmol/mol)	65 (10)	64 (10)	64 (9)	65 (8)	0.811
HbA_{1c} (%)	8.1 (0.9)	8.0 (0.9)	8.0 (0.8)	8.1 (0.7)	0.811
Fasting plasma glucose (mmol/l)	9.2 (2.3)	8.9 (1.8)	8.8 (1.8)	8.8 (1.8)	0.444
Fasting insulin (pmol/l)	64.9 (49.1)	49.7 (30.1)	50.2 (44.1)	53.0 (48.9)	0.107
Fasting C-peptide (pmol/l)	549.6 (267.5)	458.3 (189.0)	453.7 (210.1)	440.5 (162.7)	0.004
Glucose $AUC_{0-120min}$ (mmol/l·2h)	29.2 (6.0)	28.1 (5.9)	28.6 (5.5)	28.3 (4.4)	0.633
Insulin $AUC_{0-120min}$ (pmol/l·2 h)	438 (349)	345 (193)	376 (272)	366 (219)	0.148
C-peptide $AUC_{0-120min}$ (pmol/l·2 h)	2322 (1024)	2003 (690)	2066 (769)	2044 (726)	0.057
Homeostatic model assessment insulin resistance	4.4 (3.3)	3.2 (2.0)	3.3 (2.7)	3.5 (3.3)	0.032
iHOMA2S†	109.5 (69.8)	130.7 (82.8)	146.9 (97.4)	129.5 (84.6)	0.048
iHOMA2β†	36.4 (27.6)	31.9 (19.5)	31.1 (23.3)	32.4 (19.8)	0.463
Fasting free fatty acid (mmol/l)	0.67 (0.25)	0.56 (0.25)	0.58 (0.20)	0.58 (0.21)	0.013
Adipo-IR (mmol/l·pmol/l)‡	44.4 (35.4)	29.1 (24.6)	28.5 (25.7)	30.8 (23.8)	<0.001
Adiponectin (μg/ml)	6.5 (2.7)	8.0 (4.4)	7.8 (3.8)	6.8 (3.4)	0.018
Uric acid (mg/dl)	5.2 (1.3)	5.2 (1.2)	4.8 (1.1)	5.0 (1.2)	0.124
Haematocrit (%)	43.2 (4.0)	43.2 (4.0)	43.2 (3.8)	43.3 (4.1)	0.994
Urinary albumin to creatinine ratio (μmol/mol Cr)	211.2 (451.2)	135.3 (479.5)	84.3 (162.6)	72.2 (214.8)	0.046
SBP (mmHg)	131.9 (13.4)	129.0 (14.4)	128.5 (15.9)	128.9 (13.9)	0.436
DBP (mmHg)	79.2 (11.3)	77.9 (10.7)	77.4 (10.8)	78.3 (10.0)	0.749
Mean average pressure (mmHg)§	96.8 (10.9)	94.9 (10.7)	94.5 (11.6)	95.2 (9.8)	0.558
Pulse pressure (mmHg)¶	52.7 (10.8)	51.1 (12.0)	51.1 (11.1)	50.6 (12.6)	0.699
Double product‖	9681.1 (2039.0)	8248.3 (1548.7)	8165.3 (1705.9)	8219.0 (1534.9)	<0.001
Pulse rate	77.8 (12.7)	69.8 (9.9)	70.3 (10.0)	71.7 (10.4)	<0.001
Resting heart rate (bpm)	73.1 (11.2)	64.0 (10.0)	63.5 (10.4)	63.7 (9.9)	<0.001

Data are expressed as mean (SD), except **n* (%).
Analyses were performed by one-way analysis of variance or Fisher's exact test across groups.
†From the iHOMA2 model.
‡Fasting insulin (pmol/l)·fasting free fatty acid (mmol/l).
§DBP + (SBP − DBP)/3.
¶SBP – DBP.
‖SBP (mmHg)·resting heart rate (bpm).

rate independently of reductions in HbA_{1c}, weight and blood pressure.

In this investigation, a significant correlation between resting heart rate reduction and Adipo-IR change was recognized. Results of multivariate analysis suggested that higher baseline Adipo-IR levels were significantly associated with greater declines in resting heart rate. Previously, the relationship between insulin resistance and resting heart rate was examined and strong insulin resistance was found to be associated with a high resting heart rate [6]. However, this is the first study to show the relationship between insulin resistance in adipose tissue and resting heart rate. In the current study, baseline resting heart rate levels were positively correlated with baseline indices of insulin resistance in

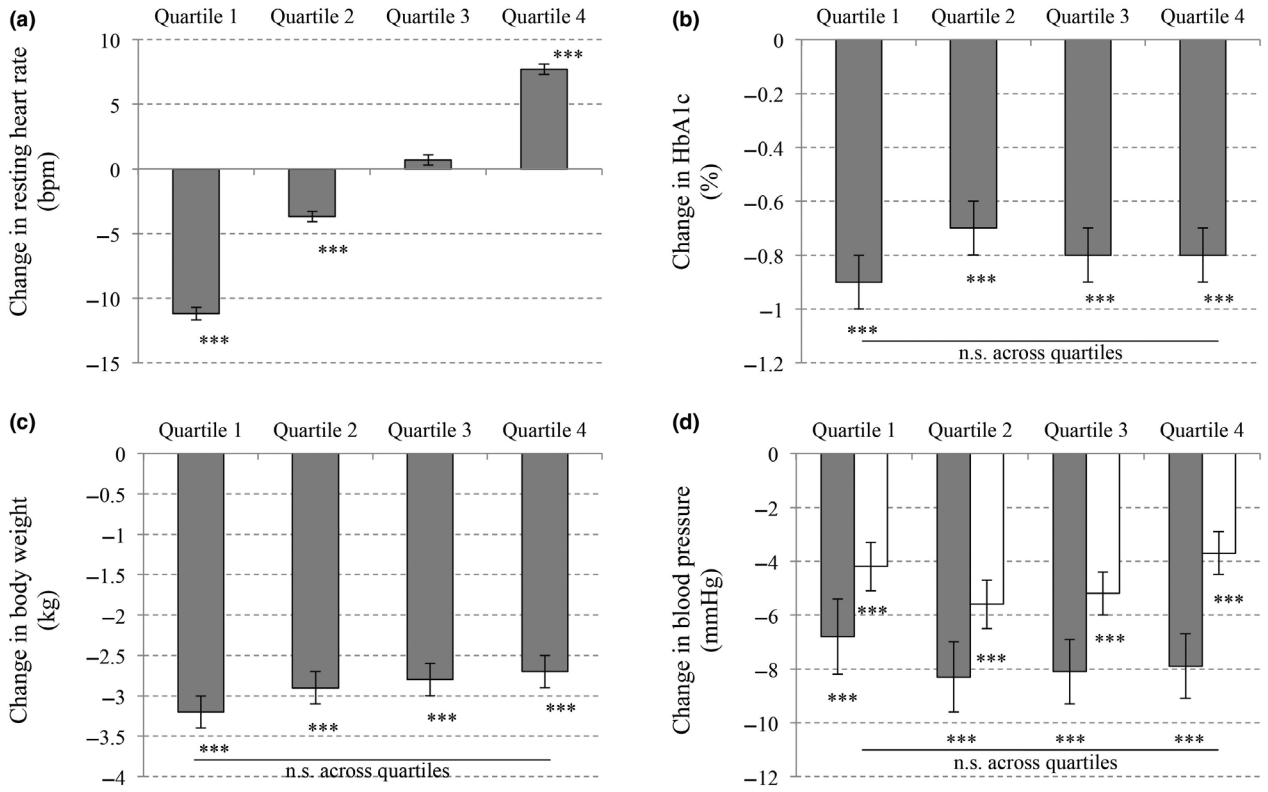

FIGURE 2 Changes in resting heart rate and other parameters from baseline at week 24 in study participants administered tofogliflozin: (a) resting heart rate, (b) HbA_{1c}, (c) body weight and (d) blood pressure (■, SBP; □, DBP). Participants were divided into four groups according to quartiles of change in resting heart rate at week 24. Data are expressed as least squares mean (SE). Analyses are performed by ANCOVA (Covariate: Baseline) to test across the groups. ***$P < 0.001$ *t*-test for least squares mean.

all study participants (Table 5). SGLT2 inhibitors reduced body weight as both monotherapy and add-on therapy [14,15,23]. It was reported that about two-thirds of body weight loss was caused by a decrease in fat mass and one-third was caused by a decrease in lean body mass, which suggests that a reduction in fat mass is the main cause of the weight loss [24,25]. Although body composition was not measured in this study, on the basis of these previous findings, it can be speculated that SGLT2 inhibitors improve insulin resistance, particularly in adipose tissue, by inducing weight loss. Results of a study of the relationship between insulin resistance (hyperinsulinaemia) and sympathetic hyperactivity [6] indicated that tofogliflozin might suppress sympathetic hyperactivity via improvement in adipose tissue insulin resistance. Furthermore, a decrease in heat production by adipocytes following reductions in resting heart rate has been reported [26]. Therefore, it may be possible to lower the resting heart rate by fat reduction through administering a SGLT2 inhibitor (adipose tissue insulin resistance improvement), thereby decreasing heat production and maintaining energy homeostasis. In addition, SGLT2 inhibitors have been reported to have anti-inflammatory effects in clinical situations [27,28]. Experimentally, SGLT2 inhibitors were reported to improve baroreceptor reflex sensitivity [29]. In particular, Yoshikawa *et al*. [29] showed that the heart rate is optimized during the active period (sympathetic dominant) and is not affected during the inactive period (parasympathetic dominant). There is a possibility that anti-inflammatory activity and sympathetic nerve activity are related. In this study, Adipo-IR, that is, insulin resistance in adipose tissue, was evaluated as the product of fasting insulin and free fatty acids. Henceforth, it will be necessary to accurately, that is, directly, evaluate adipose tissue insulin resistance by the glucose clamp method or similar methods, and evaluate the relationship between adipose tissue insulin resistance and the heart rate or sympathetic nerve activity.

The EMPA-REG OUTCOME study also suggested a reduction in resting heart rate [10]. An improvement in the prognosis of cardiovascular disease following a reduction in heart rate by drug interventions was reported [3]. This was supported by interventional studies reporting that reductions in heart rate through correction of sympathetic nervous system activity were related to an improved prognosis [30]. The results of the current investigation indicated that SGLT2 inhibitors may reduce resting heart rate in individuals with

Table 3 Change in variables according to quartiles of change in resting heart rate at week 24 after administration of tofogliflozin

	Quartile 1 (Δ resting heart rate < −5) (*n* = 76)	Quartile 2 (−5 ≤ Δ resting heart rate < −1) (*n* = 84)	Quartile 3 (−1 ≤ Δ resting heart rate < 4) (*n* = 92)	Quartile 4 (4 ≤ Δ resting heart rate) (*n* = 91)	*P*-value
eGFR (ml min^{-1} 1.73m^{-2})	−0.2 (1.0)	−1.2 (1.0)	0.3 (0.9)	−0.2 (0.9)	0.741
Waist circumference (cm)	−3.0 (0.4)***	−2.4 (0.4)***	−2.2 (0.4)***	−2.3 (0.4)***	0.486
BMI (kg/m^2)	−1.2 (0.1)***	−1.1 (0.1)***	−1.1 (0.1)***	−1.0 (0.1)***	0.519
Fasting plasma glucose (mmol/l)	−2.1 (0.1)***	−1.9 (0.1)***	−1.7 (0.1)***	−1.7 (0.1)***	0.037
Fasting insulin (pmol/l)	−17.3 (1.8)***	−16.2 (1.7)***	−14.0 (1.6)***	−9.1 (1.6)***	0.003
Fasting C-peptide (pmol/l)	−79.7 (13.2)***	−67.6 (12.4)***	−55.8 (11.8)***	−29.6 (11.9)*	0.032
Glucose $AUC_{0-120min}$ (mmol/l·2h)	−6.8 (0.3)***	−5.9 (0.3)***	−5.9 (0.3)***	−5.5 (0.3)***	0.017
Insulin $AUC_{0-120min}$ (pmol/l·2h)	−49.2 (11.8)***	−53.3 (11.0)***	−68.1 (10.6)***	−52.0 (10.5)***	0.607
C-peptide $AUC_{0-120min}$ (pmol/l·2h)	77.6 (50.5)	70.9 (47.2)	34.2 (45.8)	114.6 (45.0)*	0.664
iHOMA2S[†]	61.0 (8.1)***	58.4 (7.7)***	40.7 (7.3)***	44.3 (7.3)***	0.156
iHOMA2β[†]	9.2 (1.5)***	6.4 (1.4)***	5.5 (1.4)***	7.1 (1.4)***	0.320
Fasting free fatty acid (mmol/l)	0.05 (0.03)	0.07 (0.02)**	0.09 (0.02)***	0.13 (0.02)***	0.108
Adipo-IR (mmol·pmol)[‡]	−12.6 (1.9)***	−5.4 (1.8)**	−2.8 (1.7)	2.3 (1.7)	<0.001
Adiponectin (μg/mL)	0.8 (0.2)***	0.8 (0.2)***	0.5 (0.2)**	0.9 (0.2)***	0.416
Uric acid (mg/dL)	−0.4 (0.1)***	−0.5 (0.1)***	−0.4 (0.1)***	−0.2 (0.1)**	0.161
Hematocrit (%)	0.4 (0.3)	0.6 (0.2)**	0.6 (0.2)*	1.0 (0.2)***	0.338
Urinary albumin to creatinine ratio (μmol/mol Cr)	−71.5 (32.2)*	−57.7 (30.4)	−49.8 (29.1)	26.2 (29.3)	0.094
Mean average pressure (mmHg)[§]	−5.1 (0.9)***	−6.5 (0.9)***	−6.2 (0.9)***	−5.1 (0.9)***	0.574
Pulse pressure (mmHg)[¶]	−2.6 (1.1)*	−2.7 (1.0)**	−2.8 (1.0)**	−4.3 (1.0)***	0.578
Double product[‖]	−1786.7 (118.8)***	−1000.2 (108.1)***	−460.6 (103.6)***	404.1 (104.0)***	<0.001
Pulse rate	−6.0 (0.8)***	−2.1 (0.8)**	0.3 (0.7)	5.1 (0.7)***	<0.001

Data are expressed as least square mean (SE). Analyses are performed by ANCOVA (covariate: baseline) to test across the groups.
*P < 0.05, **P < 0.01, ***P < 0.001 t-test for least squares mean.
[†]From the iHOMA2 model.
[‡]Fasting insulin (pmol/l)·fasting free fatty acid (mmol/l).
[§]DBP + (SBP − DBP)/3.
[¶]SBP – DBP.
SBP (mmHg)·resting heart rate (bpm).

Table 4 Baseline predictors that influenced the change in resting heart rate at week 24

Factor	Regression coefficient	*P*-value
iHOMA2beta (higher 1 unit)	0.06	0.020
eGFR (ml min^{-1} 1.73m^{-2}) (higher 1 ml min^{-1} 1.73m^{-2})	0.07	0.001
Adipo-IR (pmol/l·mmol/l) (higher 1 pmol/l·mmol/l)	−0.07	0.002
Resting heart rate (bpm) (higher 1 bpm)	−0.24	<0.001
Tofogliflozin (vs. placebo)	−2.35	0.048

Factors remained through stepwise variable selection with P < 0.05.
Potential baseline predictors were tofogliflozin (vs. placebo), angiotensin II receptor blocker (use vs. nonuse), angiotensin converting enzyme inhibitor (use vs. nonuse), calcium channel blocker (use vs. nonuse), β-blocker (use vs. nonuse), diuretics (use vs. nonuse), age, sex, duration of diabetes, DBP, HbA_{1c}, fasting plasma glucose, BMI, haematocrit, eGFR, uric acid, Adipo-IR, HOMA-IR, iHOMA2S, iHOMA2β, adiponectin and resting heart rate.

an elevated resting heart rate due to improved insulin resistance in adipose tissue and fasting insulin reduction, and as a result may prevent the onset of cardiovascular disease. Participants in the current study were individuals with type 2 diabetes who were enrolled in phase 2/3 studies that compared tofogliflozin with placebo, and who had previously been treated by diet and exercise only. In an actual clinical setting, some concomitant therapies, including anti-hyperglycaemia and anti-hypertensive treatments, are needed to control cardiovascular risk factors in people with type 2 diabetes. An increase in resting heart rate has been reported for glucagon-like peptide 1 (GLP-1) receptor agonists that resulted in weight loss, unlike SGLT2 inhibitors [31]. It can be speculated that elevation in the resting heart rate caused by GLP-1 receptor agonists is the result of stimulation of the GLP-1 receptor in the sinoatrial node. Thus, it is quite interesting that the influence on resting heart rate differs between drugs producing similar reductions in blood glucose, weight and cardiovascular composite events. In our exploratory analysis (Table 6), a greater reduction in resting heart rate was observed in participants administered a concomitant β-blocker than in those without that agent, although the effect of concomitant anti-hypertensive drugs was not observed in the multivariate analysis. We could not clarify the effect of concomitant anti-hypertensive agents on resting heart rate because the number of participants using concomitant anti-hypertensive agents was so small. Therefore,

Table 5 Correlations between resting heart rate and variables at baseline in study participants

Variable	N	Pearson correlation	Pearson P-value	Spearman correlation	Spearman P-value
Duration of diabetes (years)	419	−0.1365	0.005	−0.1258	0.010
eGFR (ml min^{-1} $1.73m^{-2}$)	419	0.1707	<0.001	0.1315	0.007
Body weight (kg)	419	0.1239	0.011	0.1828	<0.001
Waist circumference (cm)	419	0.1408	0.004	0.1759	<0.001
BMI (kg/m^2)	419	0.1100	0.024	0.1542	0.002
HbA_{1c} (mmol/mol)	419	0.0986	0.044	0.1013	0.038
Fasting plasma glucose (mmol/l)	419	0.1544	0.002	0.1654	<0.001
Fasting insulin (pmol/l)	408	0.1783	<0.001	0.2011	<0.001
Fasting C-peptide (pmol/l)	419	0.1767	<0.001	0.1784	<0.001
Glucose $AUC_{0-120min}$ (mmol/l·2h)	419	0.2004	<0.001	0.2045	<0.001
Insulin $AUC_{0-120min}$ (pmol/l·2h)	399	0.1186	0.018	0.1084	0.030
C-peptide $AUC_{0-120min}$ (pmol/l·2h)	419	0.1168	0.017	0.1106	0.024
HOMA-IR	408	0.2011	<0.001	0.2317	<0.001
iHOMA2β*	408	0.0771	0.120	0.0503	0.311
iHOMA2S*	408	−0.1533	0.002	−0.2136	<0.001
Fasting free fatty acid (mmol/l)	419	0.3042	<0.001	0.2476	<0.001
Adipo-IR (mmol/l·pmol/l)†	408	0.2774	<0.001	0.2877	<0.001
Adiponectin (μg/ml)	419	−0.1161	0.018	−0.1242	0.011
Uric acid (mg/dl)	419	0.0797	0.103	0.0777	0.113
Haematocrit (%)	419	0.1487	0.002	0.1524	0.002
Urinary albumin to creatinine ratio (μmol/mol Cr)	419	0.0579	0.237	0.1687	<0.001
SBP (mmHg)	419	0.1343	0.006	0.1332	0.006
DBP (mmHg)	419	0.2040	<0.001	0.2255	<0.001
Mean average pressure (mmHg)‡	419	0.1959	<0.001	0.2062	<0.001
Pulse pressure (mmHg)§	419	−0.0243	0.621	−0.0486	0.321
Double product¶	419	0.8605	<0.001	0.8310	<0.001
Pulse rate	419	0.8096	<0.001	0.7967	<0.001

HOMA-IR, Homeostatic model assessment insulin resistance.
*From the iHOMA2 model.
†Fasting insulin (pmol/l)·fasting free fatty acid (mmol/l).
‡DBP + (SBP − DBP)/3.
§SBP − DBP.
¶SBP (mmHg)·resting heart rate (bpm).

Table 6 Changes in resting heart rate with or without concomitant anti-hypertensive agents in recipients of tofogliflozin

concomitant drugs	Group	n	LSM (SE)	p
Anti-hypertensive drugs	No	215	−0.7 (0.5)	0.149
	Yes	128	−1.9 (0.7)**	
Angiotensin II receptor blocker	No	250	−0.8 (0.5)	0.232
	Yes	93	−1.9 (0.8)*	
Angiotensin converting enzyme inhibitor	No	338	−1.1 (0.4)**	0.340
	Yes	5	−4.3 (3.4)	
Calcium channel blocker	No	266	−0.8 (0.5)	0.122
	Yes	77	−2.3 (0.9)**	
β-blocker	No	332	−1.0 (0.4)*	0.036
	Yes	11	−5.8 (2.3)*	
Diuretics	No	328	−1.1 (0.4)*	0.410
	Yes	15	−2.7 (2.0)	

Analyses were performed by ANCOVA (covariate: baseline) to test between the groups at week 24.
*$P < 0.05$, **$P < 0.01$ *t*-test for least squares mean.

further prospective studies are required to assess differences in the influence on resting heart rate of various drugs, including anti-hyperglycaemia and anti-hypertension treatments.

This study has several limitations that must be considered. It was not a prospective study; therefore, a long-term prospective study remains necessary. The number of participants was not sufficient to consider the effects of anti-hypertensive agents in both the placebo and tofogliflozin groups, nor were humoral factors affecting sympathetic and parasympathetic nervous activity measured. Fluctuations in the heart rate were not observed using 24-h Holter monitoring in any of the participants. In the future, it will also be necessary to evaluate adipose tissue insulin resistance using the glucose clamp or a similar method. Body composition and inflammatory markers were not measured. The relationship between weight loss and adipocytes, and the relationship between proinflammatory cytokines and sympathetic overactivity should be investigated in the future. Furthermore, because none of the participants in the present study had heart disease, individuals with heart disease and a long duration of diabetes must be studied.

The SGLT2 inhibitor tofogliflozin corrected resting heart rate levels in accordance with baseline levels. The correction of high resting heart rates may be attributed to the improvement of adipose tissue insulin resistance, leading to the correction of hyperinsulinaemia.

Funding sources

No grants or fellowships supported this research.

Competing interests

T.N. is an employee of Kowa Co., Ltd. Y.M. reports no conflicts of interest for the work presented in this manuscript. A.Y. and H.Su. are employees of Kowa Co., Ltd. K.F. has received donations for research from Eli Lilly and Takeda. T.A., M.I. and S.T. report no conflicts of interest for the work presented in this manuscript. H.So. has received donations for research from Astellas, Eli Lilly, Kowa, Kyowa Hakko Kirin, MSD, Japan Blood Products Organization, Boehringer Ingelheim, Pfizer, Novartis, Sumitomo Dainippon, Otsuka, Sanofi, Mitsubishi Tanabe, Asahi Kasei, Meiji Seika, Eisai, Yakult, Takeda, Taishotoyama and Daiichi Sankyo. KK. has been an advisor to and received honoraria for lectures from Astelas, Novo Nordisk Pharma, Sanwa Kagaku Kenkyusho, Takeda, Taisho Pharmaceutical, MSD, Kowa, Kissei, Sumitomo Dainippon Pharma, Novartis, Mitsubishi Tanabe Pharma, Nippon Boehringer Ingelheim, Daiichi Sankyo and Sanofi.

Acknowledgments

We sincerely acknowledge all participants in the tofogliflozin studies. The original phase 2 and phase 3 studies of tofogliflozin were funded by Chugai Pharmaceutical Co., Ltd.

Author contributions

T.N., Y.M. and H.So. contributed to the conception and design of this study. T.N. contributed to interpretation of data, writing of the first draft and revision of the manuscript for important intellectual content. H.So., A.Y., K.F., M.I., T.A. and K.K. revised the manuscript for important intellectual content. H.Su., T.N. and S.T. created the database, performed statistical analyses, contributed to the interpretation of the data and revised the manuscript for important intellectual content. H.So supervised this study. All authors have read and approved the final manuscript for submission.

References

1 Menown IB, Davies S, Gupta S, Kalra PR, Lang CC, Morley C *et al*. Resting heart rate and outcomes in patients with cardiovascular disease: where do we currently stand? *Cardiovasc Ther* 2013; **31**: 215–223.
2 Nanchen D, Stott DJ, Gussekloo J, Mooijaart SP, Westendorp RG, Jukema JW *et al*.; PROSPER Group. Resting heart rate and incident heart failure and cardiovascular mortality in older adults: role of inflammation and endothelial dysfunction: the PROSPER study. *Eur J Heart Fail* 2013; **15**: 581–588.
3 Johansen CD, Olsen RH, Pedersen LR, Kumarathurai P, Mouridsen MR, Binici Z *et al*. Resting, night-time, and 24 h heart rate as markers of cardiovascular risk in middle-aged and elderly men and women with no apparent heart disease. *Eur Heart J* 2013; **34**: 1732–1739.
4 Hillis GS, Woodward M, Rodgers A, Chow CK, Li Q, Zoungas S, Patel A *et al*. Resting heart rate and the risk of death and cardiovascular complications in patients with type 2 diabetes mellitus. *Diabetologia* 2012; **55**: 1283–1290.
5 Hillis GS, Hata J, Woodward M, Perkovic V, Arima H, Chow CK *et al*. Resting heart rate and the risk of microvascular complications in patients with type 2 diabetes mellitus. *J Am Heart Assoc* 2012; **1**: 1–11.
6 Bemelmans RH, Wassink AMJ, van der Graaf Y, Nathoe HM, Vernooij JW, Spiering W *et al*. Risk of elevated resting heart rate on the development of type 2 diabetes in patients with clinically manifest vascular diseases. *Eur J Endocrinol* 2012; **166**: 717–725.
7 Facchini FS, Stoohs RA, Reaven GM. Enhanced sympathetic nervous system activity. The linchpin between insulin resistance, hyperinsulinemia, and heart rate. *Am J Hypertens* 1996; **9**: 1013–1017.
8 Abdul-Ghani MA, Norton L, DeFronzo RA. Role of sodium-glucose cotransporter 2 (SGLT 2) inhibitors in the treatment of type 2 diabetes. *Endocr Rev* 2011; **32**: 515–531.
9 Merovci A, Solis-Herrera C, Daniele G, Eldor R, Fiorentino TV, Tripathy D *et al*. Dapagliflozin improves muscle insulin sensitivity but enhances endogenous glucose production. *J Clin Invest* 2014; **124**: 509–514.
10 Zinman B, Lachin JM, Inzucchi SE. Empagliflozin, cardiovascular outcomes, and mortality in type 2 diabetes. *N Engl J Med* 2015; **373**: 2117–2128.
11 Neal B, Perkovic V, Mahaffey KW, de Zeeuw D, Fulcher D, Erondu N *et al*.; CANVAS Programme Collaborative Group. Canagliflozin and cardiovascular and renal events in type 2 diabetes. *N Engl J Med* 2017; **377**: 644-657.
12 Wiviott SD, Raz I, Bonaca MP, Mosenzon O, Kato ET, Cahn A *et al*.; DECLARE–TIMI 58 Investigators. Dapagliflozin and cardiovascular outcomes in type 2 diabetes. *N Engl J Med* 2019; **380**: 347–357.
13 Perkovic V, Jardine MJ, Neal B, Bompoint S, Heerspink HJL, Charytan DM *et al*.; CREDENCE Trial Investigators. Canagliflozin and renal outcomes in type 2 diabetes and nephropathy. *N Engl J Med* 2019; **380**: 2295–2306.
14 Kaku K, Watada H, Iwamoto Y, Utsunomiya K, Terauchi Y, Tobe K *et al*.; Tofogliflozin 003 Study Group. Efficacy and safety of monotherapy with the novel sodium/glucose cotransporter-2 inhibitor tofogliflozin in Japanese patients with type 2 diabetes mellitus: A combined Phase 2 and 3 randomized, placebo-controlled, double-blind, parallel-group comparative. *Cardiovasc Diabetol* 2014; **13**: 65.
15 Tanizawa Y, Kaku K, Araki E, Tobe K, Terauchi Y, Utsunomiya K *et al*. Long-term safety and efficacy of tofogliflozin, a selective inhibitor of sodium-glucose cotransporter 2, as monotherapy or in combination with other oral antidiabetic agents in Japanese patients with type 2 diabetes mellitus: multicenter, open-label, rand. *Expert Opin Pharmacother* 2014; **15**: 749–766.
16 Hill NR, Levy JC, Matthews DR. Expansion of the homeostasis model assessment of β-cell function and insulin resistance to enable clinical trial outcome modeling through the interactive adjustment of physiology and treatment effects: IHOMA2. *Diabetes Care* 2013; **36**: 2324–2330.
17 Saponaro C, Gaggini M, Carli F, Gastaldelli A. The subtle balance between lipolysis and lipogenesis: a critical point in metabolic homeostasis. *Nutrients* 2015; **7**: 9453–9474.
18 Søndergaard E, De Ycaza AEE, Morgan-Bathke M, Jensen MD. How to measure adipose tissue insulin sensitivity. *J Clin Endocrinol Metab* 2017; **102**: 1193–1199.

19 Matsuo S, Imai E, Horio M, Yasuda Y, Tomita K, Nitta K *et al*. Revised equations for estimated GFR from serum creatinine in Japan. *Am J Kidney Dis* 2009; **53**: 982–992.
20 Mancia G, Fagard R, Narkiewicz K, Redon J, Zanchetti A, Böhm M *et al*. 2013 ESH/ESC guidelines for the management of arterial hypertension: the Task Force for the management of Arterial Hypertension of the European Society of Hypertension (ESH) and of the European Society of Cardiology (ESC). *Eur Heart J* 2013; **34**: 2159–2219.
21 Cherney DZ, Perkins BA, Soleymanlou N, Har R, Fagan N, Johansen OE *et al*. The effect of empagliflozin on arterial stiffness and heart rate variability in subjects with uncomplicated type 1 diabetes mellitus. *Cardiovasc Diabetol* 2014; **13**: 28.
22 Chilton R, Tikkanen I, Cannon CP, Crowe S, Woerle HJ, Broedl UC *et al*. Effects of empagliflozin on blood pressure and markers of arterial stiffness and vascular resistance in patients with type 2 diabetes. *Diabetes Obes Metab* 2015; **17**: 1180–1193.
23 Terauchi Y, Tamura M, Senda M, Gunji R, Kaku K. Efficacy and safety of tofogliflozin in Japanese patients with type 2 diabetes mellitus with inadequate glycaemic control on insulin therapy (J-STEP/INS): results of a 16-week randomized, double-blind, placebo-controlled multicentre trial. *Diabetes Obes Metab* 2017; **19**: 1397–1407.
24 Bolinder J, Ljunggren Ö, Kullberg J, Johansson L, Wilding J, Langkilde AM *et al*. Effects of dapagliflozin on body weight, total fat mass, and regional adipose tissue distribution in patients with type 2 diabetes mellitus with inadequate glycemic control on metformin. *J Clin Endocrinol Metab* 2012; **97**: 1020–1031.
25 Bolinder J, Ljunggren O, Johansson L, Wilding J, Langkilde AM, Sjostrom CD *et al*. Dapagliflozin maintains glycaemic control while reducing weight and body fat mass over 2 years in patients with type 2 diabetes mellitus inadequately controlled on metformin. *Diabetes Obes Metab* 2014; **16**: 159–169.
26 Lee ZS, Critchley JA, Tomlinson B, Young RP, Thomas GN, Cockram CS *et al*. Urinary epinephrine and norepinephrine interrelations with obesity, insulin, and the metabolic syndrome in Hong Kong Chinese. *Metabolism* 2001; **50**: 135–143.
27 Garvey WT, Van Gaal L, Leiter LA, Vijapurkar U, List J, Cuddihy R *et al*. Effects of canagliflozin versus glimepiride on adipokines and inflammatory biomarkers in type 2 diabetes. *Metabolism* 2018; **85**: 32–37.
28 Heerspink HJL, Perco P, Mulder S, Leierer J, Hansen MK, Heinzel A *et al*. Canagliflozin reduces inflammation and fibrosis biomarkers: a potential mechanism of action for beneficial effects of SGLT2 inhibitors in diabetic kidney disease. *Diabetologia* 2019; **62**: 1154–1166.
29 Yoshikawa T, Kishi T, Shinohara K, Takesue K, Shibata R, Sonoda N *et al*. Arterial pressure lability is improved by sodium-glucose cotransporter 2 inhibitor in streptozotocin-induced diabetic rats. *Hypertens Res* 2017; **40**: 646–651.
30 Paul L, Hastie CE, Li WS, Harrow C, Muir S, Connell JM *et al*. Resting heart rate pattern during follow-up and mortality in hypertensive patients. *Hypertension* 2010; **55**: 567–574.
31 Marso SP, Daniels GH, Brown-Frandsen K, Kristensen P, Mann JF, Nauck MA *et al*. Liraglutide and cardiovascular outcomes in type 2 diabetes. *N Engl J Med* 2016; **375**: 311–322.