REVIEW

Gliflozins in the Treatment of Non-diabetic Experimental Cardiovascular Diseases

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Summary

A new class of antidiabetic drugs - gliflozins (inhibitors of sodium glucose cotransporter-2; SGLT-2i) stimulate glucose and sodium excretion, thereby contributing to improved glycemic control, weight loss and blood pressure reduction in diabetic patients. Large clinical trials in patients with type 2 diabetes treated with empagliflozin, canagliflozin or dapagliflozin have demonstrated their excellent efficacy in improving many cardiovascular outcomes, including the reduction of death from cardiovascular diseases, non-fatal myocardial infarction or stroke, and hospitalization for heart failure. Moreover, the beneficial effects of SGLT-2i were also demonstrated in the decrease in proteinuria, which leads to a lower risk of progression to end-stage renal disease and thus a delay in initiation of the renal replacement therapy. Unexpectedly, their cardioprotective and renoprotective effects have been demonstrated not only in patients with diabetes but also in those without diabetes. Recently, much effort has been focused on patients with heart failure (either with reduced or preserved ejection fraction) or liver disease. Experimental studies have highlighted pleiotropic effects of SGLT-2 inhibitors beyond their natriuretic and glycosuric effects, including reduction of fibrosis, inflammation, reactive oxygen species, and others. Our results in experimental non-diabetic models of hypertension, chronic kidney disease and heart failure are partially consistent with these findings. This raises the question of whether the same mechanisms are at work in diabetic and non-diabetic conditions, and which mechanisms are responsible for the beneficial effects of gliflozins under non-diabetic conditions. Are these effects cardio-renal, metabolic, or others? This review will focus on the effects of gliflozins under different pathophysiological conditions, namely in hypertension, chronic kidney disease, and heart failure, which have been evaluated in non-diabetic rat models of these diseases.

Key words

SGLT-2 inhibitor • Hypertension • Chronic kidney disease • Heart failure • Liver disease • Rat

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Introduction

A new class of antidiabetic drugs - gliflozins (inhibitors of sodium glucose cotransporter-2; SGLT-2) enhance glucose and sodium excretion, thereby contributing to improved glycemic control, weight loss and blood pressure reduction in diabetic patients. Their history dates back to the first half of the 19th century, when the first naturally occurring non-selective SGLT inhibitor, phlorizin, was exctracted from apple bark tree [1]. It induced not only glycosuria by acting on the renal SGLT-2 transporter, but also diarrhoea by inhibiting the intestinal SGLT-1 transporter. More than 15 years years ago, a first synthetic analog of phlorizin, dapagliflozin, was tested in the treatment of diabetic patients [2], followed shortly by empagliflozin and canagliflozin, all with high selectivity for SGLT-2 transporter [3,4]. The mechanism by which glucose is returned to the blood from the ultrafiltrate is a two-step process [5]. The first step is the reabsorption of glucose together with sodium from the glomerular filtrate against the concentration gradient via the sodium-glucose cotransporter-2 located in the S1 segment of the proximal tubule of kidney. The energy required for this process is generated by Na^+/K^+ ATPase. In the second step, glucose is transported back into circulation by GLUT-2 transporter accross the basolateral membrane of proximal tubule. Under the physiological conditions, 90 % of the filtered

glucose is reabsorbed in this part of the kidney. Inhibition of SGLT-2 transporter results in a substantial loss of glucose to the urine [6]. Diabetes is associated with glomerular hyperfiltration due to the disturbances in tubulo-glomerular feedback (TGF) mechanism. Importantly, SGLT-2 inhibitors are able to modulate TGF by increasing sodium delivery to the macula densa, ultimately leading to afferent arteriolar constriction [7]. Although the effects of gliflozins were originally restricted to the kidney and heart, the pleiotrophic effects of gliflozins have now been demonstrated in many other organs, such as the liver, pancreas or adipose tissue [8,9].

The beneficial cardiovascular effects of gliflozins were first demonstrated in diabetic patients with established cardiovascular disease (CVD) in EMPA-REG OUTCOME [3] trial with empagliflozin, which resulted in impressive improvement in many cardiovascular outcomes, including reductions of death from cardiovascular diseases, non-fatal myocardial infarction or stroke. Importantly, there was a significant reduction in hospitalization for heart failure. Two other clinical trials with canagliflozin (CANVAS) [4] or dapagliflozin (DECLARE-TIMI) [10] confirmed that these benefits are not unique to empagliflozin, but are class effects of all SGLT-2 inhibitors. Moreover, the CREDENCE trial [11] demonstrated significant reduction in proteinuria, which led to a lower risk of progression to end-stage renal disease and thus to a delay in the initiation of renal replacement therapy. Later, the benefits of gliflozin therapy were demonstrated not only in patients with diabetes but also in those without diabetes. The DAPA-CKD trial [12] and the EMPA-KIDNEY [13,14] were the trials, that showed renoprotection after canagliflozin or empagliflozin treatment in patients with chronic kidney disease with or without diabetes. In addition, patients with heart failure (HF) also benefited significantly from SGLT-2 inhibition, with the DAPA-HF trial [14] enrolling HF patients treated with dapagliflozin. Subsequently, trials in selected populations of HF patients with reduced ejection fraction (EMPEROR-REDUCED) [14] and preserved ejection fraction (EMPEROR-PRESERVED) [15] demonstrated not only cardiovascular but also renal benefits of empagliflozin treatment. Together, these clinical trials enrolled approximately one hundred thousands of patients with various diagnoses and demonstrated improvements of cardiovascular and renal parameters. The question is whether the same mechanisms are at work in diabetic and non-diabetic conditions, and which mechanisms are responsible for these beneficial effects of gliflozins [16]. Are these effects rather cardio-renal, metabolic, or others? This review will focus on the effects of gliflozins under different pathophysiological conditions, namely hypertension, chronic kidney disease and heart failure, as evaluated in non-diabetic rat models of these diseases.

Experimental models of hypertension

Ren-2 transgenic rats

The Ren-2 transgenic rat (TGR) is a model of angiotensin II-dependent hypertension resulting from the insertion of the mouse Ren-2 gene into the genome of normotensive Hannover-Sprague Dawley rats [17]. This hypertension develops early in life in heterozygous animals, but they survive well throughout life. The 8-week treatment with empagliflozin in adult 6-month-old heterozygous TGR resulted in the reduction of their body weight and the size of fat depots despite their higher food consumption (Table 1) [18]. Moreover, telemetric blood

Table 1. Empagliflozin effects in three non-diabetic hypertensive models

	TGR	HHTG	SHR-CRP
Body weight	↓	↓	↓ (young)
Fat mass	↓	↓	↓ (adult)
Hyperphagia	↑	↑	NA
Proteinuria	↔	NA	↓ (young)
Liver function	↑	↑	↑ (adult)
Cardiac function	↔	↔	↑ (adult)
Inflammation	↓	↓ (liver)	↓ (young) kidneys
Oxidative stress	↓ (kidneys)	↓ (liver)	↓ (young) kidneys
Blood pressure	↓	↔	↔

TGR – Ren-2 transgenic rats, HHTG – hereditary hypertriglyceridemic rats, SHR-CRP – Spontaneously hypertensive rats expressing human C-reactive protein

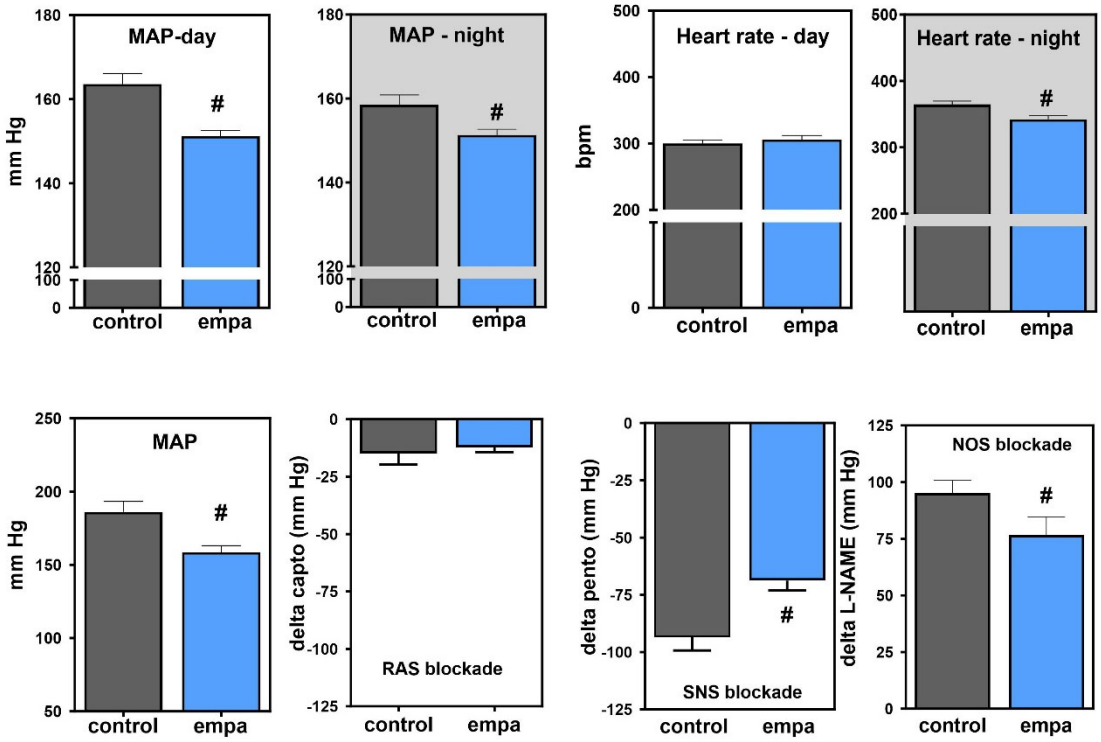

Fig. 1. Ren-2 transgenic rats. The effect of empagliflozin treatment on day and night blood pressure and heart rate measured by radiotelemetry (upper panel), and on mean arterial pressure (MAP) and its changes induced by sympathetic nervous system (SNS) blockade, renin-angiotensin system (RAS) blockade, and nitric oxide synthase (NOS) blockade in conscious rats of control and empagliflozin-treated Ren-2 transgenic rats (empa) (lower panel). # $p<0.05$ vs. control untreated group. Data are means ± SEM.

pressure recording demonstrated a significant reduction of both daytime and nighttime blood pressure, which was associated with a decrease of heart rate during the active (dark) part of the day (Fig.1 upper panels). Our results showed that this BP decrease was mainly due to the attenuation of sympathetic vasoconstriction, with no effect of angiotensin II-dependent vasoconstriction (Fig.1 lower panels).

Unexpectedly, we did not find any effects of empagliflozin on renal function (with the exception of decreased plasma urea) or cardiac function. Regarding the metabolic effects of empagliflozin, there were significant reductions in plasma insulin and non-esterified fatty acid levels, as well as liver and myocardial triglyceride content. Importantly, empagliflozin exerted anti-inflammatory and antioxidant effects (reduction of plasma leptin, TNF-α and reduced/oxidized glutathione ratio in kidneys).

Hereditary hypertriglyceridemic rat

Hereditary hypertriglyceridemic rats (HHTG) are a non-obese prediabetic rat model [19] with several signs of metabolic syndrome, namely hypertriglyceridemia and impaired glucose tolerance, represented by elevated non-fasting glucose and impaired oral glucose tolerance. Our study in adult 6-month-old HHTG [20] demonstrated that empagliflozin treatment not only improved glucose tolerance, as manifested by reduced fasting and non-fasting glucose, but also increased insulin sensitivity in skeletal muscles. Moreover, ectopic triglycerides and lipotoxic diacylglycerols in the liver were reduced after empagliflozin treatment. This was associated with decreased expression of lipogenic enzymes (Fasn and Scd1) and the lipogenic transcription factor Srebf1 in the liver. We also demonstrated changes in gene expression of the cytochrome P450 family involved in lipid metabolism. The decrease in hepatokines FGF21 and fetuin-A, as well as the attenuation of oxidative stress and increased antioxidant enzyme activity (superoxide dismutase and glutathione peroxidase) contributed to the beneficial hepato-protective effects of empagliflozin in this particular model. Similar to TGR rats, empagliflozin reduced body weight and fat mass in HHTG rats, independent of their hyperphagia. In contrast to TGR, telemetric BP monitoring did not detect any effect of SGLT-2 inhibition on blood pressure or heart rate (Fig. 2 upper panel) or on the major vasoactive systems contributing to BP maintenance (Fig. 2. lower panel). Similarly, we did not observe any effect on cardiac parameters evaluated by echocardiography.

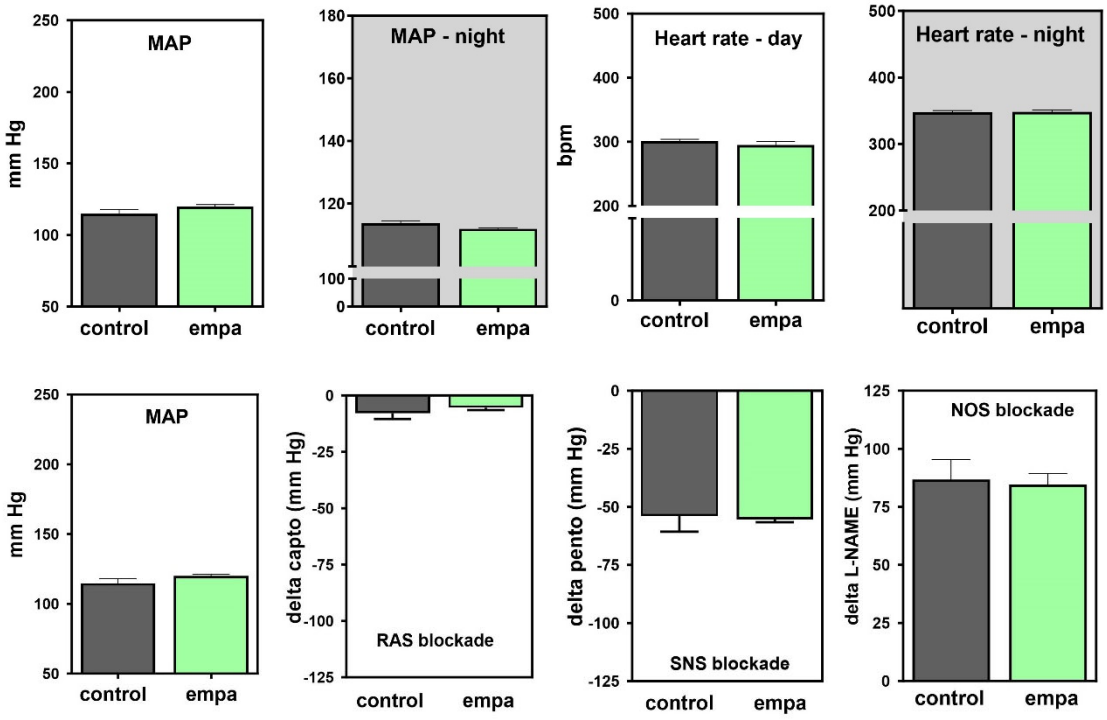

Fig. 2. Hereditary hypertriglyceridemic rats. The effect of empagliflozin treatment on day and night blood pressure and heart rate measured by radiotelemetry (upper panel), and on mean arterial pressure (MAP) and its changes induced by sympathetic nervous system (SNS) blockade, renin-angiotensin system (RAS) blockade, and nitric oxide synthase (NOS) blockade in conscious rats of control and empagliflozin-treated (empa) hereditary hypertriglyceridemic rats. Data are means ± SEM.

Spontaneously hypertensive rats expressing human C-reactive protein

Spontaneously hypertensive rats expressing human C-reactive protein (SHR-CRP) are a non-diabetic model of metabolic syndrome with severe hypertension, systemic inflammation, metabolic and hemodynamic disturbances, and target organ injury [21]. We analyzed the effect of empagliflozin treatment in both young (3-month-old) and adult (12-month-old) rats [22]. The beneficial effects of empagliflozin included not only reductions in body weight and fat mass, but also reduced ectopic fat accumulation in the liver and kidneys, the former being associated with decreased inflammation. Reduction of albuminuria was observed only in young SHR-CRP and was accompanied by attenuated oxidative stress. In contrast to adult TGR and HHTG rats, empagliflozin improved cardiac function in adult SHR-CRP by preventing the time-dependent increase in diastolic left ventricle wall thickness (both anterior and posterior) assesed by echocardiography (Fig. 3). In conclusion, empagliflozin exerted renoprotective effects in young SHR-CRP, which could be attributed to reduced renal lipid deposition and attenuation of renal oxidative stress and inflammation. In contrast to young SHR-CRP rats, the beneficial effects of empagliflozin in adult rats were related to cardiac and hepatic function.

Experimental models of chronic kidney disease

In our study [23], we evaluated renal function in relation to hypertension-induced end-organ damage in three models of chronic kidney disease with different pathophysiological background – Fawn-hooded hypertensive rats (FHH), in which hypertension, proteinuria, and focal glomerulosclerosis develop at a young age [24], and in two models of experimentally induced kidney damage – uninephrectomized rats on high salt intake (UNX+ HS) or rats with a stenosis of renal artery – two-kidney, one-clip (2K1C) Goldblatt hypertension [25].

Unexpectedly, we were unable to demonstrate renoprotective effects of empagliflozin treatment in any of these three models of chronic kidney disease [23]. Instead, a trend toward increased proteinuria was detected in all three groups following empagliflozin treatment, which was significant in FHH rats. Compared to the untreated group, the percentage increase in proteinuria during the eight weeks of empagliflozin treatment was 281 vs 201 %

in FHH, 247 vs. 227 % in UNX+HS, and 219 vs. 153 % in 2K1C model. Consistent with this, plasma MCP-1 levels were not improved by treatment. Moreover, the kidneys of all three models were substantially heavier than those of untreated rats (Table 2). Our tail-cuff blood pressure measurement did not show a blood pressure lowering effect of empagliflozin in the treated CKD groups (Fig. 4). The typical effects of SGLT-2 inhibition, namely reduction in body weight and fat mass, were detected only in FHH rats, with no effect in UNX+HS or 2K1C models (Table 2).

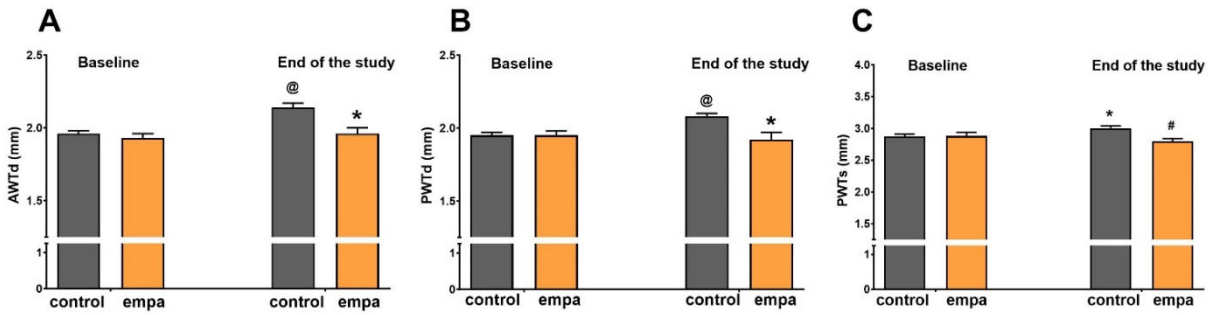

Fig. 3. Spontaneously hypertensive rats expressing human CRP. Anterior (**A**) and posterior (**B**) diastolic left ventricle wall thickness, systolic left ventricle wall thickness (**C**) at the beginning (baseline) and at the end of the experiment evaluated by echocardiography in adult control and empagliflozin-treated (empa) spontaneously hypertensive rats expressing human C-reactive protein. # p<0.05 vs. control untreated group, @ p<0.05 vs. respective baseline value. Data are means ± SEM.

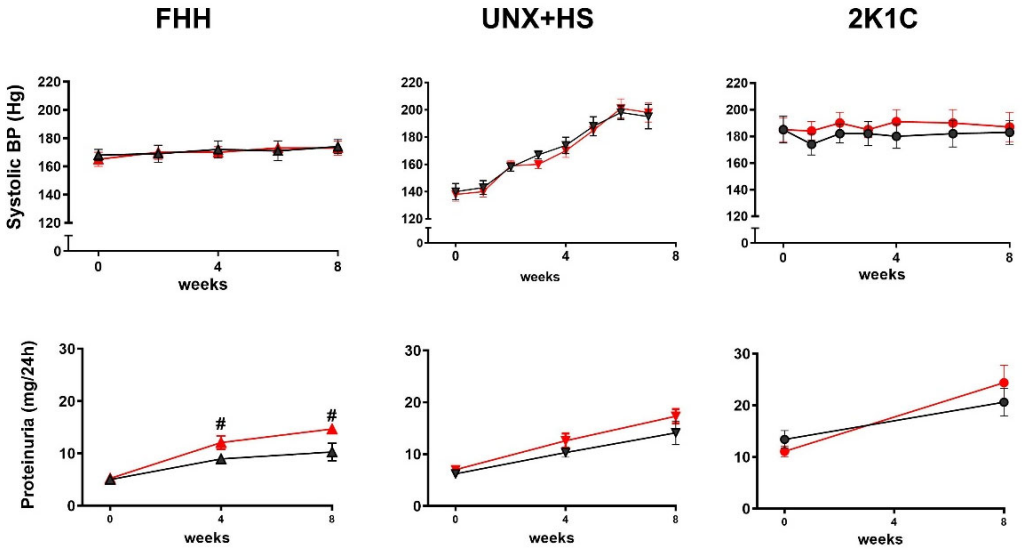

Fig. 4. The effect of empagliflozin treatment on systolic blood pressure measured by tail-cuff plethysmography (upper panel) and on proteinuria (lower panel) in control (black) and empagliflozin-treated (red) Fawn-hooded rats (FHH), uninephrectomized rats fed high-salt diet (UNX+HS) and in two-kidney one-clip (2K1C) Goldblatt hypertension. # p<0.05 vs. control untreated group. Data are means ± SEM.

Table 2. Empagliflozin effects in three non-diabetic models of chronic kidney disease

	FHH	UNX+HS	2K1C
Body weight	↓	↔	↔
Fat mass	↓	↔	↔
Relative kidney weight	↑	↑	↑
Proteinuria	↑	↔	↔
Oxidative stress	↔	↔	↔
Blood pressure	↔	↔	↔

Heart failure

Spontaneously hypertensive rats fed a high-fat diet

In this study [26], we used adult (6-month-old) male spontaneously hypertensive rats (SHR) fed a high-fat diet (60% fat) for 4 months to induce heart failure. Since aging in SHR is naturally associated with progression to heart failure with preserved ejection fraction, relatively old animals were used in the study. In addition to cardiac dysfunction (both systolic and diastolic) observed in 50 % of the untreated animals, high-fat diet feeding induced mild liver steatosis, increased liver triglycerides and cholesterol levels, and worsened glucose tolerance in this diet-induced obesity model. Empagliflozin administered during the last two months of high-fat diet reduced body weight gain and improved glucose tolerance. In addition, hepato-protective effects included reduced ectopic lipid accumulation, lipoperoxidation, and inflammation. Importantly, this was associated with a reduction of pro-inflammatory HETEs while increasing anti-inflammatory EETs levels in the liver. While empagliflozin had no beneficial effect on kidney function, it significantly improved cardiac function (systolic, diastolic and pumping) without affecting blood pressure (Fig. 5 and Table 3).

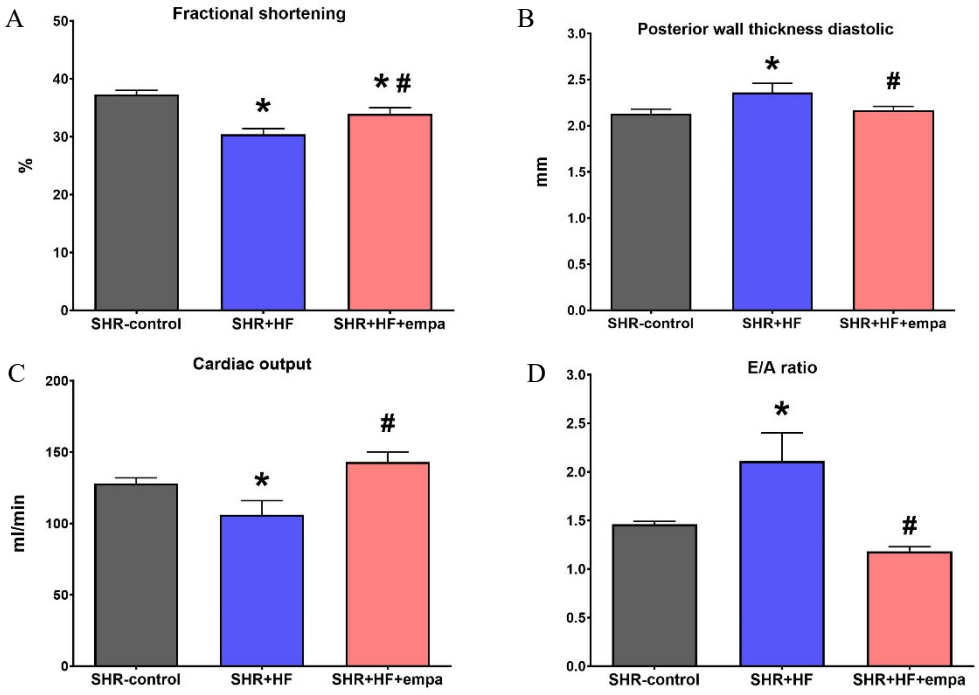

Fig. 5. Spontaneously hypertensive rats on high fat diet. The effect of empagliflozin treatment on fractional shortening (**A**), diastolic posterior wall thickness (**B**) cardiac output (**C**) and E/A ratio (**D**) in control spontaneously hypertensive rats (SHR), SHR fed a high-fat (SHR+HF) diet and empagliflozin-treated SHR-HF (SHR+HF+empa). * $p<0.05$ vs. control group fed a low-fat diet, # $p<0.05$ vs. untreated high-fat group. Data are means ± SEM.

Table 3. Empagliflozin effects in heart failure model

	SHR+HF
Body weight	↓
Fat mass	↔
Proteinuria	↔
Liver function	↑
Cardiac function	↑
Inflammation	↓
Oxidative stress	↓ (liver)
Blood pressure	↔

Discussion

Recently, several reviews on the effects of SGLT-2 inhibitors in non-diabetic animal models have been published [8,27-29] highlighting the main effects of gliflozins found in these studies. We would like to compare our results with these data and critically analyze the reasons for discrepancies between our and other studies.

Regarding the reduction of body mass and fat mass, our studies in hypertensive models are consistent with most other experimental studies [30], although even an increase in body weight has been demonstrated in Dahl

salt hypertensive rats after dapagliflozin treatment [31]. We also observed hyperphagia in TGR and HHTG rats, especially at the beginning of empagliflozin treatment. The increased food consumption is often associated with SGLT-2 inhibition and is probably due to the profound glucose excretion resulting in caloric loss. Interestingly, using a bioimpedance method, it has been shown that the weight loss during empagliflozin treatment contributes to the reduction of visceral and subcutaneous adipose tissue without affecting the lean body weight [32]. Moreover, our study in HHTG rats [20] demonstrated reduced hepatic lipid accumulation accompanied by decreased gene expression of the lipogenic enzymes Scd1, Fasn and lipogenic transcription factor Srebf1, which may also contribute to the reduction of lipid depots and body weight. On the other hand, in chronic kidney disease models, the effect on body weight and fat mass reduction was seen only in Fawn-hooded rats but not in uninephrectomized or 2K1C rats. This is in contrast to other experimental studies of CKD that have reported substantial reductions in body weight [33-35]. In SHR fed a high-fat diet, a model of heart and liver failure, body weight gains were reduced but there was no effect on fat mass. Whether these ambivalent responses are due to the fact that non-diabetic animals are less responsive to SGLT-2 inhibition than diabetic or pre-diabetic animals is debatable. The possible influence of different routes of application (food, drinking water, gavage), dose and type of SGLT-2 inhibitors cannot be excluded. Another explanation for different results could be the rat strain used.

Inconsistent results were also obtained regarding the effects of gliflozin treatment on cardiovascular function. We observed a moderate reduction in BP (15 mm Hg) and a significant reduction of nocturnal heart rate (22 bpm) after empagliflozin treatment in TGR [18], whereas there was no effect on BP level in HHTG rats [20], with BP measured telemetry in both experiments. The BP-lowering effect of empagliflozin in TGR was due to the reduced sympathetic vasoconstriction. On the other hand, tail-cuff BP monitoring demonstrated no gliflozin effect either in our three CKD models [23] or in SHR on high-fat diet [26]. Interestingly, we did not find an effect of empagliflozin treatment on cardiac function measured by echocardiography in TGR [18] and HHTG rats [20], but there was partial improvement of cardiac function in adult SHR-CRP [22]. Importantly, in SHR empagliflozin prevented the progression of high-fat diet-induced cardiac dysfunction (both systolic, diastolic and pumping) and restored cardiac parameters to the levels found in control SHR rats fed a low-fat diet [26]. Substantial (30 mm Hg) BP reductions were reported in 5/6 NX Sprague Dawley rats after both empagliflozin [33] and dapagliflozin [36] and in 5/6 NX Wistar rats on high-salt diet [37]. In contrast, Zhang *et al.* [34] found no BP effect in 5/6NX Sprague Dawley rats treated with dapagliflozin whereas a moderate BP reduction was reported in UNX Wistar rats treated with luseogliflozin [38]. We cannot provide a clear explanation for these discrepant results because the opposite results were obtained in the same strain and with the same type of gliflozin. However, the duration of the study, the age of animals, and last but not least, the dose of SGLT-2 inhibitor must be taken into account. In any case, one of the possible explanations for the antihypertensive effects of gliflozins could be the improvement of renal function and morphology due to reduced fibrosis [30,35,39], attenuated inflammation [33,40] or reduced oxidative stress [18,22], phenomena often reported in experimental studies following SGLT-2 inhibitors. The reduced fibrosis, inflammation and oxidative stress have also reported in several models of heart failure induced by myocardial infarction [41-44]. These effects probably precede the changes in proteinuria, as they have been described in many studies, whereas the functional improvement of renal function, such as improvement of creatinine clearance, proteinuria, or blood urea nitrogen, has been less frequently reported [35,45,46]. Perhaps these effects would have been more pronounced if treatment had been extended to months. In fact, the clinical trials with gliflozins, which usually lasted several years, generally showed nephroprotective effects in both diabetic and non-diabetic patients [4,13,47-49]. There is one short-term study supporting this hypothesis, which also failed to show the antiproteinuric effects in patients with focal segmental glomerulosclerosis after short (eight-week) dapagliflozin treatment [36].

It is generally accepted that the metabolic effects of SGLT-2 inhibition play an important role in mediating the beneficial effects of gliflozins. These effects have also been demonstrated in our experimental non-diabetic models [18,20]. We believe that a higher degree of metabolic dysfunction in the experimental model is associated with the higher efficacy of gliflozin treatment. Consistent with this, we have demonstrated significant metabolic effects in the prediabetic model, in hereditary hypertriglyceridemic rats, and in TGR rats, in which we have observed important signs of metabolic syndrome, such as increased body and adipose tissue weight, together with increased plasma insulin and leptin levels. In HHTG

rats [20], we found that empagliflozin modulated the expression of genes related to lipid synthesis (Fasn, Scd1) and fatty acid metabolism (Pparγ), as well as the transcription factor Nrf2 in the liver. The latter alteration may not only contribute to the reduction of oxidative stress, but also to the regulation of lipid metabolism by inhibiting lipogenesis. Recently, we have analyzed the relationship between metabolic effects and cardiac function in the spontaneously hypertensive rats feed a high-fat diet (a model of heart and liver failure) [26]. We hypothetize that cardioprotective effects – especially those affecting the systolic function – are secondary to hepatoprotective effects induced by SGLT-2 inhibition. Using correlation analysis, we demonstrated that ejection fraction and fractional shortening correlated with several metabolic parameters (ectopic lipid accumulation, lipoperoxidation, oxidative stress, 14,15-EETs and 20-HETEs). As many of these metabolic parameters were related to liver function, we suggest its direct or indirect influence on cardiac function. Whether the same is true in the opposite way remains to be determined in future studies.

In conclusion, SGLT-2 inhibitors have great potential in the treatment of both diabetic and non-diabetic patients due to their pleiotropic effects that go far beyond their hypoglycemic effects. However, it is clear from the previous paragraphs that there are still many issues that need to be addressed before their general acceptance in clinical practice.

Conflict of Interest

There is no conflict of interest.

Acknowledgements

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