Cardiomyocyte ATP Production, Metabolic Flexibility, and Survival Require Calcium Flux through Cardiac Ryanodine Receptors in Vivo**

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Background: Intracellular Ca\textsuperscript{2+} release has been implicated in ATP production in vitro.

Results: In vivo deletion of RyR2 reduces Ca\textsuperscript{2+}, ATP, and oxidative metabolism, leading to metabolic reprogramming and cell death.

Conclusion: RyR2 maintains cardiomyocyte ATP production and survival in vivo.

Significance: This work links heart metabolism to function via Ca\textsuperscript{2+} release from intracellular stores.

Intracellular Ca\textsuperscript{2+} fluxes regulate an enormous number of processes with the specificity of responses often being ensured by spatial limitation of Ca\textsuperscript{2+} ions and proximity to targets. Frequently, this takes the form of Ca\textsuperscript{2+} signals within the nanoscale space between adjacent organelles (1). For example, in vitro studies have shown that Ca\textsuperscript{2+} flux through channels such as ryanodine receptors and IP\textsubscript{3} receptors mediate privileged communication between endoplasmic reticulum/sarcoplasmic reticulum (ER/SR) and mitochondria and that this paces cellular metabolism by stimulating oxidative ATP production via interaction with TCA cycle enzymes (2–4). Work from Foskett and colleagues (5) recently demonstrated that knocking out IP\textsubscript{3} receptors in DT40 cells causes an energy-deficient state and mTor-independent autophagy. This concept has not been extended to other cell types or to the in vivo situation.

Ryanodine receptor Ca\textsuperscript{2+} channels (e.g. RyR2) have been observed in close proximity to mitochondria, and beat-to-beat calcium transients have been observed in cardiomyocyte mitochondria (6–8). Furthermore, we and others (9–12) have demonstrated in vitro that RyR2-mediated Ca\textsuperscript{2+} flux supports ATP levels in other cell types. In pancreatic β-cells, blocking RyR2 also induces calpain-10-dependent, caspase-3-independent, programmed cell death that is associated with presenilin-1-dependent up-regulation of hypoxia-inducible factor 1β (10, 11). To date, analysis of cellular energetics and survival in the context of reduced ER/SR Ca\textsuperscript{2+} channel expression has not been extended to an in vivo system. Ideally, the hypothesis that intracellular Ca\textsuperscript{2+} release is required to sustain energy metabolism in vivo would be tested using tissue-specific, inducible deletion of ER/SR Ca\textsuperscript{2+} channels.

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Ryr2 Reproduces Many Hallmarks of Heart Failure, Suggesting a Causal, Ryr2-pendent Cell Death and Cardiac Hypertrophy in Mice Following Metabolic Pathways. We further observed atypical calpain-10-dependent cell death and cardiac hypertrophy in mice following Ryr2 knock-out. Acute in vivo deletion of cardiomyocyte Ryr2 reproduces many hallmarks of heart failure, suggesting a causal, upstream role for this channel in the pathogenesis of this disease.

**EXPERIMENTAL PROCEDURES**

**Experimental Animals—**Ryr2 “floxed” mice were generated as described (22). Tamoxifen-inducible, cardiomyocyte-specific Ryr2 knock-out mice were generated by crossing mice harboring Ryr2 alleles containing flanking loxP sites (C57Bl6 Ryr2<sup>flox/flox</sup> mice) with mice expressing inducible Cre-recombinase under the control of the α-myosin heavy chain promoter (C57Bl6 mer-Cre-mer mice) (23). Tamoxifen was injected into the intraperitoneal cavity of 8–16-week-old Ryr2<sup>flox/flox</sup> mer-mer mice or controls for three consecutive days at 3 mg per 40 g of body weight. Protocols were approved by the University of British Columbia Animal Care Committee.

**Ex Vivo Analysis of Cardiac Metabolism—**Myocardial substrate utilization was measured in working hearts as detailed elsewhere (24–26). Briefly, working hearts were perfused with Krebs–Henseleit solution containing 11 mm glucose, 1.5 mm lactate, 0.15 mm pyruvate, 0.6 mm palmitate, and 20 milli-units/ml insulin. Glycolysis, as well as myocardial rates of oxidation of palmitate, glucose, and lactate were determined by the quantitative collection of <sup>3</sup>H<sub>2</sub>O or <sup>14</sup>CO<sub>2</sub> produced by hearts perfused with either KH solution containing [9,10-<sup>3</sup>H]palmitate and [U-<sup>14</sup>C]lactate or [U-<sup>14</sup>C]glucose, and 5-<sup>3</sup>H]glucose.

**Light and Electron Microscopy—**Paraformaldehyde-fixed ventricular tissue sections were stained with H&E and Masson’s trichrome to assess fibrosis. Apoptosis and cell death of adult ventricular myocytes was assessed in heart tissue sections using the TUNEL assay from Roche Applied Science according to the manufacturer’s instructions. Images were taken with a Retiga-2000R camera (Q-Imaging). TUNEL-positive cells were normalized to tissue area.

Live cell imaging was conducted on cardiomyocytes isolated from cRyr2KO and control mice using Langendorff reverse perfusion to introduce collagenase in to the cardiac vasculature as detailed elsewhere (27). To assess cytosolic Ca<sup>2+</sup> levels, isolated cardiomyocytes were loaded with 5 μM Fura-2-AM (Invitrogen) for 30 min and washed twice for 15 min before imaging. Fura-2-AM-loaded cells were excited at 340 and 380 nm and measured for emission at 510 nm using a imaging system equipped with a 10× air objective (0.3 numerical aperture) on a Zeiss Axiovert-200 M microscope with a CoolSnapHQ2 CCD Camera (Intelligent Imaging Innovations, Denver, CO). Ratiometric images were quantified using Slidebook software (Intelligent Imaging Innovations) to measure relative cytosolic Ca<sup>2+</sup> (28). To assess mitochondrial Ca<sup>2+</sup> levels, isolated cardiomyocytes were loaded with 5 μM Rhod-2-AM (Invitrogen) for 30 min and washed for 15 min before imaging. In some studies, 5 μM Mitotracker Deep Red (FM) (Invitrogen) was co-loaded. Loaded cells were imaged using the Axiovert-200 M microscope described above and a 40× oil objective (1.3 numerical aperture). Rhod-2 fluorescence was used to measure relative mitochondrial Ca<sup>2+</sup> (29), and Mitotracker was used to assess relative mitochondrial content for normalization. The co-loading of these dyes did not alter Ca<sup>2+</sup> or cell viability (data not shown). All live-cell microscopy occurred within 8 h of isolation, and only rod-shaped cardiomyocytes with typical cell morphology were considered in analysis.

**Gene, Protein, and Metabolite Analysis—**Total RNA was isolated from heart tissue using TRIzol, followed by cleanup using the RNeasy kit (Qiagen). After reverse transcription (Super-Script III; Invitrogen), TaqMan quantitative RT-PCR was conducted using probes from Applied Biosystems and PerfeCTa quantitative PCR SuperMix (Quanta) on a StepOnePlus thermocycler (Applied Biosystems). SYBR Green quantitative RT-PCR was conducted using PerfeCTa SYBR Green quantitative PCR SuperMix (Quanta). Relative gene expression changes were analyzed by the 2<sup>−ΔΔCT</sup> method and plotted in normalized relative units. Hypoxanthine-guanine phosphoribosyltransferase and cyclophilin were used as internal controls after ensuring that they were not altered in cRyr2KO cardiomyocytes. Primer details are available in supplemental Tables S1–S3.

Western blot experiments were performed on lysates from mechanically disrupted hearts, homogenized, and sonicated in ice-cold lysis buffer. Samples were quantified, boiled with loading dye, and 15–50 μg of protein was used in SDS-PAGE electrophoresis. Proteins were then transferred to PVDF membranes using standard semi-dry transfer (for protein smaller than 120 kDa) or wet transfer (for proteins larger than 120 kDa) and subsequently treated with targeted primary and horseradish peroxidase-conjugated secondary antibodies. Bands were visualized using an enhanced chemiluminescence detection kit and quantified by densitometry. Antibody details can be found in supplemental Table S4. Cardiac ATP levels were assessed using standardized high-performance liquid chromatography methods (30).
Statistical Analysis—Data are expressed as mean ± S.E. unless otherwise indicated. Results were considered statistically significant when $p \leq 0.05$ using the Student’s $t$ test. All experiments were repeated on at least three cRyr2KO mice and at least three of their tamoxifen-injected littermate controls.

RESULTS

Ryr2 Deletion Reduces Ca$^{2+}$ Flux in Cardiac Myocytes—cRyr2KO mice showed normal Ryr2 expression until they were injected with tamoxifen, a synthetic agonist of the modified estrogen receptor:Cre fusion protein (Mer-Cre-Mer) under the control of a \\textit{cmhry6} promoter, which allowed temporally controlled targeted gene deletion of Ryr2 in cardiomyocytes. Tamoxifen-injected littermate control mice (Ryr2\textsuperscript{lox/lox}) were used throughout the study; thus, any differences between cRyr2KO and control mice cannot be attributed to tamoxifen.

In our hands, this model consistently displays a >90% decrease in whole heart Ryr2 mRNA after only 3 days of tamoxifen injections (Fig. 1A) (22). This leads to a time-dependent reduction in RYR2 protein levels to 51 ± 21% at 4 days post-tamoxifen and 24 ± 22% at 10 days post-tamoxifen in cRyr2KO mouse hearts (22). No effects were seen in the expression of Ryr1, Ryr3, or any of the IP$_3$ receptor Ca$^{2+}$ release channels (Fig. 1A). As expected for cells lacking their major Ca$^{2+}$ release channel and without compensatory up-regulation of other SR channels, steady-state cytosolic Ca$^{2+}$ was reduced in isolated, acutely cultured, non-contracting cRyr2KO cardiomyocytes (Fig. 1B). We predicted that the loss of RYR2 channels juxtaposed with mitochondria would result in lower average Ca$^{2+}$ levels in mitochondria. Indeed, we observed a significant decrease in steady-state mitochondrial Ca$^{2+}$ in isolated cRyr2KO cardiomyocytes (Fig. 1C). This result was not dependent on the normalization of the Rhod2 signal to Mitotracker fluorescence (used to control for mitochondrial number). Together, these studies suggest that loss of RYR2 in cardiomyocytes reduces Ca$^{2+}$ flux from the SR into the mitochondria via the cytosol.

Ryr2 Supports ATP Production in Vivo—In vitro studies have shown that Ca$^{2+}$ flux from ER/SR stimulates metabolism in adjacent mitochondria (2, 3, 12). We used cRyr2KO hearts to test the hypothesis that SR-to-mitochondria Ca$^{2+}$ fluxes are required for ATP production and oxidative metabolism in vivo. Indeed, ATP levels were decreased in flash-frozen cRyr2KO hearts (Fig. 2A). Consistent with this model, metabolic profiling of cRyr2KO working hearts revealed significant reductions in glucose, palmitate, and lactate oxidation (Fig. 2B). These effects were specific to mitochondrial metabolism, as we did not observe a reduction in the rate of glycolysis (Fig. 2C). In fact, glycolysis was increased in cRyr2KO working hearts, suggesting a compensatory mechanism and a switch toward a heart failure-like phenotype (19). These observations were made in hearts with identically oxygenated perfusate and with similar amounts of coronary artery perfusion indicating that the cardiomyocytes had similar access to metabolic substrates (Fig. 2D). Together, these data provide evidence that Ca$^{2+}$ flux through RYR2 channels is essential for basal mitochondrial metabolism in the heart.

Ryr2 Deletion Leads to a Hypoxic/Ischemic State—Our previous \textit{in vitro} work on pancreatic β-cells demonstrated that the chemical inhibitor of RyRs, ryanodine, caused a state of ATP depletion and a presenilin-1-dependent induction of hypoxia-inducible factor (11). The hypoxia-inducible factor system is a master sensor of oxidative energy use and may therefore coordinate the response to energy deprivation. Here, we also observed an increase in the levels of presenilin-1 and presenilin-2, as well as Hif1α and Hif1β (Fig. 3, A and B). A dramatic loss of uncoupling protein gene expression suggested that cRyr2KO cardiomyocytes were reprogrammed to conserve and salvage energy (Fig. 3C). Similar to the \textit{in vitro} observations of others (5), we observed increased autophagy, based on increased levels of LC3-II, even under conditions of nutrient availability (Fig. 3D). Together, these observations suggest that cardiomyocytes display complex signs of energy stress and compensation when ATP levels are reduced following RYR2 reduction.

Reprogramming of Metabolism in cRyr2KO Hearts—Given the severity of the metabolic changes, we next tested whether core transcriptional pathways had been affected by the reduction in RYR2-mediated Ca$^{2+}$ fluxes. We focused our efforts on so-called “master regulator” pathways that modulate the expression of numerous metabolic genes in heart and other tissues. For example, we observed down-regulation of Sirt1 and Foxo1 (Fig. 4A), genes that coordinate cardiac stress responses and metabolism (31–33). Heart failure is associated with metabolic inflexibility and a switch away from fatty acid metabolism (34). Ca$^{2+}$ signals can act via calcineurin-dependent activation of the Ppargc1a (Pgc1α)/Ppara system to sustain fatty acid oxidation (35, 36). In hearts with reduced RYR2, we observed a striking down-regulation of Ppargc1a and Ppara as well as Ppary (Fig. 4B). Phosphoenolpyruvate carboxykinase 1 (Pck1), a target of Pgc1α, was virtually eliminated from cRyr2KO hearts (Fig. 4G). The Pparg target gene Cebpa was also robustly inhibited (Fig. 4C). Carbohydrate responsive element-binding protein (Chrebp), a master regulator of complex lipid metabolism, was dramatically decreased in cRyr2KO hearts (Fig. 4C). Accordingly, expression of ATP-citrate lyase (Acly) and acetyl-CoA carboxylase β (Acacb) were decreased significantly; expression of stearoyl-coenzyme A desaturase 1 (Scd1) was virtually eliminated (Fig. 4, D and E). The reduction in lipogenic enzymes was associated with down-regulation of fat transporters (Fig. 4F). At the same time, we observed a dramatic decrease in expression of genes encoding hormone sensitive lipase (Hsl), adiponutrin (Adpn), and adipose triglyceride lipase (Atg1) (Fig. 4H). Atg1 is essential for degrading lipid droplets and mice lacking this gene have fatty, hypertrophied, and failing hearts (37). Interesting, we noted reduced expression of the genes encoding the GLUT4 glucose transporter and phosphoenolpyruvate carboxykinase 1 (Fig. 4, G and I). Together, these data suggest that the use of lipids and other substrates by the cRyr2KO heart may be abrogated and provide novel insight into the link between Ca$^{2+}$ and metabolism.

Ryr2 Deletion Induces Programmed Cell Death—Heart failure is associated with an increase in cardiomyocyte death, which can be apoptotic, non-apoptotic, and/or associated with ER stress depending on the model (21). cRyr2KO hearts contained numerous TUNEL-positive cells, which were virtually absent in controls (Fig. 5, A and B). This cell death was not
caused by ER stress because we observed a decrease in CHOP expression and other ER stress markers (Fig. 5, C and D). In some cell types, RYR2 hyperactivity contributes to classical caspase-3-dependent apoptosis (28). However, we discovered previously that inhibiting RYR2-mediated Ca^{2+} flux with ryanodine caused caspase-3-independent programmed cell death (10). cRyr2KO hearts did not exhibit a significant increase in caspase-3 cleavage (Fig. 5E), confirming our hypothesis that

FIGURE 1. Acute cardiac-specific Ryr2 gene ablation without compensation from related Ca^{2+} channels. A, SR/ER Ca^{2+} release channel mRNA levels in heart tissue 10 days after tamoxifen injection. Data are normalized to cyclophilin D (Cyc) levels (n = 6). White bars, control mice; Black bars, cRyr2KO mice. B, reduced basal cytosolic Ca^{2+} levels in isolated, acutely cultured, unstimulated cardiomyocytes from cRyr2KO mice. Data are from at least three independent cell preparations (control cells n = 124; cRyr2KO cells n = 210). C, reduced basal mitochondrial luminal Ca^{2+} levels in isolated, unstimulated cardiomyocytes from cRyr2KO mice. Data are from four independent cell preparations (control cells (n = 170); cRyr2KO cells (n = 180); *, p ≤ 0.05). White scale bars indicate 20 μm. *, p ≤ 0.05. RU, relative units; IP3R, IP3 receptor.
RYR2 suppresses an atypical form of non-apoptotic cell death. We observed a reduction in the expression of the prosurvival gene \textit{Bcl2}, perhaps further contributing to cell death (Fig. 5F).

We have previously shown that calpain-10 mRNA and small molecular weight isoforms of calpain-10 are up-regulated in cells with depressed energy state resulting from ryanodine
Ryr2 Deletion Causes Cardiac Hypertrophy and Fibrosis—
cRyr2KO mice represented an ideal tool to test the hypothesis
that acute cardiomyocyte stress and dysfunction is sufficient to
induce cardiac hypertrophy. We observed an increase in gross
heart volume and mass (Fig. 6A), and heart fibrosis in cRyr2KO
mice (Fig. 6B), already evident 10 days after tamoxifen injec-
tion. Many chronic models of cardiac hypertrophy result in a
re-expression of fetal genes, including cardioprotective growth
factors. In our acute model, brain natriuretic peptide transcrip-
tion was significantly increased, and adult β-mysin heavy
chain gene (Mhy6) was attenuated (Fig. 6, C and D), but we did
not find a simultaneous up-regulation of the fetal β-mysin
heavy chain gene (Mhy7; Fig. 6D). Interestingly, we observed a
reduction of cleaved Notch1 (Fig. 6E), which plays a key role in
maintaining cardiac progenitors (39). Heart failure can be asso-
ciated with an increase in cardiac inflammation and the local
release of proinflammatory cytokines (40). We observed a
dramatic 20-fold increase in interleukin-6 transcription in
cRyr2KO hearts (Fig. 6F). Ryr2 may therefore be upstream of
some, but not all, aspects of cardiac inflammation.

Next, we examined pathways that could translate the effects
of stress into a hypertrophic pathology. Atf3, a stress gene acti-
vated by ischemia/hypoxia (41, 42), was increased ~2.5-fold in
cRyr2KO hearts (Fig. 6G). Cardiac Atf3 overexpression is suf-
cient to induce cardiomyopathy, hypertrophy, and myocyte
death (43). We also observed a dramatic loss of several genes

FIGURE 4. Conditional Ryr2 knock-out results in metabolic reprogramming at the transcriptional level. A–I, expression of key metabolic gene mRNA in
whole heart was measured 4 days post-tamoxifen using Taqman RT-quantitative PCR. We measured mRNA expression levels of sirtuin 1 (SIRT1; A), forkhead box
O1 (FOXO1; A), peroxisome proliferator-activated receptor γ, coactivator 1 α (Ppargc1a; B), peroxisome proliferator-activated receptor γ (Pparg; B), peroxisome
proliferator-activated receptor α (Ppara; B), CCAAT/enhancer binding protein (CEBP) α (Cebpα; C), carbohydrate response element binding protein (ChREBP; C), ATP citrate lyase (ACLY; D), acetyl-CoA carboxylase beta (ACACB; D), stearoyl-coenzyme A desaturase 1 (SCD1; E), fatty acid binding protein 4, adipocyte (Fabp4; F), CD36 molecule (CD36; F), phosphoenolpyruvate carboxykinase 1, cytosolic (Pck1; G),
hormone-sensitive lipase (HSL; H), adiponutrin (ADPN; H), adipose triglyceride lipase (ATGL; H), fatty acid synthase (FASN; H), and solute carrier family 2
(facilitated glucose transporter), member 4 (GLUT4; I). White bars, control; black bars, cRyr2KO throughout (n = 3). All data were collected 4 days after the first
tamoxifen injection. *, p ≤ 0.05. RU, relative units.
RYR2 Controls ATP Production and Cardiomyocyte Survival

A. Control vs. cRyr2KO

B. TUNEL-positive cells/mm²Control vs. cRyr2KO

C. CHOP and Tubulin

D. mRNA expression levels for ATP4, Btp, CHOP, Gadd34, and Sxbp1

E. Cl. Casp3 and Tubulin

F. mRNA expression levels for Bax, Bcl2, and BclX

G. Calpain 10, 56 kDa, 32 kDa, 30 kDa, 10 kDa

H. mRNA expression levels for INSRI and Ptpn1

I. mRNA expression levels for Adipoq, Adipor1, and Adipor2

J. mRNA expression levels for LepR

K. INSRIβ, Tubulin, p-ERK, ERK, pAktT308, Akt, pAktS473, pAkt/Ark

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negatively regulated by Atf3, including Glut4 (Fig. 4I). cRyr2KO also resulted in a near complete loss of Klf15 (Fig. 6H), which, based on knock-out studies, is sufficient to induce heart failure and fibrosis (44, 45). Klf15 is a positive regulator of Lcep1 and Pparg (46), genes that were decreased in cRyr2KO hearts. Thus, acute Ryr2 knock-out and the concomitant energy-deficient state have profound effects on transcriptional hubs, controlled, at least in part, by hypoxia-inducible factors, Klf15, Atf3, and Foxo1/Sirt1.

Throughout this study, we assessed the survival of cRyr2KO mice. Despite molecular and/or physical compensation, cRyr2KO mice invariably met their humane end point after a period that could range from days to weeks, as we have shown previously (22). Thus, the cardiac expression of the Ryr2 gene is essential for survival in adult mice.

**DISCUSSION**

The purpose of this study was to test the hypothesis that Ca\(^{2+}\) flux from intracellular stores, in this case through cardiomyocyte SR RYR2 channels, is required to maintain cellular energy homeostasis and survival in vivo. We used tissue-specific inducible gene ablation to examine the in vivo functions of Ryr2 in the adult mouse heart. This model circumvents the embryonic lethality of Ryr2 KO mice in the adult mouse heart. This model circumvents the embryonic lethality of Ryr2 KO mice. Our in vivo mouse studies support the concept, previously proposed after in vitro studies (5), that intracellular Ca\(^{2+}\) release paces mitochondrial metabolism and protects cells from atypical programmed cell death caused by energy depletion (Fig. 6I). Our data extend this concept to include the Ca\(^{2+}\)-dependent control of key transcription factor networks that modulate metabolic substrate utilization. Our results also demonstrate that partial loss of cardiomyocyte RYR2 protein is sufficient to recapitulate many of the characteristics of human heart failure, providing new mechanistic insight into this devastating condition.

In the physiological state, the cardiomyocyte is always working. Our analysis of metabolism and survival were conducted in vivo and in ex vivo working heart models. The single-cell Ca\(^{2+}\) measurements were the only experiments in this study not conducted in working cardiomyocytes. Nevertheless, these measurements provide some interesting information and suggest a role for RYR2 channels in cardiomyocyte Ca\(^{2+}\) homeostasis. Taken at face value, these data suggest that basal Ca\(^{2+}\) flux through RYR2 channels normally supports a significant component of resting Ca\(^{2+}\) levels in both the cytosolic and mitochondrial compartments of isolated cardiomyocytes. Indeed, microscopic and submicroscopy RYR2-mediated Ca\(^{2+}\) release events have previously been implicated in the diastolic SR Ca\(^{2+}\) leak into the cytosol (49), and subthreshold RYR2 release events are known to increase Ca\(^{2+}\) in nearby mitochondria (50). Future studies will be required to assess Ca\(^{2+}\) in these compartments in beating cardiomyocytes, ideally within the intact heart.

One of the key findings of our study is that cRyr2KO mouse hearts had decreased ATP levels, as well as lower substrate utilization when compared with control hearts. This can be attributed to defects in the utilization of metabolic substrates via oxidative ATP generation. The Ca\(^{2+}\) dependence of the TCA cycle was proposed many years ago based on the finding that Ca\(^{2+}\) in the matrix of isolated mitochondria stimulates several enzymes in the pathway, including pyruvate dehydrogenase, which controls entry of glucose carbon into the TCA cycle (4, 51, 52). Similarly, emerging evidence suggests that the F1F0 ATP synthase is also directly regulated by mitochondrial Ca\(^{2+}\) such that the maximal rate of oxidative ATP production at given mitochondrial membrane potential is dependent on Ca\(^{2+}\) concentration (12). As such, the rate and efficacy of many energy-producing substrates via the TCA cycle could be affected by mitochondrial Ca\(^{2+}\) dynamics. Collectively, these enzymes are thought to rely on Ca\(^{2+}\) signals from across nanoscale spaces between ER/SR and mitochondria. Local microdomains of concentrated Ca\(^{2+}\) are required to access the mitochondria and the duration of the mitochondrial Ca\(^{2+}\) transients is limited by rapid export mechanisms (2, 3, 6). Our results suggest that ablation of RYR2 in vivo disrupts this signaling pathway, leading to a decrease in TCA cycle enzymatic activity and, consequently, a diminished ability to sustain cellular ATP levels. Inability to employ the TCA cycle likely promotes the metabolic inflexibility observed in heart failure, as the utilization of fat as fuel requires oxidative metabolism (19). Indeed, decreased RYR2 expression or function is associated with several models of metabolic cardiomyopathy (15–18). Our data support the concept that decreased RYR2 function in cardiac pathology may directly contribute to metabolic inflexibility seen in heart failure.

Our data also provide evidence for a role for RYR2 and Ca\(^{2+}\) in regulating lipid utilization and ATP production by transcriptional reprogramming. It is well established that failing hearts switch from metabolizing free fatty acids toward carbohydrate utilization (19). Knock-out and overexpression studies show that changes in transcriptional regulators and enzymes involved in lipid metabolism are sufficient to cause cardiac hypertrophy and steatosis (35, 37, 53). Our data suggest a role of the cytoprotective and metabolism-controlling Sirt1/Foxo1/Pgc1α axis in hearts lacking Ryr2. RYR2 deficiency is sufficient to flip a transcriptional metabolic “master switch,” which down-regulates lipid utilization pathways and effectors. We

**FIGURE 5. Acute Ryr2 ablation causes calpain-10 dependent programmed cell death.** A, representative images of TUNEL staining (arrows) of fixed heart sections (scale bar, 100 μm). B, quantification of TUNEL-positive nuclei (control, n = 4; cRyr2KO, n = 5). White bars, control; black bars, cRyr2KO throughout. C, ER stress gene expression (n = 3). D, CHOP (31 kDa) protein expression (n = 7). E, cleaved caspase-3 (CL Casp3; 19 kDa) protein levels. +, a positive cleaved caspase-3 control sample (thapsigargin-treated MIN6 cells). Individual blot lanes are biological replicates throughout (n = 3). F, cell death effector gene expression 4 days post tamoxifen injection (n = 3). G, calpain-10 protein levels and expression patterns. Calpain-1 (80 kDa) and calpain-2 (80 kDa) levels are also displayed. White bars, control; red bars, cRyr2KO. H, insulin receptor (INSR) and Ptpn1 gene expression levels (n = 3). I, adiponectin and adiponectin receptor (AdipoR1) mRNA expression (n = 3). J, leptin receptor (LeptR) mRNA (n = 3). K, expression and/or phosphorylation states of insulin receptor (INSR) 190 and 95 kDa, Akt (60 kDa), and Erk (42 and 44 kDa) (n = 3). All analyses carried out 4 days post-tamoxifen treatment. Protein quantification plots represent relative expression normalized to either total protein or to tubulin (Tub; 50 kDa). TUNEL data were collected 10 days following tamoxifen treatment; all other data collected 4 days following the first tamoxifen injection. *p < 0.05. S473, Ser-473; T308, Thr-308.
also found reduced insulin signaling and dramatic loss of Glut4, which might be expected to have deleterious effects given the cardioprotective effect of glucose uptake (54). Therefore, our study suggests that acute Ryr2 deletion disrupts both lipid and carbohydrate metabolism indirectly via transcriptional programming, as well as directly at the mitochondria.
In our previous studies of other cell types, loss of metabolism-pacing Ca\(^{2+}\) communication between RyR channels and mitochondria caused cellular ATP levels to decline and induced the presenilin-dependent expression of key hypoxia response genes (3, 11). Similarly, cRyr2KO mice had significantly increased proteins levels of presenilin-1, presenilin-2, HIF1α, and HIF1β, and they displayed numerous signs of energy starvation with an associated increase in compensatory mechanisms for energy conservation and optimization. This included decreased expression of uncoupling proteins and down-regulated carbohydrate and lipid storage pathways. Thus, cRyr2KO hearts exist in a pseudohypoxic stress state. Hypoxia-inducible factors are known to increase Atfβ and decrease Klf15 expression, a combination of events that is sufficient to drive cardiomyopathy, hypertrophy, fibrosis, and cell death (41, 43, 45, 55). Although we cannot rule out these parallel mechanisms of cardiac dysfunction in other models of heart failure, our evidence does demonstrate that disruption of RYR2-dependent metabolism is sufficient to cause heart failure.

Pharmacological studies have shown that Ca\(^{2+}\) released through RyRs or IP\(_3\) receptors into adjacent mitochondria modulates apoptosis (9, 50). When in excess, Ca\(^{2+}\) transfer from SR/ER to mitochondria triggers caspase-3-dependent apoptosis (28). We and others (9, 10, 56) have shown that constitutive intracellular Ca\(^{2+}\) signals are required to stimulate cellular respiration and prevent a hypoxia-like state with calpain-dependent, caspase-3-independent cell death. Interestingly, calpain-10 is localized to mitochondria (57) where it is poised to sense metabolic state. Our data indicate that disruption of RYR2 function, via its effects on mitochondrial calcium signaling and cellular metabolism, is also sufficient to cause cell death in vivo. Additional work will be required to further elucidate the mechanisms involved in this atypical cell death mode. We found evidence of autophagy in cRyr2KO hearts similar to the data on cells lacking IP\(_3\) receptors (5), suggesting that the control of metabolism and autophagy by SR/ER-to-mitochondria Ca\(^{2+}\) shuffling, both the barely perceptible Ca\(^{2+}\) microsignaling from stochastic channel opening as well as the larger global Ca\(^{2+}\) signals found in excitable cells such as cardiomyocytes, may be a ubiquitous metabolic control system.

Our inducible knock-out approach was designed to mitigate chronic compensatory gene expression induced by lifelong genetic alterations. Accordingly, our data were not confounded by compensation from other ER/CR Ca\(^{2+}\) release channels. We speculate that this may be the reason for the discrepancy between our observations of cardiac hypertrophy and the results obtained in mice with chronic Ryr2 haploinsufficiency, which were protected from pressure overload-induced hypertrophy (48). Interestingly, increased levels of cell death were reported in Ryr2\(^{+/−}\) mice. The majority of in vivo studies of Ryr2 have used point mutation knock-in mice, typically with gain-of-function mutations causing “leaky channels” (58, 59). Studies have focused on the susceptibility of these mice to tachycardic arrhythmia. Recently, a loss-of-function knock-in model was created (Ryr2\(^{ADA/ADA}\) mice) and reported to undergo calcineurin-independent hypertrophy (60). Additional studies are required to determine whether the hypertrophy observed in our cRyr2KO hearts is mediated through a similar mechanism. Given the fact that basal cytosolic Ca\(^{2+}\) appears to be lower in cRyr2KO cardiomyocytes, one might speculate that a calcineurin-independent pathway is involved.

Although ours is the first in vivo study to target an intracellular Ca\(^{2+}\) channel and examine metabolism, our conclusions are well supported by more reductionist studies on the roles of RYR2 in cardiac metabolism (2–5, 12), cell survival (10, 11, 48), and cardiac dysfunction (15–17). In our model, deletion of Ryr2 rapidly led to many alterations in cardiac phenotype. Because our only manipulation was Ryr2 deletion, all of the observed changes must ultimately be downstream of RYR2 action in cardiomyocytes, either immediately downstream or indirectly downstream. Future studies are required to elucidate the specific mechanisms involved and the temporal order in which these events occur.

In summary, our results illustrate that Ca\(^{2+}\) flux through the ERCR/Ryr2 Ca\(^{2+}\) channels is required to maintain mitochondrial Ca\(^{2+}\), oxidative metabolism, metabolic transcriptional pathways, and cellular survival in the mouse heart in vivo. These observations, which support concepts advanced from in vitro studies, demonstrate a paramount role for RYR2 in cardiomyocyte energetics. This creates a paradigm in which RYR2 Ca\(^{2+}\) fluxes have simultaneous roles in the regulation of heart rate and rhythmicity (22), as well as effects on metabolic mitochondrial pacing, and transcriptional programming in cardiomyocytes. This comprehensive model may explain how functional and metabolic demands of cardiomyocytes can be soquisitely coupled to ensure energy needs are met.

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FIGURE 6. Cardiac hypertrophy and fibrosis in cRyr2KO hearts. A, heart size, heart wet weight relative to body mass, and total body mass 10 days following tamoxifen treatment (control, n = 6; cRyr2KO, n = 8, \(p < 0.05\)). The image is representative. B, Masson’s trichrome staining of cardiac tissue sections collected 10 days following tamoxifen treatment. Lower panels are higher magnification images of top panels. Images are representative (control, n = 4; cRyr2KO, n = 5; scale bar, 100 μm). C and D, Anp, Bnp, Mhy6, and Mhy7 gene expression 4 and 10 days after tamoxifen treatment (n = 3). E, cleaved Notch1 (110 kDa) protein at 4 days post-tamoxifen. Individual blot lanes are biological replicates throughout (n = 3). F, inflammatory gene expression 4 days post-tamoxifen (n = 3). G and H, gene expression levels of Atf3 and Klf15 assessed 4 days post-tamoxifen (n = 3). Protein quantification plots represent relative expression normalized to either total protein or to tubulin (50 kDa). I, a schematic summarizing the observed effects of Ryr2 deletion on cardiomyocyte energy metabolism. The top panel depicts a normal situation where calcium from the SR/ER enters the mitochondria via RYR2, where it stimulates oxidative metabolism and maintains a normal cardiac transcriptional program. The bottom panel depicts a situation where there is reduced RYR2 signaling resulting in less calcium entering the mitochondria, which blunts oxidative ATP metabolism. This activates hypoxia-inducible transcription factors, which, along with alternate pathways, alters the cardiac transcriptional programming in a manner associated with cardiac pathology, *, \(p < 0.05\). HRE, hypoxia-response element.
REFERENCES

1. Johnson, J. D., Broun, M. J., White, S. A., and Luciani, D. S. (2012) Nano-
spaces between endoplasmic reticulum and mitochondria as control cen-
tres of pancreatic β-cell metabolism and survival. *Protoplasma* **249**, 549–58

2. Giacominelli, M., Drago, I., Bortolozzi, M., Scorzo, M., Gianelle, A., Pizzo, P., and Pozzan, T. (2010) Ca
superscript 2+ hot spots on the mitochondrial surface are generated by Ca
superscript 2+ mobilization from stores, but not by activation of store-
operated Ca
superscript 2+ channels. *Mol. Cell** **38**, 280–290

3. Liu, T., and O’Rourke, B. (2008) Enhancing mitochondrial Ca
superscript 2+ uptake in myocytes from failing hearts restores energy supply and demand match-
ing. *Circ. Res.* **103**, 279–288

4. Denton, R. M., and McCormack, J. G. (1990) Ca
superscript 2+ as a second messenger within mitochondria of the heart and other tissues. *Annu. Rev. Physiol.* **52**, 451–466

5. Cárdenas, C., Miller, R. A., Smith, I., Bui, T., Molgó, J., Müller, M., Vais, H., Cheung, K. H., Yang, J., Parker, I., Thompson, C. B., Birnbaum, M. J., Hallows, K. R., and Foskett, J. K. (2010) Essential regulation of cell bioen-
ergetics by constitutive InsP3 receptor Ca
superscript 2+ transfer to mitochondria. *Cell** **142**, 270–283

6. Kettlewell, S., Cabrero, P., Nicklin, S. A., Dow, J. A., Davies, S., and Smith, G. L. (2009) Changes of intra-mitochondrial Ca
superscript 2+ in adult ventricular cardiomyocytes examined using a novel fluorescent Ca
superscript 2+ indicator tar-
ged to mitochondria. *J. Mol. Cell Cardiol.* **46**, 891–901

7. Salnikov, V., Lukyanenko, Y. O., Lederer, W. J., and Lukyanenko, V. (2009) Distribution ofryanodine receptors in rat ventricular myocytes. *J. Muscle Res. Cell Motil.* **30**, 161–170

8. Lukyanenko, V., Ziman, A., Lukyanenko, A., Salnikov, V., and Lederer, W. J. (2007) Functional groups ofryanodine receptors in rat ventricular cells. *J. Physiol.* **583**, 251–269

9. Tsuobi, T., da Silva Xavier, G., Holz, G. G., Jouvaille, L. S., Thomas, A. P., Misler, S., Bell, G. I., and Polonsky, K. S. (2004) RyR2 and calpain-10 delineate a novel apoptosis pathway in pancreatic islets. *J. Biol. Chem.* **279**, 24794–24802

10. Dör, V., Kalynyak, T. B., Bychikvsa, Y., Frey, M. H., Tee, M., Jeffrey, K. D., Nguyen, V., Luciani, D. S., and Johnson, J. D. (2008) Glucose and endo-
plasmic reticulum calcium channels regulate HIF-1-β via presenilin in pan-
creatic β-cells. *J. Biol. Chem.* **283**, 9909–9916

11. Glancy, B., and Balaban, R. S. (2012) Role of mitochondrial Ca
superscript 2+ in the regulation of cellular energetics. *Biochemistry* **51**, 2959–2973

12. Kandili, H. B., Tuncay, E., Zeydanli, E. N., Sozmen, N. N., and Turan, B. (2011) Age-related regulation of excitation-contraction coupling in rat heart. *J. Physiol. Biochem.* **67**, 317–330

13. Assayag, P., Charlemagne, D., Marty, I., de Leiris, J., Lopaschuk, G. D. (2005) Myocardial substrate metabolism in the normal and failing heart. *Physiol. Rev.* **85**, 1093–1129

14. Crossman, D. J., Ruygrok, P. N., Ruygrok, P. R., Soeller, C., and Cannell, M. B. (2011) Changes in the organization of excitation-contraction cou-
pling structures in failing human heart. *PLoS One* **6**, e17901

15. Braun, M. J., Asghari, P., Wambolt, R. B., Bohunek, L., Smits, C., Philit, M., Kieffer, T. J., Lakatta, E. G., Boheler, K. R., Moore, E. D., Allard, M. F., and Johnson, J. D. (2012) Cardiac ryanodine receptors control heart rate and rhythmicity in adult mice. *Cardiovasc. Res.* **96**, 372–380

16. Sohal, D. S., Nghiêm, M., Crackower, M. A., Witt, S. A., Kimball, T. R., Tymitz, K. M., Penninger, J. M., and Molkentin, J. D. (2001) Temporally regulated and tissue-specific gene manipulations in the adult and embry-
onic heart using a tamoxifen-inducible Cre protein. *Circ. Res.* **89**, 20–25

17. Allard, M. F., Schönekess, B. O., Henning, S. L., English, D. R., and Lopas-
chuk, G. D. (1994) Contribution of oxidative metabolism and glycolysis to ATP production in hypertrophied hearts. *Am. J. Physiol.* **267**, H1742–H1750

18. Belke, D. D., Larsen, T. S., Lopaschuk, G. D., and Severson, D. L. (1999) Glucose and fatty acid metabolism in the isolated working mouse heart. *Am. J. Physiol.* **277**, R1210–1217

19. Khairallah, M., Labarthe, F., Bouchard, B., Danialou, G., Petrof, B. J., and Des Rosiers, C. (2004) Profiling substrate fluxes in the isolated working mouse heart using 13C-labeled substrates: focusing on the origin and fate of pyruvate and citrate carbons. *Am. J. Physiol. Heart Circ. Physiol.* **286**, H1461–1470

20. O’Connell, T. D., Rodrigo, M. C., and Simpson, P. C. (2007) Isolation and culture of adult mouse cardiac myocytes. *Methods Mol. Biol.* **357**, 271–296

21. Luciani, D. S., Gwiazda, K. S., Yang, T. L., Kalynyak, T. B., Bychikvsa, Y., Frey, M. H., Jeffrey, K. D., Sampaio, A. V., Underhill, T. M., and Johnson, J. D. (2009) Roles of IP3R and RyR Ca
superscript 2+ channels in endoplasmic reticu-
lar stress and β-cell death. *Diabetes* **58**, 422–432

22. Trolinger, D. R., Cawio, W. E., and Lemsart, J. J. (1997) Selective loading of Rhod 2 into mitochondria shows mitochondrial Ca
superscript 2+ transients during the contractile cycle in adult rabbit cardiac myocytes. *Biochem. Biophys. Res. Commun.* **236**, 738–742

23. Aly, A., and Park, G. (1992) Rapid determination of creatine, phospho-
creatic, purine bases and nucleotides (ATP, ADP, AMP, GTP, GDP) in heart biopsies by gradient ion-pair reversed-phase liquid chromatography. *J. Chromatogr.* **575**, 19–27

24. Buteau, J., Shlien, A., Foisy, S., and Accili, D. (2007) Metabolic diapause in pancreatic β-cells expressing a gain-of-function mutant of the forkhead protein FoxO1. *J. Biol. Chem.** **282**, 287–293

25. Planavila, A., Iglesias, R., Giralt, M., and Villarroya, F. (2011) SIRT1 acts in association with PPARD to protect the heart from hypertrophy, metabolic dysregulation, and inflammation. *Cardiovasc. Res.** **89**, 276–284

26. Nemoto, S., Fergusson, M. M., and Finkel, T. (2004) Nutrient availability regulates SIRT1 through a forkhead-dependent pathway. *Science** **306**, 2105–2108

27. Lionetti, V., Stanley, W. C., and Recchia, F. A. (2011) Modulating fatty acid oxidation in heart failure. *Cardiovasc. Res.* **90**, 202–209

28. Son, N. H., Yu, S., Tuinei, J., Arai, K., Hamai, H., Homma, S., Shulman, G. I., Abel, E. D., and Goldberg, I. J. (2010) PPARY-induced cardioprotect-
ivity in mice is ameliorated by PPARY deficiency despite increases in fatty acid oxidation. *J. Clin. Invest.** **120**, 3443–3454

29. Schaeffer, P. J., Wende, A. R., Magee, C. J., Neilson, J. R., Leone, T. C., Chen, F., and Kelly, D. P. (2004) Calcineurin and calcium/calmodulin-de-
pendent protein kinase activate distinct metabolic gene regulatory pro-
grams in cardiac muscle. *J. Biol. Chem.* **279**, 39593–39603

30. Haemmerle, G., Lass, A., Zimmermann, R., Gorkiewicz, G., Meyer, C., Rozman, I., Heldmaier, G., Maier, R., Theussl, C., Eder, S., Kratky, D., Wagner, E. F., Klingenspor, M., Hoefler, G., and Zechner, R. (2006) Defec-
tive lipolysis and altered energy metabolism in mice lacking adipose trig-
lyceride lipase. *Science** **312**, 734–737

31. Regazzetti, C., Peraldi, P., Grémeaux, T., Najem-Lendom, R., Ben-Sahra, I., Cormont, M., Bost, F., Le Marchand-Brustel, Y., Tanti, J. F., and Gioretti-
Peraldi, S. (2009) Hypoxia decreases insulin signaling pathways in adi-

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RYR2 Controls ATP Production and Cardiomyocyte Survival

408. Yamamoto, K., Sakaguchi, M., Medina, R. J., Niida, A., Sakaguchi, Y., Mende, U.,
Niu, Y., Jiang, H., Takano, H., Toko, H., Yao, A., Takeshima, H., Akazawa,
H., Shiojima, I., Wang, Y., Komuro, I., and Ge, J. (2011) Ryanodine recep-
tor type 2 is required for the development of pressure overload-induced
cardiac hypertrophy. Hypertension 58, 1099–1110
409. Santiago, D. I., Curran, J. W., Bera, D. M., Lederer, W. J., Stern, M. D., Rios,
E., and Shannon, T. R. (2010) Ca sparks do not explain all ryanodine
receptor-mediated SR Ca leak in mouse ventricular myocytes. Biophys. J.
98, 2111–2120
410. Pacher, P., Thomas, A. P., and Hajnöczky, G. (2002) Ca\textsuperscript{2+} marks: mini-
ture calcium signals in single mitochondria driven by ryanodine receptors.
Proc. Natl. Acad. Sci. U.S.A. 99, 2380–2385
411. Denton, R. M., and McCormack, J. G. (1985) Ca\textsuperscript{2+} transport by mamma-
lian mitochondria and its role in hormone action. Am. J. Physiol. 249,
E543–554
412. Wan, B., LaNoue, K. F., Cheung, J. Y., and Scaduto, R. C., Jr. (1989) Regu-
atation of citric acid cycle by calcium. J. Biol. Chem. 264, 13430–13439
413. Ueno, M., Suzuki, J., Zeninariu, Y., Takahashi, S., Koizumi, T., Noriki, S.,
Yamaguchi, O., Otsu, K., Shen, W. J., Kraemer, F. B., and Miyamori, I.
(2008) Cardiac overexpression of hormone-sensitive lipase inhibits myo-
cardial steatosis and fibrosis in streptozotocin diabetic mice. Am. J.
Physiol. Endocrinol. Metab. 294, E1109–1118
414. Liao, R., Jain, M., Cui, L., D’Agostino, J., Aiello, F., Luptak, I., Ngoy, S.,
Mortensen, R. M., and Tian, R. (2002) Cardiac-specific overexpression of
GLUT1 prevents the development of heart failure attributable to pressure
overload in mice. Circulation 106, 2125–2131
415. Cullingford, T. E., Butler, M. J., Marshall, A. K., Tham els, L., Sugden, P. H.,
and Clerk, A. (2008) Differential regulation of Kruppel-like factor family
transcription factor expression in neonatal rat cardiac myocytes: effects of
endothelin-1, oxidative stress and cytokines. Biochim. Biophys. Acta 1783,
1229–1236
416. Rutter, G. A., Burnett, P., Rizzuto, R., Brini, M., Murgia, M., Pozzan, T.,
Tavare, J. M., and Denton, R. M. (1996) Subcellular imaging of intramito-
chondrial Ca\textsuperscript{2+} with recombinant targeted aequorin: Significance for the
regulation of pyruvate dehydrogenase activity. P. Natl. Acad. Sci. U.S.A.
93, 5489–5494
417. Arrington, D. D., Van Vleet, T. R., and Schnellmann, R. G. (2006) Calpain
10: a mitochondrial calpain and its role in calcium-induced mitochondrial
dysfunction. Am. J. Physiol. Cell Physiol. 291, C1159–1171
418. Lehnhart, S. E., Mongillo, M., Bellinger, A., Lindegger, N., Chen, B. X.,
Hsueh, W., Reiken, S., Wronska, A., Drew, L. J., Ward, C. W., Lederer,
W. J., Kass, R. S., Morley, G., and Marks, A. R. (2008) Leaky Ca\textsuperscript{2+} release
channel/ryanodine receptor 2 causes seizures and sudden cardiac death in
mice. J. Clin. Invest. 118, 2230–2245
419. George, C. H., Jundi, H., Thomas, N. L., Fry, D. L., and Lai, F. A. (2007)
Ryanodine receptors and ventricular arrhythmias: emerging trends in muta-
tations, mechanisms and therapies. J. Mol. Cell Cardiol. 42, 34–50
420. Yamaguchi, N., Chakraborty, A., Pasek, D. A., Molkentin, J. D., and Meis-
ger, G. (2011) Dysfunctional ryanodine receptor and cardiac hypertrophy:
role of signaling molecules. Am. J. Physiol. Heart Circ. Physiol. 300,
H2187–H2195