Effects of different exercise modalities on cardiac dysfunction in heart failure with preserved ejection fraction

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Abstract

Aims Heart failure with preserved ejection fraction (HFpEF) is an increasingly prevalent disease. Physical exercise has been shown to alter disease progression in HFpEF. We examined cardiomyocyte Ca2+ homeostasis and left ventricular function in a metabolic HFpEF model in sedentary and trained rats following 8 weeks of moderate-intensity continuous training (MICT) or high-intensity interval training (HIIT).

Methods and results Left ventricular in vivo function (echocardiography) and cardiomyocyte Ca2+ transients (CaTs) (Fluo-4, confocal) were compared in ZSF-1 obese (metabolic syndrome, HFpEF) and ZSF-1 lean (control) 21- and 28-week-old rats. At 21 weeks, cardiomyocytes from HFpEF rats showed prolonged Ca2+ reuptake in cytosolic and nuclear CaTs and impaired Ca2+ release kinetics in nuclear CaTs. At 28 weeks, HFpEF cardiomyocytes had depressed CaT amplitudes, decreased sarcoplasmic reticulum (SR) Ca2+ content, increased SR Ca2+ leak, and elevated diastolic [Ca2+] following increased pacing rate (5 Hz). In trained HFpEF rats (HIIT or MICT), cardiomyocyte SR Ca2+ leak was significantly reduced. While HIIT had no effects on the CaTs (1–5 Hz), MICT accelerated early Ca2+ release, reduced the amplitude, and prolonged the CaT without increasing diastolic [Ca2+] or cytosolic Ca2+ load at basal or increased pacing rate (1–5 Hz). MICT lowered pro-arrhythmogenic Ca2+ sparks and attenuated Ca2+-wave propagation in cardiomyocytes. MICT was associated with increased stroke volume in HFpEF.

Conclusions In this metabolic rat model of HFpEF at an advanced stage, Ca2+ release was impaired under baseline conditions. HIIT and MICT differentially affected Ca2+ homeostasis with positive effects of MICT on stroke volume, end-diastolic volume, and cellular arrhythmogenicity.

Keywords Excitation–contraction coupling; Exercise; HFpEF; Metabolic syndrome

Introduction

Obesity-related heart failure (HF) with preserved ejection fraction (HFpEF) is a highly prevalent condition with significant morbidity and mortality, yet pharmacological therapies for this condition remain elusive. Physical exercise has been proposed as an approach to mitigate the course of the disease. For example, in the randomized clinical Ex-DHF pilot study, investigating the impact of supervised exercise training in HFpEF, diastolic dysfunction was partly mitigated associated with improved quality of life.1 The mechanisms by which physical exercise alters...
disease progression in HFP EF are not understood. In a hypertensive rat model of HFP EF, high-intensity interval training (HIIT), initiated before the onset of HFP EF, was associated with improved skeletal muscle performance. In contrast, in a rat model of obesity-related HFP EF, neither HIIT nor moderate-intensity continuous training (MICT) treadmill exercise improved impaired contractile forces in skeletal muscle.

In HF with reduced ejection fraction (HFrEF), depressed Ca\textsuperscript{2+} transients (CaTs) in cardiomyocytes contribute to contractile dysfunction, and exercise training can improve left ventricular (LV) cardiomyocyte Ca\textsuperscript{2+} homeostasis. In an animal model of cardiorenal HFP EF, cardiac remodelling and contractile dysfunction have also been linked to impaired Ca\textsuperscript{2+} homeostasis in LV cardiomyocytes. Similarly, in a model of obesity-related HFP EF, overt HF was associated with altered cytosolic Ca\textsuperscript{2+}. The role of exercise training on cardiomyocyte Ca\textsuperscript{2+} homeostasis has not been evaluated yet.

Vasculature and heart adaption to exercise is highly dependent on the intensity, duration, and frequency of exercise training. Both MICT and HIIT have been associated with T-tubular reverse remodelling and differential contractile in vitro response in the setting of hypertensive heart disease. Moreover, especially MICT has been shown to improve regional cardiac function and reduce cardiomyocyte cross-sectional area. Here, we compared the two popular exercise programmes, MICT and HIIT, and investigated molecular Ca\textsuperscript{2+}-related mechanisms of in vitro dysfunction in LV myocytes from a well-characterized obesity-related HFP EF model.

We hypothesized that LV myocytes from HFP EF rats have impaired Ca\textsuperscript{2+} handling when compared with control. Furthermore, we hypothesized that both exercise programmes improve LV function, measured by echocardiography, and normalize LV cardiomyocyte Ca\textsuperscript{2+} handling.

Methods

Animal model

ZSF-1 rats were acquired (Charles River Laboratories; at 8 weeks of age) and kept in identical conditions of 12 h light/dark cycles and free access to food and water. The model is based on a leptin receptor mutation leading to a lean (ZSF\textsuperscript{+/-}; CT) and obese (ZSF\textsuperscript{+/-}; HFP EF) phenotype. At 20 weeks, the obese rats have repeatedly been shown to develop clinical signs of HFP EF.

All procedures were performed in accordance with the ethical standards laid down in the 1964 Declaration of Helsinki and its later amendments. All procedures were approved by the Norwegian Animal Research Authority in accordance with the Use of Laboratory Animals by the European Commission Directive 86/609/EEC.

Study design

The study design is visualized in Figure 1. The pathological HFP EF phenotype was validated in vivo, and Ca\textsuperscript{2+} cycling of LV cardiomyocytes was investigated at 20–21 weeks in CT and HFP EF (n = 10 per group). Subsequently, HFP EF rats were randomly assigned to undergo either MICT or HIIT or to remain sedentary (Sed.) for 8 weeks until final in vivo and in vitro evaluation at 28 weeks (n = 4 per group). The experimenter was blinded for all in vivo and in vitro experiments.

Training

High-intensity interval training was performed on a treadmill at an inclination of 25° three times per week (four intervals at 90% VO\textsubscript{2peak} for 4 min, with 3 min of recovery at 60% VO\textsubscript{2peak}) for 8 weeks. MCT was performed on a treadmill at an inclination of 25° five times per week (60% VO\textsubscript{2peak} for 1 h, followed by 10 min of running at 40% to 50% VO\textsubscript{2peak}) for 8 weeks.

Echocardiography

Transthoracic echocardiography (Vevo 2,100; VisualSonics, Ontario, Canada) was performed as previously described in detail. In brief, lightly anaesthetized rats (1.5–2% isoflurane) and spontaneously breathing rats in supine position were imaged using a 24 MHz transducer. Diastolic and systolic volumes, as well as global longitudinal strain (speckle tracking), were calculated from images obtained in parasternal long-axis view.

Single-cell isolation

Isolated cardiomyocytes were acquired in 21- and 28-week-old rats by enzymatic digestion using a Langendorff system as previously described in detail. In brief, animals were sacrificed by cervical dislocation, the heart was excised, and the aorta was cannulated. The heart was mounted to the Langendorff system and perfused with nominally Ca\textsuperscript{2+}-free perfusion buffer containing highly purified collagenase (Liberase) at 37°C until satisfactory digestion of the LV was reached. LV tissue was dispersed, cardiomyocytes were allowed to settle, and external [Ca\textsuperscript{2+}] increased to 2 mmol/L in a stepwise manner. LV cardiomyocytes were placed on laminin-coated coverslips for subsequent in vitro experiments.
Solution and chemicals

Chemicals were obtained from Sigma-Aldrich (St. Louis, MO, USA) unless noted otherwise. The fluorescent Ca²⁺ indicator Fluo-4 AM was obtained from Thermo Fisher Scientific (Waltham, MA, USA). Tyrode’s solution contained (in mmol/L): 130 NaCl, 4 KCl, 2 CaCl₂, 1 MgCl₂, 10 glucose, and 10 HEPES, pH adjusted to 7.4 with NaOH. Tyrode’s solution without sodium and Ca²⁺ (0Na0Ca) contained (in mmol/L): 130 LiCl, 4 KCl 1 MgCl₂, 10 glucose, and 10 HEPES, pH adjusted to 7.4 with LiOH.

Confocal Ca²⁺ measurements

Cells were loaded with Fluo-4 AM as previously described. Confocal line scan images were recorded along the longitudinal axis of the cell at 1041 or 870 lines per second (pixel size: 0.12 μm) using a ×40 oil-immersion objective lens with a Zeiss LSM 510 system. The cells were stimulated in an electrical field using a pair of platinum electrodes (voltage: ~50% above threshold) at varying frequencies (according to the experimental protocol), and CaTs were recorded.

Experimental protocol

Left ventricular cardiomyocytes of 21-week-old rats were perfused at 37°C with Tyrode’s solution containing 2 mmol/L Ca²⁺ and stimulated at 1 Hz for 3 min, allowing them to reach a steady state of Ca²⁺ cycling. CaTs were recorded for the last 10 s. Pacing was increased to 2, 3, and 5 Hz and decreased to 1 Hz. Perfusion was immediately changed to 0Na0Ca solution, and electrical pacing was paused. The cell was imaged for 10 s to record spontaneous Ca²⁺ release events (sparks and waves) and quantify the change in cytosolic resting [Ca²⁺]. $\Delta F/F_0$ during 0Na0Ca was used as a measure of cytosolic Ca²⁺ leak. Perfusion was changed to 0Na0Ca solution containing 20 mmol/L caffeine, and the caffeine-induced CaTs were recorded. For a graphical outline of the experimental protocol, refer to Figures 3A and 4A.

Image analysis

Changes in Ca²⁺ are expressed as the amplitude $\Delta F/F_0$, where $F$ represents time-dependent Fluo-4 fluorescence levels under steady-state conditions during electrical stimulation, and $\Delta F = F - F_0$. Tau of a mono-exponential fit of the decay of CaTs was obtained as a parameter of Ca²⁺ removal. $F_{50}$ was defined as 50% of the CaT amplitude, and the corresponding time to $F_{50}$ ($T_{F50}$) was calculated as an indicator of early release (ER).

Transient amplitudes of caffeine-induced contractures ($\Delta F/F_0$) were determined as an indicator of sarcoplasmic reticulum (SR) Ca²⁺ content and tau of Ca²⁺ decay as in indicator of NCX forward-mode function.

For early site analysis

Scan lines along the longitudinal axis were grouped into 1 μm intervals, indicating active couplons. ER was defined to be smaller than the average $T_{F50}$ of the control.
Figure 2  Representative samples of the experimental protocol (A). Left ventricular cardiomyocytes of 21-week-old rats were exposed at 1 Hz electrical stimulation, and cytosolic Ca\textsuperscript{2+} transients were analysed for their Ca\textsuperscript{2+} release amplitude (B), time to half peak (TF\textsubscript{50}; C), time to peak (TTP; D), and Ca\textsuperscript{2+} decay (tau; E). Consecutively, cells were paced at 3 Hz, and cytosolic Ca\textsuperscript{2+} transients were analysed for their Ca\textsuperscript{2+} release amplitude (F), time to half peak (TF\textsubscript{50}; G), time to peak (TTP; H), and Ca\textsuperscript{2+} decay (tau; I). Nuclear Ca\textsuperscript{2+} transients were recorded at 1 Hz electric pacing, and cytosolic Ca\textsuperscript{2+} transients were analysed for their Ca\textsuperscript{2+} release amplitude (J), time to half peak (TF\textsubscript{50}; K), time to peak (TTP; L), and Ca\textsuperscript{2+} decay (tau; M) determined. Statistical analysis: two-tailed, unpaired Student’s t-test. P-values: 1 < 0.027, 2 < 0.0001, 3 < 0.035, and 4 < 0.027. n = cells from 10 animals per group. HFpEF, heart failure with preserved ejection fraction.
Figure 3  Representative samples of the experimental protocol (A). Left ventricular cardiomyocytes of 28-week-old rats were exposed to varying pacing frequencies by electrical stimulation, and Ca²⁺ transients were analysed for their time to peak (TTP; B), Ca²⁺ release amplitude (C), Ca²⁺ decay (tau; D), and diastolic Ca²⁺ (E). Ca²⁺ leak from the sarcoplasmic reticulum (SR; F) during sodium–Ca²⁺ exchanger inactivation. Ca²⁺ load of the SR quantified by application of caffeine (G) and subsequent Ca²⁺ decay (H). Statistical analysis: two-way ANOVA followed by post hoc Bonferroni (B–D: 1–5 Hz); two-tailed, unpaired Student’s t-test (B–D: 1 Hz rec., F–H). P-values: 1≥0.0001, 2<0.0001, 3<0.001, 4<0.01, 5<0.02, 6<0.007, 7<0.047, 8<0.02, 9<0.039, and 10<0.025. n = cells from four animals per group. (B–E) CT, n = 23; heart failure with preserved ejection fraction (HFpEF), n = 19.
**Figure 4** Representative examples of the experimental protocol (A). Ca$^{2+}$ transients (CaTs) were analysed for their Ca$^{2+}$ release amplitude (B), the area under the curve (AUC; C), Ca$^{2+}$ decay during CaTs (tau CaT; D), and diastolic Ca$^{2+}$ (E). Ca$^{2+}$ leak from the sarcoplasmic reticulum (SR; F) during sodium–Ca$^{2+}$ exchanger inactivation. Ca$^{2+}$ load of the SR quantified by application of caffeine (G) and subsequent Ca$^{2+}$ decay (H). Statistical analysis: paired, two-way (B–E: 1–5 Hz) and one-way ANOVA (B–E: 1–5 Hz rec., F–H) followed by post hoc Bonferroni vs. Sed. P-values: $^*_{0.016}$, $^*_{0.004}$, $^*_{0.006}$, $^*_{0.007}$, $^*_{0.005}$, $^*_{0.04}$, $^*_{0.049}$, $^*_{0.046}$, $^*_{0.028}$, and $^*_{0.006}$. n = cells from four animals per group. (B–E) Sed., n = 20; moderate-intensity continuous training (MICT), n = 22; and high-intensity interval training (HIIT), n = 27.
group (CT, $T_{50} < 10.5$ ms), and an ER site was defined to be an active couplon with ER events in at least three out of 10 consecutive stimulation cycles. The fraction of ER events ER sites in 10 consecutive cycles was quantified as the probability of ER.

**Code availability**

Image analysis was mostly performed with the freely available software ImageJ (http://imagej.nih.gov). Calcium transient analysis was performed with custom code (Interactive Data Language), which is not publicly available.

The analyser was blinded towards group and/or treatment for all in vivo and in vitro experiments.

**T-tubular network**

T-tubules were visualized as previously described. In brief, two-dimensional images of LV cardiomyocytes were obtained after staining with the fluorescence probe di-8-butyl-aminonaphthyl-ethylene-pyridinium-propyl-sulfonate, subjected to local thresholding, and the fraction of signal positive pixels in relation to the cell surface was taken as a measure of T-tubular density.

**Western blotting**

Left ventricular tissue samples were homogenized at 4°C in lysis buffer (in mmol/L: 20 Tris–HCl (pH 7.4), 137 NaCl, 20 NaF, 1 sodium pyrophosphate, 50 β-glycerophosphate, 10 EDTA, 1 EGTA, 1 PMSF, 10% glycerol, 1% NP 40, 4 μg/mL aprotinin, 4 μg/mL pepstatin A, and 4 μg/mL leupeptin); 30 μg of tissues homogenates was run on 4–12% Bis–Tris polyacrylamide gels and transferred to nitrocellulose membranes for 1 h. Proteins on membrane were stained with Ponceau S. Non-specific binding was blocked with 5% dried milk in Tris-buffered saline (pH 7.4) containing 0.1% Tween 20. Membranes were probed with anti-phospho-Thr17 PLB, anti-phospho-Ser16 PLB, anti-SERCA2a (Badrilla, Leeds, UK), and anti-PLB (Santa Cruz, Heidelberg, Germany) overnight at 4°C. Anti-rabbit IgG linked with IRDye 680RD or anti-mouse linked with 800CW (LI-COR, Lincoln, Nebraska, USA) were used as a secondary antibody. The signal was detected with Odyssey CLx System. The band intensities and total proteins stain were determined by Image Studio software (LI-COR).

**Data analysis and statistics**

Results are shown as mean ± standard error. Individual data points are shown where spatially feasible. Statistical tests,
Results

Cardiomyocytes of early heart failure with preserved ejection fraction animals (21 weeks) show impaired Ca$^{2+}$ reuptake

Freshly isolated cardiomyocytes of 21-week-old animals were stimulated at 1 and 3 Hz pacing frequencies, and CaTs of the cytosolic and nuclear compartment were examined (Figure 2A). Compared with controls, cytosolic CaTs of early HFpEF animals did not show alterations in Ca$^{2+}$ release amplitude (Figure 2B) and release kinetics (Figure 2C and 2D), but Ca$^{2+}$ reuptake was prolonged (Figure 2E) at 1 Hz pacing. Similar results were observed at 3 Hz (Figure 2F–2I). In the nucleus, diastolic Ca$^{2+}$ and Ca$^{2+}$ release amplitudes remained unaltered in early HFpEF (Figure 2J and 2K), but Ca$^{2+}$ release and reuptake kinetics were significantly slower (Figure 2L–2M). Differences could be detected neither in cell size nor in T-tubular density (Supporting Information, Figure S1).

Cardiomyocytes of heart failure with preserved ejection fraction animals (28 weeks) show impaired Ca$^{2+}$ release

At 28 weeks, CaTs of LV cardiomyocytes were analysed during increasing pacing frequencies and during recovery to the initial pacing frequency (Figure 3A). Compared with controls, time to peak Ca$^{2+}$ (TTP; Figure 3B) was unchanged in HFpEF at lower frequencies 1–3 Hz but significantly prolonged at 5 Hz. Both groups showed a significantly increased TTP Ca$^{2+}$ upon recovery to 1 Hz vs. initial 1 Hz, with no significant difference between the groups. The Ca$^{2+}$ amplitude was significantly decreased in HFpEF at 1–3 Hz vs. CT and after recovery to 1 Hz (Figure 3C). No difference in cytosolic Ca$^{2+}$ removal kinetics could be detected at various frequencies (Figure 3D).

Diastolic Ca$^{2+}$ and sarcoplasmic reticulum leak are increased, and sarcoplasmic reticulum load decreased after pacing in heart failure with preserved ejection fraction

Diastolic Ca$^{2+}$ of HFpEF cardiomyocytes rose to a similar extent as CT with increasing pacing frequencies from 1 to 5 Hz but showed an impaired recovery to baseline values vs. CT with return to 1 Hz stimulation (Figure 3E). Resting cardiomyocytes from HfEF (following stimulation) showed a significantly increased rate of cytosolic Ca$^{2+}$ accumulation (Figure 3F), suggesting SR Ca$^{2+}$ leak. In line with this finding, a decreased SR Ca$^{2+}$ load could be detected in HFpEF (Figure 3G). In addition, HFpEF showed a faster Ca$^{2+}$ decay following caffeine-induced contractures (Figure 3H), indicating enhanced NCX forward-mode function.

Moderate-intensity continuous training but not high-intensity interval training is associated with a lower Ca$^{2+}$ transient amplitude and prolonged Ca$^{2+}$ transient decay

The same protocol (as Figure 3A) was employed to study changes of intracellular Ca$^{2+}$ cycling in LV cardiomyocytes after 8 weeks of MICT or HIIT vs. sedentary animals (Sed.). In HFpEF animals that had performed MICT, CaT amplitude was lower at 2–5 Hz vs. HFpEF Sed. (Figure 4B), but Ca$^{2+}$ transient decay was lower at 2–5 Hz in the MICT group (Figure 4C). This may in part be mediated by a slowed Ca$^{2+}$ decay at 1–3 Hz in MICT (Figure 4D).

Moderate-intensity continuous training and high-intensity interval training protect from pacing-induced diastolic Ca$^{2+}$ increase

Neither MICT nor HIIT had an influence on diastolic [Ca$^{2+}$] with increased pacing rate (2–5 Hz, Figure 4E). However, restitution of diastolic cytosolic Ca$^{2+}$ with return to 1 Hz stimulation was improved with MICT and HIIT vs. Sed. A lower diastolic SR Ca$^{2+}$ leak from the SR in the HIIT (significant) and MICT (trend) groups vs. Sed. was identified as a possible contributor to this phenomenon (Figure 4F). SR Ca$^{2+}$ load was unchanged MICT and HIIT (Figure 4G), as was the decay of the caffeine transient (Figure 4H).

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spontaneous Ca\textsuperscript{2+} release events (Figure 6A). MICT, but not HIIT, lowered the incidence of sparks (Figure 6B). A statistically relevant difference in the incidence of waves was not observed (OS: 0.020 ± 0.012 vs. MICT: 0.024 ± 0.016 vs. HIIT: 0.017 ± 0.012 waves per second per 100 \(\mu\)m, n.s.). Assessment of Ca\textsuperscript{2+}-wave propagation velocity revealed a significant reduction in MICT vs. Sed. (Figure 6C). Wave propagation velocity of HIIT could not be assessed because of the very low wave incidence (two waves in 27 measured cells).

**Discussion**

In this study, we investigated the effect of two different exercise modalities on myocardial function in vitro and in vivo in a model of metabolic HFpEF.\textsuperscript{15} Exercise training has been shown to improve diastolic dysfunction in human HFpEF.\textsuperscript{1} While a positive effect of chronic low-intensity exercise has been previously reported in afterload-dependent HFpEF, the effect of different exercise regimes on metabolic HFpEF remained elusive.\textsuperscript{22} At the age of 28 weeks, we found the cytosolic CaT amplitude in LV cardiomyocytes to be significantly reduced, despite a preserved ejection fraction.\textsuperscript{15} Lower cytosolic Ca\textsuperscript{2+} release in HFpEF was related to a decreased SR Ca\textsuperscript{2+} load and an increased diastolic SR Ca\textsuperscript{2+} leak (Supporting Information, Figure S3). Also, in this study, we show that MICT and HIIT significantly reduced diastolic SR Ca\textsuperscript{2+} leak in HFpEF, associated with a significantly improved stroke volume in MICT. We found that MICT and HIIT affected cytosolic CaTs differently: only MICT synchronized early cytosolic Ca\textsuperscript{2+} release and reduced the CaT amplitude and the rate of Ca\textsuperscript{2+} decay.

The cellular pathomechanisms of HFpEF are not well understood. However, in a variety of animal models and in human myocardium, diastolic dysfunction in HFpEF has been linked to alterations in the cytosolic CaTs in LV cardiomyocytes.\textsuperscript{6,7,23–25} CaT amplitudes have been reported as higher (abdominal aortic banding model,\textsuperscript{24} or hypertrophic heart rat\textsuperscript{26}), unchanged (ZSF-1 rat\textsuperscript{25}), or lower (aortic banding rat\textsuperscript{27}) as compared with control animals, suggesting that adaptation of the CaT may depend on the pathological trigger of HFpEF and probably the disease stage. In accordance, we have shown earlier in a cardiorenal model of HFpEF that an unchanged CaT amplitude in early HFpEF may deteriorate...
with progressive remodelling despite preserved ejection fraction.\textsuperscript{6,7} Indeed, also in the present model, a normal systolic Ca\textsuperscript{2+} amplitude has been reported at earlier disease stages,\textsuperscript{25} indicating similar dynamic adaptations in Ca\textsuperscript{2+} homeostasis with HFpEF disease progression. Disease stage-dependent adaptations in cardiomyocyte Ca\textsuperscript{2+} signalling were also observed in atrial cardiomyocytes in this HFpEF model.\textsuperscript{13,28}

In the present model of advanced metabolic HFpEF, we identified a lower SR Ca\textsuperscript{2+} load and increased SR Ca\textsuperscript{2+} leak as a contributing mechanism for reduced CaT amplitudes. Modelling of human myocardium suggested that a concentrically hypertrophied ventricular wall can maintain a preserved EF despite reduced sarcomere shortening at the cardiomyocyte level.\textsuperscript{29}

Interestingly, also in the cardiorenal model of HFpEF deterioration of the CaT amplitude was associated with the occurrence of SR Ca\textsuperscript{2+} leak and a reduced SR Ca\textsuperscript{2+} load,\textsuperscript{7} suggesting a common cellular pathomechanism in advanced stages of HFpEF.

In untrained conditions, diastolic Ca\textsuperscript{2+} was unchanged at baseline, and this was confirmed at stimulation frequencies close to the \textit{in vivo} heart rate (i.e. 5 Hz, Figure 3E). Interestingly, cytosolic [Ca\textsuperscript{2+}] remained significantly elevated vs.

\begin{figure}
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\caption{Ca\textsuperscript{2+} transients were analysed for their time to reach maximum amplitude (A) and their time to reach half-maximum amplitude (TF\textsubscript{50}; B). Example of the spatial distribution of early and late Ca\textsuperscript{2+} release in left ventricular cardiomyocytes (shown: Sed.), as well as their consecutive beat-to-beat variation (C). The amount of early release sites was quantified (>3/10 early release events; D), and their probability of early release was determined in 10 consecutive cycles (E). Statistical analysis: either a two-way (A–E: 1–5 Hz) or one-way (A–E: 1 Hz rec.) ANOVA followed by a post hoc Bonferroni vs. Sed. (A–C), \textit{P}-values: 10.0494, 20.03, 30.02, and 40.03. \textit{n} = cells from four animals per group. (A, B) Sed., \textit{n} = 20; moderate-intensity continuous training (MIC), \textit{n} = 22; and high-intensity interval training (HIIT), \textit{n} = 27. (D, E) Sed., \textit{n} = 22; and HIIT, \textit{n} = 27.

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control during the recovery period after 5 Hz pacing, indicating impaired Ca^2+ removal in HFP EF following cellular stress.

Moderate-intensity continuous training and HIIT have both been proven to be effective interventions to reduce endothelial dysfunction in the ZSF-1 metabolic HFPEF model. HIIT was associated with improved clinical outcome in human HFP EF, however potentially related to non-cardiac training effects. In the present study, MICT and HIIT significantly reduced resting SR Ca^{2+} leak in LV cardiomyocytes. As we and others have shown earlier in other types of HF, a reduction in SR Ca^{2+} leak may attenuate cardiac remodelling and deterioration of contractile function in vivo.

High-intensity interval training had no significant effect on the CaT in LV cardiomyocytes at low or elevated pacing frequencies in this metabolic HFP EF model. While this observation argues against a positive effect of HIIT on active (i.e. Ca^{2+}-dependent) cardiomyocyte contraction and relaxation, our results do not exclude beneficial effects of HIIT on diastolic function, especially because in vivo parameters like SV and EDV showed a trend towards improvement upon HIIT. Moreover, HIIT decreased SR Ca^{2+} leak and, as opposed to MICT, had no effect on CaT tau (i.e. cytosolic Ca^{2+} removal). Indeed, in previous studies, positive effects of HIIT on diastolic function were attributed to decreased stiffness or improved cardiac vagal tone.

In MICT, additional parameters of in vivo function were improved as both SV and EDV increased upon training. Interestingly, CaT amplitudes were smaller in LV cardiomyocytes from MICT. In addition, CaT decay was prolonged. However, as opposed to HFP EF models, where impaired contractility is frequently associated with Ca^{2+} overload, total cytosolic Ca^{2+} exposure (area under the curve) in our trained HFP EF model was unchanged and diastolic Ca^{2+} even decreased during the recovery period after high-frequency stimulation, which argues against cellular Ca^{2+} overload in MICT. It is of note that slowed Ca^{2+} decay in MICT occurred without a detectable increase in end-diastolic [Ca^{2+}] also at higher pacing frequencies. This might lead to an increased cytosolic availability of Ca^{2+} during systole. Ca^{2+} sensitization and a prolonged exposure of myofilaments to Ca^{2+} are used in the clinic for the treatment of HFr EF, and components of systolic dysfunction are often also observed in HFP EF. Following this concept, the Ca^{2+} sensitizer levosimendan is currently evaluated in vivo in the clinic for the treatment of HFr EF, and components of systolic dysfunction are often also observed in HFP EF. As the decay of the caffeine-induced Ca^{2+} release as a measure for Na^+/Ca^{2+} exchanger-dependent Ca^{2+} extrusion was unchanged, slower cytosolic Ca^{2+} removal in MICT might be related to altered SERCA activity (see also Supporting Information, Figure S2).

In contrast to HIIT, MICT also significantly increased the number and open probability of functional early Ca^{2+} release sites (dyads) within the cardiomyocytes resulting in an accelerated early rise in cytosolic [Ca^{2+}] and suggesting an improved gain of Ca^{2+}-induced Ca^{2+} release.

Training also reduces arrhythmias in the setting of HF. Here, we show that MICT significantly decreased pro-arrhythmic Ca^{2+} sparks and slowed Ca^{2+} wave propagation.

In conclusion, we show a novel pattern of Ca^{2+} dysregulation in a metabolic model of HFP EF. In addition, MICT and HIIT improved SR Ca^{2+} leak in cardiomyocytes, but only MICT was associated with profound effects on the cytosolic CaT and improved stroke volume in vivo.

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Conflict of interest

None declared.

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Supporting information

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

Figure S1. (A) Cell surface of isolated LV cardiomyocytes quantified from two-dimensional light microscopic images. (B) Representative example of the t-tubular network visualized by fluorescence probe di-8-ANNEPS in isolated LV cardiomyocytes and (C) quantification of t-tubular density after thresholding. Statistical analysis: Two-tailed, unpaired students t-test.

Figure S2. (A) Original images of Western Blot analysis showing LV myocardial expression of (B) sarcoplasmic/endoplasmic reticulum calcium ATPase (SERCA), (C) total...
phospholamban (PLB_{total}), (D) ratio of phosphorylated PLB at serine 16 (p-PLBSer16) to PLB_{total} and (E) ratio of phosphorylated PLB at threonine 17 (p-PLBThr17) to PLB_{total}. Statistical analysis: one-way ANOVA followed by a post-hoc Fisher's LSD test vs. Sed. p-values: 0.034, 0.046, 0.045, 0.028, 0.036. n = animals.

**Figure S3.** Correlation of calcium transient (CaT) amplitude with (A) sarcoplasmic reticulum (SR) Ca^{2+} load and (B) SR leak. p-values (deviation from zero): 0.0002, 0.013.

**Table S1.** List of used equipment and chemicals.

**Table S2.** Distribution of measured LV cardiomyocytes in 28-week-old animals.

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**References**

1. Edelmann F, Gelbrich G, Dungen HD, Frohling S, Wachter R, Stahrenberg R, Binder I, Töpper A, Lashki DJ, Schwarz S, Herrmann-Lingen C. Exercise training improves exercise capacity and diastolic function in patients with heart failure with preserved ejection fraction with preserved ejection fraction result: results of the Ex-DHF (Exercise training in Diastolic Heart Failure) pilot study. *J Am Coll Cardiol* 2011; 58: 1780–1791.

2. Bowen TS, Rolim NP, Fischer T, Baekkerud FH, Medeiros A, Werner S, Branstad E, Rognmo O, Mangner N, Linke A, Schuler G. Heart failure with preserved ejection fraction induces molecular, mitochondrial, histological, and functional alterations in rat respiratory and limb skeletal muscle. *Eur J Heart Fail* 2015; 17: 263–272.

3. Bowen TS, Herz C, Rolim NPL, Berre AO, Halle M, Kricke A, Linke A, Da Silva GJ, Wislöff U, Adams V. Effects of endurance training on detrimental structural, cellular, and functional alterations in skeletal muscles of heart failure with preserved ejection fraction. *J Card Fail* 2018; 24: 603–613.

4. Lu L, Mei DF, Wang S, Lentzner B, Gustein DE, Zwas D, Homma S, Yi GH, Wang J. Exercise training normalizes altered calcium-handling proteins during development of heart failure. *J Appl Physiol (1985)* 2002; 92: 1524–1530.

5. Qin R, Murakoshi N, Xu D, Tajiri K, Feng D, Stujanna EN, Yonebayashi S, Nakagawa Y, Shimano H, Nogami A, Koike A, Aonuma K, Ieda M. Exercise training reduces ventricular arrhythmias through restoring calcium handling and sympathetic tone in myocardial infarction mice. *Physiol Rep* 2019; 7: e13972.

6. Primessnig U, Schonleitner P, Holl A, Pfeiffer S, Bracic T, Rau T, Kapl M, Stojakovic T, Glasnov T, Leineweber K, Waku P. Novel pathomechanisms of cardiomyocyte dysfunction in a model of heart failure with preserved ejection fraction. *Eur J Heart Fail* 2016; 18: 987–997.

7. Primessnig U, Bracic T, Levjoki J, Otsooja L, Polinello P, Falcke M, Pieske B, Heinzl FR. Long-term effects of Na^{+}/Ca^{2+} exchanger inhibition with ORM-11035 improves cardiac function and remodeling without lowering blood pressure in a model of heart failure with preserved ejection fraction. *Eur J Heart Fail* 2019; 21: 1543–1552.

8. Miranda-Silva D, Wust RGI, Conceicao G, Goncalves-Rodrigues P, Goncalves N, Goncalves A, Kuster DW, Leite-Moreira AF, van der Velden J, de Sousa Beleza JM, Magalhães J. Disturbed cardiac mitochondrial and cytosolic calcium handling in a metabolic risk-related rat model of heart failure with preserved ejection fraction. *Acta Physiol (Oxf)* 2019; 228: e13378.

9. Haykowsky MJ, Liang Y, Pechter D, Jones LW, McAlister FA, Clark AM. A meta-analysis of the effect of exercise training on left ventricular remodeling in heart failure patients: the benefit depends on the type of training performed. *J Am Coll Cardiol* 2007; 49: 2329–2336.

10. Krzesiak A, Cognard C, Sebille S, Carre G, Bosquet I, Delpech N. High-intensity intermittent training is as effective as moderate continuous training, and not deleterious, in cardiomyocyte remodeling of hypertensive rats. *J Appl Physiol (1985)* 2019; 126: 903–915.

11. Sturgeon K, Muthukumarana G, Ding D, Bajulaiye A, Ferrari V, Libonati JR. Modelling in a metabolic risk-related rat model of heart failure with preserved ejection fraction: altered calcium-handling proteins. *J Card Fail* 2017; 23: 1524–1530.

12. Hamdani N, Franssen C, Lourenco A, Callewaert G, Bach D, Wakula P, Pieske BM, Heinzel FR, Hohendanner F. Isolation of atrial cardiomyocytes from a rat model of metabolic syndrome-related heart failure with preserved ejection fraction. *Eur J Heart Fail* 2018; 20: 137–148.

13. Hohendanner F, Bode D, Guthof T, Pieske BM, Heinzel FR, Bito V, Biesmans L, Wu M, Detre E, von Wegner F, Claus P, Dymarkowski S, Maes F, Bogaert J, Ramdakers F, D’hooge J, Sipido K. Remodeling of T-tubules and reduced synthesis of Ca^{2+} release in myocardic cardiomyocytes. *Am J Physiol 1989; 257*: C147–C152.

14. Callewaert G, Cleemann L, Morad M. Caffeine-induced Ca^{2+} release activates Ca^{2+} extrusion via Na^{+}/Ca^{2+} exchanger in cardiac myocytes. *Am J Physiol 1989; 257*: C147–C152.

15. Heinzl FR, Bito V, Volders PG, Antoons G, Mubagwa K, Sipido KR. Spatial and temporal inhomogeneities during Ca^{2+} release from the sarcoplasmic reticulum in pig ventricular myocytes. *Circ Res 2002; 91*: 1023–1030.

16. Hohendanner F, Walther S, Maxwell JT, Kettlevell S, Awad S, Smith GL, Lonchyna VA, Blatter LA, Jostosol 1,4,5-trisphosphate induced Ca^{2+} release and excitation–contraction coupling in atrial myocytes from normal and failing hearts. *J Physiol 2015; 593*: 1459–1477.

17. Hiemstra JA, Veteto AB, Lambert MD, Olver TD, Ferguson BS, McDonald KS, Emter CA, Domeier TL. Chronic
27. Roe AT, Aronsen JM, Skardal K, Hamdani N, Linthout S, Tschöpe C, Schenrath F, Soltani S, Stamm C, Duisterhoef V, Rolim N, Wislaff U, Knosalla C, Fialk V, Pieske BM, Heinzel FR, Hoheinder F. The role of fibroblast—cardiomyocyte interaction for atrial dysfunction in HPeF and hypertensive heart disease. *J Mol Cell Cardiol* 2019; 131: 53–65.

28. Bode D, Lindner D, Schwarzl M, van Linthout S, Hamdani N, Spillmann F, Van Linthout S, Ljubojevic S, Negri S, Arnstein G, Gutschi EM, Harrap SB, Delbridge LMD. Cardiomyocyte functional etiology in heart failure with preserved ejection fraction. *Front Physiol* 2015; 6: 78.

29. Schmiederer Z, Rolim N, Bowen TS, Linke A, Wislöff U, Adams V, OptimEx Study Group. Endothelial function is disturbed in a hypertensive diabetic animal model of HPeF: moderate continuous vs. high-intensity interval training. *Int J Cardiol* 2018; 273: 147–154.

30. Angadi SS, Mookadam F, Lee CD, Tucker PJ, Hamdani N, Moukadam F, Lee CD, Tucker PJ, Hamdani N, Spillmann F, Van Linthout S. Cardiac contractility modulation: mechanisms of action in heart failure with reduced ejection fraction and beyond. *Eur J Heart Fail* 2019; 21: 14–22.

31. Lima JB, Silveira ADD, Pollesello P, Parissis JT. Use of levosimendan in acute heart failure. *Eur Heart J Suppl* 2018; 20: 12–110.

32. Kraigher-Kainer E, Shah AM, Gupta DK, Santos A, Claggott B, Pieske B, Zile MR, Voors AA, LeFkowitz MP, Packer M, McMurray J, Solomon SD, PARACOUGHT Investigators. Impaired systolic function by strain imaging in heart failure with preserved ejection fraction. *J Am Coll Cardiol* 2014; 63: 447–456.

33. Reid CJ, Danes VR, Bell JR, Raajmakers AJA, Ip WTK, Chandramouli C, Harding DJ, Soeller C, Mellor KM, Kalman JM, Harrap SB, Delbridge LMD. Cardiomyocyte functional etiology in heart failure with preserved ejection fraction is distinctive—a new preclinical model. *J Am Heart Assoc* 2018; 7.

34. Roe AT, Aronsen JM, Skardal K, Hamdani N, Linke WA, Danielsen HE, Sejersted OM, Sjaastad I, Louch WE. Increased passive stiffness promotes diastolic dysfunction despite improved Ca²⁺ handling during left ventricular concentric hypertrophy. *Cardiovasc Res* 2017; 113: 1161–1172.

35. Bode D, Lindner D, Schwarzl M, Westermann D, Deissler P, Primessnig U, Hegemann N, Blatter LA, van Linthout S, Tschöpe C, Schenkath F, Soltani S, Stamm C, Duisterhoef V, Rolim N, Wislaff U, Knosalla C, Fialk V, Pieske BM, Heinzel FR, Hoheinder F. The role of fibroblast—cardiomyocyte interaction for atrial dysfunction in HPeF and hypertensive heart disease. *J Mol Cell Cardiol* 2019; 131: 53–65.

36. Rouhana S, Roy J, Finan A, Rodrigues da Araujo G, Bideaux P, Scheuermann V, Saliba Y, Reboul C, Cazorla O, Aimond F, Richard S, Thireau J, Fares N. Early calcium handling imbalance in pressure overload-induced heart failure with nearly normal left ventricular ejection fraction. *Biochim Biophys Acta Mol Basis Dis* 2019; 1865: 230–242.

37. Miranda-Silva D, Wust RCI, Conceicao G, Goncalves-Rodrigues P, Goncalves N, Goncalves A, Kuster DW, Leite-Moreira AF, van der Velden J, de Sousa Beleza JM, Magalhaes J. Disturbed cardiac mitochondrial and cytosolic calcium handling in a metabolic risk-related rat model of heart failure with preserved ejection fraction. *Acta Physiol* (Oxf) 2020; 228: e13378.

38. Curl CL, Danes VR, Bell JR, Raajmakers AJA, Ip WTK, Chandramouli C, Harding DJ, Soeller C, Mellor KM, Kalman JM, Harrap SB, Delbridge LMD. Cardiomyocyte functional etiology in heart failure with preserved ejection fraction is distinctive—a new preclinical model. *J Am Heart Assoc* 2018; 7.

39. Roe AT, Aronsen JM, Skardal K, Hamdani N, Linke WA, Danielsen HE, Sejersted OM, Sjaastad I, Louch WE. Increased passive stiffness promotes diastolic dysfunction despite improved Ca²⁺ handling during left ventricular concentric hypertrophy. *Cardiovasc Res* 2017; 113: 1161–1172.

40. Bode D, Lindner D, Schwarzl M, Westermann D, Deissler P, Primessnig U, Hegemann N, Blatter LA, van Linthout S, Tschöpe C, Schoenrath F, Soltani S, Stamm C, Duisterhoef V, Rolim N, Wislaff U, Knosalla C, Fialk V, Pieske BM, Heinzel FR, Hoheinder F. The role of fibroblast—cardiomyocyte interaction for atrial dysfunction in HPeF and hypertensive heart disease. *J Mol Cell Cardiol* 2019; 131: 53–65.

41. Adeniran I, MacVeer DH, Saffo JC, Zhang H. Abnormal calcium homeostasis in heart failure with preserved ejection fraction is related to both reduced contractile function and incomplete relaxation: an electromechanically detailed biophysical modeling study. *Front Physiol* 2015; 6: 78.

42. Schmiederer Z, Rolim N, Bowen TS, Linke A, Wislöff U, Adams V, OptimEx Study Group. Endothelial function is disturbed in a hypertensive diabetic animal model of HPeF: moderate continuous vs. high-intensity interval training. *Int J Cardiol* 2018; 273: 147–154.

43. Lima JB, Silveira ADD, Pollesello P, Parissis JT. Use of levosimendan in acute heart failure. *Eur Heart J Suppl* 2018; 20: 12–110.

44. Kraigher-Kainer E, Shah AM, Gupta DK, Santos A, Claggott B, Pieske B, Zile MR, Voors AA, LeFkowitz MP, Packer M, McMurray J, Solomon SD, PARACOUGHT Investigators. Impaired systolic function by strain imaging in heart failure with preserved ejection fraction. *J Am Coll Cardiol* 2014; 63: 447–456.

45. ClinicalTrial.gov identifier: NCT03541603.

46. ClinicalTrial.gov identifier: NCT03624010.

47. Heinzel FR, MacQuaide N, Bideaux P, Pollesello P, Parissis JT. Use of levosimendan in acute heart failure. *Eur Heart J Suppl* 2018; 20: 12–110.

48. Grau Putxet M, Guerchaz-Cazalis K, Despas F, Puyol P, Bideaux P, Gales C, Vaccaro A, Bouquet M, Gallinier M, SchéNARD JM. High-intensity interval exercise improves vagal tone and decreases arrhythmias in chronic heart failure. *Med Sci Sports Exerc* 2013; 45: 1861–1867.