Effect of Sodium Glucose Co-Transporter-2 Inhibition on the Aldosterone/Renin Ratio in Type 2 Diabetes Mellitus

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Key words

sodium glucose co-transporter-2, type 2 diabetes mellitus, hyperaldosteronism, aldosterone, renin

received 01.06.2018
accepted 06.11.2018

Bibliography

DOI https://doi.org/10.1055/a-0794-7026
Published online: 6.12.2018
Horm Metab Res 2019; 51: 91–99

ISSN 0018-5043

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ABSTRACT

The aldosterone to renin ratio (ARR) is recommended for case detection of primary aldosteronism (PA). Several factors including medications can undermine its diagnostic accuracy. The objective was to explore the effect of Sodium Glucose Co-Transporter-2 Inhibition on the ARR in patients with type 2 diabetes mellitus (T2DM) who were prescribed a Sodium Glucose Co-Transporter-2 Inhibitor (SGLT-2i) as part of routine clinical care. The primary outcomes were intra-individual changes in aldosterone, renin and ARR. Participants were recruited at routine diabetes outpatient visits as part of a prospective longitudinal study. Eligible participants were prescribed standard doses of empagliflozin and sampled at baseline (pre-SGLT-2i) and at their next routine outpatient visit (post-SGLT-2i). After a mean of 198 (± 87) days on SGLT-2i treatment (n = 20), there was a significant reduction in HbA_{1c}, BMI, eGFR and serum triglycerides and a significant increase in serum creatinine and sodium. Compared with baseline, there was a significant increase in median direct renin concentration (mIU/l) [40.3 (6.2–249.5) vs. 70.2 (7.0, 551.0) (p = 0.005)] and no significant change in median plasma aldosterone concentration (pmol/l) [296 (101, 685) vs. 273 (101, 794) (p = 0.541)] with a significant reduction in median ARR (pmol/mIU) [6.9 (0.6–70.7) vs. 5.3 (0.2–39.3) (p = 0.007)]. The proportion of participants with a screen positive ARR decreased from 20 % (pre-SGLT-2i) to 5 % (post-SGLT-2i) (p = 0.248). Although performed in a relatively small cohort of medically complex patients, the study indicates that SGLT-2i therapy has the potential to cause false-negative screening for PA in the setting of T2DM. Future confirmatory studies should include patients with confirmed PA.

Abbreviations

ARR	Aldosterone to renin ratio
PA	Primary aldosteronism
SGLT-2i	Sodium Glucose Co-Transporter-2 Inhibitor
HbA_{1c}	Glycated haemoglobin
BMI	Body mass index
eGFR	Estimated glomerular filtration rate
PCT	Proximal convoluted tubule
GUH	Galway University Hospitals
NUIG	National University of Ireland, Galway
EDTA	Ethylenediaminetetraacetic acid
DRC	Direct renin concentration
CRP	C-Reactive protein
uACR	Urinary albumin to creatinine ratio

CKD-EPI	Chronic kidney disease epidemiology collaboration
ISO	International Organisation for Standardisation
WHR	Waist-to-hip ratio
ICMA	Immunochemiluminometric assay
CV_A	Coefficient of variation (analytical)
SD	Standard deviation
RAAS	Renin-angiotensin-aldosterone-system
PRA	Plasma renin activity
OLETF	Otsuka Long-Evans Tokushima Fatty
LETO	Long-Evans Tokushima Otsuka

Introduction

Primary aldosteronism (PA) represents a group of disorders characterised by aldosterone hypersecretion that is inappropriately high for sodium status, not suppressible by sodium loading and partially or completely autonomous of the renin-angiotensin system [1]. It is the most common cause of secondary hypertension [2]. Until recently it was felt that < 1 % of patients with mild to moderate essential hypertension had PA [3] but the most recent evidence suggests that this may be > 5–10 % [4]. In patients with resistant hypertension and type 2 diabetes mellitus (T2DM), the prevalence of PA is approximately 14 % [5]. Diabetes is more prevalent in patients with PA than age, sex and blood pressure matched hypertensive controls [6, 7]. Early detection of this potentially treatable and curable condition [8] is essential because patients with PA have increased cardiovascular morbidity and mortality [9].

The aldosterone to renin ratio (ARR) is recommended for case detection of PA [1, 10]. ARR is a highly variable test and accurate interpretation mandates that all factors that can interfere with renin and aldosterone measurement and confound results are accounted for [11]. Factors which can interfere with the ARR include age [12], gender [13], potassium concentration [14], timing system [1], posture [15] and medications such as β-blockers [16–18], potassium wasting diuretics and central agonists [1]. Current guidelines focus on avoiding the risks associated with a missed diagnosis of PA (due to the potential to reduce the associated cardiovascular morbidity and mortality) and places a lower value on avoiding falsely classifying a patient as having PA and exposing them to unnecessary diagnostic tests [1]. Thus, it is imperative to know if a particular medication has the potential to cause false-negative screens.

Sodium-Glucose Cotransporter-2 inhibitors (SGLT-2is) are a new class of medications which have been introduced into treatment algorithms for patients with T2DM [19]. SGLT-2 is a protein located in the proximal convoluted tubule (PCT) that is responsible for approximately 90 % of the reabsorption of glucose filtered by the kidney [20]. In the EMPA-REG outcome study, treatment with empagliflozin was associated with a reduction in blood pressure and reduced hospitalisation from heart failure [21]. The exact mechanism behind these beneficial effects is not completely understood. It is postulated that they are due to the combined effect of an osmotic diuresis and mild natriuresis [22, 23]. We hypothesised that the osmotic diuresis and natriuresis may stimulate renin production due to decreased circulating volume and juxtaglomerular apparatus pressure, and loss of water and sodium. While the increase in renin may lead to stimulation of aldosterone secretion through increased angiotensin II production, a state of equipoise exists as to whether it would be in proportion to the increase in renin secretion. The ARR, derived from the measurement of aldosterone and renin is particularly sensitive to changes in the denominator renin [10]. A small increase in renin values for example at low levels (typical of patients with PA) can result in a significant change in the ARR leading to the possibility of false-negative screens.

To our knowledge there is no reported study evaluating the effect of SGLT-2is on the ARR. The objective of this study was to determine the effect of Sodium Glucose Co-Transporter-2 Inhibition on the ARR in patients with T2DM who were prescribed a SGLT-2i as part of routine clinical care.

Subjects and Methods

Ethical approval was granted by the Clinical Research Ethics Committee, Galway University Hospitals (GUH, Ref: C.A. 1404) and the National University of Ireland, Galway Research Ethics Committee (NUIG, Ref: 16-July-05).

Study design

This study, a subset of a prospective longitudinal cohort study evaluating novel biomarkers in participants with diabetes, investigated the primary outcomes of intra-individual changes in plasma aldosterone, renin and the ARR. This design in which each participant acted as his or her own control provides good statistical power despite the limitation of a relatively small sample size.

Participants were identified and recruited by convenience consecutive sampling at routine diabetes outpatient visits to the Centre for Diabetes, Endocrinology and Metabolism, GUH between June 2016 and November 2017. Eligible participants were prescribed standard doses of a single SGLT-2i (empagliflozin) as part of routine clinical care. Empagliflozin was prescribed as either a single daily dose (10 mg) or split dose (5 mg bd) in combination with metformin (participants already prescribed this medication but not in combination). Empagliflozin could be increased to the maximum dose (either a single daily dose of 25 mg or a split dose of 12.5 mg bd). Participants were sampled at baseline (prior to initiation of an SGLT-2i) and at their next routine outpatient visit (post initiation of an SGLT-2i).

The inclusion criteria were written informed consent, age ≥ 18 years, known diagnosis of T2DM, estimated glomerular filtration rate (eGFR) ≥ 60 ml/min/1.73 m^2, prescribed salt restricting diet, haemoglobin > 10 g/dl within 3 months of study enrolment or no history of anaemia, no active infection, cancer, acute cardiovascular event or haematological condition at time of study enrolment and no contraindications to SGLT-2is. The exclusion criteria were lack of tolerance to SGLT-2is for the study duration due to side effects such as complicated urinary tract infection or genital infections, acute kidney injury, diabetic ketoacidosis or an event consistent with volume depletion, poor medication adherence and withdrawal or addition of an agent that could markedly effect the ARR during the study period such as spironolactone, eplerenone, amiloride, triamterene, potassium-wasting diuretics or products derived from liquorice root such as confectionary liquorice and chewing tobacco [1]. Participants who were on these medicines at baseline were not excluded as this study reflects the realities of a real-life clinical cohort who would be prescribed an SGLT-2i. If the participant was prescribed the medicine

at both time points, it was assumed that the impact on the ARR would be similar.

Sampling strategy

The ARR test was carried out mid-morning with the participant in the seated, upright position and following 2 h ambulation. The participant was seated for 10–15 min prior to venesection. Blood (20 ml) was drawn from each participant and collected in appropriate specimen tubes (Becton Dickinson plastic vacutainer®): potassium ethylenediaminetetraacetic acid (EDTA) containing tubes (plasma) for measurement of aldosterone/direct renin concentration (DRC)/glycated haemoglobin (HbA_{1c})/haematology profile and plain tubes (serum) for the measurement of urea and electrolytes, bicarbonate, liver function tests, ketones and C-reactive protein (CRP). The eGFR was calculated using the Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI) equation. A second void midstream urine sample was collected to measure the urinary albumin to creatinine ratio (uACR) and sodium. Plasma for aldosterone/renin and serum for ketone measurements were delivered to the laboratory at room temperature (19 ± 2 °C) for processing (centrifugation, separation and freezing at –80 °C pending batch analyses). All tests were processed in a medical testing laboratory accredited to ISO (International Organisation for Standardisation) 15189: 2012 standards.

Weight was measured using a Tanita® scale and height using a Seca® wall-mounted stadiometer as per departmental protocol. Body Mass Index (BMI) (kg/m^2) was calculated by dividing weight in kilograms (kg) by height in metres squared (m^2). Blood pressure and heart rate were measured using a validated oscillometric device (Vital Signs Monitor 300 Series: Welch Allyn, Beaverton, OR, USA) following 5–10 min seated rest. Waist-to-hip ratio (WHR) is the ratio of waist circumference (measured at the midpoint between the lower margin of the last palpable rib and the top of the iliac crest) and hip circumference (measured around the widest portion of the buttocks).

Analytical method

Renin and aldosterone were measured in EDTA plasma using the Immunodiagnostic Systems (IDS-iSYS) automated specialty immunoassay analyser. Both assays are accredited to ISO15189:2012 standards for medical testing laboratories at GUH. The direct renin concentration (DRC) assay is a sandwich immunochemiluminometric assay (ICMA) that employs 2 monoclonal antibodies, a magnetic particle solid-phase capture antibody and an acridinium-labelled tag antibody. Renin concentration is directly proportional to light (expressed in relative light units) emitted by the acridinium label and measured by the system luminometer. The DRC assay is calibrated to the WHO International Standard 68/356. The inter-assay precision expressed as coefficient of variation (CV_A %), at a mean DRC of 14 mIU/l, 100.3 mIU/l and 390.2 mIU/l was 7.7, 8.4 and 4.9 % respectively.

The aldosterone (PAC) assay is a competitive one-site ICMA referenced to liquid chromatography-tandem mass spectrometry that uses a biotinylated monoclonal antibody bound to streptavidin-coated magnetic particles. Acridinium-labelled aldosterone competes with sample aldosterone for the limited amount of biotinylated antibody. Aldosterone concentration is inversely proportional to light emitted by the acridinium label and measured by the system luminometer. The inter-assay CV_A % at a mean aldosterone of 238 pmol/l, 442 pmol/l and 1648 pmol/l was 9.71, 9.37 and 3.83 %, respectively.

The ARR was calculated from temporally-paired PAC and DRC results as follows: PAC in pmol/l divided by DRC in mIU/l to give the ARR in pmol/mIU. The decision threshold for screen positive PA for both males and females in our institution is > 25 pmol/mIU. Notwithstanding, there is overlap in the ARR between individuals with and without PA. Assuming adherence to pretesting criteria (off potentially interfering medications) an ARR < 25 pmol/mIU makes PA highly unlikely. The likelihood of PA increases significantly as the ARR rises > 35 pmol/mIU and these patients require further investigation [24]. We have previously determined that method and gender-specific decision thresholds may be more appropriate; but these would require validation in a large external cohort [13].

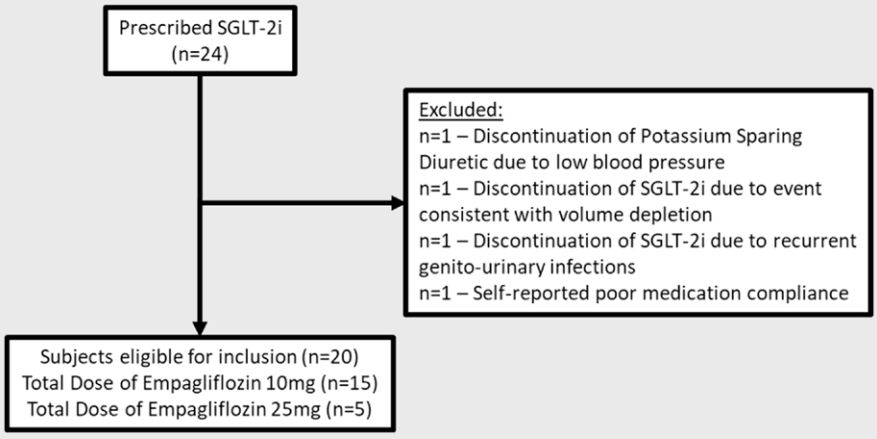
Statistical analyses

GraphPad Prism Version 6.01 for Windows and Minitab® 17.1.0 were used to analyse these data. Continuous data were represented using the mean and standard deviation (SD) when the data was normally distributed and the median and range (min-max) if the data was not normally distributed. Comparison of means (pre- and post-SGLT-2i) was performed using the paired t-test. Non-parametric data were compared using the Wilcoxin matched pairs signed rank test. The 95 % confidence interval of the mean was used to give an indication of the effect size (post–pre-SGLT-2i). Use of 95 % confidence interval of the median was avoided as it does not provide insight into effect size. Categorical data were summarised with frequencies (percentages). Comparisons of proportions were performed using the McNemar's test. All analyses were 2-tailed and $p < 0.05$ was deemed statistically significant.

Results

In total, 24 participants were eligible for inclusion in this study (▶ **Fig. 1**). Of these, 4 participants were excluded from the study due to discontinuation of therapy (n = 2), poor adherence to medication (n = 1) and discontinuation of potassium sparing diuretic (n = 1). Mean time between sampling was 198 (± 87) days. Demographics, anthropometric measurements and results of biochemical, metabolic, urinary and haematological parameters are shown in ▶ **Table 1**, **2** and ▶ **Fig. 2**. As expected, there was a significant reduction in HbA_{1c} (▶ **Fig. 2a**), BMI (▶ **Fig. 2b**), eGFR (▶ **Fig. 2c**) and serum triglycerides with a significant increase in serum creatinine and sodium (▶ **Table 2**). Furthermore, there were significant increases in blood urea, haemoglobin and haematocrit that may suggest a haemoconcentration effect (▶ **Table 2**). There were no significant changes in urinary ACR (▶ **Fig. 2d**), blood pressure (▶ **Fig. 2e, f**) or urinary sodium (▶ **Fig. 2g**). ▶ **Table 3** outlines the medications the participants were taking at baseline and following SGLT-2i. In total, 14 (70 %) participants had a diagnosis of hypertension at baseline.

DRC, aldosterone and ARR measurements are outlined in ▶ **Table 2**. Compared with baseline, there was a significant increase in median DRC (mIU/l) [40.3 (6.2–249.5) vs. 70.2 (7.0, 551.0)] (▶ **Fig. 3a**) and no significant change in median PAC (pmol/l) [296 (101, 685) vs. 273 (101, 794)] (▶ **Fig. 3b**). Consequently, there was a significant decrease in median ARR (pmol/mIU) compared to



▶ **Fig. 1** Recruitment Schematic.

▶ **Table 1** Clinical demographics of the patient population.

	Pre-SGLT-2i	Post-SGLT-2i	Mean Difference (post - pre)×	p-Value
	n = 20	n = 20		
Male no. (%)	16 (80)		N/A	N/A
Age (years)*	58.45 (8.99)	59.03 (9.08)	0.57 (0.46, 0.69)	<0.001
BMI (kg/m^2)*	31.0 (5.5)	30.0 (5.6)	−0.9 (−1.3, −0.5)	<0.001
Waist/hip ratio*	0.99 (0.11)	0.99 (0.11)	0.00 (0.00, 0.00)	0.615
SBP (mmHg)*	133 (13)	129 (14)	−4 (−11, 4)	0.3
DBP (mmHg)*	75 (9)	74 (8)	−1 (−6, 4)	0.649
Pulse rate (beats per min)*	73 (12)	73 (14)	0 (−4, 5)	0.863
Current smoker no. (%)^	3 (15)	3 (15)	N/A	N/A
Duration of diabetes (years)*	9.79 (7.32)	10.37 (7.35)	0.57 (0.46, 0.69)	<0.001

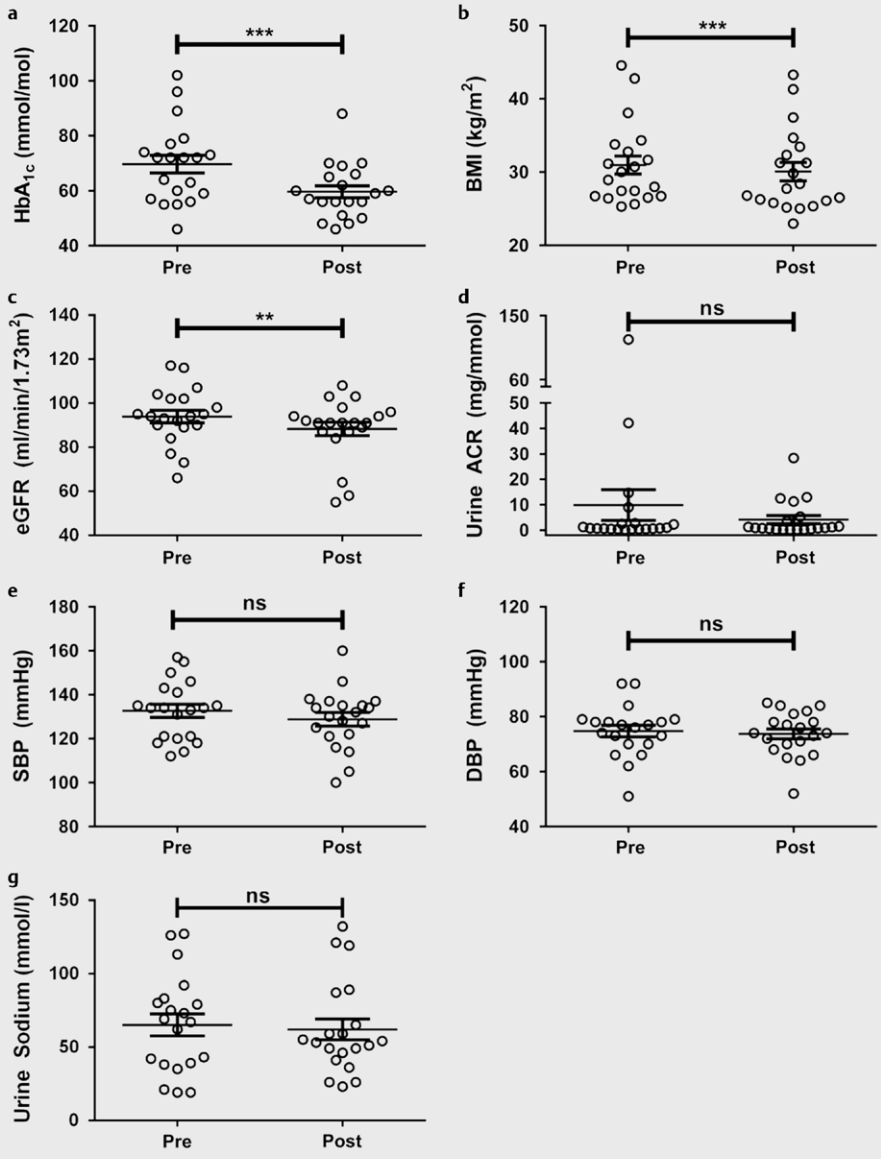
BMI: Body mass index; SBP: Systolic blood pressure; DBP: Diastolic blood pressure; N/A: Not available; * Mean (standard deviation), paired *t*-test used to compare means between groups; ^ Number (%); × Mean Difference (95 % Confidence Interval).

baseline [6.9 (0.6–70.7) pmol/mIU vs. 5.3 (0.2–39.3) pmol/mIU] (▶ **Fig. 3c**). The proportion of participants with screen positive ARR decreased from 4/20 (20 %) to 1/20 (5 %) (p = 0.248). The single participant who remained screen positive had a drop in ARR from 71 pmol/mIU to 39 pmol/mIU. Furthermore, a participant who was borderline screen positive prior to SGLT-2i (24 mIU/l) became clearly screen negative (18 mIU/l). The 4 participants who were screen positive for PA at baseline are at low risk of having PA: only one of the 4 participants was taking an antihypertensive and all participants had blood pressure < 150/100 mmHg, no hypokalaemia, no known adrenal incidentaloma and no family history of early onset hypertension or stroke at a young age.

Discussion

After treatment with the SGLT-2i, empagliflozin, we found a significant reduction in ARR levels when calculated using plasma renin measured as DRC. This was due to a significant increase in DRC with no change in aldosterone level. SGLT-2is mediate their effects by inhibiting SGLT-2 in the PCT supressing the cotransport of glucose coupled with sodium from the lumen of the PCT [25] with an associated osmotic diuresis and mild natriuresis. Multiple theories exist as to the exact mechanism underlying the osmotic diuresis and/or natriuretic effect of SGLT-2is that leads to a reduction in intravascular volume, arterial blood pressure and weight and improved cardiovascular outcomes [21, 26, 27].

The inhibition of sodium-coupled glucose absorption in the PCT results in an increased distal delivery of sodium to the macula densa (which would be expected to suppress renin production). The restoration of tubuloglomerular feedback has been cited as the putative mechanism to mediate hyperfiltration [28]. Furthermore, the metabolic abnormalities associated with T2DM may cause activation of the renin-angiotensin-aldosterone-system (RAAS) [29]. In patients, who have not been prescribed a SGLT-2i as HbA_{1c} increases renin increases [30]. While there was a significant reduction in HbA_{1c} amongst our cohort, DRC increased suggesting that the intravascular volume depletion from SGLT-2 inhibition may override these effects resulting in increased renin production. We also found a reduction in BMI and eGFR with an increase in serum sodium, urea, haemoglobin and haematocrit that reflect haemoconcentration. SGLT-2is have previously been associated with an increase in haematocrit and haemoglobin [31]. Healthy renal fibroblasts produce erythropoietin that decreases after tubular injury and may be associated with hyperglycemia. Fibroblasts can regenerate following resolution of a mild renal tubular injury and it is postulated that this may also occur following SGLT-2i therapy, which could contribute to the rise in haematocrit and haemoglobin [31]. Interestingly, there was no change in spot urinary sodium concentration before or after treatment with SGLT-2is. Unexpectedly, despite changes in markers of intravascular volume there was no significant change in systolic or diastolic blood pressure.



▶ **Fig. 2** **a** HbA_{1c}; **b** BMI; **c** eGFR; **d** Urine ACR; **e** SBP; **f** DBP; **g** Urine Sodium pre SGLT-2i and post SGLT-2i. For illustration, data is represented as means with error bars indicating the SEM. * $p<0.05$; ** $p<0.01$; *** $p<0.001$; NS: Not significant. For HbA_{1c}, BMI, eGFR, SBP, and DBP comparison is made using paired *t*-test. For Urine ACR and Sodium pairwise comparison is made using Wilcoxin matched pairs signed rank test.

Previous in vivo studies provide conflicting evidence regarding the effect of SGLT-2i therapy on the RAAS. In a 10 week follow-up study of subtotally nephrectomised non-diabetic rats following oral administration of a selective SGLT-2i, TA-1887, Li et al. noted a trend towards an increase in plasma renin activity (PRA) ($p=0.087$) [32]. Gallo et al. showed that oral empagliflozin increased both plasma and intrarenal renin activity in diabetic and non-diabetic mice [33]. Aldosterone levels were not measured in these studies. In contrast, there was no difference in plasma renin activity or serum aldosterone levels between Otsuka Long-Evans Tokushima Fatty (OLETF) rats treated with dapagliflozin, OLETF rats treated with voglibose (alpha glucosidase inhibitor), OLETF rats (diabetic control) and Long-Evans Tokushima Otsuka (LETO) rats (non-diabetic control) after 12 weeks of therapy [34].

Similar to our study, Lambers Heerspink et al. found that SGLT-2i (dapagliflozin) therapy for 12-weeks in patients with T2DM is associated with an increase in renin (PRA) and in contrast to our study an increase in serum aldosterone. There was a 33.3 % increase in PRA from baseline compared to a 14.8 % increase in aldosterone suggesting that if the ARR had been calculated it may have been significantly reduced. Changes in PRA and aldosterone levels with SGLT-2is were compared to changes seen with hydrochlorthiazide. While the change in aldosterone was similar to that seen in the hydrochlorthiazide treatment arm the change in PRA was not as pronounced [35]. Cherney et al. noted a significant increase in aldosterone with no significant change in PRA in participants with type 1 diabetes treated with empagliflozin with and without renal hyperfiltration [23]. The ARR was not calculated. It is worth noting that in this study, partici-

▶ **Table 2** Biochemical, metabolic, haematological, urinary and renin and aldosterone measurements.

	Pre-SGLT-2i	Post-SGLT-2i	Mean Difference (post - pre)×	p-Value
	n = 20	n = 20		
Biochemical Parameters				
CRP (mg/l)§	1.3 (0.5–12.2)	1.6 (0.5–8.4)	−0.7 (−1.7, 0.3)	0.213
Sodium (mmol/l)*	139 (2)	140 (2)	1 (0, 2)	0.034
Potassium (mmol/l)*	4.5 (0.3)	4.5 (0.4)	0.0 (−0.2, 0.2)	0.675
Chloride (mmol/l)*	99 (2)	99 (3)	0 (−1, 2)	0.513
Bicarbonate (mmol/l)*	23 (2)	25 (2)	2 (1, 2)	0.003
Urea (mmol/l)*	5.3 (1.6)	6.5 (2.2)	1.2 (0.4, 2.0)	0.005
Creatinine (µmol/l)*	74 (15)	80 (17)	6 (3, 10)	0.001
eGFR (ml /min/1.73 m^2)*	94 (13)	88 (14)	−6 (−9, −2)	0.002
ALT (U/l)§	23 (16–64)	23 (12–54)	−2 (−8, 3)	0.088
β-Hydroxybutyrate (mmol/l)*	<0.6 (0.1)	<0.6 (0.1)	0.0 (0.0, 0.0)	0.999
Metabolic Parameters				
HbA_{1c} (mmol/mol)*	70 (14)	60 (10)	−10 (−15, −5)	<0.001
Cholesterol (mmol/l)*	3.8 (0.8)	3.9 (0.8)	0.1 (−0.2, 0.3)	0.631
LDL (mmol/l)*	1.8 (0.8)	1.9 (0.8)	0.1 (−0.2, 0.3)	0.52
HDL (mmol/l)*	1.1 (0.3)	1.2 (0.4)	0.1 (0.0, 0.2)	0.01
Triglycerides (mmol/l)*	2.2 (1.4)	1.9 (1.1)	−0.3 (−0.6, 0.0)	0.038
Haematological Parameters				
Haemoglobin (g/dl)*	14.1 (1.4)	14.5 (1.8)	0.5 (0.0, 0.9)	0.032
Haematocrit (l/l)*	0.42 (0.03)	0.44 (0.05)	0.02 (0.00, 0.03)	0.002
Urinary Parameters				
ACR (mg/mmol)§	0.8 (0.2–116.4)	0.9 (0.2–28.4)	−5.7 (−15.4, 3.9)	0.712
Sodium (mmol/l)§	68 (19–127)	54 (23–132)	−3 (−16, 10)	0.655
Renin & Aldosterone Measurements				
Aldosterone (pmol/l)§	296 (101, 685)	273 (101, 794)	25 (−59, 110)	0.541
DRC (mIU/l)§	40.3 (6.2–249.5)	70.2 (7.0, 551.0)	107.1 (27.0, 187.1)	0.005
ARR using DRC (pmol/mIU)§	6.9 (0.6–70.7)	5.3 (0.2–39.3)	−5.7 (−10.5, −0.9)	0.007

CRP: C-reactive protein; eGFR: Estimated glomerular filtration rate; ALT: Alanine aminotransferase; HbA_{1c}: Glycated haemoglobin; LDL: Low-density lipoprotein; HDL: High-density lipoprotein; DRC: Direct renin concentration; *Mean (standard deviation), paired *t*-test used for pairwise comparison; § Median (min-max), Wilcoxin matched pairs signed rank test used for pairwise comparison; ×Mean difference (95 % Confidence Interval).

pants were sampled under steady state euglycemic or hyperglycemic clamp conditions (established using insulin and glucose infusions) after only 8 weeks of empagliflozin therapy. Furthermore, in this study, participants were advised to adhere to a high-sodium (>140 mmol/d) and moderate protein diet (<1.5 g/kg/d) in the days leading up to sampling, to avoid volume contraction and RAAS activation from sodium depletion. A relatively high salt diet for 7 days prior to sampling is similar to the oral sodium loading test used as a confirmatory test for PA. A combination of being volume replete with sodium loading and simultaneous infusion of fluid (glucose and insulin) would be expected to suppress aldosterone production. The complex sampling protocol (with varying rates of infusion of insulin and glucose) may interfere with the interpretation of the effect of SGLT-2i on aldosterone and renin. Cortisol was not measured; thus the potential acute impact of adrenocorticotrophic hormone (ACTH) on aldosterone in this study could be missed.

In our cohort, aldosterone did not increase in parallel with renin increase. Blockade of RAAS with Angiotensin-converting enzyme (ACE) inhibitors or Angiotensin II receptor blockers (ARBs) may have prevented the increase in renin stimulating aldosterone release. While the increase in renin may stimulate the conversion of angiotensinogen to angiotensin I, ACE-inhibitors or ARBs inhibit the production of aldosterone either by preventing the conversion of angiotensin I to angiotensin II (ACE-inhibitors) or preventing angiotensin II from binding to angiotensin II receptors (ARBs). The proportion of participants on ACE-inhibitors or ARBs is similar to that in Heerspink et al.'s dapagliflozin study where an increase in renin out of proportion to that of aldosterone was observed [35]. Furthermore, previous studies have shown that increased adipose tissue is associated

▶ **Table 3** Medications.

	Pre-SGLT-2i	Post-SGLT-2i	p-Value
	n = 20	n = 20	
Insulin Therapy			
Long acting insulin no. (%)*	5 (25)	3 (15)	0.48
Short acting insulin no. (%)	1 (5)	1 (5)	0.999
Pre-mixed insulin no. (%)*	2 (10)	3 (15)	0.999
Insulin pump no. (%)*	1 (5)	1 (5)	0.999
Oral Hypoglycaemic Agents			
Metformin no. (%)*	19 (95)	17 (85)	0.48
Sulfonylurea no. (%)*	8 (40)	5 (25)	0.248
GLP-1 Agonist no. (%)*	6 (30)	6 (30)	0.999
DPP IV Inhibitor no. (%)*	4 (20)	3 (15)	0.999
Antihypertensives*			
ACE inhibitor no. (%)*	7 (35)	7 (35)	0.999
ARB no. (%)*	4 (20)	5 (25)	0.999
Ca^{2+} Channel blocker no. (%)*	7 (35)	7 (35)	0.999
β-Blocker no. (%)*	5 (25)	5 (25)	0.999
Thiazide diuretic no. (%)*	4 (20)	4 (20)	0.999
Loop diuretic no. (%)*	1 (5)	1 (5)	0.999
No. of antihypertensives§	1.5 (0–4)	1.5 (0–4)	0.999
Other Meds			
Statin no. (%)*	17 (85)	18 (90)	0.999
Fibrate no. (%)*	3 (15)	3 (15)	0.999
Aspirin no. (%)*	11 (55)	11 (55)	0.999

GLP-1: Glucagon-like peptide-1; DPP IV: Dipeptidyl peptidase IV; ACE: Angiotensin-converting-enzyme; ARB: Angiotensin II receptor blocker
* Number (%). McNemar's test was used to compare proportions between the groups; § Median (min-max), Wilcoxin matched pairs signed rank test used for pairwise comparison.

with increased circulating aldosterone [36–39] and weight loss is associated with a reduction in plasma aldosterone and renin [40]. If part of the weight loss in our study was due to a reduction in body fat in addition to volume depletion, a reduction in aldosterone (and renin) may have also been expected.

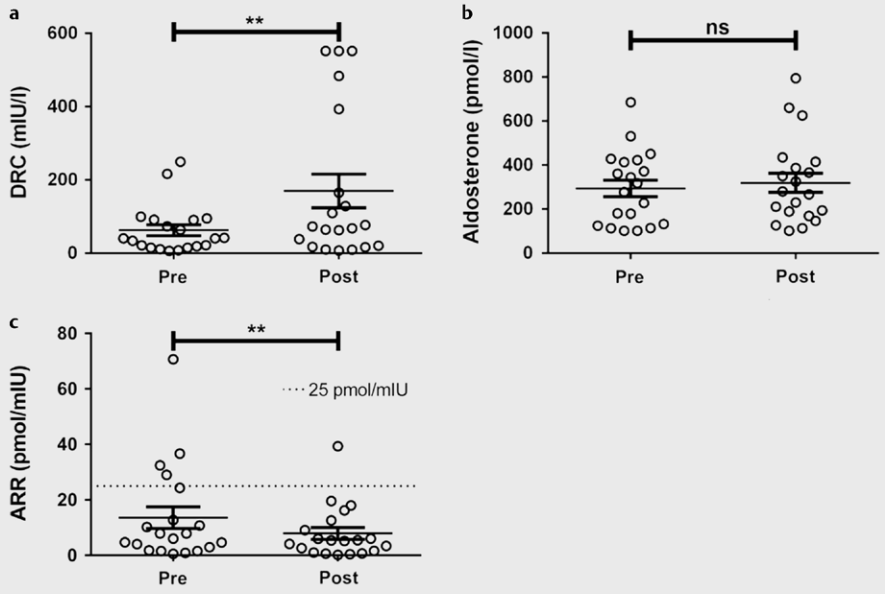
Of importance, the proportion of participants who were screen positive for PA decreased from 20 to 5 % – although this was not statistically significant. In addition, the participant who remained positive following SGLT-2i had almost a 50 % decrease in the ARR. This coupled with the increase in renin and the decrease in ARR suggests that measuring ARR for case detection of PA in the context of SGLT-2i increases the possibility of missing cases of true PA. The current guidelines put a high value on avoiding the risks associated with a missed diagnosis of PA [1].

We recommend withdrawal of SGLT-2is prior to measuring the ARR. Although we have not established the optimal withdrawal duration in this study, there are multiple studies suggesting when volume status may return to normal following discontinuation of SGLT-2is. In the EMPA-REG outcome study, eGFR normalised a median of 34 days after discontinuation of SGLT-2is [41]. Withdrawal of SGLT-2is for 4-5 weeks could be considered, guided by markers that may suggest volume depletion returning to baseline (such as eGFR, urea, haemoglobin and haematocrit). This timeline requires further study.

Although we have demonstrated for the first time that SGLT-2is have the potential to cause a significant reduction in the ARR due to an increase in renin with no significant change in aldosterone, it must be acknowledged that this study has several limitations including a relatively small sample size (n = 20). Notwithstanding, we used a study design that provides good statistical power; each study participant acted as his/her own control. Our findings however, need to be validated in a larger cohort. Furthermore, it is worth noting that 4 out of 24 patients (16.67 %) who enrolled in this study had to stop their medication due to an adverse side effect profile or due to non-compliance. While the inclusion of a clinical cohort with T2DM (with their complex pathophysiology, multiple interfering medications at different doses and varying adherence to prescribed therapy) may be considered a limitation, it is worth noting that our study represents a real-world clinical scenario for screening patients for PA. By selecting participants at risk of PA, we felt we were more likely to identify patients who would be screen positive thereby providing the opportunity to determine if SGLT-2is would increase the risk of false-negative screens. To date, despite appropriate investigations and follow-up, no patient in our cohort has a confirmed diagnosis of PA. Much of the baseline literature on medication interference with the ARR has been derived from patients without PA [42–44]. Thus, the fact that our study was carried out in patients without PA does not preclude making clear conclusions about the potential effects of SGLT-2is on the ARR. The varying lengths of patient follow-up is also a potential study limitation. The minimum time to patient follow-up was appreciable at 111 days. From this study we cannot, therefore, determine how long SGLT-2i would need to be discontinued to minimise the risk of false-negative results. Clearly further study is required, both in patients who have SGLT-2i therapy discontinued to determine the optimal duration of withdrawal for ARR sampling and in patients with PA.

Conclusions

This study provides evidence that SGLT-2is have the potential to cause false-negative screens for PA in patients with T2DM. Treatment with the SGLT-2i empagliflozin for an average of 198 (± 87) days is associated with an increase in DRC and no increase in aldosterone with a resultant decrease in ARR in participants with medically stable T2DM. There was a decrease in the proportion of participants who were screen positive for ARR. Lowered ARR consequent to SGLT-2i therapy can result in false-negative screens. To minimise the potential risk to patients of missing a case of PA, we suggest the withdrawal of SGLT-2i therapy for approximately 4–5 weeks prior to screening for PA in patients with T2DM. However, as discussed above, further studies to support our findings are required and should include patients with confirmed PA.



▶ **Fig. 3** **a** DRC; **b** Aldosterone; **c** ARR pre SGLT-2i and post SGLT-2i. For illustration, data is represented as means with error bars indicating the SEM. ** $p < 0.01$; NS: Not significant. Pairwise comparison is made using Wilcoxin matched pairs signed rank test. 25 pmol/mIU: decision threshold for screen positive for PA.

Author Contributions

TPG: Conception, medical assessment, recruitment and consenting of participants, sample collection, processing, biobanking, data assembly, statistical analysis and interpretation; MNI: Processing, biobanking, data assembly, statistical approach and interpretation; LB: Processing of laboratory samples, accuracy of laboratory methods, analysis and interpretation; MB: Medical assessment, recruitment and consenting of participants, analysis and interpretation; MDG: study design, statistical approach and interpretation; PMOS: Conception, design, quality, accuracy of laboratory methods, data acquisition, assembly, statistical approach, analysis and interpretation. TPG: First draft of manuscript. All authors reviewed, edited and approved the final version of the manuscript.

Acknowledgements

We wish to express our gratitude to all volunteers and patients who made this study possible. Special thanks to the scientific, nursing and medical staff at the Centre for Diabetes, Endocrinology and Metabolism, and the Department of Clinical Biochemistry at Saolta University Health Care Group (SUHCG), Galway University Hospitals (GUH).

Funding

TPG is supported by a Hardiman Scholarship from the College of Medicine, Nursing and Health Science, National University of Ireland, Galway and a bursary from the Royal College of Physicians of Ireland. The authors are supported by grants from the European Commission [Horizon 2020 Collaborative Health Project NEPHSTROM (grant number 634086; TPG, NI, MDG) and FP7 Collaborative Health Project VISICORT (grant number 602470; MDG)] and from Science Foundation Ireland [REMEDI Strategic Research Cluster (grant number 09/SRC-B1794; MDG) and CÚRAM Research Centre (grant number 13/RC/2073; MDG)] and by the European Regional Development Fund. The materials presented, and views expressed here are the responsibility of the author(s) only. The EU Commission takes no responsibility for any use made of the information set out.

Conflict of Interest

The authors declare that they have no conflict of interest.

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