Empagliflozin reduces Ca/calmodulin-dependent kinase II activity in isolated ventricular cardiomyocytes

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Abstract

Aims The EMPA-REG OUTCOME study showed reduced mortality and hospitalization due to heart failure (HF) in diabetic patients treated with empagliflozin. Overexpression and Ca²⁺-dependent activation of Ca²⁺/calmodulin-dependent kinase II (CaMKII) are hallmarks of HF, leading to contractile dysfunction and arrhythmias. We tested whether empagliflozin reduces CaMKII-activity and improves Ca²⁺-handling in human and murine ventricular myocytes.

Methods and results Myocytes from wild-type mice, mice with transverse aortic constriction (TAC) as a model of HF, and human failing ventricular myocytes were exposed to empagliflozin (1 μmol/L) or vehicle. CaMKII activity was assessed by CaMKII-histone deacetylase pulldown assay. Ca²⁺ spark frequency (CaSpF) as a measure of sarcoplasmatic reticulum (SR) Ca²⁺ leak was investigated by confocal microscopy. [Na⁺]i was measured using Na⁺/Ca²⁺-exchanger (NCX) currents (whole-cell patch clamp). Compared with vehicle, 24 h empagliflozin exposure of murine myocytes reduced CaMKII activity (1.6 ± 0.7 vs. 4.2 ± 0.9, P < 0.05, n = 10 mice), and also CaMKII-dependent ryanodine receptor phosphorylation (0.8 ± 0.1 vs. 1.0 ± 0.1, P < 0.05, n = 11 mice), with similar results upon TAC. In murine myocytes, empagliflozin reduced CaSpF (TAC: 1.7 ± 0.3 vs. 2.5 ± 0.4 1/100 μm⁻¹ s⁻¹, P < 0.05, n = 4 mice) but increased SR Ca²⁺ load and Ca²⁺ transient amplitude. Importantly, empagliflozin also significantly reduced CaSpF in human failing ventricular myocytes (1 ± 0.2 vs. 3.3 ± 0.9, P < 0.05, n = 4 patients), while Ca²⁺ transient amplitude was increased (F/F₀: 0.53 ± 0.05 vs. 0.36 ± 0.02, P < 0.05, n = 3 patients). In contrast, 30 min exposure with empagliflozin did not affect CaMKII activity nor Ca²⁺-handling but significantly reduced [Na⁺]i.

Conclusions We show for the first time that empagliflozin reduces CaMKII activity and CaMKII-dependent SR Ca²⁺ leak. Reduced Ca²⁺ leak and improved Ca²⁺ transients may contribute to the beneficial effects of empagliflozin in HF.

Keywords Empagliflozin; Heart failure; CaMKII; Calcium; Ca leak

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Background

Treatment of diabetic patients with cardiovascular disease with the novel anti-diabetic drug empagliflozin, a selective inhibitor of sodium-dependent glucose transporter 2 (SGLT2) in the renal proximal tubules, reduced the incidence of cardiovascular death by 38% (EMPA-REG OUTCOME trial, n = 7020 patients). The underlying mechanisms are hotly discussed. Surprisingly, the beneficial effects were not driven by a reduction in ischaemic endpoints (stroke or myocardial infarction) but by a reduction in the risk of hospitalization for heart failure (HF). In addition, empagliflozin exerts the same beneficial cardiovascular effects after adjustment for ischaemic risk factors (blood pressure, low-density...
lipoprotein cholesterol, HbA1c). In a mediation analysis of this trial, changes in haematocrit (and haemoglobin) but not HbA1c appeared to be the variables with the largest impact on the risk of cardiovascular death. This argues strongly against glycaemic control as the underlying cardio-protective mechanism. As a consequence, large Phase III trials are currently initiated that investigate empagliflozin in non-diabetic patients with HF (EMPEROR and EMPERIAL trial programs). Interestingly, in a non-diabetic mouse model of afterload-induced HF, empagliflozin prevented worsening of left ventricular function. Overexpression and activation of Ca\(^{2+}\)/calmodulin-dependent kinase II (CaMKII) are hallmarks of HF and also found in diabetic patients, leading to increased diastolic Ca\(^{2+}\) leak from the sarcoplasmic reticulum (SR) and reduced SR Ca\(^{2+}\) load, causing contractile dysfunction and arrhythmias. Interestingly, CaMKII is activated by increased cytosolic Ca\(^{2+}\) concentrations.

**Aims**

We tested whether empagliflozin inhibits CaMKII activity and diastolic SR Ca\(^{2+}\) leak in cultured human and murine ventricular cardiomyocytes.

**Methods**

Experiments conform to the Declaration of Helsinki and were approved by local authorities. Left ventricular myocardium was acquired from explanted hearts of heart transplant recipients with end-stage HF (patient characteristics in Table S1). Atrial tissue was acquired from patients undergoing coronary artery bypass grafting. Written consent had been given prior to tissue donation. For some experiments, C57BL/6 mice (8–12 weeks) were used, and HF was induced by transverse aortic constriction (TAC; 5 weeks, as previously described). Echocardiographic data on heart structure and function of TAC mice are shown in Table S2. Isolated cardiomyocytes were exposed to either vehicle or 1 μmol/L empagliflozin. This concentration is in the range of the typical plasma concentration upon 25 mg q.d. oral treatment of patients. CaMKII activity was measured by CaMKII–histone deacetylase (HDAC4) pulldown normalized to CaMKII expression. Cell isolation, culture, Western blots, and measurement of Na\(^{+}\) and Ca\(^{2+}\) handling were employed as previously described. Cardiomyocyte survival after 30 min or 24 h exposure to empagliflozin or vehicle control was not significantly different. Whole-cell voltage-clamp patch-clamp experiments were performed as described previously, and subsarcolemmal [Na\(^{+}\)] was calculated using the Nernst equation. Liquid chromatography–mass spectrometry (LC–MS) from SGLT2 gel bands from non-failing and failing human myocardium and high-resolution selected reaction monitoring (HR-SRM) for SGLT2 from human failing myocardium, and murine kidney and myocardium were performed as previously described. Data are shown as mean ± SEM; normality was tested using the Shapiro–Wilk normality test and parametric (ANOVA or (paired) Student’s t-test) or non-parametric (Mann–Whitney test or Wilcoxon signed-rank test) tests were used as appropriate.

An extended methods section can be found in the Supporting Information.

**Results**

CaMKII activity was measured using CaMKII pulldown by HDAC4 binding. Interestingly, 24 h exposure of murine wild-type (WT) ventricular cardiomyocytes to empagliflozin (1 μmol/L) significantly reduced CaMKII activity from 4.2 ± 0.9 to 1.6 ± 0.7 (P < 0.05, n = 10 mice, Figure 1A). As CaMKII is a critical regulator of Ca\(^{2+}\) handling, we assessed SR Ca\(^{2+}\) load (caffeine transients, 10 mmol/L) and diastolic Ca\(^{2+}\) leak (Ca\(^{2+}\) sparks) in isolated murine WT ventricular cardiomyocytes. Interestingly, compared with vehicle, 24 h empagliflozin exposure significantly increased SR Ca\(^{2+}\) load (Figure 1B). Without post-translational modifications of cardiac ryanodine receptor (RyR2) that directly reduce their open probability, increased luminal (SR) Ca\(^{2+}\) would lead to increased Ca\(^{2+}\) leak. However, compared with vehicle, 24 h empagliflozin exposure did not increase Ca\(^{2+}\) spark frequency (CaSpF) as a measure of Ca\(^{2+}\) leak but significantly reduced the SR Ca\(^{2+}\) leak/SR Ca\(^{2+}\) load ratio (Figure 1C).

Accordingly, CaMKII-dependent phosphorylation of RyR2 (at serine 2814) was significantly decreased, while the protein kinase A (PKA)-dependent phosphorylation (at S2809) was unaltered upon 24 h exposure to empagliflozin (Figure 1D). Interestingly, phosphorylation of phospholamban (PLN) at threonine 17, perhaps the most frequently studied CaMKII phospho-site, was significantly reduced by 24 h empagliflozin exposure (Figure 1E).

In order to test if empagliflozin also affects CaMKII-dependent SR Ca\(^{2+}\) leak in HF, ventricular myocytes were isolated from mice with HF upon TAC and from explanted hearts of patients with end-stage HF. Intriguingly, compared with vehicle, 24 h empagliflozin exposure significantly reduced CaSpF in TAC myocytes and human failing ventricular cardiomyocytes (Figure 2A). This empagliflozin effect was not restricted to HF. Treatment with empagliflozin also reduced CaSpF in human atrial cardiomyocytes (Figure 2A). Accordingly, 24 h empagliflozin exposure significantly reduced CaMKII activity (CaMKII pulldown by HDAC binding) and CaMKII-dependent RyR2 and PLN phosphorylation in...
failing TAC cardiomyocytes (Figure 2B) and CaMKII-dependent RyR2 phosphorylation in human failing ventricular myocytes (Figure 2C). PKA-dependent RyR2 phosphorylation (at 2809), on the other hand, was not affected by empagliflozin in both in TAC and human failing cardiomyocytes (Figure 2B and C). Expression of neither RyR2 nor PLN was significantly altered.

Increased SR Ca\textsuperscript{2+} load can lead to increased systolic SR Ca\textsuperscript{2+} release and improved contractile function. We show here that, compared with vehicle, 24 h empagliflozin exposure significantly increased Ca\textsuperscript{2+} transient amplitude (CaTransAmpl) in both healthy murine ventricular myocytes and failing ventricular myocytes from TAC mice and human failing hearts (Figure 3A). This increase was less pronounced...
Figure 2  (A) Mean data for CaSpF in failing murine ventricular myocytes (TAC, left panel), human atrial myocytes (middle panel), or human failing ventricular cardiomyocytes (right panel, original line scans also shown). *P < 0.05 Mann–Whitney test. (B) Left panel: original Western blots of CaMKII–HDAC pulldown, total HDAC, CaMKII expression, and GADPH, as well as mean data of CaMKII activity normalized to CaMKII expression from lysates of murine TAC ventricular cardiomyocytes exposed to empagliflozin (Empa, 1 μmol/L) for 24 h. Middle panel: original Western blots and mean data for phospho-S2814/RyR2 ratio and phospho-S2809/RyR2 ratio from murine TAC ventricular cardiomyocytes after 24 h empagliflozin (E) or vehicle (V) exposure. Right panel: original Western blots and mean data for phospho-T17/PLN ratio, as well as PLN and SERCA expression from murine ventricular cardiomyocytes after 24 h empagliflozin (E) or vehicle (V) exposure. (C) Original Western blots and mean data for phospho-S2814/RyR2 ratio, phospho-S2809/RyR2 ratio, and RyR2 expression from human failing ventricular cardiomyocytes. (B and C) *P < 0.05 Wilcoxon signed-rank test or paired t-test as appropriate. CaMKII, Ca2+/calmodulin-dependent kinase II; CaSpF, Ca2+ spark frequency; HDAC, histone deacetylase; PLN, phospholamban; TAC, transverse aortic constriction.

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Figure 3  (A) Mean data for Ca^{2+} transient amplitude (CaTransAmpl) in healthy murine ventricular myocytes (left panel), failing murine ventricular myocytes (TAC, middle panel, both Fura-2), or human failing ventricular cardiomyocytes (right panel, Fluo-4) elicited by electrical field stimulation at indicated frequencies. (B) Mean data of subsarcolemmal [Na^+]i in murine WT CM (left panel, calculated from Na^{+}/Ca^{2+}-exchanger (NCX) current measured by whole-cell patch clamp), diastolic [Ca^{2+}]i (Fura-2, middle panel) in murine WT CM (electrical field stimulation at indicated frequencies), and CaMKII activity (CaMKII–HDAC pulldown) in human failing ventricular tissue after 30 min incubation with empagliflozin. (C) Mean data of [Na^+]i, [SBFI (sodium-binding benzofuran isophthalate = sodium indicator) fluorescence, left panel] and diastolic [Ca^{2+}]i (Fura-2, right panel) in murine WT CM after 24 h empagliflozin exposure. (D) Representative chromatographic images of HR-SRM analyses of human and murine SGLT2 protein. Two proteotypic SGLT2 peptides that are identical between mouse and human were used for highly sensitive detection of SGLT2 (top panel, VCGTEVGCSNIAYPR; bottom panel, GTVGGYFLAGR). For each peptide, six transitions (y4, 8, 9, 10, 11, 13 or y4, 6, 7, 8, 9, respectively) were detected. In contrast to clear SGLT2 expression in mouse kidney (at retention times of 65 or 72.5 min), no signal was detected neither in WT or TAC mouse hearts nor in human failing myocardium. Of note, the signal at 71.5 min retention time in human HF is unspecific. *P < 0.05 (Mann–Whitney test), #P < 0.05 two-way ANOVA. CaMKII, Ca^{2+}/calmodulin-dependent kinase II; HDAC, histone deacetylase; HR-SRM, high-resolution selected reaction monitoring; TAC, transverse aortic constriction.

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A

WT mice

| Frequency (Hz) | Empa, n=41 (13) | Vehicle, n=40 (13) |
|---------------|-----------------|-------------------|
| 0.5           | 0.6             | 0.2              |
| 1.0           | 0.4             | 0.1              |
| 2.0           | 0.2             | 0.0              |

TAC in mice

| Frequency (Hz) | Empa, n=13 (4) | Vehicle, n=17 (4) |
|---------------|-----------------|-------------------|
| 0.5           | 0.5             | 0.2              |
| 1.0           | 0.4             | 0.1              |
| 2.0           | 0.2             | 0.0              |

Human failing ventricular CM

| Frequency (Hz) | Empa, n=12(3) | Vehicle Empa, n=13(3) |
|---------------|----------------|-----------------------|
| 0.5           | 0.5             | 0.2                    |
| 1.0           | 0.4             | 0.1                    |
| 2.0           | 0.2             | 0.0                    |

B

30 min Empagliflozin

| [Na]^+ (mmol/l) | Empa, n=16 (4) | Vehicle, n=16 (4) |
|----------------|-----------------|-------------------|
| 6             | 8               | 6                 |
| 7             | 9               | 7                 |

Diastolic [Ca^{2+}]i (FURA F340/F380)

| Frequency (Hz) | Empa, n=16 (4) | Vehicle, n=16 (4) |
|---------------|-----------------|-------------------|
| 0.5           | 2.0             | 1.5               |
| 1.0           | 1.5             | 1.0               |
| 2.0           | 1.0             | 0.5               |

CaMKII–activity

| Frequency (Hz) | Empa, n=11 | Vehicle Empa, n=11 |
|---------------|------------|--------------------|
| 0.5           | 1.0        | 0.5                |
| 1.0           | 0.5        | 0.0                |
| 2.0           | 0.0        | -0.5               |

C

24h Empagliflozin

| SBFi (F340/F380) | Empa, n=23 (7) | Vehicle, n=26 (7) |
|-----------------|-----------------|-------------------|
| 1.0             | 1.2             | 1.1               |

Diastolic [Ca^{2+}]i (FURA baseline)

| Frequency (Hz) | Empa, n=38 (11) | Vehicle, n=37 (11) |
|---------------|-----------------|-------------------|
| 0.5           | 2.0             | 1.5               |
| 1.0           | 1.5             | 1.0               |
| 2.0           | 1.0             | 0.5               |

D

Mouse kidney

| Retention time (min) | Intensity (uAU) |
|----------------------|-----------------|
| 0                    | 150             |
| 1                    | 100             |
| 2                    | 50              |
| 3                    | 25              |

WT mouse

| Retention time (min) | Intensity (uAU) |
|----------------------|-----------------|
| 0                    | 150             |
| 1                    | 100             |
| 2                    | 50              |
| 3                    | 25              |

TAC in mice

| Retention time (min) | Intensity (uAU) |
|----------------------|-----------------|
| 0                    | 150             |
| 1                    | 100             |
| 2                    | 50              |
| 3                    | 25              |

Human HF

| Retention time (min) | Intensity (uAU) |
|----------------------|-----------------|
| 0                    | 150             |
| 1                    | 100             |
| 2                    | 50              |
| 3                    | 25              |
in WT compared with β-adrenergic stimulation (isoproterenol (ISO) 10⁻⁷ M). At 1 Hz, ISO increased CaTransAmpl 2.1 ± 0.65-fold relative to vehicle (n = 9) but empagliﬂozin increased 1.6 ± 0.35-fold only (n = 9 vs. 13 mice).

In contrast to 24 h exposure, acute empagliﬂozin exposure for 30 min did not reduce CaSpF (in healthy murine ventricular myocytes, TAC myocytes, or human failing ventricular myocytes) or SR Ca²⁺ load (murine ventricular myocytes; data not shown). Furthermore, acute empagliﬂozin exposure for 30 min also did not alter diastolic Ca²⁺ concentration (WT myocytes) or CaMKII activity (human failing ventricular tissue; Figure 3B, n = 11 patients). This suggests that direct inhibitory effects of empagliﬂozin on CaMKII or RyR2 are unlikely. Interestingly, consistent with previous data,⁵,⁶ acute (30 min) empagliﬂozin exposure signiﬁcantly reduced subsarcolemmal [Na⁺], (measured by Na⁺/Ca²⁺-exchanger (NCX) currents; Figure 3B). Therefore, empagliﬂozin may rather directly impair Na⁺ entry or elimination. To test if empagliﬂozin may inhibit SGLT2 in the heart, which is a Na⁺ transporter, we analysed SGLT2 expression in ventricular myocardium from three patients with end-stage HF and three non-failing donor hearts by LC–MS. No SGLT2 expression was detected using this methodology (data not shown). We further analysed SGLT2 expression by HR-SRM in murine WT myocardium and kidney, TAC myocardium, and human failing myocardium. In contrast to a robust SGLT2 expression in murine kidney, no SGLT2 signal was detected in myocardium (Figure 3D), full data set linked in the Supporting Information), which is in accordance with previous data.¹³ Thus, cardiac SGLT2 inhibition can be excluded as potential mechanism of action of empagliﬂozin on the heart. Baartscheer et al. recently proposed that empagliﬂozin may reduce cardiomyocyte [Na⁺], and [Ca²⁺], by inhibition of Na⁺/H⁺ exchange (NCX);¹⁴ As CaMKII is activated by increased diastolic [Ca²⁺], which may be a consequence of Na⁺ entry, we also measured Na⁺ and diastolic Ca²⁺. Interestingly, 24 h empagliﬂozin exposure signiﬁcantly reduced cytosolic [Na⁺], and diastolic [Ca²⁺], (Figure 3C).

Conclusions

We show for the first time that empagliﬂozin potently reduces CaMKII activity in isolated failing and non-failing murine ventricular myocytes. More importantly, empagliﬂozin also reduced CaMKII-dependent phosphorylation of RyR2 in not only murine but also failing human ventricular myocytes. This results in a signiﬁcantly reduced SR Ca²⁺ leak and improved contractility as measured by signiﬁcantly increased Ca²⁺ transient amplitude in murine (TAC) and human failing ventricular myocytes. The mechanism by which empagliﬂozin reduces CaMKII activity has yet to be discerned, but our data demonstrate that empagliﬂozin may be useful in the treatment of pathologies with increased CaMKII activity, such as HF.

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Conflict of interest

Julian Mustroph, Olivia Wagemann, Charlotte M. Lücht, Maximilian Trum, Karin P. Hammer, Can Martin Sag, Simon Lebek, Daniel Tarnowski, Jörg Reinders, Filippo Perbellini, Cesare Terracciano, Christof Schmid, Simon Schoppka, Michael Hilker, York Zausig, Steffen Pabel, Samuel T. Sossalla, Frank Schweda, and Stefan Wagner declare that they have no conflict of interest. Lars S. Maier gives talks for Boehringer Ingelheim, the company that sells empagliﬂozin.

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Supporting information

Additional supporting information may be found online in the Supporting Information section at the end of the article.

Table S1. Clinical characteristics of patient, from which atrial biopsies were obtained (upper table), or from which left ventricular tissue of explanted hearts (after heart transplantation, lower table) was used. y = yes, n = no, Nr = number, CAD = coronary artery disease, ICM = ischemic cardiomyopathy, DCM = dilated cardiomyopathy, EF = ejection fraction in %, LVEDD = left ventricular enddiastolic diameter in mm, m = male, SR = sinus rhythm, CABG = coronary artery bypass graft, valves = valvular dysfunction, MR = mitral regurgitation, TR = tricuspid regurgitation x = indicated severity degree according to international standards, Art Hyp = arterial hypertension, KD = kidney disease, GFR = glomerular filtration rate in ml/min, NTproBNP in pg/ml (internal cutoff <150 pg/ml).

Table S2. Echocardiographic data obtained from 16 mice. OP = TAC surgery, bpm = beats per minute, LV = left ventricle, AWThd = anterior wall thickness in diastole, PWThd = posterior wall thickness in diastole, LVIDs = left ventricular internal diameter in systole, LVIDd = left ventricular internal diameter in diastole, *P < 0.05 versus respective pre-OP-measurement (Student’s t-test).
References

1. Zinman B, Wanner C, Lachin JM, Fitchett D, Bluhmki E, Hantel S, Mathieu M, Devis M, Johansen OE, Woerle HJ, Broedl UC, Inzucchi SE. Empagliflozin, cardiovascular outcomes, and mortality in type 2 diabetes. *N Engl J Med* 2015; 373: 2117–2128.

2. Fitchett D, Zinman B, Wanner C, Lachin JM, Hantel S, Salsali A, Johansen OE, Woerle HJ, Broedl UC, Inzucchi SE. Heart failure outcomes with empagliflozin in patients with type 2 diabetes at high cardiovascular risk: results of the EMPA-REG OUTCOME trial. *Eur Heart J* 2016; 37: 1526–1534.

3. Inzucchi SE, Zinman B, Fitchett D, Wanner C, Ferrannini E, Schumacher M, Schmoor C, Ohneberg K, Johansen OE, George JT, Hantel S, Bluhmki E, Lachin JM. How does empagliflozin reduce cardiovascular mortality? Insights from a mediation analysis of the EMPA-REG OUTCOME trial. *Diabetes Care* 2018; 41: 356–363.

4. Wanner C, Lachin JM, Inzucchi SE, Fitchett D, Mathieu M, George J, Woerle HJ, Broedl UC, von EM, Zinman B, EMPA-REG OUTCOME Investigators. Empagliflozin and clinical outcomes in patients with type 2 diabetes mellitus, established cardiovascular disease, and chronic kidney disease. *Circulation* 2018; 137: 119–129.

5. Byrne NJ, Parajuli N, Levasseur JL, Boisvenue J, Beker DL, Masson G, Fedak PWM, Verma S, Dyck JRB. Empagliflozin prevents worsening of cardiac function in an experimental model of pressure overload-induced heart failure. *JACC Basic Transl Sci* 2017; 2: 347–354.

6. Toischer K, Hartmann N, Wagner S, Fischer TH, Herting J, Danner BC, Sag CM, Hasenfuss G, Maier LS, Sossa S. Role of late sodium current as a potential arrhythmogenic mechanism in the progression of pressure-induced heart disease. *J Mol Cell Cardiol* 2013; 61: 111–122.

7. Scheen AJ. Pharmacokinetic and pharmacodynamic profile of empagliflozin, a sodium glucose co-transporter 2 inhibitor. *Clin Pharmacokinet* 2014; 53: 213–225.

8. Kreusser MM, Lehmann LH, Keranov S, Hoting M-O, Oehl U, Kohlhaas M, Reil J-C, Neumann K, Olson EN, Dobrev D, Maack C, Maier LS, Gröne H-J, Katus HA, Olson EN, Backs J. Cardiac CaM kinase II genes δ and γ contribute to adverse remodeling but redundantly inhibit calcineurin-induced myocardial hypertrophy. *Circulation* 2014; 130: 1262–1273.

9. Wagner S, Ruff HM, Weber SL, Bellmann S, Sowa T, Schulte T, Anderson ME, Grandi E, Bers DM, Backs J, Belardinelli L, Maier LS. Reactive oxygen species-activated Ca/calmodulin kinase IIδ is required for late INa augmentation leading to cellular Na and Ca overload. *Circ Res* 2011; 108: 555–565.

10. Hofhuis J, Bersch K, Büßenschütt R, Drzymalski M, Liebetanz D, Nikolaev VO, Wagner S, Maier LS, Gärtner J, Klinge I, Thoms S. Dysferlin mediates membrane tubulation and links T-tubule biogenesis to muscular dystrophy. *J Cell Sci* 2017; 130: 841–852.

11. Armoundas AA, Hobai IA, Tomaselli GF, Winslow RL, O’Rourke B. Role of sodium–calcium exchanger in modulating the action potential of ventricular myocytes from normal and failing hearts. *Circ Res* 2003; 93: 46–53.

12. Shannon TR. Quantitative assessment of the SR Ca²⁺ leak–load relationship. *Circ Res* 2002; 91: 594–600.

13. von Lewinski D, Rainer PP, Gasser R, Huber M-S, Khaﬁga M, Wilhelm B, Haas T, Mächler H, Rössl U, Peske B. Glucose-transporter-mediated positive inotropic effects in human myocardium of diabetic and nondiabetic patients. *Metabolism* 2010; 59: 1020–1028.

14. Baartscheer A, Schumacher CA, Wüst RCI, Fiolet JWT, Steinen GJM, Coronel R, Zuurbier CJ. Empagliflozin decreases myocardial cytoplasmic Na⁺ through inhibition of the cardiac Na⁺/H⁺ exchanger in rats and rabbits. *Diabetologia* 2017; 60: 568–573.