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SGLT2 inhibitor ertugliflozin decreases elevated intracellular sodium, and improves energetics and contractile function in diabetic cardiomyopathy

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Abstract

Background: Elevated myocardial intracellular sodium ($[Na^+]_i$) was shown to decrease mitochondrial calcium ($[Ca^{2+}]_{MITO}$) via mitochondrial sodium/calcium exchanger (NCXMITO), resulting in decreased mitochondrial ATP synthesis. The sodium-glucose co-transporter 2 inhibitor (SGLT2i) ertugliflozin (ERTU) improved energetic deficit and contractile dysfunction in a mouse model of high fat, high sucrose (HFHS) diet-induced diabetic cardiomyopathy (DCMP). As SGLT2is were shown to lower $[Na^+]_i$ in isolated cardiomyocytes, we hypothesized that energetic improvement in DCMP is at least partially mediated by a decrease in abnormally elevated myocardial $[Na^+]_i$.

Methods: Forty-two eight-week-old male C57BL/6J mice were fed a control or HFHS diet for six months. In the last month, a subgroup of HFHS-fed mice was treated with ERTU. At the end of the study, left ventricular contractile function and energetics were measured simultaneously in isolated beating hearts by ^{31}P NMR (Nuclear Magnetic Resonance) spectroscopy. A subset of untreated HFHS hearts was perfused with vehicle vs. CGP 37157, an NCX_{MITO} inhibitor. Myocardial $[Na^+]_i$ was measured by ^{23}Na NMR spectroscopy.

Results: HFHS hearts showed diastolic dysfunction, decreased contractile reserve, and impaired energetics as reflected by decreased phosphocreatine (PCr) and PCr/ATP ratio. Myocardial $[Na^+]_i$ was elevated > 2-fold in HFHS (vs. control diet). ERTU reversed the impairments in HFHS hearts

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CRediT authorship contribution statement

Ivan Luptak, Wilson S. Colucci and James A. Balschi designed the study. Ivan Luptak and Tomas Baka interpreted the data. Wilson S. Colucci provided the funding. Ivan Luptak and Tomas Baka wrote the first draft of the manuscript. Ivan Luptak, Dominique Croteau, Sara Young, Huamei He, Jordan M. Chambers and Fuzhong Qin collected the data. Tomas Baka conducted the statistical analyses, and Wilson S. Colucci, James A. Balschi, Marcello Panagia and David R. Pimentel provided relevant intellectual contribution to the development of the manuscript. All authors critically revised the manuscript and gave final approval of the version to be submitted for publication.

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to levels similar to or better than control diet and decreased myocardial $[Na^+]_i$ to control levels. CGP 37157 normalized the PCr/ATP ratio in HFHS hearts.

Conclusions: Elevated myocardial $[Na^+]_i$ contributes to mitochondrial and contractile dysfunction in DCMP. Targeting myocardial $[Na^+]_i$ and/or NCX_{MITO} may be an effective strategy in DCMP and other forms of heart disease associated with elevated myocardial $[Na^+]_i$.

Keywords

Cardiac energetics; Contractile function; Diabetic cardiomyopathy; Ertugliflozin; Myocardial intracellular sodium; Sodium-glucose co-transporter 2 (SGLT2); inhibitor

1. Introduction

Obesity-related type 2 diabetes, which frequently leads to heart failure (HF), predisposes to a diabetic cardiomyopathy (DCMP) characterized by mitochondrial and contractile dysfunction [1,2]. Sodium-glucose co-transporter 2 inhibitors (SGLT2is) improve HF outcomes in patients with or without diabetes [3]. Thus, the beneficial effect of SGLT2is may be unrelated to improved glucose control [4,5]. It has been suggested that SGLT2is act at least in part by lowering abnormally elevated myocardial intracellular sodium ($[Na^+]_i$) [6,7]. Interestingly, elevated myocardial $[Na^+]_i$ was found in various forms of heart disease, including failing [8] and diabetic hearts [9]. It was shown that the pathological induction of the cardiac voltage-gated sodium channel to generate late/persistent Na^+ current (iNaL) significantly contributes to myocardial $[Na^+]_i$ elevation and is involved in the pathophysiology of HF and arrhythmias [10,11]. SGLT2i empagliflozin directly inhibited the INaL current in cardiomyocytes from a murine model of transverse aortic constriction-induced HF [12] and from a two-hit murine model of HF with preserved ejection fraction induced by combining high-fat diet and nitric oxide synthase inhibition [13].

We previously reported hallmarks of type 2 diabetes, including increased body weight and fat mass, impaired glucose tolerance, increased fasting glycemia and insulinemia and increased insulin resistance, in mice fed a high fat, high sucrose (HFHS) diet for 2, 4 and 8 months [14-16]. Furthermore, using ^{31}P NMR (Phosphorus-31 Nuclear Magnetic Resonance) spectroscopy we showed that mice fed HFHS diet develop DCMP characterized by left ventricular (LV) hypertrophy, energetic and contractile dysfunction [17], all of which were prevented by the SGLT2i, ertugliflozin (ERTU) [15]. Here, we hypothesized that myocardial $[Na^+]_i$ is abnormally elevated in DCMP and is lowered by ERTU. Given that elevated $[Na^+]_i$ impairs mitochondrial energetics due to Ca^{2+}-efflux from mitochondria via a mitochondrial sodium/calcium exchanger (NCX_{MITO}) [6,7], we further hypothesized that the ERTU-induced normalization of elevated $[Na^+]_i$ would improve myocardial energetics and contractile function. To assess the role of mitochondrial Na^+/Ca^{2+} exchange, we tested the ability of the NCX_{MITO} inhibitor, CGP 37157, to correct energetics in hearts from DCMP mice with elevated $[Na^+]_i$.

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2. Materials and methods

2.1. Experimental animals and study design

Eight-week-old male C57BL/6J mice (Jackson Laboratory, Bar Harbor, ME, USA) were fed ad libitum a control diet (CD; 10 % kcal lard, 0 % sucrose; product No. D09071703; Research Diets, New Brunswick, NJ, USA) or high fat, high sucrose diet (HFHS; 58 % kcal lard, 28 % kcal sucrose; product No. D09071702; Research Diets) for six months [15, 17]. Forty-two mice were randomized by body weight and divided into 3 groups: i) CD + placebo (water); ii) HFHS + placebo (water); and iii) HFHS + ertugliflozin (ERTU; 0.5 mg/g of diet; Merck, Kenilworth, NJ, USA) in the last month (Fig. 1a) [15]. Mice were housed under standard laboratory conditions with controlled temperature, humidity, and light. The protocol was approved by the Institutional Animal Care and Use Committee at Boston University School of Medicine.

2.2. Determination of left ventricular contractile function and high-energy phosphates in isolated beating hearts by ^{31}P NMR spectroscopy

Left ventricular (LV) contractile function and high-energy phosphates were measured simultaneously in isolated retrograde-perfused Langendorff heart preparation as we have described previously [15, 17]. Hearts were perfused with Krebs-Henseleit buffer containing 0.5 mmol/l pyruvate and 10 mmol/l glucose. In order to measure LV pressure and adjust LV volume, a water-filled balloon was inserted into the LV. The LV developed pressure (DevP) was calculated as: DevP = systolic pressure – end-diastolic pressure (LVEDP). Perfused hearts were placed in a 10-mm glass tube in a 9.4 T vertical bore magnet and maintained at 37 °C throughout the protocol. After stabilization for 30 min, balloon volume was adjusted to achieve an LVEDP of 8–9 mmHg, and held constant during the protocol. LV workload was changed by increasing the concentration of $CaCl_2$ in the Krebs-Henseleit buffer from 2 to 4 mmol/l and increasing the pacing rate from 450 to 600 bpm. Hearts were perfused at baseline workload (450 bpm, 2 mmol/l $CaCl_2$ for 2 measurements (8 min each measurement) and at high workload (600 bpm, 4 mmol/l $CaCl_2$) for another 2 measurements. The measurements were averaged for each condition. Rate pressure product (RPP = DevP × heart rate) was calculated to estimate the work performed. Simultaneously, [ATP], [phosphocreatine (PCr)], [inorganic phosphate (Pi)], and intracellular pH (pH) were measured by ^{31}P NMR using a Varian spectrometer at 161.4 MHz (Varian, Palo Alto, CA, USA) as we have described previously [17]. Each ^{31}P NMR spectrum resulted from the average of 208 free induction decay signals over 8 min. n = 6 (CD), 9 (HFHS), and 4 (HFHS + ERTU) for this measurement.

To determine whether elevated $[Na^+]_i$ contributes to impaired cardiac energetics in DCMP, untreated CD and HFHS hearts were perfused with or without CGP 37157 (1 μmol/l), an NCX_{MITO} inhibitor, n = 5 (CD), and 6 (HFHS) for this measurement.

2.3. Determination of $[Na^+]_i$ in isolated beating hearts by ^{23}Na NMR spectroscopy

$[Na^+]_i$ in isolated beating hearts was determined by ^{23}Na NMR spectroscopy [18]. Briefly, hearts were perfused with Krebs-Henseleit buffer containing 10 mmol/l glucose, 0.5 mmol/l pyruvate, 118 mmol/l NaCl, 5.9 mmol/l KCl, 1.2 mmol/l $MgSO_4$), 25 mmol/l $NaHCO_3$, 3.4

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mmol/l $CaCl_2$, and 3.5 mmol/l $TmDOTP^{5-}$ (thulium(III) 1,4,7, 10-tetraazacyclododecane-N,N',N",N'"-tetra(methylene-phosphonate); shift reagent). A water-filled balloon was inserted into the LV; the balloon volume was adjusted to achieve an LVEDP of 8–9 mmHg, and held constant during the protocol. Perfused hearts were placed in a 10-mm glass tube in a 9.4 T vertical bore magnet and maintained at 37 °C throughout the protocol. ^{23}Na NMR spectra were acquired using a Varian spectrometer at 105.5 MHz. Each ^{23}Na NMR spectrum resulted from the average of 268 free induction decay signals over 1 min. The average of five subsequent ^{23}Na NMR spectra was used for quantification.

Quantification was performed relative to a reference resonance of a capillary containing 12 μL of 736 mmol/l Na^+ and 20 mmol/l $TmDOTP^{5-}$ placed next to the heart. At the end of the protocol, hearts were dried at 60 °C for 48 h to determine the heart dry weights. Finally, $[Na^+]_i$ was calculated as follows: $[Na^+]_i$ = (NMR area)/(NMR standard area) × (Na^+ standard content)/V_i], where V_i is intracellular volume determined from dry weight and ^{31}P NMR as described previously [18, 19]. n = 4 (CD), 5 (HFHS), and 3 (HFHS + ERTU) for this measurement.

2.4. Statistical analysis

Data are expressed as mean ± SEM. The differences among groups were determined using 1- or 2-way ANOVA with Tukey's or Bonferroni's correction for multiple comparisons as appropriate. Specific statistical tests are indicated in the figure legend. A value of $p < 0.05$ was considered statistically significant. The statistical analysis was performed using GraphPad Prism 9 software (GraphPad, San Diego, CA, USA).

3. Results

As expected, HFHS-fed mice developed obesity, myocardial hypertrophy (Table 1), diastolic dysfunction (higher LVEDP at high workload by 59 %), and decreased contractile reserve (lower DevP and RPP at high workload by 32 % and 31 %, respectively) (Fig 1b). Contractile dysfunction was associated with impaired energetics as reflected by decreased PCr and PCr/ATP ratio at baseline (by 32 % and 30 %, respectively) and with high work (by 30 % and 23 %, respectively) (Fig. 1c). ERTU induced weight loss and reversed the myocardial hypertrophy (Table 1) as well as impairments in diastolic function, contractile reserve and cardiac energetics to levels similar to or better than control diet (CD) (Fig. 1b, c). Myocardial $[Na^+]_i$ was elevated > 2-fold in HFHS (vs. CD) hearts, and decreased to control levels with ERTU (Fig. 2). In HFHS hearts, CGP 37157 improved the PCr/ATP ratio back to control levels, suggesting that increased mitochondrial Na^+/Ca^{2+} exchange contributes to energetic dysfunction in DCMP (Fig. 3).

4. Discussion

Our observations show that 1) myocardial $[Na^+]_i$ is elevated in a model of fully developed DCMP; 2) one-month treatment with ERTU corrects both the elevated $[Na^+]_i$ and the associated impairments in energetic and contractile function; and 3) acute inhibition of NCX_{MITO} protects myocardial energetics, further suggesting that mitochondrial dysfunction in this model is related to elevated $[Na^+]_i$ leading to decreased $[Ca^{2+}]_{MITO}$ (Fig. 4).

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Sodium homeostasis is central to myocardial physiology - a low myocardial $[Na^+]_i$ (5–10 mmol/l) facilitates normal excitability and contractility. Conversely, pathological remodeling and HF are associated with elevated $[Na^+]_i$, arrhythmias and contractile dysfunction. Maack and O'Rourke describe a causal mechanism of $[Na^+]_i$-dependent mitochondrial Ca^{2+} efflux resulting in decreased ATP and increased reactive oxygen species production [6]. Mismatch between ATP supply and demand may eventually lead to "energy starvation", a known characteristic of the failing heart [1,6]. Mitochondrial abnormalities associated with HF include a preferential switch of energy substrate from fatty acid oxidation to carbohydrates. Interestingly, a recent report linked pathologic substrate reprogramming to elevated $[Na^+]_i$ and subsequent Ca^{2+} efflux from mitochondria [20]. This makes the $[Na^+]_i$-lowering effect of ERTU consistent with our recent finding of ERTU-dependent metabolic reprogramming to promote fatty acid oxidation and simultaneously improve energetics [15]. Indeed, in the current report *ERTU not only normalized elevated myocardial $[Na^+]_i$ but also reversed cardiac energetic and contractile dysfunction in DCMP*. The ability of the NCX_{MITO} inhibitor CGP 37157 to acutely improve energetics in HFHS hearts further supports the view that mitochondrial Na^+/Ca^{2+} exchange may contribute to the metabolic effect of elevated $[Na^+]_i$. Moreover, the rapid onset of the CGP 37157 effect in our study corresponds to the previously published metabolic reversal also detected as early as 30 min after administration of CGP 37157 [20].

One of the limitations of this study is the exclusive use of male mice. Considering sexual dimorphism in the incidence and outcomes of DCMP in clinical studies [21], such investigations should be a focus of further studies. The whole-body diabetic status of mice fed HFHS diet has been fully described in previous reports [14-16] and is demonstrated by development of obesity in the current study. Here we focused on the morphologic and metabolic characterization of the diabetic cardiomyopathy and how ERTU treatment improves myocardial hypertrophy, diastolic dysfunction and myocardial sodium and energetics. These results fully correspond to our prior findings [15-17] and attest to the model's reproducibility.

In summary, our results suggest that elevated myocardial $[Na^+]_i$ contributes to mitochondrial and contractile dysfunction in DCMP. While the role of mitochondria in the pathophysiology of DCMP is appreciated, there is a dearth of mitochondria-targeted therapies [1]. Our findings suggest that targeting myocardial $[Na^+]_i$ and/or NCX_{MITO} may be a clinically-useful approach. Furthermore, these findings support the role of $[Na^+]_i$ in mediating mitochondrial dysfunction, and suggest that SGLT2 inhibition may be an effective strategy in other forms of heart disease associated with elevated myocardial $[Na^+]_i$.

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Declaration of Competing Interest

Dr. Colucci is supported by an investigator-initiated grant (IISP-57335) from Merck Pharmaceuticals (Kenilworth, NJ, USA). The remaining authors have no disclosures to report.

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Data Availability

Data will be made available on request.

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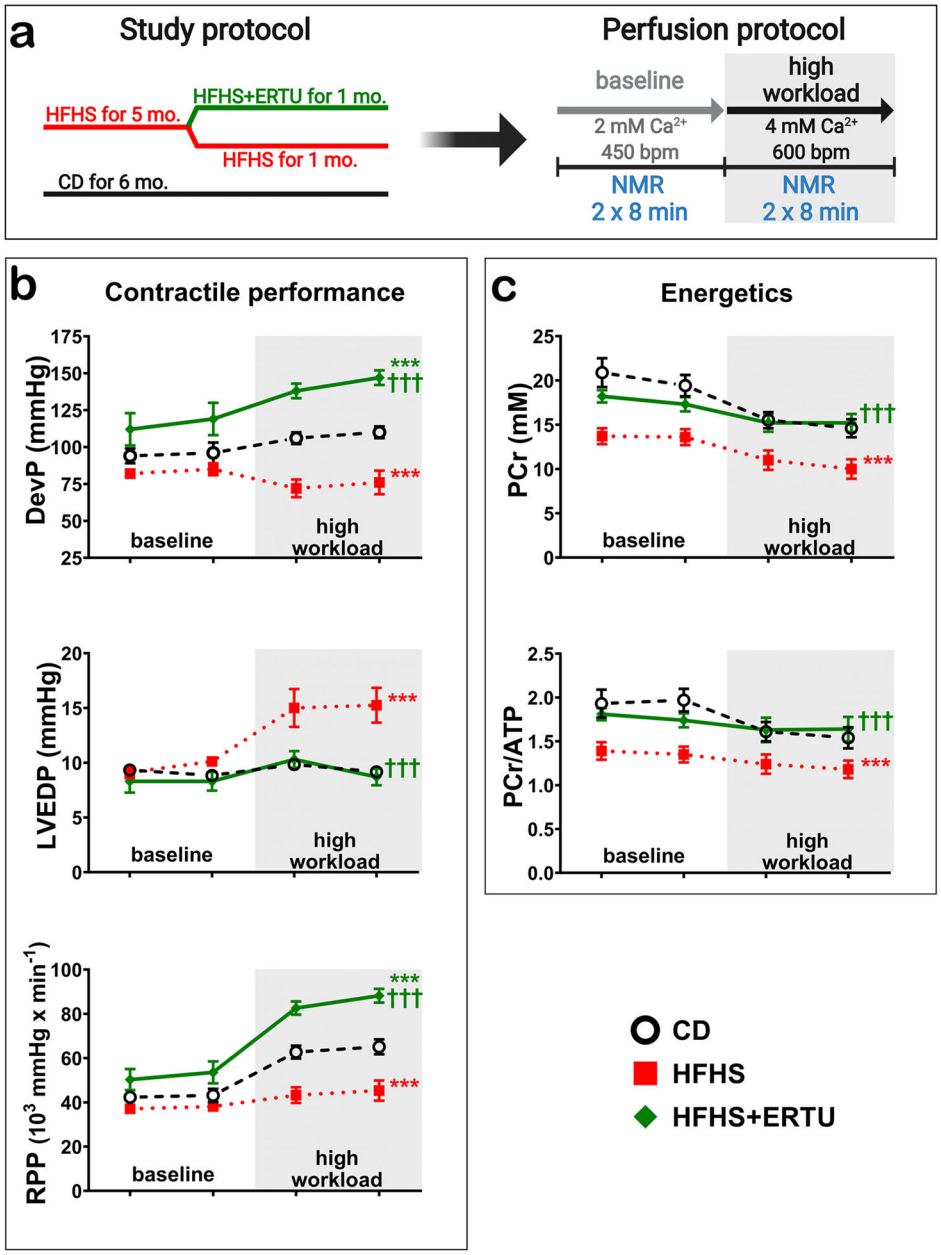

Fig. 1. Study design

(a): 8-week-old male C57BL/6J mice were fed a control (CD) or high fat, high sucrose diet (HFHS) for six months. Ertugliflozin (ERTU) was added to HFHS in the last month. Left ventricular contractile function and energetics were measured in isolated hearts by ^{31}P NMR spectroscopy at baseline (2 mM Ca^{2+}, paced at 450 bpm) and high work demand (4 mM Ca^{2+}, 600 bpm). Myocardial $[Na^+]_i$ was measured by ^{23}Na NMR spectroscopy. CD in black, HFHS in red, HFHS + ERTU in green. **ERTU improves cardiac function (b):** HFHS hearts showed diastolic dysfunction (increased left ventricular end-diastolic pressure, LVEDP) and decreased contractile reserve, assessed by rate pressure product, RPP. **ERTU improves cardiac energetics (c):** Phosphocreatine (PCr) concentration and PCr/ATP were lower in HFHS during all workloads. ERTU treatment normalized LVEDP, contractile reserve and

energetics in HFHS. n = 6 (CD), 9 (HFHS), and 4 (HFHS + ERTU). ***P < 0.001 vs. CD, †††P < 0.001 vs. HFHS. 2-way ANOVA followed by Tukey's multiple comparisons test.

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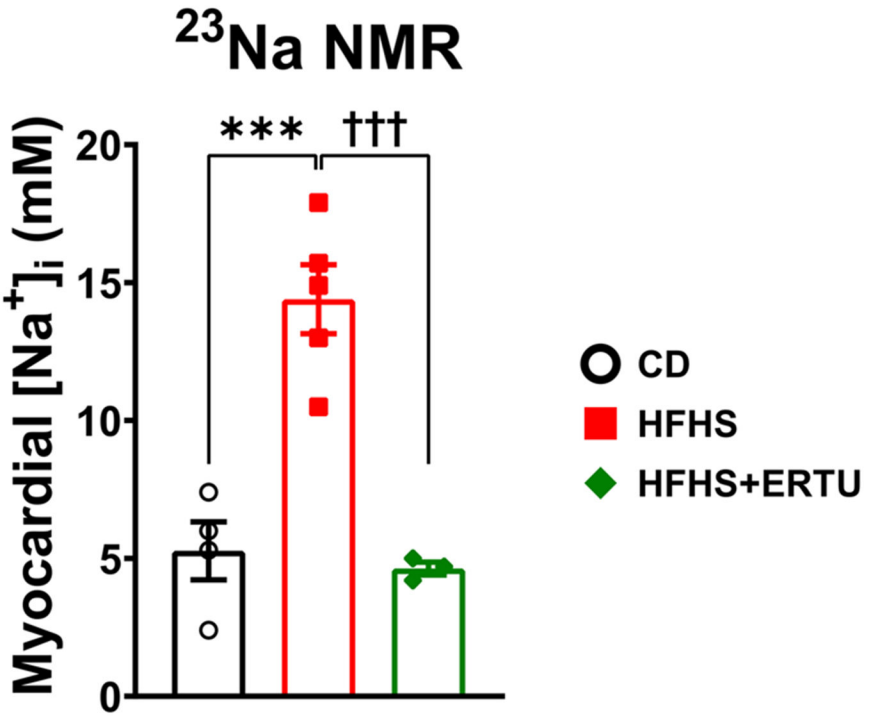

Fig. 2. ERTU decreases elevated $[Na^+]_i$ in DCMP:

$[Na^+]_i$ was elevated 2-fold in HFHS, and normalized by ERTU treatment. n = 4 (CD), 5 (HFHS), and 3 (HFHS + ERTU). 1-way ANOVA followed by Tukey's multiple comparisons test.

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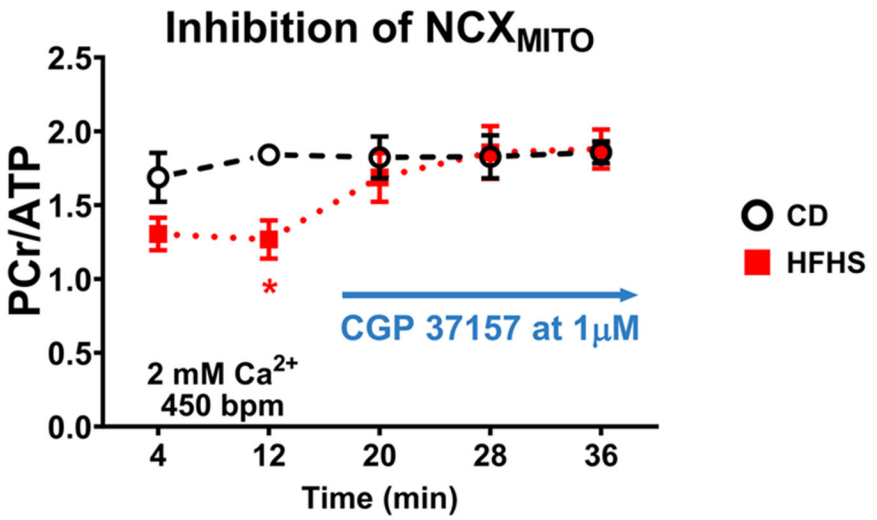

Fig. 3. Elevated $[Na^+]_i$ contributes to impaired cardiac energetics:

Perfusion with 1 μmol/l CGP 37157, an NCX_{MITO} inhibitor, improved the PCr/ATP ratio in HFHS hearts to the level of CD, implying a detrimental effect of elevated myocardial $[Na^+]_i$ on $[Ca^{2+}]_{MITO}$-dependent ATP production in DCMP. n = 5 (CD), and 6 (HFHS). *P < 0.05 vs. CD. 2-way ANOVA followed by Bonferroni's multiple comparisons test.

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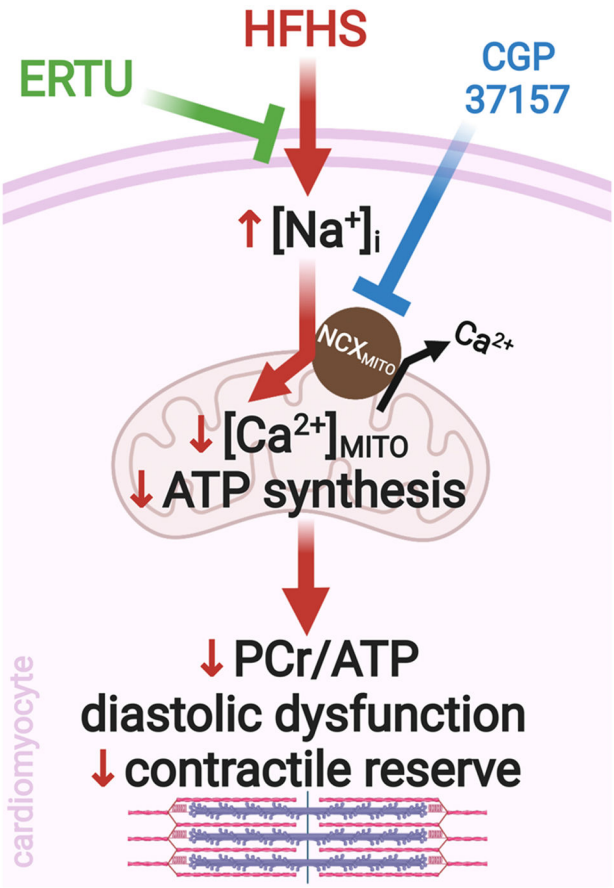

Fig. 4. Schematic representation of ERTU's effect on intracellular sodium, cardiac energetics and function in DCMP.

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Table 1

ERTU decreases elevated body weight and heart weight in DCMP.

HFHS-fed mice developed obesity and myocardial hypertrophy; both reversed by ERTU treatment. **P < 0.01 vs. CD, ***P < 0.001 vs. CD, †P < 0.05 vs. HFHS, †††P < 0.001 vs. HFHS. (n = 7–14) 1-way ANOVA followed by Bonferroni's multiple comparisons test.

	CD	HFHS	HFHS + ERTU
Body weight (g)	38.6 ± 2.2	50.3 ± 1.9**	43.4 ± 1.9†
Heart weight (mg)	119.0 ± 4.0	157.2 ± 4.2***	128.0 ± 2.9†††