Empagliflozin enhances human and murine cardiomyocyte glucose uptake by increased expression of GLUT1

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Abbreviations

2-DG	2-Deoxyglucose
HF	Heart failure
SGLT2	Sodium–glucose cotransporter 2
TAC	Transverse aortic constriction
WT	Wild-type

To the Editor: The results of the Empagliflozin, Cardiovascular Outcome Event Trial in Type 2 Diabetes Mellitus Patients (EMPA-REG OUTCOME) showed that the sodium–glucose cotransporter 2 (SGLT2) inhibitor empagliflozin reduced mortality and hospitalisation due to heart failure (HF) in high-risk diabetic patients [1]. We have previously reported that empagliflozin improved contractile function in human cardiomyocytes isolated from individuals with HF, as well as myocytes from a mouse model of HF by transverse aortic constriction (TAC) [2]. However, we have shown that SGLT2 is not expressed in healthy or failing myocardium.

Cardiomyocytes mainly metabolise fatty acids to generate energy for contraction [3]. However, HF is characterised by fatty

acid oxidation dysfunction, rendering myocytes more dependent on glucose metabolism [3]. The major pathway of glucose uptake in the cardiomyocyte is via glucose transporters of the GLUT family [3]. However, GLUT1 expression has been shown to be reduced in human HF [4]. In addition, transgenic overexpression of GLUT1 protects mice from the development of HF [5]. In the present study we investigated whether empagliflozin affects glucose transporter expression and glucose metabolism in cultured murine and human ventricular cardiomyocytes.

For detailed methods, please refer to the electronic supplementary material (ESM). Experiments conform to the Declaration of Helsinki and were approved by local authorities. Left ventricular myocardium was obtained from explanted hearts of heart transplant recipients with end-stage HF. Written consent had been given prior to tissue donation. The clinical characteristics of the donors can be found in ESM Table 1. For some experiments, male C57BL/6J mice (8–12 weeks old, Charles River, Sulzfeld, Germany; housed according to German animal laws) were used and HF was induced by TAC (at 5 weeks, as previously described [6]; see ESM Methods for details). Left ventricular function was assessed by echocardiography (ESM Methods, ESM Table 2).

Cardiomyocytes were isolated and cultured as described previously [2] (see ESM Methods for details) and exposed to a physiologically relevant concentration of empagliflozin (1 µmol/l [7]) or vehicle control (dimethyl sulfoxide; DMSO). Cell viability after 24 h exposure to empagliflozin was not different from vehicle control and unaffected by treatment with the GLUT inhibitor fasentin (50 µmol/l; ESM Fig. 1). However, the inclusion of non-vital cells in our experiments might limit the extrapolation of our data to the in vivo situation.

Western blots were performed using cell lysates. After denaturation (30 min at 37°C) in 1% (vol./vol.) β-mercaptoethanol, proteins were separated on 8% (wt/vol.) SDS-polyacrylamide gels, transferred to a membrane and incubated with primary GLUT antibodies at 4°C overnight (for details, see ESM Methods).

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Secondary horseradish peroxidase (HRP)-conjugated donkey anti-rabbit and sheep anti-mouse IgG antibodies were incubated for 1 h at room temperature. For detection, Immobilon Western

Chemiluminescent HRP Substrate was used (see ESM Methods for details). We focused on the expression of GLUT1 and GLUT4 as they are the most abundant GLUT transporters in

Fig. 1 Empagliflozin (E) exposure for 24 h significantly increased GLUT1 expression in isolated murine WT ventricular cardiomyocytes (**a, b**), cardiomyocytes from mice with HF due to TAC (**d, e**), and isolated human ventricular cardiomyocytes from individuals with end-stage HF undergoing transplantation (**g, h**) vs vehicle control (V). In contrast, GLUT4 expression was unaltered in murine WT (**c**) and TAC (**f**) cardiomyocytes, and human HF ventricular cardiomyocytes (**i**). Empagliflozin exposure (24 h) significantly increased intracellular glucose concentration in murine WT (**j**) and TAC (**k**) cardiomyocytes, and human HF ventricular cardiomyocytes (**l**). Data are shown as scatter plots with the mean indicated. In addition, we report spaghetti graphs for paired data from each specimen to enable better evaluation of the empagliflozin effect. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, paired t test. (**m**) Glucose uptake (assessed as 2-DG uptake) was significantly increased by 24 h empagliflozin exposure in isolated cardiomyocytes from mice with HF due to TAC, which was blocked by the GLUT inhibitor fasentin (F, 50 $\mu\text{mol/l}$). ** $p < 0.01$ vs vehicle, * $p < 0.05$ vs vehicle, † $p < 0.05$ vs empagliflozin. GAPDH, glyceraldehyde 3-phosphate dehydrogenase

the heart and are responsible for handling the bulk of glucose transport [3]. One sample from Fig. 1c was excluded from the analysis due to failure of the loading control (GAPDH).

Glucose concentration was evaluated in wild-type (WT) and TAC murine cardiomyocytes and failing human ventricular cardiomyocytes using the Abcam Glucose Assay Kit (ab65333). In brief, after culture, cardiomyocytes were settled, supernatant was discarded, and cells were washed. Perchloric acid/potassium hydroxide deproteinisation was performed as described by the manufacturer. After addition of samples to well plates, reaction buffer from the kit was added and fluorescence was analysed using a Tecan plate reader (Tecan Group, Männedorf, Switzerland). For further details, please refer to the ESM Methods.

Glucose uptake was evaluated for TAC murine cardiomyocytes using the Abcam Glucose Uptake Assay Kit (ab136956) (see ESM Methods for details). In brief, after culture, cells were washed with PBS and starved in Tyrode's solution with 10 mmol/l mannitol (instead of glucose) for 40 min. 2-Deoxyglucose (2-DG; 10 mmol/l) was added and in some cases fasentin 50 $\mu\text{mol/l}$ was added to inhibit GLUT1 (experimental groups: vehicle, empagliflozin, vehicle + fasentin, empagliflozin + fasentin) for 20 min. 2-DG was omitted in respective negative controls. The rest of the protocol was performed as detailed in the manufacturer's protocol. After addition of reaction mix, fluorescence was measured using a Tecan plate reader.

As all mice were from the same background and for TAC were subjected to the same surgical technique, no specific randomisation was performed. Cells from the cardiomyocyte isolations were split into equal parts and cultured with either vehicle or empagliflozin (+/– fasentin). Investigators were blinded with respect to group assignment. Outcome assessment was unblinded.

For all data, normality was tested using the Shapiro–Wilk normality test. All data showed normal distribution. Data points from each specimen were generated in pairs, as each cell isolation was split and cardiomyocytes exposed to vehicle and empagliflozin. Thus, the paired Student's t test was used to test significance. Data are shown as scatter plots with the mean indicated. In addition, we

report spaghetti graphs for paired data from each specimen to enable better evaluation of the empagliflozin effect.

Here, we show for the first time that empagliflozin exposure (24 h) resulted in significantly increased GLUT1 expression in murine and human ventricular cardiomyocytes, while expression of GLUT4 was unaltered (Fig. 1a–i). Expression of other cardiac GLUT transporters (GLUT8, GLUT10, GLUT12) was also unaffected by empagliflozin exposure (data not shown).

Increased empagliflozin-dependent GLUT1 expression was not only observed in healthy WT murine myocytes (Fig. 1a–c), but also in isolated ventricular cardiomyocytes of mice with HF induced by TAC (Fig. 1d–f), and, importantly, also in isolated human ventricular myocytes of individuals with end-stage HF (Fig. 1g–i).

To investigate if increased GLUT1 expression with empagliflozin may affect intracellular glucose availability, we measured intracellular glucose concentration in isolated ventricular myocytes. Interestingly, 24 h empagliflozin exposure significantly increased glucose concentration in ventricular myocytes isolated from WT mice, mice with TAC-induced HF and human failing ventricular myocytes (Fig. 1j–l, respectively).

To test if the increased glucose concentration was due to increased GLUT1-dependent glucose uptake in empagliflozin-exposed cardiomyocytes, we measured the uptake of glucose analogue 2-DG, which cannot be metabolised by the cell and allows for precise measurement of glucose uptake [8]. Since the empagliflozin effects are most relevant in HF, we used isolated TAC cardiomyocytes for these experiments.

Importantly, empagliflozin-exposure for 24 h significantly increased 2-DG uptake in TAC cardiomyocytes compared with vehicle control (Fig. 1m).

To test whether the increased glucose-uptake was indeed due to increased GLUT1 expression, we used the specific GLUT inhibitor fasentin (50 $\mu\text{mol/l}$). While this inhibitor may also affect GLUT4, the unchanged GLUT4 expression allows for assessment of the GLUT1 dependent uptake. Inhibition of GLUT1 with fasentin completely prevented the empagliflozin-dependent stimulation of glucose uptake (Fig. 1m), while expression of GLUT4 was not affected by fasentin (data not shown).

In summary, we show here that empagliflozin directly induces upregulation of GLUT1 expression in isolated failing human and murine cardiomyocytes. Moreover, increased empagliflozin-dependent GLUT1 expression enhanced glucose uptake and the intracellular glucose concentration. Our results may explain, at least in part, the beneficial effects of empagliflozin in HF.

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Data availability Original data can be made available upon request.

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Contribution statement JM, CML, OW, TS, KPH, CMS, DT, AH, SP, BEB, SS, LSM and SW contributed to analysis and interpretation of data, drafting and revising the article, and approved the final study. JM, TS and SW designed the experiments and performed data acquisition. JM and SW are both responsible for the integrity of the work as a whole.

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References

1. Zinman B, Wanner C, Lachin JM et al (2015) Empagliflozin, cardiovascular outcomes, and mortality in type 2 diabetes. *N Engl J Med* 373(22):2117–2128. <https://doi.org/10.1056/NEJMoa1504720>
2. Mustroph J, Wagemann O, Lucht CM et al (2018) Empagliflozin reduces Ca/calmodulin-dependent kinase II activity in isolated ventricular cardiomyocytes. *ESC Heart Fail* 5(4):642–648. <https://doi.org/10.1002/ehf2.12336>
3. Shao D, Tian R (2015) Glucose transporters in cardiac metabolism and hypertrophy. *Compr Physiol* 6(1):331–351. <https://doi.org/10.1002/cphy.c150016>
4. Razeghi P, Young ME, Ying J et al (2002) Downregulation of metabolic gene expression in failing human heart before and after mechanical unloading. *Cardiology* 97(4):203–209. <https://doi.org/10.1159/000063122>
5. Liao R, Jain M, Cui L et al (2002) Cardiac-specific overexpression of GLUT1 prevents the development of heart failure attributable to pressure overload in mice. *Circulation* 106(16):2125–2131. <https://doi.org/10.1161/01.CIR.0000034049.61181.F3>
6. Toischer K, Hartmann N, Wagner S et al (2013) Role of late sodium current as a potential arrhythmogenic mechanism in the progression of pressure-induced heart disease. *J Mol Cell Cardiol* 61:111–122. <https://doi.org/10.1016/j.yjmcc.2013.03.021>
7. Baartscheer A, Schumacher CA, Wüst RCI et al (2017) Empagliflozin decreases myocardial cytoplasmic Na⁺ through inhibition of the cardiac Na⁺/H⁺ exchanger in rats and rabbits. *Diabetologia* 60(3):568–573. <https://doi.org/10.1007/s00125-016-4134-x>
8. Liu L, Cui S, Zhang R et al (2017) MiR-421 inhibits the malignant phenotype in glioma by directly targeting MEF2D. *Am J Cancer Res* 7(4):857–868