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ORIGINAL RESEARCH

Acute and Chronic Effects of SGLT2 Inhibitor Empagliflozin on Renal Oxygenation and Blood Pressure Control in Nondiabetic Normotensive Subjects: A Randomized, Placebo-Controlled Trial

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BACKGROUND: The sodium/glucose cotransporter 2 inhibitor empagliflozin has cardiorenal protective properties through mechanisms beyond glucose control. In this study we assessed whether empagliflozin modifies renal oxygenation as a possible mechanism of renal protection, and determined the metabolic, renal, and hemodynamic effects of empagliflozin in nondiabetic subjects.

METHODS AND RESULTS: In this double-blind, randomized, placebo-controlled study, 45 healthy volunteers underwent blood and urine sampling, renal ultrasound, and blood-oxygenation-level–dependent magnetic resonance imaging before and 180 minutes after administration of 10 mg empagliflozin (n=30) or placebo (n=15). These examinations were repeated after 1 month of daily intake. Cortical and medullary renal oxygenation were not affected by the acute or chronic administration of empagliflozin, as determined by 148 renal blood-oxygenation-level–dependent magnetic resonance imaging examinations. Empagliflozin increased glucosuria (24-hour glucosuria at 1 month: +50.1±16.3 g). The acute decrease in proximal sodium reabsorption, as determined by endogenous fractional excretion of lithium (–34.6% versus placebo), was compensated at 1 month by a rise in plasma renin activity (+28.6%) and aldosterone (+55.7%). The 24-hour systolic and diastolic ambulatory blood pressures decreased significantly after 1 month of empagliflozin administration (–5.1 and –2.0 mm Hg, respectively). Serum uric acid levels decreased (–28.4%), hemoglobin increased (+1.7%), and erythropoietin remained the same.

CONCLUSIONS: Empagliflozin has a rapid and significant effect on tubular function, with sustained glucosuria and transient natriuresis in nondiabetic normotensive subjects. These effects favor blood pressure reduction. No acute or sustained changes were found in renal cortical or medullary tissue oxygenation. It remains to be determined whether this is the case in nondiabetic or diabetic patients with congestive heart failure or kidney disease.

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Key Words: blood pressure ■ BOLD-MRI ■ empagliflozin ■ renal oxygenation ■ SGLT2

Empagliflozin is a sodium/glucose cotransporter 2 inhibitor (SGLT2i) used for the treatment of type 2 diabetes mellitus (T2DM) with the unique property of decreasing blood glucose independently from insulin, by enhancing renal glucose excretion. The recent 2019 European Society of Cardiology guidelines developed

CLINICAL PERSPECTIVE

What Is New?

- This is the first study to examine the acute and chronic effects of sodium/glucose cotransporter 2 inhibition on renal oxygenation, as determined by renal blood-oxygenation-level–dependent magnetic resonance imaging.
- Acute and sustained sodium/glucose cotransporter 2 inhibition with empagliflozin does not alter renal oxygenation in nondiabetic normotensive subjects in spite of significant tubular and blood pressure effects.

What Are the Clinical Implications?

- The observed metabolic changes are similar to the effects known in type 2 diabetic subjects, namely increased natriuresis, glucosuria, decreased blood pressure and uric acid levels, and increased hemoglobin; these effects will likely be beneficial to a larger population of nondiabetic subjects.
- Renal tissue oxygenation, renal function, and electrolytes did not change, compatible with a favorable renal profile of this drug in nondiabetic subjects
- Endogenous lithium clearance studies show that natriuresis was the result of reduced reabsorption of sodium in proximal tubuli, illustrating why sodium/glucose cotransporter 2 inhibitors have added value on top of more distally active diuretics, such as loop diuretics.

Nonstandard Abbreviations and Acronyms

AP	acute phase
BOLD-MRI	blood oxygenation-level–dependent magnetic resonance imaging
CP	chronic phase
SGLT2	sodium/glucose cotransporter 2
SGLT2i	sodium/glucose cotransporter 2 inhibitor
T2DM	type 2 diabetes mellitus

in collaboration with the European Association for the Study of Diabetes recommend SGLT2i as initial therapy for patients with T2DM at high risk of cardiovascular disease to reduce cardiovascular and renal events and hospitalization for heart failure. The guidelines also recommend empagliflozin to reduce mortality in those with established cardiovascular disease.[1]

Although landmark studies with SGLT2 inhibitors included T2DM individuals, there is growing evidence that the cardiorenal protective effects of SGLT2 inhibitors are due to mechanisms beyond glucose control. Randomized, controlled trials and observational studies with SGLT2 inhibitors showed renal protective effects independent from glucose control.[2,3] Very recently, the Dapagliflozin and Prevention of Adverse Outcomes in Heart Failure study demonstrated a significant decrease in worsening of heart failure or death in nondiabetic individuals with reduced ejection fraction.[4] Rapid forced diuresis with SGLT2 inhibition has salutary hemodynamic effects on the heart in people with high cardiovascular risk. The effect on the kidney is more complex. SGLT2 inhibitors decrease the concomitant active reabsorption of sodium and glucose in the proximal tubule. They also induce an acute and reversible decrease in estimated glomerular filtration rate and albuminuria followed by a long-term slowing of estimated glomerular filtration rate decline and albuminuria progression.[3,5] Several potential mechanisms may explain the beneficial effects of SGLT2i on renal disease progression, such as a decrease in glomerular hyperfiltration, a reduction in blood pressure, and an increase in urinary sodium excretion.[6–8] Because active sodium reabsorption is the main determinant of renal oxygen consumption, many have hypothesized that SGLT2 inhibition may lead to a decrease in cortical oxygen utilization and an increase in tissue oxygenation, and thereby protect kidney function.[9,10] Recent studies have shown a significant association between cortical renal tissue oxygenation and the progression of chronic kidney disease in humans.[11] It is currently not known whether the nephroprotective effects of SGLT2 inhibitors are related to improved renal oxygenation.

Mechanistic studies involving cardiorenal interactions are important because of the growing interest of SGLT inhibition in nondiabetic individuals. The primary aim of this study was therefore to explore the acute and sustained effects of empagliflozin on renal tissue oxygenation as measured noninvasively with blood-oxygenation-level–dependent magnetic resonance imaging (BOLD-MRI) in nondiabetic normotensive subjects, and the secondary aims were to assess the effect of empagliflozin on blood pressure control and renal tubular function in nondiabetic subjects. Another reason to investigate nondiabetic subjects was to avoid the confounding factor of blood glucose fluctuations in diabetes mellitus on the BOLD-MRI signal, as recently described by our group.[12,13]

METHODS

The details of the study protocol have been published elsewhere.[14] This monocentric research project was approved by the local institutional review committee (ethics committee of the Canton de Vaud, Switzerland) and conducted according to the principles of the Declaration of Helsinki. Written informed

consent was obtained from all participants. The data that support the findings of this study are available from the corresponding author upon reasonable request.

Trial Design

This is a double-blind, randomized, placebo-controlled study addressing the acute and chronic renal effects of 10 mg empagliflozin given orally in healthy, nonmedicated volunteers.

Study Participants

In brief, a total of 45 healthy volunteers, 18 to 50 years of age, were recruited. Inclusion criteria were absence of diabetes mellitus based on glycated hemoglobin (<6.5%) and oral glucose tolerance (<7 mmol/L fasting, <11.1 mmol/L after 75 g of glucose) testing, a Chronic Kidney Disease Epidemiology Collaboration–based estimated glomerular filtration rate >60 mL/min per 1.73 m², a urine albumin/creatinine ratio <3.3 mg/mmol, a normal urine dipstick, normal hematology and chemistry results, and a normal renal ultrasound. Among the exclusion criteria were a history of substance abuse and claustrophobia or another contraindication for MRI (pregnancy, implanted metallic devices). Each group was randomized to placebo (n=15) or empagliflozin (n=30). The randomization procedure was done by the hospital's pharmacy on the first day of the acute phase. A 2:1 randomization was chosen to compensate for the possible higher dropout rate in the empagliflozin group due to side effects. Empagliflozin 10 mg or placebo tablets were identical in size and stored in similar boxes containing 30 tablets. Investigators (research nurses, doctors, and technicians) were blinded to treatment.

Intervention

At baseline, each subject underwent 24-hour ambulatory blood pressure monitoring (Diasys, Physicor, Geneva, Switzerland) and a 24-hour urine collection without any treatment. Renal ultrasound was performed using an Aplio XG device (Toshiba Medical Systems, Volketswil, Switzerland). The renal resistive indexes were measured on three segmental arteries (superior, middle, and inferior) in each kidney and averaged. Volunteers were instructed not to smoke or drink alcohol or have any caffeine-containing beverage during the study days. On the following morning, after a light breakfast at home at 7:00 AM, the volunteer arrived at the study center at 9:00 AM and stayed the whole day (acute phase [AP]). Serial blood and urine collections were performed at arrival and after a standardized hydration protocol at T0(AP), as well as 90 (T90(AP)) and 180 minutes (T180(AP)) after receiving the first oral administration of placebo or

empagliflozin. Renal BOLD-MRI measurements were performed at T0(AP) and T180(AP) minutes. At completion of the study day, volunteers left the center and continued taking the tablet once a day in the morning for 4 weeks. During this period, they were examined once per week and received a telephone call on another day each week for safety reasons. On the day before taking the last tablet, both 24-hour ambulatory blood pressure monitoring and 24-hour urine collection were repeated. On the last day, similar assessments were performed throughout the day at the study center (chronic phase [CP]) before and after the last empagliflozin or placebo tablet, as during the AP.

BOLD-MRI Analysis

BOLD-MRI uses the paramagnetic properties of deoxyhemoglobin. Increases in its outcome value R2* (apparent relaxation rate, expressed per second) corresponded to higher local deoxyhemoglobin levels and thus lower oxygenation.[15] Twelve T2*-weighted MR images were acquired at one coronal slice within a single breath-hold on a 3-T whole-body magnetic resonance system (Magnetom Prisma, Siemens Medical Systems, Erlangen, Germany), using a previously described modified multiecho data image combination sequence for BOLD-MRI analysis. This procedure was repeated 10 times. Images were imported for further analysis in MATLAB version 7.11 (The MathWorks, Natick, MA).[16] To minimize bias, we only included BOLD-MRIs that had a minimum of 8 of 10 series with excellent image quality. After image selection, the 12-layer concentric objects method was used to analyze the images, as reported elsewhere.[16]

The mean R2* value of the outer 3 layers of the two kidneys was used as a proxy of renal cortical oxygenation, and the mean R2* value of the inner (eighth through tenth) layers was used to report medullary oxygenation. The slope of the linear part of the R2* curve was expressed as the change in hertz per percentage change of depth, and its regression coefficient, β, was calculated using linear least-square regression.

Biochemical Measurements

Plasma and urine samples were analyzed for glucose, urea nitrogen, creatinine, urate, and sodium, using routine clinical chemistry methods on a Cobas 8000 device (Roche Diagnostics System, Basel, Switzerland). Proximal renal sodium handling was assessed by determination of fractional excretion of endogeneous lithium (FE_{Li}), a proxy of proximal sodium reabsorption, as described previously.[17] The fractional excretion of lithium (FE_{Li}) and sodium (FE_{Na}) were assessed using the standard formula

Table 1. Clinical Characteristics of Enrolled Volunteers by Randomization

	Placebo (n=15)	Empagliflozin (n=30)	
Age, y	34.1±10.6	32.7±7.5	NS
Weight	86.5±21.8	84.4±18.9	NS
BMI, kg/m^2	28.1±4.7	28.2±5.3	NS
Sex, males/females	8/7	19/11	NS
Ethnicity (%) African, Asian, white, other	20/0/73.3/6.7	6.7/6.7/66.7/20	NS
Office systolic blood pressure, mm Hg	118.2±9.6	120.9±12.2	NS
Office diastolic blood pressure, mm Hg	71.5±7.5	71.9±7.9	NS
Pulse rate, bpm	68.0±11.4	69.7±9.8	NS
eGFR, mL/min per 1.73 m^2	113.1±12.4	112.9±11.5	NS
Urinary albumin/creatinine ratio <3 mg/mmol, %	100	100	NS
Fasting plasma glucose, mmol/L	5.0±0.7	5.0±0.4	NS
Plasma glucose 2 h after 75 g glucose, mmol/L	4.4±1.2	5.5±1.7	0.03
HbA1C, %	5.4±0.3	5.4±0.3	NS

Data expressed as mean±SD. Placebo and empagliflozin groups compared using unpaired two-sample t tests. BMI indicates body mass index; bpm, beats per minute; eGFR, estimated glomerular filtration rate; HbA1C, glycated hemoglobin; and NS, not statistically significant.

$(FE_x=(U_x \times P_{creatinine}) / (P_x \times U_{creatinine}))$, with U and P representing urine and plasma concentrations of sodium or lithium.

Hormones

Plasma renin activity was measured using a commercial radioimmunoassay kit for the quantitative determination of angiotensin I in human plasma, whereas aldosterone quantification in blood was performed with the Aldo-Riact RIA kit (both kits from CISBio International, Yvette, France). Total plasma erythropoietin concentrations were determined using a solid-phase sandwich ELISA kit (Human Erythropoietin Quantikine IVD ELISA Kit; R&D Systems, Minneapolis, MN).

Outcomes

The primary outcome measures were the acute and chronic effects of empagliflozin on renal tissue oxygenation as measured by BOLD-MRI. The secondary outcomes were the effects of empagliflozin on body weight, office blood pressure and 24-hour blood pressure measurements, renal tubular function, erythropoietin, hematocrit, and ultrasound-assessed renal resistance indexes and length.

Statistical Analysis

Sample size calculation was based on the assumption that empagliflozin would improve oxygenation by 10% (corresponding to an approximate decrease in cortical R2* of 2 per second) with a sigma (SD) of 5% (1 per second). As empagliflozin influences sodium reabsorption in proximal tubuli, we based this estimation and SD on our previous BOLD-MRI studies that

examined the influence of tubular sodium handling on tissue oxygenation. Dietary interventions that altered tubular sodium transport (a change in dietary sodium intake) induced R2* changes of ≈10%, albeit in the medulla.[18] Another study showed that furosemide induced medullary changes of R2*≈15%, and cortical changes of ≈5%.[19] Previous intervention studies have not often focused on drugs with mainly cortical actions, with the exception of a study by Schachinger et al,[20] where intravenous perfusion of angiotensin II altered cortical T2* (and thus R2*) by ≈10%. Finally, in animal studies with direct measurement of oxygenation by microelectrodes, cortical PO$_2$ increased by ≈20% after phlorizin in streptozotocin-induced diabetic rats, whereas no changes were seen in control Sprague-Dawley rats. On the basis of these data, we therefore decided to choose 10% as expected R2* change. This also seemed to be a clinically significant value. As such, the sample size required to demonstrate a significant effect with a power of 90% and an α type I error of 5% was 15 subjects per group[14]; 10 subjects per group were needed to have a power of 80%.

Statistical analysis was performed using STATA version 14.0 (StataCorp, College Station, TX). Quantitative variables are expressed as mean±SD, and qualitative variables are expressed as number of volunteers and percentage. Test of normality was performed followed by ANOVA or Student t test when appropriate. For repeated measurements, we performed a repeated-measures ANOVA that analyzes between-subjects (treatment) and within-subjects (time) effects. The treatment×time interaction was used to examine the treatment influences over time. In case of a significant treatment×time interaction by ANOVA, a test for simple effects was performed for analysis of the effect

RESULTS

From March 2017 to October 2018, 79 healthy volunteers were screened, among whom 34 were excluded because of exclusion criteria and 45 were randomized to placebo (n=15) or empagliflozin 10 mg (n=30). All subjects completed the AP, whereas 13 subjects in the placebo group (86.7%) and 27 in the empagliflozin group (90%) completed the CP (Figure S1).

Baseline characteristics of the study groups are presented in Table 1. Average age, weight, body mass index, sex distribution, office blood pressure, and heart rate did not differ between groups. Blood glucose was similar at baseline in both groups but slightly higher in the empagliflozin group 2 hours after 75 g of glucose.

RENAL BOLD-MRI

Table 2 and Figure 1 present the effects of empagliflozin or placebo on renal tissue oxygenation, as measured by the BOLD-MRI technique. Thirteen volunteers completed the first- and last-dose assessment in the placebo group and 27 in the empagliflozin group. Based on the strict criteria of image selection, 12 and 21 volunteers, respectively, passed the image selection criteria at all 4 sessions, as compared with 15 and 26 volunteers before and after the first dose. As such, a total of 148 BOLD-MRI exams were included in the analysis (Figure S2). Table 2 summarizes the mean cortical and medullary R* values and the slope of the right and left kidney R2* curves. Figure 1 illustrates the curves before and 180 minutes after the first (Figure A, B) and last tablet (Figure C and D) and at baseline (before drug intake) and after 1 month of treatment (Figure E and F). Empagliflozin and placebo effects on cortical and medullary R2* values did not differ 180 minutes after the first tablet or 180 minutes after the last tablet. After 1 month of treatment there was also no change in cortical R2* with placebo or empagliflozin (+0.4±0.6 and −0.4±0.2, respectively). After 1 month of treatment there was no change in medullary R2* with placebo and empagliflozin (+0.29±0.50 and −0.03±0.33, respectively). The slopes of right and left kidney R2* curves did not differ by treatment.

BIOCHEMICAL AND RENAL EFFECTS DURING AP AND CP

The biochemical and hormonal variations from baseline to 90 and 180 minutes after the first tablet (AP) and after the last tablet (CP after 1-month treatment) are detailed in Table 3.

Blood

Plasma glucose remained the same with placebo and empagliflozin 180 minutes after the first and last tablet. Overall, treatment had no significant acute effect on hemoglobin, hematocrit, plasma creatinine, sodium, potassium, and urate levels, although there was a clear trend toward an increase in hemoglobin and creatinine after the first dose of empagliflozin. The observed decrease in urea on first and last drug administration was most probably due to the hydration protocol as similar changes were observed with placebo.

Spot urine

The increase in urinary glucose/creatinine ratio was rapid and significant already 90 minutes after the first dose of empagliflozin, and rose higher at 180 minutes. After 1 month of treatment with empagliflozin, glucose/creatinine ratio was already high at baseline and increased further at 180 minutes after the last empagliflozin tablet. There was a nonsignificant decrease in FE_{Na} and FE_{Li} at 90 and 180 minutes with placebo suggesting sodium retention per protocol due to the fasting

Table 2. Renal BOLD-MRI at Baseline Before and After First Tablet and After 1-Month Therapy Before and After Last Tablet

| BOLD-MRI | Placebo | | | | Empagliflozin | | | | ANOVA |
| | Baseline | | 1-Month Treatment | | Baseline | | 1-Month Treatment | | |
	T0(AP)	T180(AP)	T0(CP)	T180(CP)	T0(AP)	T180(AP)	T0(CP)	T180(CP)	
Outer R2* (s⁻¹)*	20.6±1.3	21.5±1.9	21.0±2.4	20.5±1.3	20.1±1.9	20.5±2.1	19.8±1.8	20.3±2.2	0.09
Inner R2* (s⁻¹)†	23.9±1.7	24.7±2.0	24.2±1.9	24±1.6	23.9±1.7	24.6±2.1	23.9±1.5	24.4±1.9	0.7
Slope R (%)‡	12±6	11±5	12±5	12±4	13±4	13±3	12±3	13±5	0.5
Slope L (%)‡	12±5	13±5	12±5	12±4	12±5	12±5	13±5	11±4	0.7

For comparison, data from subjects having completed all four BOLD-MRIs are presented (n=12 placebo; n=21 empagliflozin). Data show results of repeated-measures analysis with *P* value for time×treatment effect. BOLD-MRI indicates blood-oxygen-level–dependent magnetic resonance imaging; T0(AP), time 0 min (acute phase); T180(AP), time 180 min (acute phase); T0(CP), time 0 (chronic phase); T180(CP), time 180 min (chronic phase).

Outer R2 = mean R2* of the three most superficial, cortical layers of renal parenchyma.

†Inner R2* = mean R2* of the eighth to tenth layer (corresponding to medulla) of renal parenchyma.
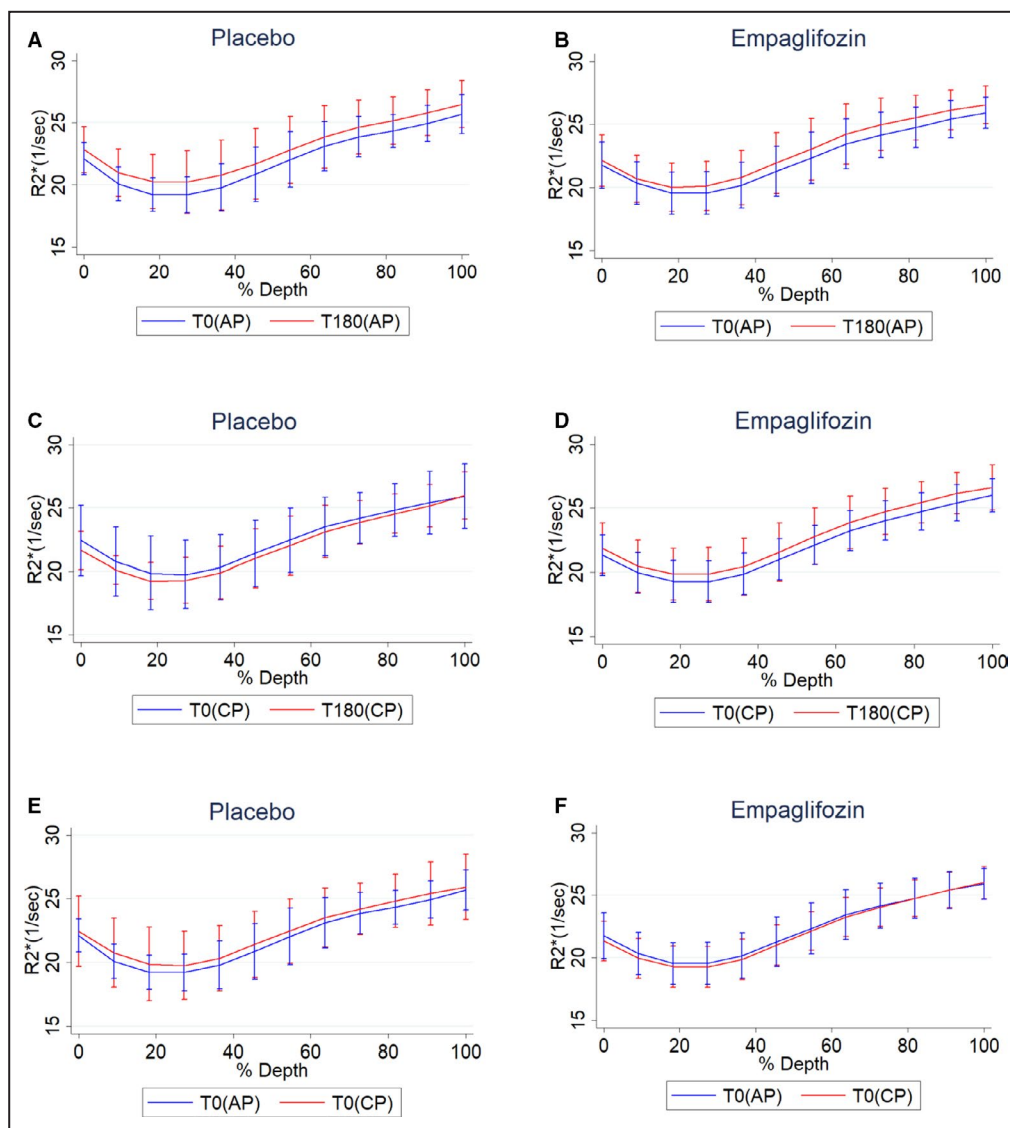
‡Slope = hertz per percent depth.

Figure 1. Placebo and empagliflozin R2* profile graphs summarizing the mean±SD R2* of the 12 layers. The percentage scale refers to the relative depth of each layer. The deepest layers are in the medulla and have a higher R2*, and thus lower oxygenation. **A** and **B**, Curves before and after the first tablet (acute phase, T0(AP) and T180(AP)). **C** and **D**, Curves before and after the last tablet (chronic phase, T0(CP) and T180(CP)). **E** and **F**, Curves at baseline and after 1 month of treatment (T0(AP) and T0(CP)).

state. In contrast, there was a sharp and significant increase in FE_{Na} and a moderate increase in FE_{Li} after the first empagliflozin tablet, suggesting a natriuretic effect of empagliflozin due to reduced reabsorption of sodium in the proximal tubule. Increases in FE_{Na} and FE_{Li} were more modest after 1 month of therapy. For comparison, the percent changes in FE_{Li} from T0(AP) and T180(AP) were −21.8% and +12.8% with placebo and empagliflozin (+34.6% empagliflozin-induced difference), respectively, showing proximal sodium retention per protocol with placebo but increase in proximal sodium excretion with empagliflozin that persisted after 1 month but to a milder degree. Likewise, the percent changes in FE_{Na} from T0(AP) and T180(AP) were −11.5% and +35.6%

with placebo and empagliflozin (+47.1% empagliflozin-induced difference), respectively, and from T0(CP) and T180(CP) of −13.0% and +8.0% (+21% empagliflozin-induced difference).

CLINICAL, BIOCHEMICAL, AND RENAL EFFECTS BEFORE AND AFTER 1-MONTH TREATMENT

Changes in Body Weight and Blood Pressure

Weight did not change with placebo (Table 4). There was a clear trend toward a decrease in weight in the empagliflozin group, which was significant after 1

Table 3. Mean ± SD at Different Timepoints (T0, T90, T120)

3a. Acute Phase

	Placebo			Empagliflozin			Time	Treatment	TimexTreatment
	T0(AP)	T90(AP)	T180(AP)	T0(AP)	T90(AP)	T180(AP)			
Blood									
Fasting glucose, mmol/L	4.8±0.4	NA	4.7±0.3	4.8±0.4	NA	4.7±0.4	0.06	0.9	0.7
Hemoglobin, g/L	145.6±12.3	145.6±13.1	145.9±11.7	143.6±11.4	143.2±11.5	144.8±12.6	0.2	0.6	0.4
Hematocrit, %	42.3±3.1	42.0±3.5±1.3	42.3±3.2	41.4±3.1	41.6±3.3	41.8±3.2	0.4	0.5	0.7
Sodium, mmol/L	139.7±1.2	139.8±1.5	139.5±1.3	139.3±1.7	139.3±1.4	139.5±1.4	0.9	0.5	0.4
Potassium, mmol/L	3.9±0.3	3.8±0.2	3.8±0.2	4.0±0.2	4.0±0.5	3.9±0.3	0.4	0.1	0.9
Urate, μmol/L	269±70	272±71	273±71	299±72	299±74	297±70	0.6	0.2	0.07
Creatinine, μmol/L	74.0±10.7	74.1±10.6	72.9±9.4	74.9±12.2	76.9±12.4	76.3±12.6	0.3	0.5	0.2
Urea, mmol/L	4.4±1.0	4.0±0.9*	3.9±0.9*	4.0±2.0	3.8±0.9*	3.7±0.9*	<0.0001	0.4	**0.02**
Urine									
Volume	0	637±192	357±192	0	650±253	432±248	NA	0.5	0.6
Glucose/creatinine	0.04±0.02	0.04±0.02	0.05±0.01	0.04±0.01	6.3±2.8*	20.5±5.1*	**<0.0001**	**<0.0001**	**<0.0001**
Sodium/creatinine ratio	18.1±7.6	15.8±7.3	16.4±7.2	19.3±7.7	19.0±6.7	25.4±8.1*	**0.002**	**0.04**	**0.0002**
FE_{Na}	9.5±3.8	8.1±3.2	8.4±3.3	10.1±3.7	10.3±3.8	13.7±4.6*	**0.003**	**0.01**	**0.0002**
FE_{Li}	17.0±9.5	13.3±4.1	13.3±5.6	13.2±4.9	14.6±9.2	14.9±7.4	0.6	0.9	**0.04**

3b. Chronic Phase

	Placebo			Empagliflozin			Time	Treatment	TimexTreatment
	T0(CP)	T90(CP)	T180(CP)	T0(CP)	T90(CP)	T180(CP)			
Blood									
Fasting glucose, mmol/L	4.8±0.3	NA	4.7±0.3	4.7±0.4	NA	4.7±0.7	0.3	0.7	0.5
Hemoglobin, g/L	145.4±11.0	142.8±12.5	144.5±12.8	145.5±12.4	144.3±11.3	145.6±10.6	**0.04**	0.7	0.2
Hematocrit, %	42.0±3.0	41.5±3.3	41.5±3.4	42.2±3.2	41.9±2.9	42.6±2.9	0.1	0.5	0.07
Sodium, mmol/L	139.8±1.6	139.8±1.3	140.5±1.5	139.2±1.6	138.4±2.0	138.9±1.5	0.09	**0.02**	0.2
Potassium, mmol/L	4.0±0.4	3.8±0.2	3.9±0.4	4.0±0.3	4.1±0.5	3.9±0.2	0.4	0.5	0.1
Urate, mmol/L	286±91	288±92	291±91	218±53	220±52	223±49	**0.01**	**0.005**	0.9
Creatinine, μmol/L	74.3±11.6	72.9±11.3	74.4±12.1	76.3±12.5	77.2±12.6	76.9±13.4	0.8	0.5	0.4
Urea, mmol/L	4.0±0.9	3.8±0.8*	3.6±0.7*	4.1±0.8	3.9±0.8*	3.8±0.8*	**<0.0001**	0.6	0.1
Urine									
Volume	0	577±180	414±230	0	496±181	322±141	NA	0.1	0.2
Glucose/creatinine	0.05±0.02	0.04±0.01	0.05±0.01	10.2±6.8	9.6±4.5	15.7±4.7*	**0.01**	**<0.0001**	**0.01**

(Continued)

Table 3. Continued

	Placebo			Empagliflozin			Time	Treatment	Time×Treatment
	T0(CP)	T90(CP)	T180(CP)	T0(CP)	T90(CP)	T180(CP)			
3b. Chronic Phase									
Sodium/creatinine ratio	20.8±8.4	18.9±8.3	19.9±5.2	16.2±7.1	14.0±4.8*	17.3±6.1	0.1	0.08	**0.04**
FE_{Na}	10.8±3.9	9.6±3.8	9.4±2.7	8.7±3.6	7.7±3.1	9.4±3.4	0.08	0.1	**0.049**
FE_{Li}	14.5±9.4	11.2±4.0	11.2±2.9	14.3±5.1	13.0±4.9	15.6±7.5	0.1	0.2	0.1

Data show results of repeated-measures analysis of variance, effect of time and treatment and effect of time×treatment. FE_{Li}, fractional excretion of lithium; FE_{Na}, fractional excretion of sodium AP (acute phase); CP (chronic phase).

*values indicate significance vs T0 within treatment group when time×treatment interaction was significant.

and 2 weeks. At 4 weeks, however, the differences in weight were no longer significant (Table 4). Office systolic and diastolic blood pressures and heart rate were similar at baseline and after 1 month of placebo. Office systolic blood pressure decreased by an average of 4.0 mm Hg with empagliflozin ($P=0.05$), but office diastolic blood pressure and heart rate did not differ. Twenty-four-hour ambulatory blood pressure measurements showed significant decreases in systolic (mean±SD: placebo, +2.8±6.3 mm Hg; empagliflozin, −5.1±6.7 mm Hg; $P=0.0005$) and diastolic (placebo, +1.5±4.4 mm Hg; empagliflozin, −2.0±5.5 mm Hg; $P=0.03$) blood pressure with empagliflozin (Figure 2).

Biochemical and hormonal changes after 1-month treatment

Creatinine clearance, 24-hour urinary sodium excretion, sodium, potassium, urate, and hormonal parameters (plasma renin activity, aldosterone) were not different between groups at baseline (Table 4). Fasting plasma glucose, insulin, and Homeostatic Model of Insulin Resistance insulin resistance index did not change in both groups. Urinary glucose/creatinine ratio increased with empagliflozin in all volunteers. Total urinary glucose excretion ranged between 0.4±0.2 to 0.5±0.3 mmol at baseline or with placebo, whereas it increased to 285.4±85.8 mmol (51.4±15.4 g) after 1-month treatment with empagliflozin.

Fasting hemoglobin and hematocrit were similar at baseline in both groups. After 1-month treatment, hemoglobin and hematocrit levels increased with empagliflozin. Erythropoietin levels did not change from baseline to 1-month therapy with empagliflozin (60.3±30.8 pg/mL at baseline and 60.3±25.1 pg/mL after 1-month treatment).

Plasma sodium, potassium, and creatinine levels remained the same in both groups. Creatinine clearance did not change. Plasma uric acid levels decreased significantly with empagliflozin (−86±36 µmol/L, $P<0.0001$).

Total 24-hour urinary sodium excretions were 182±63 mmol and 203±93 mmol at baseline, respectively, in the placebo and empagliflozin groups and did not change significantly after 1-month treatment. Twenty-four-hour FE_{Na} did not vary after 1 month of treatment, whereas FE_{Li} increased significantly with empagliflozin (Table 4). Plasma aldosterone levels increased significantly with empagliflozin (+36.9±61.3 pmol/L, $P=0.002$), as did plasma renin activity (+0.18±0.41 ng/mL per hour, $P=0.02$).

Significant 24-hour blood pressure changes and biochemical changes in renal BOLD-MRI measurements are presented in Figure 2.

Table 4. Effects of 1-Month Treatment on Clinical and Biochemical Parameters

	Placebo				Empagliflozin 10 mg				Placebo vs Empagliflozin
4a. Clinical	Baseline T0(AP)	1 Month T0(CP)	Delta	P Value	Baseline T0(AP)	1 Month T0(CP)	Delta	P Value	P Value*
Weight, kg	88.9±22.8	89.2±22.8	+0.3±0.9	0.3	87.8±17.4	87.4±17.8	−0.5±1.9	0.2	0.2
Office SBP, mm Hg	118.8±10.3	120.4±9.9	+1.6±10.8	0.6	120.5±12.3	116.6±13.9	−4.0±12.1	0.05	0.08
Office DBP, mm Hg	72.4±8.6	71.4±9.6	−1.0±7.4	0.6	73.7±11.3	71.1±9.3	−2.6±10.5	0.2	0.6
Pulse, bpm	61.1±9.5	59.5±6.9	−1.5±5.8	0.4	63.1±8.8	65.3±10.9	+2.1±12.2	0.4	0.3
24-h BP measurement									
24-h SBP (mm Hg)	111.9±8.4	114.8±11.0	+2.9±6.3	0.1	117.1±9.1	112.0±8.5	−5.1±6.7	**0.0003**	**0.0005**
24-h DBP, mm Hg	71.5±6.8	73±8.1	+1.5±4.4	0.2	72.8±5.9	70.9±6.4	−2.0±5.5	**0.04**	**0.03**
24-h pulse, mm Hg	74.1±12.4	72.5±12.7	−1.5±9.5	0.6	75.5±8.3	73.8±7.7	−1.6±9.7	0.4	0.9
4b. Blood	Baseline T0(AP)	1 Month T0(CP)	Delta	P Value	Baseline T0(AP)	1 Month T0(CP)	Delta	P Value	P Value*
Hemoglobin, g/L	145.6±12.3	145.4±11.0	−0.2±3.7	0.6	143.6±11.4	146.1±12.6	+2.5±11.4	**0.02**	0.06
Hematocrit, %	42.3±3.4	42.0±3.0	−0.3±1.2	0.8	41.7±3.2	42.3±3.3	+0.6±1.9	0.06	0.06
Fasting plasma glucose, mmol/L	4.81±0.40	4.84±0.34	+0.02±0.24	0.4	4.82±0.37	4.72±0.35	−0.1±0.38	0.9	0.2
Insulin, mmol/L	6.87±3.04	6.90±3.32	+0.02±2.46	0.9	6.79±5.24	7.78±5.10	+0.99±4.42	0.3	0.5
HOMA-IR	1.5±0.6	1.5±0.7	+0.0±0.6	0.9	1.5±1.2	1.7±1.1	+0.2±1.0	0.4	0.6
Sodium, mmol/L	139.5±1.3	139.8±1.6	+0.2±1.5	0.3	139.4±1.7	139.2±1.6	−0.3±1.8	0.8	0.4
Potassium, mmol/L	3.9±0.3	4.0±0.4	+0.2±0.4	0.09	4.0±0.2	4.0±0.3	+0.0±0.3	0.4	0.2
Urate, µmol/L	275±73	286±91	+11±36	0.2	303±70	218±53	−86±36	**<0.0001**	**<0.0001**
Creatinine, µmol/L	74.7±11.3	74.3±11.6	−0.4±8.6	0.4	76.6±11.4	76.3±12.5	−0.3±6.9	0.4	0.5
Urea, mmol/L	4.3±0.9	4.0±0.9	−0.3±0.9	0.2	4.0±0.9	4.1±0.8	+0.1±0.7	0.4	0.05
Aldosterone, pmol/L	42.7±25.7	55.5±27.2	+12.8±27.4	0.06	66.3±55.8	103.3±83.4	+36.9±61.3	**0.002**	0.09
Plasma renin activity, ng/mL per hour	0.48±0.24	0.43±0.25	−0.05±0.22	0.5	0.63±0.44	0.81±0.44	+0.18±0.41	**0.02**	**0.04**

(Continued)

Table 4. Continued

4c. 24-h Urine Collection	Placebo				Empagliflozin 10 mg				Placebo vs Empagliflozin
	Baseline T0(AP)	1 Month T0(CP)	Delta	P Value	Baseline T0(AP)	1 Month T0(CP)	Delta	P Value	P Value*
Creatinine clearance, mL/min	145±28	159±46	+14±46	0.1	152±52	147±55	−5.0±51	0.6	0.2
Glucose/creatinine, mmol/mmol	0.03±0.01	0.03±0.02	+0.01±0.02	0.3	0.03±0.01	19.94±7.33	+19.91±7.32	**<0.0001**	**<0.0001**
24-h glucose excretion, mmol	0.4±0.2	0.6±0.3	+0.1±0.2	0.2	0.5±0.03	285.4±85.8	+285.9±85.7	**<0.0001**	**<0.0001**
Na/creatinine, mmol/μmol	12.8±5.0	14.3±6.6	+1.5±7.4	0.5	13.1±5.6	12.5±3.1	−0.6±5.0	0.5	0.3
FE_{Na}	0.66±0.21	0.66±0.14	+0.005±0.26	0.9	0.70±0.25	0.66±0.16	−0.4±0.26	0.4	0.6
24-h sodium excretion, mmol	182±63	229±122	+46±119	0.2	203±93	188±81	−15±105	0.5	0.05
FE_{Li}	13.6±7.0	11.3±5.3	−2.2±7.9	0.3	12.9±5.1	15.0±7.0	+2.1±7.2	0.08	**0.049**

Data show results of paired t test within treatment groups. AP indicates acute phase; bpm, beats per minute; CP, chronic phase; DBP, diastolic blood pressure; FE_{Li}, fractional excretion of lithium; FE_{Na}, fractional excretion of sodium; HOMA-IR, Homeostatic Model of Insulin Resistance; and SBP, systolic blood pressure.

*Comparison of changes in placebo vs empagliflozin treatment groups using unpaired two-sample t test.

Renal Ultrasound

Renal resistance indexes remained the same with placebo and empagliflozin (delta differences [mean±SD]: right kidney: placebo, −0.001±0.132; empagliflozin, −0.014±0.147; left kidney: placebo, −0.005±0.053; empagliflozin, −0.017±0.080). Renal lengths also did not change with placebo and empagliflozin (delta differences: right kidney: placebo, −0.47±4.46; empagliflozin, 0.97±5.47; left kidney: placebo, 1.75±6.31; empagliflozin, −1.04±5.69).

DISCUSSION

This study has shown that, in healthy normotensive and nondiabetic subjects, empagliflozin decreases 24-hour systolic blood pressure by 5 mm Hg with significant renal tubular effects (Tables 3 and 4, Figure 2), but with no alteration of renal tissue oxygenation in the cortex or in the medulla (Table 2, Figure 1). This observation was made despite the fact that empagliflozin increased urinary glucose and proximal sodium excretion both acutely and chronically, as reflected by changes in fractional excretion of endogenous lithium (Tables 3 and 4). Chronically, however, the increase in aldosterone contributed to counteract the natriuretic effect of empagliflozin (Table 4). Empagliflozin also increased hemoglobin, decreased serum uric acid, and stimulated the renin-angiotensin system (Table 4).

The primary hypothesis of this study, namely that empagliflozin increases cortical oxygenation by 10%, was not confirmed in these nondiabetic, normotensive subjects (Table 2, Figure 1). The absence of changes in cortical oxygenation may seem surprising in the context of a marked reduction in oxygen-consuming glucose and sodium reabsorption in the cortical proximal tubules. Although renal sodium handling is a major determinant of oxygen consumption by kidneys, a first explanation may be that changes in oxygenation were too modest to affect the BOLD-MRI signal. In this context, Prasad et al did not observe significant changes in cortical R2* values after a single dose of acetazolamide (a diuretic acting on the proximal tubules).[15] However, their study was performed in only six subjects at 1.5 T. Since then, studies performed at higher magnetic field strength have shown that changes in cortical R2* of 5% to 10% can be observed with other drugs such as angiotensin-converting enzyme inhibitors and nitric oxide synthase inhibitors.[21,22] Hence, BOLD-MRI performed at 3 T in our study should be sensitive enough to capture drug-induced changes in cortical oxygenation if they are large enough.

As oxygen-consuming sodium transport is the main determinant of renal tissue oxygenation, another possibility is that changes in sodium transport
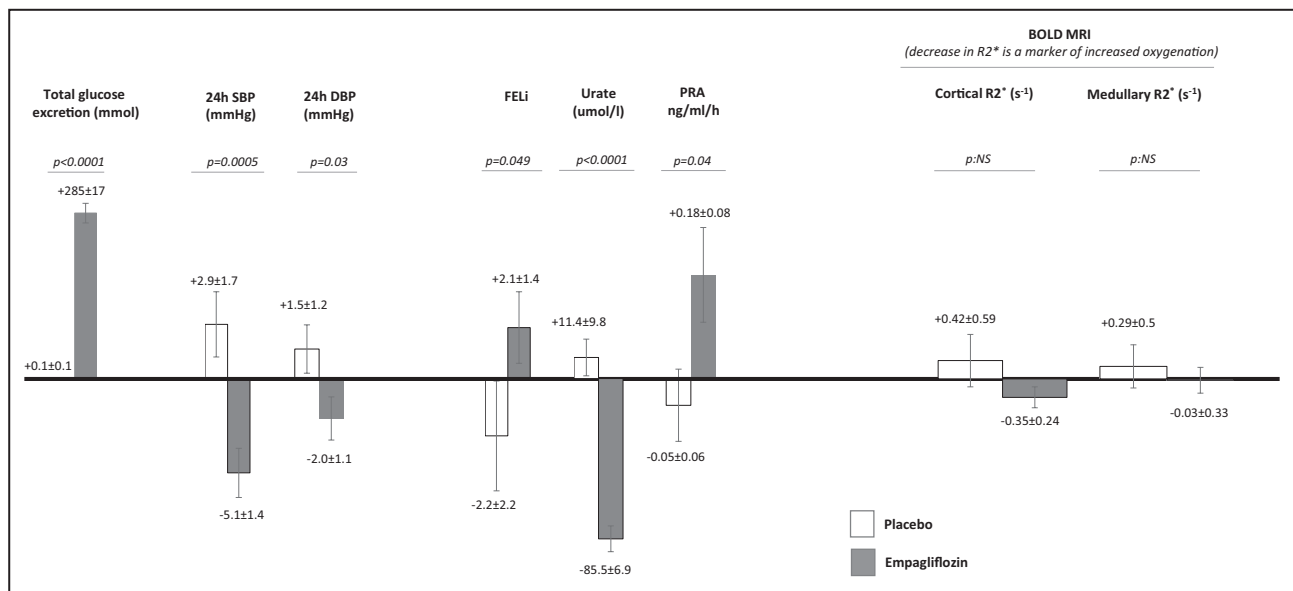
Figure 2. Summary of significant variations from baseline to 1-month treatment in placebo and empagliflozin groups. Data are expressed as mean±SEM. Delta variations in placebo and empagliflozin groups were compared by unpaired two-sample t tests. BOLD-MRI indicates blood oxygenation-level–dependent magnetic resonance imaging; DBP, diastolic blood pressure; FELi, fractional excretion of lithium; PRA, plasma renin activity; and SBP, systolic blood pressure.

with empagliflozin were not large or acute enough to alter oxygenation. Studies showed that extreme changes in sodium intake (>10-fold) altered medullary but not cortical oxygenation in healthy volunteers.[18] Furthermore, in humans, the only diuretic demonstrating short-term effects on renal tissue oxygenation thus far is intravenously administered furosemide.[12] This loop diuretic induces acute natriuresis around 8-fold higher than the 0.1-mol/min increase with empagliflozin in our study.

Our results are in line with a previous study in Sprague-Dawley rats that also showed no change in cortical PO_2 as measured with Clark microelectrodes after a single dose of phlorizin. However, medullary PO_2 decreased in these healthy rats, which was explained by the authors as a shift in active Na^+ transport from proximal to more distal nephron segments.[23] In contrast, in rats with streptozotocin-induced diabetes mellitus, phlorizin restored the decreased cortical oxygen tension to normal levels while decreasing medullary oxygen tension. In another study using a computational rat kidney model, it was predicted that SGLT2 inhibition would shift oxygen-consuming active transport to the medulla and enhance hypoxia.[9] In this sense, our data are reassuring as the shift to more distal nephrons did not lead to a decrease in medullary oxygenation measured with BOLD-MRI in humans, as previously hypothesized by Heyman et al.[24,25]

One should keep in mind that our study enrolled healthy subjects with a normal renal function and no proteinuria. We primarily included healthy subjects

to study the effects of empagliflozin in those without diabetes mellitus, but also to avoid the confounding factor of blood glucose fluctuations on the BOLD-MRI signal, as recently described by our group.[12,13] It remains to be demonstrated whether empagliflozin has a similar impact on patients with diabetes mellitus characterized by glomerular hyperfiltration and excessive sodium reabsorption in the proximal tubules.

In this study we have demonstrated that empagliflozin decreases the reabsorption of sodium in the proximal tubule using an endogenous lithium clearance technique (Table 3). Fractional excretion of lithium is a reliable marker of proximal sodium reabsorption as lithium is reabsorbed in parallel to water and sodium in the early segments of the proximal tubules. The principle of using endogenous lithium as a marker of proximal sodium reabsorption is that lithium is normally not reabsorbed in postproximal segments. Lithium transport in the thick ascending limb is not impossible and, in the more distal segment, lithium may be reabsorbed in cases of dehydration and a stimulated vasopressin system.[26] However, in our study, subjects were well hydrated per protocol and avoided distal reabsorption[27] despite a possible stimulation of vasopressin, as suggested by Eickhoff et al.[28] Moreover, the early segments of the proximal tubule in which lithium is reabsorbed are also those where SGLT2 modulates glucose reabsorption. Our observation confirms a recent observation in T2DM subjects indicating that dapagliflozin increased fractional excretion of lithium by an average of 19.6%.[28] Likewise, there was a compensatory activation of the

renin-angiotensin system. Interestingly, in our study, in spite of these compensatory mechanisms, 24-hour systolic blood pressure decreased by an average of 5.1 mm Hg after 1 month of empagliflozin in normotensive subjects. This response is even more pronounced than that seen after 3-month treatment in patients with T2DM and hypertension.[29] The mechanisms of blood pressure reduction were independent of weight control but may be related to mild volume contraction, as suggested by the increase in hemoglobin and hematocrit.

When comparing these data with those seen in T2DM patients, although the effects on 24-hour glucosuria are milder,[30] the lowering effects on systolic blood pressure[29] and plasma uric acid levels seem more pronounced.[31] However, the decrease in proximal sodium reabsorption and activation of the renin-angiotensin system are comparable.[28] Finally, we could not demonstrate any increase in erythropoietin levels at 1 month, in contrast to T2DM patients with coronary artery disease.[32] These similarities and differences should be considered when comparing our neutral results of SGLT2 inhibition on renal BOLD-MRI measurements with future studies.

Our study has both limitations and strengths. As we used very strict selection criteria for image quality and anatomic coherence to minimize bias, a certain number of subjects were excluded from analysis. In addition, the chosen threshold of a hypothetical empagliflozin-induced change of 10% in R2* was empirical. However, the observed changes in R2* were minimal between the visits, underlining the high reproducibility of BOLD-MRI. Another limitation is that our study cohort included normotensive, young healthy volunteers. It is not known whether these the results can be generalized to patients with T2DM at risk of diabetic kidney disease.

In summary, this is the first study to examine simultaneously the effects of empagliflozin on renal oxygenation, tubular sodium transport, and blood pressure in humans. We have demonstrated that empagliflozin has beneficial metabolic and blood pressure effects but does not affect renal tissue oxygenation acutely or chronically in nondiabetic subjects with normal renal function. It remains to be explored whether these results can be generalized to diabetic or nondiabetic patients with congestive heart failure or kidney disease.

ARTICLE INFORMATION

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Disclosures

Supplementary Materials

Figures S1–S2

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SUPPLEMENTAL MATERIAL

Figure S1. Flow chart of the study.
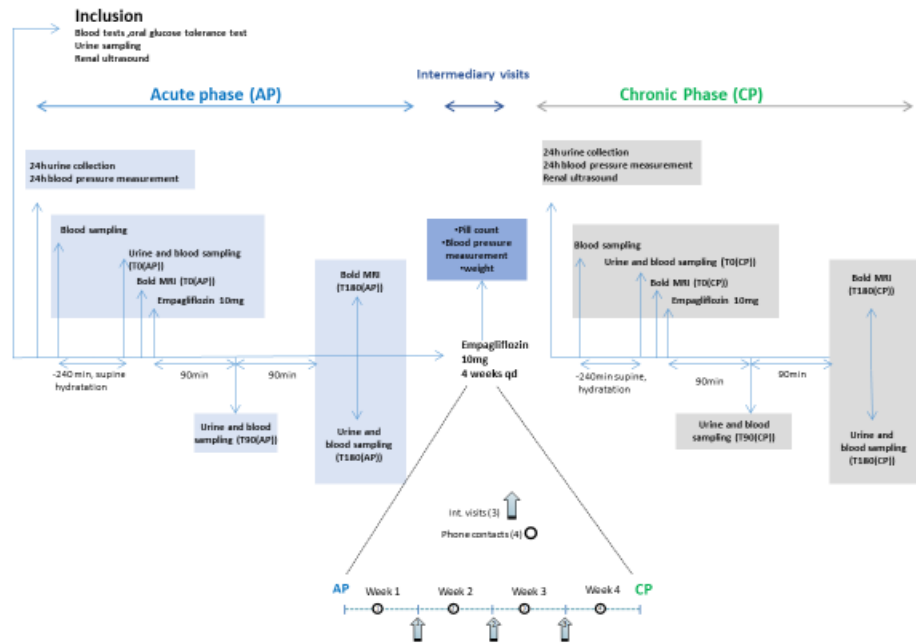
Acute phase (AP), Chronic phase (CP) with 24h urine collections, 24h ambulatory blood pressure measurements, blood and urine sampling and renal BOLD-MRI sessions. T0 (baseline before pill), T90, T120 (90 and 120 minutes after oral pill), SAE: serious adverse effect

Figure S2. Flow diagram of subjects from screening to completion of the study.
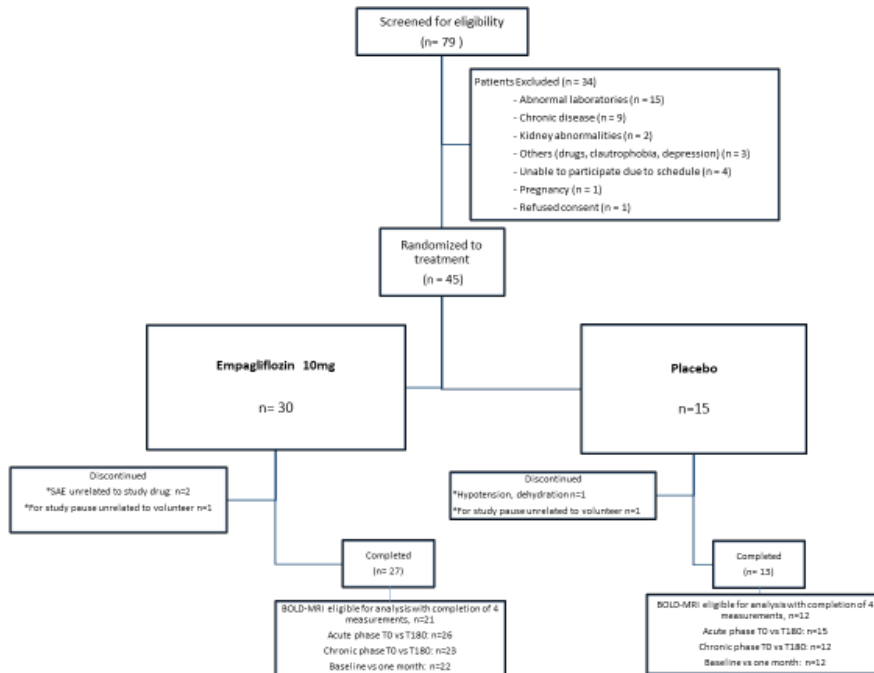
Three volunteers enrolled in the empagliflozin group did not terminate the study (2 for hospitalizations not related to empagliflozin and one due to study pause unrelated to volunteer), and two in the placebo group (one for hypotension and dehydration and the other for study pause unrelated to volunteer). Following the 2 hospitalizations, the study was paused for 2 weeks waiting for the approval from the national health regulating authorities Swissmedic and the ethical committee to confirm that these events were not related to the study drug and the permission to complete the study.