Naunyn-Schmiedeberg's Archives of Pharmacology
https://doi.org/10.1007/s00210-020-01873-7

ORIGINAL ARTICLE

# Comparative efficacy of empagliflozin and drugs of baseline therapy in post-infarct heart failure in normoglycemic rats

Marina Krasnova[1] · Alexander Kulikov[2] · Sergey Okovityi[1] · Dmitry Ivkin[1] · Andrey Karpov[3] · Elena Kaschina[4] · Alexey Smirnov[2]

Received: 9 January 2020 / Accepted: 14 April 2020


## Abstract

The study aimed to investigate the effects of the sodium-glucose co-transporter 2 (SGLT2) inhibitor empagliflozin on chronic heart failure (HF) in normoglycemic rats. The effects of empagliflozin were compared with the standard medications for HF, e.g., angiotensin-converting enzyme (ACE) inhibitor fosinopril, beta-blocker bisoprolol, and aldosterone antagonist spironolactone. Myocardial infarction (MI) was induced in male Wistar rats via permanent ligation of the left descending coronary artery. One-month post MI, 50 animals were randomized into 5 groups ($n = 10$): vehicle-treated, empagliflozin (1.0 mg/kg), fosinopril (10 mg/kg), bisoprolol (10 mg/kg), and spironolactone (20 mg/kg). All medications except empagliflozin were titrated within a month and administered per os daily for 3 months. Echocardiography, 24-hour urine volume test, and treadmill exercise tests were performed at the beginning and at the end of the study. Treatment with empagliflozin slowed the progression of left ventricular dysfunction: LV sizes and ejection fraction were not changed and the minute volume was significantly increased (from $52.0 \pm 15.5$ to $61.2 \pm 21.2$ ml/min) as compared with baseline. No deaths occurred in empagliflozin group. The 24-hour urine volume tends to be higher in empagliflozin and spironolactone groups than in vehicle and fosinopril group. Moreover, empagliflozin exhibited maximal physical exercise tolerance in comparison with all investigated groups ($289 \pm 27$ s versus $183 \pm 61$ s in fosinopril group, $197 \pm 95$ s in bisoprolol group, and $47 \pm 46$ s in spironolactone group, $p = 0.0035$ for multiple comparisons). Sodium-glucose co-transporter 2 inhibitor empagliflozin reduced progression of left ventricular dysfunction and improved tolerance of physical exercise in normoglycemic rats with HF. Empagliflozin treatment was superior with respect to physical tolerance compared with fosinopril, bisoprolol, and spironolactone.

**Keywords** Chronic heart failure · Left ventricular dysfunction · Empagliflozin · Physical exercise · Normoglycemic rats

✉ Marina Krasnova
marina.krasnova@pharminnotech.com

[1] Department of Pharmacology and Clinical Pharmacology, Center of Experimental Pharmacology Saint Petersburg Chemical Pharmaceutical University, Saint Petersburg, Russia

[2] Pavlov First Saint Petersburg State Medical University, Saint Petersburg, Russia

[3] Almazov National Medical Research Centre, Saint Petersburg, Russia

[4] Charite Universitatsmedizin Berlin, corporate member of Freie Universitat Berlin, Humboldt-Universitat zu Berlin and Berlin Institute of Health, German Centre for Cardiovascular Research, Berlin, Germany

**Abbreviations**

| | |
|---|---|
| ACE | Angiotensin-converting enzyme |
| CV | Cardiovascular |
| EF | Ejection fraction |
| FS | Left ventricular fraction shortening |
| HF | Heart failure |
| HR | Heart rate |
| IVST | Interventricular septum thickness |
| LA_ap | Left atrial antero-posterior dimension |
| LA_l | Left atrial long axis dimension |
| LA_s | Left atrial short axis dimension |
| LV | Left ventricular |
| LV EDV | Left ventricular end-diastolic |
| LV EDD | Left ventricle end-diastolic diameter |

Published online: 06 May 2020

| | |
|---|---|
| LV ESD | Left ventricle end-systolic diameters |
| LV ESV | Left ventricular end-systolic volumes |
| LVM | Left ventricular myocardium mass |
| MAPSE | Mitral annular plane systolic excursion |
| MI | Myocardial infarction |
| MV | Left ventricular minute volume |
| PWT | Left ventricular posterior wall thickness in diastole |
| RA_l | Long axis dimensions |
| RA_s | Right atrium short axis |
| RAAS | Angiotensin aldosterone system |
| RV | Right ventricle antero-posterior dimension |
| RWT | Left ventricular relative wall thickness |
| SGLT2 | Sodium-glucose co-transporter 2 |
| SV | Stroke volume |
| T2D | Type 2 diabetes |
| TAPSE | Tricuspid annular plane systolic excursion |

## Introduction

Heart failure (HF) is a major public health problem with a prevalence of over 23 million worldwide (Roger 2013). Pharmacological modulation of renin angiotensin aldosterone system (RAAS) by ACE inhibitors, the AT1 receptor antagonists and aldosterone antagonists, has been clearly shown to improve the outcome of heart failure (Heerspink et al. 2016). However, left ventricular (LV) remodeling is still observed in many patients and is related to an adverse prognosis (Konstam et al. 2011). Moreover, up to now, there are no therapies improving survival in patients with HF with preserved ejection fraction.

Recently, sodium-glucose co-transporters (SGLTs) have attracted attention for its role in cardiac protection (Kaplan 2018). The remarkable reductions in cardiovascular (CV) mortality (38%), major CV events (14%), hospitalization for heart failure (35%), and death from any cause (32%) were observed over a period of 2.6 years in patients with T2D and high CV risk in the EMPA-REG OUTCOME trial involving the SGLT2 inhibitor empagliflozin (Zinman et al. 2015). Importantly, the decrease of cardiovascular and total risk was associated with the reduction of hospitalization rates due to heart failure (35%). Empagliflozin in T2DM patients with a high risk of CV events also decreased excessive body weight, blood pressure, HbA1c, triglyceride levels, and albuminuria (Zinman et al. 2015). Multiple studies investigated the direct impact of SGLT2 inhibitors on the cardiovascular system with limited findings about pathophysiological role of SGLTs in the heart (Kaplan et al. 2018). Although SGLT2 expression is not found in heart tissue, empagliflozin may directly interfere with sodium hydrogen exchanger (NHE), decrease cardiac myocyte cytosolic sodium and calcium levels, and increase myocyte mitochondrial $Ca^{2+}$ concentration (Baartscheer et al. 2017).

In our previously experimental study, we found that treatment with empagliflozin increased tolerance to physical exercise in normoglycemic rats with post myocardial infarction HF (Kulikov et al. 2016, 2017). This effect was associated with an improvement of left ventricular dysfunction and higher minute volume of blood circulation at rest. Whether cardiac protective effects of empagliflozin are comparable or even superior to the standard HF medications such as ACE inhibitors, aldosterone antagonists, or beta-blockers requires further investigations.

Therefore, present study analyzed the effects of empagliflozin in experimental chronic HF model in normoglycemic rats in comparison with ACE inhibitor fosinopril, beta-blocker bisoprolol, and aldosterone antagonist spironolactone.

## Methods

### Ethics statement

This study was carried out in strict accordance with national and European guidelines for animal experiments with approval by the ethics commission of the regulatory authorities of the City of Saint Petersburg, Russia and Institutional Animal Care Committee at the State High Educational Institute "Saint Petersburg Chemical and Pharmaceutical University".

### Animals

Male normotensive Wistar rats (weight, 180–200 g; Rappolovo Laboratory Animal Resource Facility, St. Petersburg, Russia) were kept under standardized conditions with respect to temperature and humidity, and were housed on a 12 h light/12 h dark cycle in groups of four animals with food and water ad libitum.

### Experimental protocol

Myocardial infarction was induced by ligation of proximal left anterior descending coronary artery as previously described (Karpov et al. 2014). One-month post MI, 50 animals were randomly assigned to the following 5 groups ($n = 10$): vehicle-treated group, empagliflozin (1.0 mg/kg; *Jardiance*, Boehringer Ingelheim Pharma), fosinopril (10 mg/kg; *Monopril*, Bristol Myers Squibb), bisoprolol (10 mg/kg; *Concor*, Takeda), and spironolactone (20 mg/kg; *Verospirone*, Gedeon Richter). The therapy began 30 days after MI induction. All medications were administered per or via oral gavage daily for 3 months. Drug doses, except for empagliflozin, were titrated within a month (first week, 1/8 of the target dose; second, 1/4 of the dose; third, 1/2 of the

dose; fourth, 3/4 of the dose); then, during 2 months, experimental animals received full-dose therapy.

Echocardiography and treadmill exercise tests were performed 1 month after MI (baseline) and 4 months after MI. Body weight and 24-h urine volume were recorded.

At the end of the treatment course, $CO_2$–box was used for euthanasia of the animals.

### Rationale for daily dose of medications

Empagliflozin 1 mg/kg is the minimal dose that affects glucosuria in the rats Boehringer Ingelheim Pharmaceuticals Inc.(2013). Therefore, we considered this dose as minimally effective with lower risk of hypoglycemia and ketosis in non-diabetic animals. Fosinopril was applicated in the low-dose of 10 mg/kg with the aim to abolish the blood pressure lowering effect but to reduce ACE activity (Hayek et al. 1999). Previous studies by Gohlke et al. (1994), Gohlke and Unger (1995) demonstrated that the chronic low-dose ACE inhibitor treatment led to a pattern of changes in cardiodynamics and cardiac metabolism similar to that observed with the high dose. Moreover, fosinopril 10 mg/kg was effective in the occlusion-reperfusion model in rats (Xia et al. 2014). The daily dose of bisoprolol 10 mg/kg was chosen based on the study of Watanabe et al. (2001) on survival and cardiac functions in a chronic HF model in the rat.

We used daily dose of spironolactone 20 mg/kg because in previous study by Cezar et al. (2015), this dose decreased HF development frequency in spontaneously hypertensive rats.

### Ultrasound measurements

Transthoracic Doppler echocardiography (M-mode and B-Mode) was performed in anesthetized animals (zoletil 25 mg/kg combined with xylazine 10 mg/kg) on a heated platform with the use of high-resolution imaging system MyLab Touch SL 3116 (frequency 13 MHz; Esaote, Italy).

Following parameters were assessed in M-mode: interventricular septum thickness (IVST) and left ventricular posterior wall thickness in diastole (PWT), left ventricle end-diastolic diameter (LV EDD) and end-systolic diameters (LV ESD) (mm), and heart rate (HR, beats/min). Moreover, mitral annular plane systolic excursion (MAPSE, mm) and tricuspid annular plane systolic excursion (TAPSE, mm) values were measured in the four-chamber cross section.

Left ventricular ejection fraction by the Teichholz method (EF(T), %), LV fraction shortening (FS, %) by the formula: $100 \times (\text{LV EDD} - \text{LV ESD})/\text{LV EDD}$, left ventricular relative wall thickness (RWT, units) by the formula: $(\text{PWT} \times 2)/\text{LV EDD}$, and left ventricular myocardium mass (LVM, g) by the formula: $1.05 \times ((\text{LV EDD} + \text{IVST} + \text{PWT})^3 - \text{LV EDD}^3)$ were calculated.

In B-mode, LV end-diastolic (LV EDV, ml) and LV end-systolic volumes (LV ESV, ml) were assessed by the Simpson 2D method with the calculation of stroke volume (SV = LV EDV−LV ESV, ml) and left ventricular ejection fraction ($\text{EF(S)} = 100 \times \text{SV/LV EDV}$, %). LV minute volume was calculated as the multiplication of HR and SV (MV, ml/min). We also determined: left atrial antero-posterior dimension (LA_ap, mm), left atrial short axis dimension (LA_s, mm) and left atrial long axis dimension (LA_l, mm), right ventricle antero-posterior dimension (RV, mm), right atrium short axis (RA_s, mm), and long axis dimensions (RA_l, mm).

### Physical tolerance

Physical tolerance was examined by using the treadmill (Treadmill System for rats, TSE, Germany) at movement rate of the transporter line 0.7 m/s and the slope angle 15°. Full animal tiredness was asserted by a lack of animal reaction to electric shock (3 мA). The end of running time was reported in seconds as previously described (Kulikov et al. 2016). Treadmill exercise tests were performed 1 month after MI (baseline) and 4 months after MI (3 month on treatment).

### Glucose in serum and urine

Fasting glucose levels in blood was measured by Accu-chek (Roche Diabetes Care GmbH, Mannheim, Germany).

Glucose 24-h urine was determined by glucosooxidase method using the urine analyzer CL-50 (HTI-Diagnostics, North Attleboro, USA).

### Statistical analysis

Statistical data analysis was performed with software «Statistica for Windows 10». Because of the small size of each group and frequent abnormal statistical distribution of the studied variables, we used nonparametric criteria. To assess significance of differences between all treatment groups and vehicle, the Kruskal-Wallis test for multiple comparisons was used. To assess the differences in each group separately (data of the beginning and of the end of treatment), we used Wilcoxon's pair test.

## Results

### Cardiac remodeling and function 1 month after myocardial infarction (baseline)

One month after MI, the animals developed LV systolic dysfunction. The rats exhibited significant increase of LV EDD, LV ESD, LV EDV, and LV ESV and a decrease of FS, EF(T),

**Table 1** Echocardiographic values in 1 month after induction of myocardial infarction. All groups had low mean fraction shortening (FS) and mean ejection fraction that were measured by two different methods (EF(T) and EF(S)). Each experimental group had no differences with others in terms of the heart dimensions and functional parameters ($p > 0.05$ by Kruskal-Wallis test for each variable)

| Variable | Vehicle (group 1) $N = 10$ | Empagliflozin (group 2) $N = 10$ | Fosinopril (group 3) $N = 10$ | Bisoprolol (group 4) $N = 10$ | Spironolactone (group 5) $N = 10$ |
|---|---|---|---|---|---|
| LV EDD, mm | 8.0 ± 1.1 | 7.93 ± 1.19 | 7.85 ± 0.95 | 8.28 ± 0.89 | 8.01 ± 1.40 |
| LV ESD, mm | 6.12 ± 0.44 | 5.97 ± 1.59 | 6.14 ± 1.19 | 6.46 ± 1.50 | 6.45 ± 1.85 |
| IVST, mm | 1.30 ± 0.30 | 1.31 ± 0.46 | 1.34 ± 0.35 | 1.23 ± 0.44 | 1.34 ± 0.53 |
| PWT, mm | 1.48 ± 0.40 | 1.76 ± 0.39 | 1.60 ± 0.26 | 1.80 ± 0.41 | 1.63 ± 0.49 |
| LVM, g | 0.90 ± 0.167 | 0.90 ± 0.288 | 0.81 ± 0.168 | 0.92 ± 0.205 | 0.85 ± 0.255 |
| RWT, units | 0.46 ± 0.11 | 0.45 ± 0.13 | 0.42 ± 0.10 | 0.45 ± 0.16 | 0.43 ± 0.18 |
| FS, % | 21.0 ± 9.9 | 19.3 ± 11.0 | 21.4 ± 9.9 | 22.9 ± 11.3 | 20.9 ± 11.6 |
| EF(T), % | 46.1 ± 16.8 | 47.09 ± 11.3 | 49.7 ± 14.0 | 49.1 ± 18.7 | 45.5 ± 18.4 |
| HR, beats/min | 410 ± 25 | 424 ± 72 | 407 ± 34 | 406 ± 59 | 448 ± 32 |
| LV EDV, ml | 0.39 ± 0.12 | 0.35 ± 0.11 | 0.39 ± 0.09 | 0.42 ± 0.09 | 0.45 ± 0.11 |
| LV ESV, ml | 0.25 ± 0.10 | 0.22 ± 0.10 | 0.25 ± 0.09 | 0.27 ± 0.098 | 0.30 ± 0.12 |
| SV, ml | 0.15 ± 0.05 | 0.12 ± 0.03 | 0.14 ± 0.05 | 0.15 ± 0.05 | 0.16 ± 0.04 |
| MV, ml/min | 61.4 ± 23.0 | 52.0 ± 15.5 | 58.7 ± 21.4 | 61.5 ± 24.0 | 70.2 ± 19.2 |
| EF(S), % | 38.2 ± 14.2 | 37.4 ± 12.0 | 37.9 ± 12.9 | 36.4 ± 11.6 | 36.3 ± 12.6 |
| MAPSE, mm | 1.08 ± 0.33 | 1.08 ± 0.29 | 1.01 ± 0.32 | 1.17 ± 0.39 | 1.23 ± 0.34 |
| TAPSE, mm | 1.41 ± 0.64 | 1.56 ± 0.67 | 1.99 ± 0.75 | 1.69 ± 0.47 | 1.35 ± 0.33 |
| LA_ap, mm | 4.47 ± 0.81 | 3.60 ± 0.93 | 3.62 ± 0.45 | 4.14 ± 0.81 | 4.35 ± 0.85 |
| LA_s, mm | 5.38 ± 0.82 | 5.46 ± 0.98 | 5.23 ± 0.51 | 5.53 ± 0.61 | 5.56 ± 0.89 |
| LA_l, mm | 5.49 ± 0.53 | 4.90 ± 0.83 | 4.90 ± 0.77 | 5.36 ± 1.05 | 5.64 ± 0.63 |
| RV, mm | 3.10 ± 0.43 | 2.98 ± 0.32 | 3.13 ± 0.63 | 3.14 ± 0.33 | 2.80 ± 0.37 |
| RA_s, mm | 3.73 ± 1.35 | 4.23 ± 0.81 | 4.05 ± 0.63 | 4.12 ± 0.61 | 3.65 ± 0.76 |
| RA_l, mm | 5.64 ± 0.71 | 4.99 ± 0.86 | 4.643 ± 0.85 | 5.39 ± 0.45 | 4.99 ± 0.97 |

EF(C), MAPSE, and TAPSE values compared with normal range for intact rats of the same age (Table 1).

Comparison between experimental groups did not show any significant differences in LV EDD, LV ESD, LV EDV, LV ESV, FS, EF(T), EF(S), MAPSE, and TAPSE values. The maximum time of the treadmill exercise was also similar in all groups.

At the end of the experiment (4 months after MI−3 months on treatment), the number of deaths in each group was as follows: 1 case in vehicle group, 3 cases in fosinopril group, 1 case in bisoprolol group, and 3 cases in spironolactone group. All empagliflozin-treated animals survived.

## The body weights

The body weight significantly increased in all study groups except bisoprolol-treated rats (Fig. 1).

## Cardiac remodeling and function after 3 months on treatment

Vehicle-treated animals exhibited an increase of LV EDD, LV ESD, LV EDV, and LV ESV and a decrease of FS, EF(T), and EF(C) as compared with baseline, 1-month post MI measurements. Moreover, most atrial sizes were increased: LA_s, LA_l, and RA_l (Table 2, Figs. 2 and 3). These changes characterize progression of heart failure.

Empagliflozin-treated animals had only increased left atrial anterior-posterior dimension and left atrium long axis dimension as compared with baseline. Neither LV sizes nor ejection fraction were changed. Moreover, the minute volume significantly increased from 52.0 ± 15.5 to 61.2 ± 21.2 ml/min (Fig. 4).

Rats from fosinopril-treated group showed an increase in the left atrial anterior-posterior dimension and right atrium long axis dimension, although LV sizes and LV ejection fraction also did not significantly change. In bisoprolol group, we found an increase in the left atrial anterior-posterior dimension and deterioration of LV dysfunction exhibited by decreased FS, EF(T), and MAPSE. The animals treated with spironolactone did not have any echocardiographic changes in comparison with baseline, except for thickening of the interventricular septum (Table 2, Figs. 2 and 3). Further comparisons of echocardiographic data between each group did not show any statistically significant differences.

## Physical activity

The tolerance during the treadmill exercise as maximal activity time (MAT) was evidently decreased in 3 months in rats of all study groups compared with baseline (1 month after MI) (Fig. 5). Hereby, the maximal changes were shown in vehicle, fosinopril, bisoprolol, and spironolactone groups. So, in the final point of experiment, MAT was the highest in the rats treated with empagliflozin (289 ± 27 s). It was significantly higher than in the fosinopril group (183 ± 61 s), bisoprolol group (197 ± 95 s), and spironolactone group (47 ± 46 s; $p = 0.0035$ for multiple comparisons).

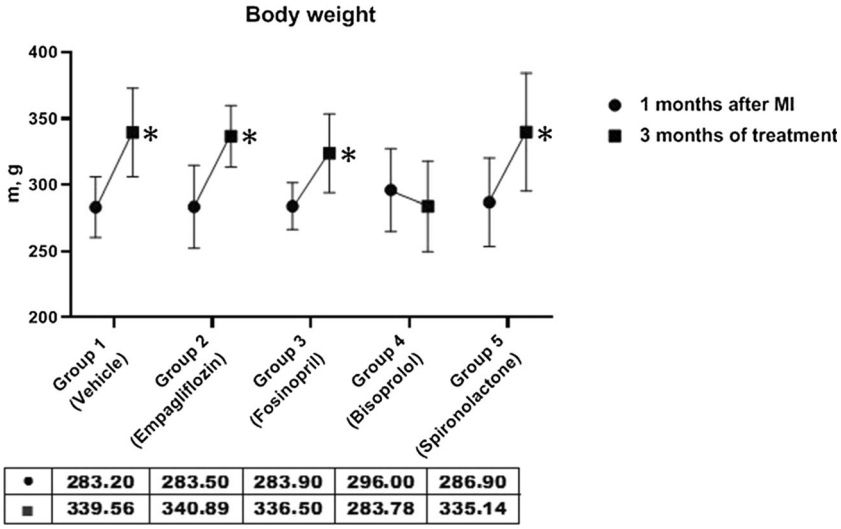


**Fig. 1** Body weight changes in experimental groups during treatment (M ± STD, g). In all groups, an increase in body weight was observed compared with baseline ($p < 0.05$ by Wilcoxon's pair test) except for the group that took bisoprolol as a treatment

## 24-h urine volume test

The data from 24-h urine volume test are presented in Fig. 6. In rats of empagliflozin and spironolactone groups, urine volume (9.8 ± 0.92 ml and 11.1 ± 1.3 ml respectively) was slightly but not significantly higher than in the vehicle (6.9 ± 1.25 ml) and in the fosinopril group (7.39 ± 0.53 ml); $p = 0.09$ for multiple comparisons.

## Glucose concentration

Glucose concentration in blood was lower after treatment with empagliflozin (3.34 ± 0.21 mmol/l versus 5.0 ± 0.26 mmol/l; $p < 0.001$) compared with vehicle-treated animals. Concomitantly, empagliflozin increased glucose 24-h urine concentration (2.8 ± 1.95 mmol/l versus 0 mmol/l in vehicle; $p < 0.05$).

**Table 2** Echocardiographic values after 3 months on treatment (4 month after induction of myocardial infarction). Each experimental group had no differences with others in terms of the heart dimensions and functional parameters ($p > 0.05$ by Kruskal-Wallis test for each variable) but some differences were observed in each of the groups when assessing changes in variables compared with baseline (see Figs. 2, 3, and 4)

| Variable | Vehicle (group 1) $N = 9$ | Empagliflozin (group 2) $N = 10$ | Fosinopril (group 3) $N = 7$ | Bisoprolol (group 4) $N = 9$ | Spironolactone (group 5) $N = 7$ |
|---|---|---|---|---|---|
| LV EDD, mm | 8.79 ± 0.44 | 8.15 ± 0.91 | 7.52 ± 0.93 | 7.70 ± 2.57 | 8.19 ± 1.25 |
| LV ESD, mm | 7.46 ± 0.79 | 6.42 ± 1.63 | 5.76 ± 1.45 | 6.61 ± 2.20 | 6.67 ± 1.71 |
| IVST, mm | 1.34 ± 0.31 | 1.43 ± 0.20 | 1.43 ± 0.60 | 1.16 ± 0.36 | 1.58 ± 0.56 |
| PWT, mm | 1.80 ± 0.29 | 1.63 ± 0.28 | 1.64 ± 0.38 | 1.66 ± 0.36 | 1.47 ± 0.36 |
| LVM, g | 0.93 ± 0.210 | 0.92 ± 0.128 | 0.788 ± 0.192 | 0.82 ± 0.326 | 0.92 ± 0.300 |
| RWT, units | 0.34 ± 0.08 | 0.39 ± 0.07 | 0.45 ± 0.15 | 0.39 ± 0.12 | 0.38 ± 0.15 |
| FS, % | 16.3 ± 6.8 | 18.4 ± 11.0 | 24.4 ± 10.9 | 13.52 ± 4.07 | 19.6 ± 10.7 |
| EF(T), % | 37.9 ± 13.1 | 43.4 ± 18.1 | 52.1 ± 18.6 | 32.6 ± 8.6 | 43.4 ± 18.9 |
| HR, beats/min | 372 ± 95 | 409 ± 31 | 394 ± 63 | 380 ± 55 | 407 ± 58 |
| LV EDV, ml | 0.53 ± 0.09 | 0.45 ± 0.12 | 0.39 ± 0.09 | 0.47 ± 0.14 | 0.55 ± 0.16 |
| LV ESV, ml | 0.36 ± 0.118 | 0.30 ± 0.12 | 0.25 ± 0.09 | 0.32 ± 0.13 | 0.39 ± 0.18 |
| SV, ml | 0.17 ± 0.06 | 0.15 ± 0.06 | 0.15 ± 0.03 | 0.15 ± 0.05 | 0.15 ± 0.04 |
| MV, ml/min | 66.1 ± 28.0 | 61.2 ± 21.2 | 59.7 ± 19.2 | 63.8 ± 19.7 | 57.6 ± 19.3 |
| EF(S), % | 32.4 ± 12.3 | 34.6 ± 14.4 | 38.8 ± 10.6 | 33.8 ± 11.0 | 29.7 ± 13.0 |
| MAPSE, mm | 1.08 ± 0.39 | 1.23 ± 0.11 | 1.07 ± 0.25 | 0.92 ± 0.19 | 0.95 ± 0.20 |
| TAPSE, mm | 1.52 ± 0.55 | 1.70 ± 0.89 | 1.86 ± 0.53 | 1.41 ± 0.28 | 1.34 ± 0.48 |
| LA_ap, mm | 5.12 ± 1.19 | 5.22 ± 0.57 | 4.51 ± 0.57 | 5.41 ± 1.06 | 5.4 ± 0.57 |
| LA_s, mm | 6.76 ± 0.72 | 6.36 ± 0.71 | 5.55 ± 0.44 | 6.27 ± 0.87 | 5.96 ± 0.24 |
| LA_l, mm | 6.60 ± 0.86 | 6.31 ± 0.71 | 5.33 ± 0.56 | 6.39 ± 1.33 | 5.84 ± 0.77 |
| RV, mm | 3.08 ± 0.53 | 2.94 ± 0.26 | 2.84 ± 0.31 | 2.98 ± 0.70 | 2.90 ± 0.35 |
| RA_s, mm | 4.24 ± 0.86 | 4.16 ± 0.78 | 4.20 ± 0.66 | 4.4 ± 0.82 | 3.78 ± 0.76 |
| RA_l, mm | 6.36 ± 0.68 | 5.52 ± 0.795 | 5.52 ± 0.82 | 5.84 ± 0.71 | 5.65 ± 0.79 |

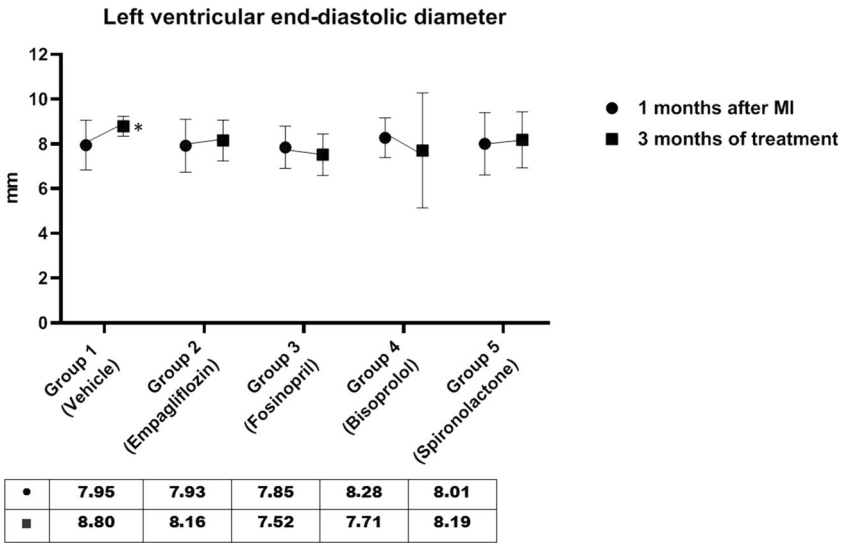


**Fig. 2** LV end-diastolic diameter changes in experimental groups during treatment (M ± STD, mm). In vehicle group, an increase of LV end-diastolic diameter was observed ($p < 0.05$ by Wilcoxon's pair test). In other groups, LV end-diastolic diameter did not significantly change

## Discussion

In the present study, we provide evidence that SGLT2 inhibition improved cardiac function and increased the tolerance to physical exercise. Administration of the SGLT2 inhibitor empagliflozin in normoglycemic rats with heart failure slowed the progression of left ventricular dysfunction and increased the maximum activity time at the treadmill. We further compared the effects of empagliflozin with the standard medications for HF such as ACE inhibitor fosinopril, beta-blocker bisoprolol, and aldosterone antagonist spironolactone.

SGLT2 inhibitors are a new class of drug being developed for the treatment of T2DM that showed cardiovascular and renal protective effects irrespective of glucose reduction (Zinman et al. 2015). Since SGLT2 is involved in the absorption/reabsorption of glucose driven by the sodium gradient across the cell membrane (Vrhovac et al. 2015), its inhibition decreases renal glucose reabsorption, promotes urinary glucose excretion, and reduces plasma glucose concentrations (Neal et al. 2017; Zinman et al. 2015).

Dapagliflozin, empagliflozin, and canagliflozin have internally and externally consistent and biologically plausible class effects on cardiovascular and cardiorenal outcomes (Cavaiola and Pettus 2018; Kluger et al. 2019). We investigated the direct impact of SGLT2 inhibitors on the cardiovascular system. SGLT2 inhibitor empagliflozin was selected because it has the highest selectivity for SGLT2 over SGLT1 (> 2500-fold) (Grempler et al. 2012) and because this compound showed positive outcomes in humans in EMPA-REG OUTCOME trial (Zinman et al. 2015).

Heart failure model was ready by 1 month after ligation of the left coronary artery. At this time point, the vehicle-treated animals developed left ventricular remodeling with systolic and diastolic dysfunction. The LV remodeling was manifested by an increase of LV dimensions (LVID and, especially, LVIDS) and decrease of cardiac contractility (EF and FS).

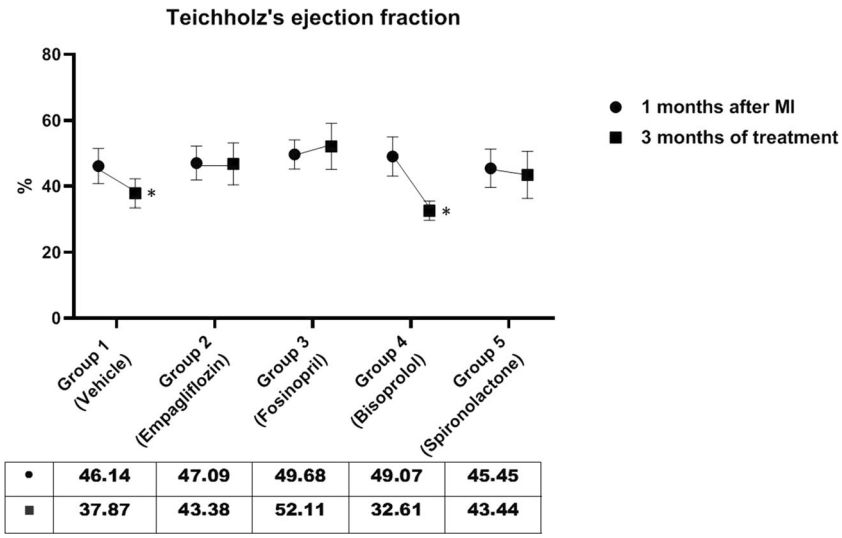


**Fig. 3** Ejection fraction (EF) changes in experimental groups during treatment (M ± STD, %). Significant decrease of EF was observed in vehicle group ($p < 0.05$ by Wilcoxon's pair test) and in bisoprolol group ($p < 0.05$ by Wilcoxon's pair test). In other groups, EF did not significantly change

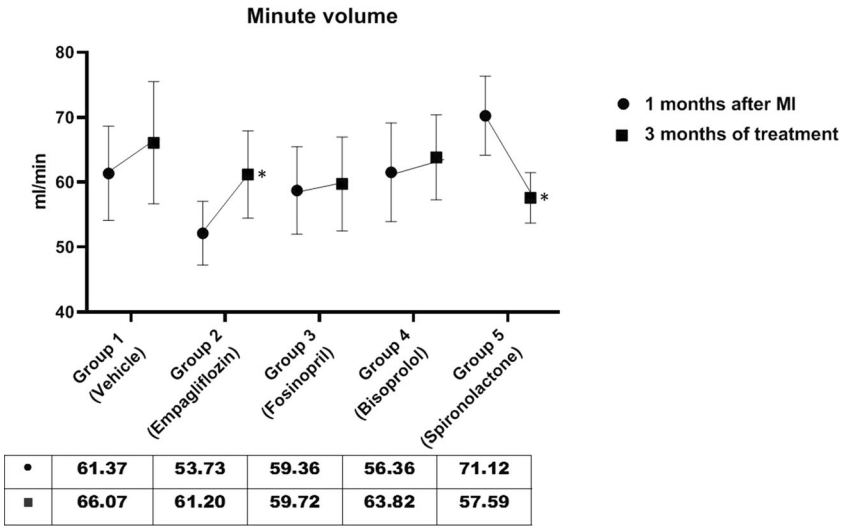


**Fig. 4** Minute volume in experimental groups during treatment (M ± STD, ml/min). Minute volume at rest in all experimental groups after 3 months of treatment did not change except rats of spironolactone and empagliflozin groups. In spironolactone-treated rats, minute volume significantly decreased ($p < 0.05$ by Wilcoxon's pair test) but in empagliflozin-treated animals, minute volume significantly increased ($p < 0.05$ by Wilcoxon's pair test)

Recently, using this model in the normoglycemic rats, we have found that SGLT2 inhibition for 3 months increased the tolerance to physical exercise (Kulikov et al. 2016; Kulikov et al. 2017). This effect was associated with an improvement of cardiac function as demonstrated by increased stroke volume, minute volume, and LV ejection fraction as well as with the improvement of renal function (Beresneva et al. 2017; Okovityi et al. 2018). The beneficial effects on myocardium of empagliflozin also have been demonstrated in studies on rats with heart failure (Yurista et al. 2019; Santos-Gallego et al. 2019; Connelly et al. 2019).

In the present study, we compared empagliflozin with the reference compounds, e.g., ACE inhibitor fosinopril, β-blocker bisoprolol, and aldosterone antagonist spironolactone.

First, we could confirm in general our previous findings: empagliflozin-treated animals exhibited only increased left atrial anterior-posterior dimension and left atrium long axis dimension after 3 months of treatment as compared with baseline. Left ventricular sizes and ejection fraction were unchanged and the minute volume was increased. These data suggest that empagliflozin administration in normoglycemic HF rats prevents development of pathological LV remodeling and functional deterioration that happened in vehicle group. Notably, only in this group, there were no death cases. Rats of fosinopril group confirm well-known beneficial effect ACE inhibitors on cardiac remodeling and function; left ventricular sizes and ejection fraction did not change except 2 atrial dimension enlargements. In contrast, bisoprolol-treated rats, despite its long-term application, had significantly decreased FS, EF(T), and MAPSE which may be interpreted as a cardio-depressive effect. This negative effect could be explained by a high dose of bisoprolol (10 mg/mg), although this dose was chosen based on previous studies (Watanabe et al. 2001) and titrated as described above. Bisoprolol-treated animals also did not gain weight as animals in the other groups. Animal of the spironolactone group did not show any significant changes of cardiac sizes and function that was not surprising but contrasted with more deaths (3 cases). Similarly, there were 3 deaths in fosinopril group.

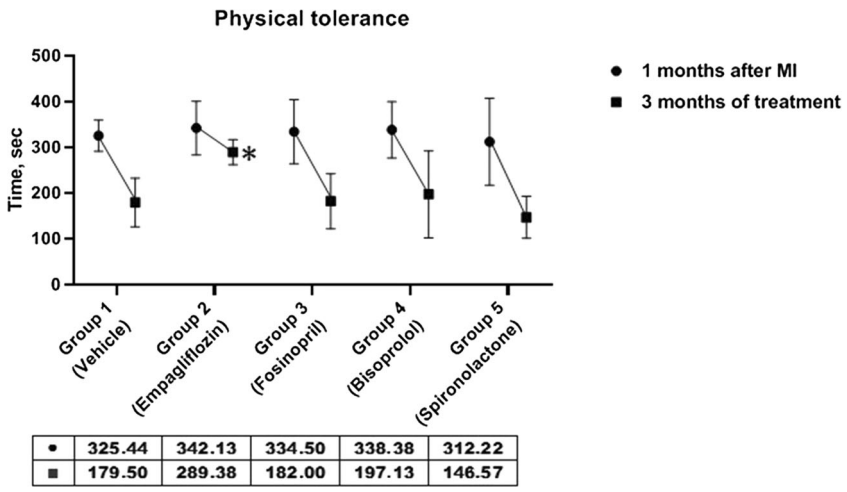


**Fig. 5** Physical tolerance changes in experimental groups during treatment (M ± STD, s). Physical tolerance as maximum activity time (MAT) on treadmill decreased in all groups after 3 months of treatment but in the rats treated with empagliflozin, MAT was significantly higher than in the fosinopril group, bisoprolol, spironolactone group, and vehicle ($p = 0{,}0036$ by Kruskal-Wallis test)

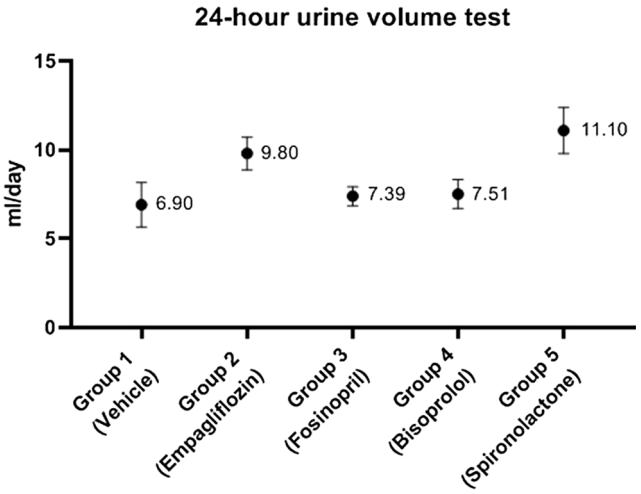


**Fig. 6** The 24-h urine volume test (M ± STD, ml/day). Urine production did not significantly differ between experimental groups ($p = 0.09$ by Kruskal-Wallis test) but a distinct tendency towards higher urine output in rats treated by empagliflozin and spironolactone can be seen

Diuretic action is one of the most evident clinical effects of empagliflozin that could be observed in HF (Heerspink et al. 2016). It can be explained by the main mechanism of action of SGLT2 inhibitors on the renal glucose reabsorption, followed by urinary glucose and water excretion (Martens et al. 2017). In our study, empagliflozin in a low dose of 1 mg/kg also induced glucosuria and, concomitantly, decreased glucose levels. Nevertheless, glucosuria was less pronounced as compared with the study by Yurista et al.(2019), who applied empagliflozin in a high dose of 30 mg/kg. We also observed a clear trend to higher 24-h urine output in animals of empagliflozin and spironolactone groups than in vehicle and fosinopril groups. Thus, due to increased water and sodium excretion, empagliflozin treatment can lead to decreased cardiac preload.

A decrease of afterload also may contribute to cardiac protection as demonstrated in humans (Martens et al. 2017). However, in our experiments, it seems not to be the main mechanism of empagliflozin because the blood pressure was not reduced (Kulikov et al. 2016).

Furthermore, empagliflozin improved treadmill-based exercise tolerance being superior to all reference compounds. This novel finding is very important in view of muscle atrophy by HF patients (Nilsson et al. 2008) because no therapy exists that directly reverses this myopathy. Notably, cardiac cachexia that affects 10–16% of patients with chronic HF is known to be an independent risk factor for mortality in HF (Anker et al. 1997; Kosiborod et al. 2017). In our study, empagliflozin increased maximum activity time at the treadmill 1.6-fold compared with vehicle. Therefore, empagliflozin may be considered a new therapeutic option for treatment muscle atrophy, and this therapy may also prevent mortality. Further research into the effects of SGLT2 inhibitors on the skeletal muscle is necessary.

Our results on the empagliflozin effects on LV remodeling are in accordance with recent findings on cellular mechanisms of SGLT2 inhibitors. It has been shown that SGLT2 inhibitors directly interfere with sodium hydrogen exchanger (NHE) preventing its activation, and thus, preventing intracellular acidosis and calcium overload in the heart and kidneys (Baartscheer et al. 2017). Other described mechanisms of empagliflozin include anti-inflammation, anti-fibrosis (Lee et al. 2017), and prevention of oxidative stress (Joubert et al. 2017) as well as increased energy supply via oxidation of β-hydroxybutirate and increased oxygen transport due to high hematocrit level (Martens et al. 2017).

## Study limitations

Based on the purpose of our study, we had to perform multiple comparisons of variables between 5 study groups which significantly reduced the power of statistical analysis. Another limitation is the lack of data on diastolic LV function. It has been previously shown an improvement of the diastolic LV function after empagliflozin treatment (Heidenreich 2015). Therefore, this important aspect requires further investigations.

Altogether, empagliflozin prevented progression of left ventricular dysfunction and improved tolerance to physical exercise in normoglycemic rats with HF. Empagliflozin treatment was superior with respect to physical tolerance compared with fosinopril, bisoprolol, and spironolactone.

Our experimental results anticipate the outcomes of ongoing clinical studies. For example, EMPA-HEART study examines empagliflozin effect on systolic and diastolic functions and LV remodeling in patients with diabetes mellitus 2 (Natali et al. 2017); EMPEROR HF clinical trial program evaluates the efficacy and safety of empagliflozin in patients with chronic heart failure, including those with and without type 2 diabetes. Such a program (DAPA-HF) just now successfully finished for another SGLT2 inhibitor dapagliflozin (EMPEROR-Preserved n.d.; EMPEROR-Reduced n.d.; McMurray et al. 2019).

It should be also mentioned that in the present study, ACE inhibitor fosinopril and aldosterone antagonist spironolactone also were very successive in preventing adverse LV remodeling. Accordingly, it is important to know whether a combination of these compounds with SGLT inhibitors is beneficial or not. Moreover, systemic action of the SGLT2 inhibitors on volume overload may activate renin excretion and, subsequently, the renin-angiotensin cascade and aldosterone production. Due to this, a combination of SGLT2 inhibitors with RAAS blockers or/and aldosterone antagonists may be needed. A combination of ACE inhibitor, beta-blocker, and aldosterone antagonist with or without the addition of

empagliflozin would be also important to compare. Future investigations should provide information that is crucial for HF treatment.

Based on our results on physical tolerance, empagliflozin may be considered a new therapeutic option for treatment of muscle atrophy in HF.

The abstract of this paper was presented at the Heart Failure 2019 & World Congress on Acute Heart Failure Congress name «Efficacy of combination therapy with empagliflozin and baseline drugs in post-infarct heart failure in normoglycemic rats» and at the Heart Failure 2018 & World Congress on Acute Heart Failure Congress name « Comparative efficacy of empagliflozin and drugs of baseline therapy in post-infarct heart failure in normoglycemic rats» as a poster presentation with interim findings. The poster's abstract was published in "Poster Abstracts" in European Journal of Heart Failure: https://esc365.escardio.org/Congress/Heart-Failure-2019-6th-World-Congress-on-Acute-Heart-Failure/Poster-Session-4-Basic-Science-and-Translational/194571-efficacy-of-combination-therapy-with-empagliflozin-and-baseline-drugs-in-post-infarct-heart-failure-in-normoglycemic-rats; https://esc365.escardio.org/Congress/Heart-Failure-2018-World-Congress-on-Acute-Heart-Failure/Poster-session-1-Basic-Science-Chronic-Heart-Failure-Treatment/173703-comparative-efficacy-of-empagliflozin-and-drugs-of-baseline-therapy-in-post-infarct-heart-failure-i-normoglycemic-rats

**Acknowledgments** We thank Kaiukov I.G., Beresneva O.N., and Galkina O.V. for their excellent technical assistance.

**Author contributions** M. Krasnova: provision of study material, collection and assembly of data, data analysis and interpretation, and manuscript writing; A. Kulikov: conception and design, collection and assembly of data, data analysis and interpretation, and manuscript writing; S. Okovityi: conception and design, administrative support, data analysis, and interpretation; D. Ivkin: collection and assembly of data and data analysis; A. Smirnov: collection and assembly of data; A. Karpov: statistical data analysis and interpretation; E. Kaschina: data analysis and interpretation, manuscript writing advising, and final approval of manuscript. All authors read and approved the manuscript.

**Funding information** This work was supported by the St. Petersburg Chemical and Pharmaceutical University, Saint Petersburg, Russia.

## References

Anker SD, Chua TP, Ponikowski P, Harrington D, Swan JW, Kox WJ, Poole-Wilson PA, Coats AJ (1997) Hormonal changes and catabolic/anabolic imbalance in chronic heart failure and their importance for cardiac cachexia. Circulation 96(2):526–534

Baartscheer A, Schumacher CA, Wüst RC, Fiolet JW, Stienen GJ, Coronel R, Zuurbier CJ (2017) Empagliflozin decreases myocardial cytoplasmic Na+ through inhibition of the cardiac $Na^+/H^+$ exchanger in rats and rabbits. Diabetologia 60(3):568–573

Beresneva ON, Kulikov AN, Parastaeva MM, Okovityi SV, Ivkin DY, Zaraiskii M (2017) The influence of empagliflozin on microRNA-21 urinary expression in Wistar rats with left coronary ligation. Nephrol Dial Transplant 32(Suppl 3):iii613–iii614. https://doi.org/10.1093/ndt/gfx174.MP504

Cavaiola TS, Pettus J (2018) Cardiovascular effects of sodium glucose cotransporter 2 inhibitors. Diabetes Metab Syndr Obes 11:133–148

Boehringer Ingelheim Pharmaceuticals Inc. (2013) Pharmacology/toxicology NDA review and evaluation. NDA 204629

Cezar MD, Damatto RL, Pagan LU, Lima AR, Martinez PF, Bonomo C, Rosa CM, Campos DH, Cicogna AC, Gomes MJ, Oliveira SA Jr, Blotta DA, Okoshi MP, Okoshi K (2015) Early spironolactone treatment attenuates heart failure development by improving myocardial function and reducing fibrosis in spontaneously hypertensive rats. Cell Physiol Biochem 36(4):1453–1466

Connelly KA, Zhang Y, Visram A, Advani A, Batchu SN, Desjardins JF, Thai K, Gilbert RE (2019) Empagliflozin improves diastolic function in a nondiabetic rodent model of heart failure with preserved ejection fraction. JACC Basic Transl Sci 4(1):27–37

Empagliflozin outcome trial in patients with chronic heart failure with preserved ejection fraction (EMPEROR-Preserved). Clinicaltrials.gov. https://clinicaltrials.gov/ct2/show/NCT03057951?term=emperor&rank=2. Accessed 20 Feb 2017

Empagliflozin outcome trial in patients with chronic heart failure with reduced ejection fraction (EMPEROR-Reduced). Clinicaltrials.gov. https://clinicaltrials.gov/ct2/show/NCT03057977?term=emperor&rank=1. Accessed 20 Feb 2017

Gohlke P, Unger T (1995) Chronic low-dose treatment with perindopril improves cardiac function in stroke-prone spontaneously hypertensive rats by potentiation of endogenous bradykinin. 76(15):41E–45E

Gohlke P, Linz W, Schölkens BA, Kuwer I, Bartenbach S, Schnell A, Unger T (1994) Angiotensin-converting enzyme inhibition improves cardiac function. Role of bradykinin. Hypertension 23(4):411–418

Grempler R, Thomas L, Eckhardt M, Himmelsbach F, Sauer A, Sharp DE, Bakker RA, Mark M, Klein T, Eickelmann P (2012) Empagliflozin, a novel selective sodium glucose cotransporter-2 (SGLT-2) inhibitor: characterisation and comparison with other SGLT-2 inhibitors. Diabetes Obes Metab 14(1):83–90

Hayek T, Attias J, Coleman R, Brodsky S, Smith J, Breslow JL, Keidar S (1999) The angiotensin-converting enzyme inhibitor, fosinopril, and the angiotensin II receptor antagonist, losartan, inhibit LDL oxidation and attenuate atherosclerosis independent of lowering blood pressure in apolipoprotein E deficient mice. Cardiovasc Res 44:579–587

Heerspink HJ, Perkins BA, Fitchett DH, Husain M, Cherney DZ (2016) Sodium glucose cotransporter 2 inhibitors in the treatment of diabetes mellitus: cardiovascular and kidney effects, potential mechanisms, and clinical applications. Circulation. 134(10):752–772

Heidenreich P (2015) Heart failure prevention and team-based interventions. Heart Fail Clin 11(3):349–358

Joubert M, Jagu B, Montaigne D, Marechal X, Tesse A, Ayer A, Dollet L, Le May C, Toumaniantz G, Manrique A, Charpentier F, Staels B, Magré J, Cariou B, Prieur X (2017) The sodium-glucose cotransporter 2 inhibitor dapagliflozin prevents cardiomyopathy in a diabetic lipodystrophic mouse model. Diabetes 66(4):1030–1040

Kaplan A, Abidi E, El-Yazbi A, Eid A, Booz GW, Zouein FA (2018) Direct cardiovascular impact of SGLT2 inhibitors: mechanisms and effects. Heart Fail Rev 23(3):419–437 https://doi.org/10.1007/s10741-017-9665-9

Karpov AA, Ivkin DY, Dracheva AV, Pitukhina NN, Uspenskaya YK, Vaulina DD, Uskov IS, Eyvazova SD, Minasyan SM, Vlasov E, Buryakina AV, Galagudza MM (2014) Rat model of post-infarct heart failure by left coronary artery occlusion: technical aspects, functional and morphological assessment. Biomedicine 1:32–48

Kluger AY, Tecson KM, Lee AY, Lerma EV, Rangaswami J, Lepor NE, Cobble ME, McCullough PA (2019) Class effects of SGLT2 inhibitors on cardiorenal outcomes. Cardiovasc Diabetol 18(1):99

Konstam MA, Kramer DG, Patel AR, Maron MS, Udelson JE (2011) Left ventricular remodeling in heart failure: current concepts in clinical significance and assessment. JACC Cardiovasc Imaging 4(1):98–108

Kosiborod M, Cavender MA, Fu AZ, Wilding JP, Khunti K, Holl RW, Norhammar A, Birkeland KI, Jørgensen ME, Thuresson M, Arya N,

Bodegård J, Hammar N, Fenici P (2017) CVD-REAL Investigators and Study Group. Lower risk of heart failure and death in patients initiated on sodium-glucose cotransporter-2 inhibitors versus other glucose-lowering drugs: the CVD-REAL study (comparative effectiveness of cardiovascular outcomes in new users of sodium-glucose cotransporter-2 inhibitors). Circulation 137(9):989–991

Kulikov AN, Okovityj SV, Ivkin DY, Karpov AA, Lisitsky DS, Lyubishin MM et al (2016) Effects of empagliflozin in an experimental model of chronic heart failure in normoglycemic rats. Russ Heart Fail J 17(6):454–460

Kulikov AA, Okovityi SV, Ivkin DY, Karpov AA, Lisitskyi DS, Lubishin MM, Alekseeva PA, Pitukhina NN, Smirnov AV, Kaiukov IG, Parusova EV (2017) Empagliflozin influence on the course of experimental heart failure in normoglycemic rats. Eur J Heart Fail 19–S1:165

Lee TM, Chang NC, Lin SZ (2017) Dapagliflozin, a selective SGLT2 inhibitor, attenuated cardiac fibrosis by regulating the macrophage polarization via STAT3 signaling in infarcted rat hearts. Free Radic Biol Med 104:298–310

Martens P, Mathieu C, Verbrugge FH (2017) Promise of SGLT2 inhibitors in heart failure: diabetes and beyond. Curr Treat Options Cardiovasc Med 19(3):23

McMurray JJV, DeMets DL, Inzucchi SE, Køber L, Kosiborod MN, Langkilde AM, Martinez FA, Bengtsson O, Ponikowski P, Sabatine MS, Sjöstrand M (2019) Solomon SD; DAPA-HF Committees and Investigators. The dapagliflozin and prevention of adverse-outcomes in heart failure (DAPA-HF) trial: baseline characteristics. Eur J Heart Fail 21(11):1402–1411

Natali A, Nesti L, Fabiani I, Calogero E, Di Bello V (2017) Impact of empagliflozin on subclinical left ventricular dysfunctions and on the mechanisms involved in myocardial disease progression in type 2 diabetes: rationale and design of the EMPA-HEART trial. Cardiovasc Diabetol 16(1):130

Neal B, Perkovic V, Mahaffey KW, de Zeeuw D, Fulcher G, Erondu N, Shaw W, Law G, Desai M, Matthews DR (2017) Canagliflozin and cardiovascular and renal events in type 2 diabetes. N Engl J Med 377(7):644–657

Nilsson KR, Duscha BD, Hranitzky PM, Kraus WE (2008) Chronic heart failure and exercise intolerance: the hemodynamic paradox. Curr Cardiol Rev 4(2):92–100

Okovityi SV, Beresneva ON, Parastaeva MM, Ivanova GT, Ivkin DY, Ivkina AS, Sipovsky VG, Zaraysky MI, Karpov AA, Kucher AG, Bogdanova EO, Sipovskaya EB, Kulikov AN, Kayukov IG (2018) Empagliflozin renal safety in normoglycemic rats failure. Nephrology (Saint-Petersburg) 22(1):83–90

Roger VL (2013) Epidemiology of heart failure. Circ Res 113(6):646–659

Santos-Gallego CG, Requena-Ibanez JA, San Antonio R, Ishikawa K, Watanabe S, Picatoste B, Flores E, Garcia-Ropero A, Sanz J, Hajjar RJ, Fuster V, Badimon JJ (2019) Empagliflozin ameliorates adverse left ventricular remodeling in nondiabetic heart failure by enhancing myocardial energetics. J Am Coll Cardiol 73(15):1931–1944

Vrhovac I, Balen Eror D, Klessen D, Burger C, Breljak D, Kraus O, Radović N, Jadrijević S, Aleksic I, Walles T, Sauvant C, Sabolić I, Koepsell H (2015) Localizations of Na(+)-D-glucose cotransporters SGLT1 and SGLT2 in human kidney and of SGLT1 in human small intestine, liver, lung, and heart. Pflugers Arch 467(9):1881–1898

Watanabe K, Ohta Y, Inoue M, Ma M, Wahed MI, Nakazawa M, Hasegawa G, Naito M, Fuse K, Ito M, Kato K, Hanawa H, Kodama M, Aizawa Y (2001) Bisoprolol improves survival in rats with heart failure. J Cardiovasc Pharmacol 38(Suppl 1):S55–S58

Xia A, Xue Z, Li Y, Wang W, Xia J, Wei T, Cao J, Zhou W (2014) Cardioprotective effect of betulinic acid on myocardial ischemia reperfusion injury in rats. Evid Based Complement Alternat Med 573745

Yurista SR, Sillje HHW, Oberdorf-Maass SU, Schouten EM, Pavez Giani MG, Hillebrands JL, van Goor H, van Veldhuisen DJ, de Boer RA, Westenbrink BD (2019) Sodium-glucose co-transporter 2 inhibition with empagliflozin improves cardiac function in non-diabetic rats with left ventricular dysfunction after myocardial infarction. Eur J Heart Fail 21(7):862–873 https://doi.org/10.1002/ejhf.1473

Zinman B, Wanner C, Lachin JM, Fitchett D, Bluhmki E, Hantel S, Fitchett D, Bluhmki E, Hantel S, Mattheus M, Devins T, Johansen OE, Woerle HJ, Broedl UC, Silvio E (2015) Empagliflozin, cardiovascular outcomes, and mortality in type 2 diabetes. N Engl J Med 373(22):2117–2128