Early Remodelling of Perinuclear Ca\(^{2+}\) Stores and Nucleoplasmic Ca\(^{2+}\) Signalling During the Development of Hypertrophy and Heart Failure

Running title: Ljubojevic et al.; Early remodelling of perinuclear Ca\(^{2+}\) stores

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Abstract:

**Background**—A hallmark of heart failure is impaired cytoplasmic Ca\(^{2+}\) handling of cardiomyocytes. It remains unknown whether specific alterations in nuclear Ca\(^{2+}\) handling – via altered excitation-transcription coupling – contribute to the development and progression of heart failure.

**Methods and Results**—Using tissue and isolated cardiomyocytes from non-failing and failing human hearts, as well as mouse and rabbit models of hypertrophy and heart failure, we provide compelling evidence for structural and functional changes of the nuclear envelope and nuclear Ca\(^{2+}\) handling in cardiomyocytes as remodeling progresses. Increased nuclear size and less frequent intrusions of the nuclear envelope into the nuclear lumen indicated altered nuclear structure that could have functional consequences. In the (peri)nuclear compartment there was also reduced expression of Ca\(^{2+}\) pumps and ryanodine receptors, and increased expression of inositol-1,4,5-trisphosphate receptors, and differential orientation among these Ca\(^{2+}\) transporters. These changes were associated with altered nucleoplasmic Ca\(^{2+}\) handling in cardiomyocytes from hypertrophied and failing hearts, reflected as increased diastolic Ca\(^{2+}\) levels with diminished and prolonged nuclear Ca\(^{2+}\) transients and slowed intranuclear Ca\(^{2+}\) diffusion. Altered nucleoplasmic Ca\(^{2+}\) levels were translated to higher activation of nuclear Ca\(^{2+}\)/calmodulin-dependent protein kinase II and nuclear export of histone deacetylases. Importantly, the nuclear Ca\(^{2+}\) alterations occurred early during hypertrophy and preceded the cytoplasmic Ca\(^{2+}\) changes that are typical of heart failure.

**Conclusions**—During cardiac remodeling, early changes of cardiomyocyte nuclei cause altered nuclear Ca\(^{2+}\) signaling implicated in hypertrophic gene program activation. Normalization of nuclear Ca\(^{2+}\) regulation may, therefore, be a novel therapeutic approach for preventing adverse cardiac remodeling.

**Key words:** heart failure, remodeling, hypertrophy, nuclear calcium
Heart failure (HF) is characterized by systolic and diastolic dysfunction and abnormalities of intracellular Ca\textsuperscript{2+} handling with disturbed excitation-contraction coupling underlying contractile failure.\textsuperscript{1} Current research focuses on better understanding the mechanisms leading to disturbed Ca\textsuperscript{2+} handling during progression from cardiac remodelling (such as hypertrophy) to failure. The Ca\textsuperscript{2+} cycle in cardiomyocytes that governs contraction and relaxation on a beat-to-beat basis consists of a transient rise in the cytoplasmic free Ca\textsuperscript{2+} concentration ([Ca\textsuperscript{2+}]\textsubscript{cyto}) and subsequent Ca\textsuperscript{2+} decay. Each cytoplasmic [Ca\textsuperscript{2+}] transient (CaT) also elicits a nucleoplasmic CaT.\textsuperscript{2} Much progress has been made towards understanding the role of altered cytoplasmic Ca\textsuperscript{2+} homeostasis in hypertrophy and HF.\textsuperscript{3-5} However, nucleoplasmic [Ca\textsuperscript{2+}] ([Ca\textsuperscript{2+}]\textsubscript{nuc}) in HF is understudied and may be critical to cardiac remodeling, because it regulates protein expression through nuclear Ca\textsuperscript{2+}-dependent regulation of gene transcription.\textsuperscript{6,7}

Our previous studies indicated that nucleoplasmic CaTs follow distinct kinetics and may be regulated quite differently from cytoplasmic CaTs.\textsuperscript{2,8} An important aspect of [Ca\textsuperscript{2+}]\textsubscript{nuc} is the nuclear envelope (NE), which not only contributes to nuclear structure and insulation from the surrounding cytoplasm, but also controls bidirectional transport of ions (including Ca\textsuperscript{2+}) and macromolecular cargo via nuclear pore complexes (NPCs). The NE is also a functional Ca\textsuperscript{2+} store, akin to the sarcoplasmic reticulum (SR), and contains Ca\textsuperscript{2+} pumps (SERCA2) and Ca\textsuperscript{2+} release channels. The regulation of [Ca\textsuperscript{2+}]\textsubscript{nuc} via Ca\textsuperscript{2+} release from the NE is also important in Ca\textsuperscript{2+}-mediated gene expression.\textsuperscript{9,10} However, it remains unknown whether specific alterations in NE and nuclear Ca\textsuperscript{2+} handling occur in cardiac disease.

Therefore, we analyzed NE structure and function during cardiac remodeling from hypertrophy to HF in animal models of pressure overload and in non-failing and failing human hearts. We demonstrate that NE structure, its molecular composition and nucleoplasmic CaTs
undergo significant changes during pressure overload-induced hypertrophy in experimental animal models and in failing human hearts. These nuclear changes precede the changes in cytoplasmic Ca\(^{2+}\) dysregulation and, thus, suggest that altered nucleoplasmic [Ca\(^{2+}\)] is an early event during remodeling and may contribute to the development and progression of cardiac hypertrophy and failure via nuclear Ca\(^{2+}\)-dependent regulation of gene expression through activation of nuclear Ca\(^{2+}\)/calmodulin-dependent protein kinase II (CaMKII) and nuclear export of histone deacetylases (HDAC). Normalization of impaired [Ca\(^{2+}\)]\(_{\text{nuc}}\) regulation may, therefore, represent a novel therapeutic target to prevent adverse cardiac remodeling.

Materials and Methods

Detailed description can be found in the Supplemental Material.

Animal models

All experimental procedures involving animals were approved by the local Animal Care and Use Committees according to the Guide for the Care and Use of Laboratory Animals prepared by the U.S. National Academy of Sciences (National Institutes of Health publication No. 85-23, revised 1996). Hypertrophy and HF was induced by transverse aortic constriction (TAC) in C57BL/6 mice or by combined aortic insufficiency and stenosis in New Zealand White rabbits.

Human myocardium

All procedures involving human myocardium were approved by the Ethical Committee of the Medical University of Graz (ref. No. 20-277 ex08/09) and were carried out in accordance with the Declaration of Helsinki. Patient characteristics are summarized in Table S1.

Cardiomyocyte isolation

Murine, rabbit and human ventricular cardiomyocytes were isolated using standard enzymatic dissociation procedures.
Confocal Ca\textsuperscript{2+} imaging of nucleoplasmic and cytoplasmic CaTs

Simultaneous imaging of nucleoplasmic and cytoplasmic CaTs occurred in cardiomyocytes loaded with Fluo-4 (Molecular Probes, Leiden, The Netherlands) using a confocal imaging system (Zeiss LSM 510 Meta or Olympus Fluoview 1000) as described previously.\textsuperscript{8} Cardiomyocytes were field-stimulated via two platinum electrodes. Isoprenaline (30 nM) and angiotensin II (ATII, 100 nM) were used to investigate the effects of β-adrenergic stimulation and IP\textsubscript{3} signaling, respectively. Cytoplasmic and nucleoplasmic fluorescence signals were transformed into calibrated [Ca\textsuperscript{2+}] using the previously described method.\textsuperscript{8}

Imaging of perinuclear Ca\textsuperscript{2+} stores

Perinuclear Ca\textsuperscript{2+} stores were visualized in cardiomyocytes loaded with the low affinity Ca\textsuperscript{2+} indicator Mag-Fluo-4/AM using a confocal imaging system (Zeiss LSM 510 Meta or Olympus Fluoview 1000). The optical slice thickness was ≤0.76 μm. 2D images at a central depth of the nuclei were collected. The longitudinal axis was drawn through the middle of the nuclei and tubular structures (longer than 1 μm) were counted along the half of the nuclear envelope, which contained more invaginations. Rapid application of caffeine (20 mM) occurred in the presence of 20 mM BDM by wash-in of caffeine for 3 s. Caffeine experiments were conducted in cardiomyocytes isolated from C57BL/6 mice that did not undergo any surgery.

Immunocytochemistry

Immunocytochemistry was performed as previously described \textsuperscript{11} using the following antibodies: mouse monoclonal anti-nuclear pore complex proteins antibody (ab60080, Abcam, Cambridge, UK), mouse monoclonal anti-SERCA2a and mouse monoclonal anti-RyR antibody (MA3-919 and MA3-916, Thermo Scientific, Rockford, IL, USA), goat polyclonal anti-IP\textsubscript{3}R2 antibody (NB100-2466, Novus Biologicals, Littleton, CO, USA), rabbit polyclonal anti-P-CaMKII
antibody (ab32678, Abcam, Cambridge, UK) and rabbit polyclonal anti-HDAC4 antibody (sc-11418, Santa Cruz Biotechnology, Santa Cruz, CA, USA). The specificity of the antibodies was confirmed in Western blots.

**Electron microscopy and immunogold labeling**

Electron microscopic analyses of the NE were performed on human ventricular endocardial trabeculae, prepared as previously described.12

For immunogold labeling, ultrathin slices of mouse ventricles were stained with primary antibodies as in *Immunocytochemistry*, except for the rabbit polyclonal anti-SERCA2a antibody (A010-20, Badrilla, Leeds, UK). Goat anti-rabbit IgG (10 nm) and rabbit anti-goat IgG (5 nm) gold conjugates were from British BioCell International (BBI, Cardiff, UK).

**FRET imaging**

FRET imaging of CaMKII activation state was performed using the FRET-based biosensor Camui as previously described.13

**Isolation of cardiac nuclei from human hearts**

A detailed protocol for isolation of cardiac nuclei from human myocardium can be found in the Supplemental Material. The final nuclear fraction (N) was tested for expression of Ca2+-regulating proteins using standard immunoblot techniques with commercially available antibodies (see section *Immunocytochemistry*; anti-Nup62 (610497, BD Transduction Laboratories, Oxford, UK) and anti-Nkx2.5 (sc-14033, Santa Cruz Biotechnology, Santa Cruz, CA, USA)). The anti-RyR (1093) antibody used was custom-made.14 For quantification, signals were normalized to GAPDH (H) or Ponceau staining (H and N).

**Drugs and solutions**

Unless otherwise indicated, all chemicals were from Sigma-Aldrich (Steinheim, Germany).
Statistics

Data are presented as mean ± SEM. Differences between data sets were evaluated with Wilcoxon's rank sum test for between group comparisons and Wilcoxon's signed rank test for within group comparisons. Correlations were determined using Spearman rank correlation. Significance was accepted at *P<0.05. Statistical analyses were performed with SPSS Version 20.

Results

Hypertrophy vs. HF in mice

Sham-operated mouse hearts showed identical left ventricular (LV) dimensions and systolic function at either 1 or 6 weeks after surgery (not shown). Assessment of LVEDD and LVESD revealed that 1 week post-TAC mice displayed concentric LV hypertrophy, while LV dilation was observed 6 weeks post-TAC (Table S2 and Figure S1a). LV systolic function progressively declined, as indicated by ejection fraction reduction of ~8% one week and ~50% six weeks post-TAC (Table S2). TAC-induced hypertrophy was confirmed by increased heart weight normalized to tibia length or body weight (N=5-10; Figure S1b and S1c). Six weeks post-TAC mice developed pulmonary edema as manifested by an elevated lung weight-to-tibia length ratio (not shown). These data indicate that pressure overload-induced myocardial remodeling was associated with an early onset of compensatory hypertrophy and subsequent progression to overt HF.

Nuclear envelope remodeling in hypertrophy and failure

In cardiomyocytes from sham-operated mice and rabbits, loading of perinuclear Ca\textsuperscript{2+} stores with the low affinity Ca\textsuperscript{2+} indicator Mag-Fluo-4 revealed a NE and tubular invaginations traversing
the nucleus (Figure 1a, left). Rapid application of caffeine (20 mM) reversibly abolished Mag-
Fluo-4 fluorescence (Figure 1c) both in the NE and its tubular structures. Fluorescence recovery
after depletion was identical in both regions, returning to ~90% of the pre-caffeine level (n=7;
Figure 1d). This implies that the NE and its tubular invaginations are functional Ca\(^{2+}\) stores
capable of releasing and re-accumulating Ca\(^{2+}\), both in the nuclear periphery and the regions
within the nucleus otherwise remote from the NE.

A significant increase in the density of tubular invaginations, calculated as the number of
invaginations per NE circumference in the central depth of the nucleus, was observed during
physiological aging in young (2-5 months) sham-operated mice (n=90 nuclei/group; Figure 1b).
In contrast, TAC-operated mice exhibited a progressive decrease in NE tubular invagination
density 1 and 7 weeks post TAC. Similar reductions were observed in cardiomyocytes from non-
failing vs. failing rabbit hearts (n=30 nuclei/group) and from non-failing vs. failing human hearts
(n=20 nuclei/group), suggesting a misrelationship between the growth of the nuclei and the NE
compartment as a general feature of HF, independent of species and etiology of HF. Average
nuclear dimensions and number of NE tubular invaginations per nucleus are summarized in
Figure S2.

In order to confirm the presence of NE invaginations in cardiac tissue (vs. isolated
myocytes), and to investigate their detailed structure, electron microscopy imaging of nuclei
from sections of human ventricular trabeculae was performed. The observed invaginations were
lined by the inner and outer nuclear membranes, interrupted by numerous NPCs (Figure 2a,
right, red arrows and inset), and filled with cytoplasm (Figure 2a, left and middle).

In summary, these data indicate that the NE of cardiomyocytes contains a network of
tubular structures (i.e. NE invaginations) that undergoes significant changes during hypertrophy
and HF. With the progression of cardiac remodeling the size of nuclei increases, whereas, at the same time, the number of NE invaginations decreases.

**Remodeling of Ca\(^{2+}\)-regulating proteins in and around the nucleus**

We also investigated (peri)nuclear expression of Ca\(^{2+}\) release channels, ryanodine receptor (RyR) and inositol-1,4,5-trisphosphate receptor (IP\(_3\)R), nuclear pore complexes (NPCs) and sarcoplasmic reticulum Ca\(^{2+}\)-ATPase (SERCA) that could have functional consequences for [Ca\(^{2+}\)]\(_{\text{nuc}}\) and HF progression.

Immunostaining of cardiomyocytes confirmed the presence of NPCs both in the NE and its invaginations (n=15; **Figure 2b, top**). In line with Mag-fluo-4 stainings (**Figure 1a**), NPC labeling showed increased nuclear dimensions with reduced number of invaginations in 7 weeks post-TAC mice and in cardiomyocytes from failing human hearts (*quantitative analyses not shown*).

In sham-operated mice and in non-failing human cardiomyocytes, IP\(_3\)R2 was found in a striated pattern throughout the cell, in close proximity to the sarcolemma (most prominent) and on the NE. In HF, accumulation of IP\(_3\)R2 in the perinuclear region was observed (n=15; **Figure 2b, middle**). Immunogold labeling revealed that IP\(_3\)R2 is localized more prominently on the inner NE surface (**Figure 2d, red arrows**) but is also seen on the outer NE, as well as in the perinuclear region (**Figure 2d, bottom**).

A punctate pattern with a striated organization was observed for RyR2, and combined with nuclei staining revealed (**Figure S3**) that RyR2 did not penetrate into the nuclei but rather formed a "cage" around them, as described previously.\(^{15}\) A large reduction of RyR2 staining in perinuclear regions, in particular in the longitudinal direction, was observed in 7 weeks post-TAC mice and in cardiomyocytes from failing human hearts (n=15; **Figure 2b, middle** and
Figure S3). Immunogold labeling confirmed that RyR was not expressed on, but only near the NE (Figure 2c, top and middle). As expected, RyR2 was seen in SR-like structures surrounding T-tubules (Figure 2c, bottom).

SERCA2a expression exhibited a characteristic network-like pattern reflecting the SR throughout the cytoplasm. In addition, SERCA2a staining was strongly positive on the NE and its invaginations in sham mouse and non-failing human cardiomyocytes. Consistent with our earlier observation of blunted invagination density in HF, such structures were absent with SERCA2a immunostaining in either murine or human failing myocytes (n=15; Figure 2b, bottom). Immunogold labeling indicated that SERCA2a was mostly expressed on the outer nuclear membrane (n=11; Figure 2c; Figure S4), and that its expression on the NE was significantly decreased in 7 weeks post-TAC mice (n=6; Figure 3c, bottom right).

Altered expression levels of Ca\(^{2+}\)-regulating proteins were confirmed by Western Blot analysis of isolated human cardiac nuclei (Figure S5). Nuclear fractions from failing human myocardium exhibited a significant decrease in SERCA2a and RyR2 expression (which was detectable most likely due to remnants of perinuclear regions sticking to the nuclei), while IP\(_3\)R2 levels were increased as compared to non-failing hearts.

**[Ca\(^{2+}\)]\(_{\text{cyto}}\) and [Ca\(^{2+}\)]\(_{\text{nuc}}\) during electrical stimulation**

Fluo-4 fluorescence signals recorded during electrically stimulated CaTs (Figure 3a) were transformed into [Ca\(^{2+}\)]\(_{\text{nuc}}\) and [Ca\(^{2+}\)]\(_{\text{cyto}}\) using our compartment-specific calibration methods (Figure 3b).\(^8\) No differences in CaTs were observed between the two groups (1 and 7 weeks) of sham-operated mice (not shown). One week post-TAC, diastolic [Ca\(^{2+}\)]\(_{\text{nuc}}\) and [Ca\(^{2+}\)]\(_{\text{cyto}}\) were significantly increased and increased further 7 weeks post-TAC (Figure 3c). The increase of diastolic [Ca\(^{2+}\)]\(_{\text{nuc}}\) was, much more pronounced than [Ca\(^{2+}\)]\(_{\text{cyto}}\) (Figure S6). Peak systolic [Ca\(^{2+}\)]
remained unchanged in either compartment (not shown). Early in hypertrophy CaTs were slowed and CaT amplitudes were reduced only in the nucleoplasm. At seven weeks post-TAC, similar changes of CaTs also occurred in the cytoplasm (Figure 3d-e).

The NE invaginations and SERCA2/RyR2 distribution suggest the following working model of nuclear CaTs. The rise in \([\text{Ca}^{2+}]_{\text{nuc}}\) is driven mainly by RyR2-dependent rise in perinuclear \([\text{Ca}^{2+}]_{\text{cyto}}\), and the primarily cytosolic facing SERCA2 distribution suggests that \(\text{Ca}^{2+}\) may largely have to diffuse out of the nucleus to be pumped back into the NE and SR. These aspects would slow the rise and fall of nuclear vs. cytosolic CaTs (as observed) and could be exacerbated by the reduction in invaginations during the progression to HF. We measured this diffusional delay during development of HF in our TAC mice by measuring CaTs at the surface and center of both the nucleus and cytosol (Figure 3f-j and Figure S7). Kinetics of cytosolic CaTs were spatially uniform and unaltered 1 week post-TAC (n=10; Figure S7). However, at seven weeks post-TAC there was some slowing of the central CaT vs. the sub-sarcolemmal CaT (Figure S7), presumably caused by the reported reduction in T-tubular density that occurs in HF.16 In contrast, nuclear CaT propagation velocity (difference in time to peak between sub-nucleolemmal and central nucleoplasmic region divided by the distance) was already slowed significantly at 1 week post-TAC (Figure 3j, right). Selective slowing of the central nucleoplasmic CaTs 1 week post-TAC (Figure 3i-j) led also to a more pronounced increase in diastolic central \(\text{Ca}^{2+}\) already 1 week post-TAC (Figure 3h). This observation might be particularly relevant at high stimulation frequencies, when diastolic \(\text{Ca}^{2+}\) is more affected than \(\text{Ca}^{2+}\) due to its slower kinetics.8 In the rabbit HF model we also observed elevated diastolic \(\text{Ca}^{2+}\) and slowed kinetics of \(\text{Ca}^{2+}\) rise (Figure S8).

Importantly, in human myocytes (both for moderate and severe HF) we saw quite similar
effects as in the 1- and 7-week post-TAC mice (Figure 4). Although a low number of moderately failing hearts is a limitation of the study, our results suggests that early alterations in [Ca\textsuperscript{2+}] transients that occur selectively in the nucleus might be clinically relevant for the progression of cardiac remodeling in HF patients.

**Correlation of NE invaginations with nuclear [Ca\textsuperscript{2+}] transient kinetics**

In mouse and rabbit cardiomyocytes, we simultaneously quantified NE invaginations (with Mag-fluo-4) and spatiotemporal aspects of cytoplasmic and nucleoplasmic CaTs (with rhod-2). Figure 5a shows that nuclear CaT propagation is slowed in cardiomyocytes with lower NE invagination density. Correlation analysis indicates a strong inverse correlation between the density of NE invaginations and Ca\textsuperscript{2+} propagation to and from the nucleus (time to peak and DT\textsubscript{50}) in both sham and HF conditions (Figure 5b). This means that there is indeed a functional link between NE structure and nuclear CaTs kinetics.

**Frequency-dependent changes of nucleoplasmic [Ca\textsuperscript{2+}] transients**

Figure 6a-b show recordings in which stimulation frequency was gradually increased from 0.5 to 5 Hz (mouse cells) or from 0.2 to 1 Hz (rabbit cells). In all 3 groups of mice diastolic [Ca\textsuperscript{2+}] in the nucleus and cytoplasm rose with an increase in stimulation frequency (Table S3). However, during early remodeling (TAC 1 week) diastolic [Ca\textsuperscript{2+}]\textsubscript{nuc} was already elevated at very low stimulation rate (in contrast to diastolic [Ca\textsuperscript{2+}]\textsubscript{cyto}). At faster stimulation rates, diastolic [Ca\textsuperscript{2+}]\textsubscript{nuc} also rose much more prominently than [Ca\textsuperscript{2+}]\textsubscript{cyto}. In failing cardiomyocytes (TAC 7 weeks), these changes in nucleoplasmic and cytoplasmic diastolic [Ca\textsuperscript{2+}] were exacerbated. Systolic peak [Ca\textsuperscript{2+}] at 1 and 7-weeks post-TAC was comparable to the sham group in both compartments (at most frequencies). The increase in diastolic [Ca\textsuperscript{2+}] resulted in a progressive decline in the CaT amplitude with faster stimulation rates. The reduction of CaT amplitude started at lower
frequency in the nucleoplasmic vs. cytoplasmic compartment. Similar changes were observed in cardiomyocytes from non-failing vs. failing rabbit hearts (Figure 6b).

In further experiments, we investigated whether β-adrenergic stimulation can diminish or prevent the disproportionate rise in $[\text{Ca}^{2+}]_{\text{nuc}}$ in hypertrophied and failing cardiomyocytes to increased stimulation frequencies (Figure 6a, right). β-adrenergic stimulation greatly accelerates CaT decay and, thereby, might blunt the frequency-dependent increases in diastolic $[\text{Ca}^{2+}]$. Application of isoprenaline (30 nM) resulted in a robust increase of both nucleoplasmic and cytoplasmic CaTs in control cardiomyocytes. However, the preferential rise in diastolic $[\text{Ca}^{2+}]_{\text{nuc}}$ was still observed, and again was more pronounced as HF progressed (1- vs. 7-week post-TAC). Average data and statistical analysis related to Fig 6a are summarized in Table S3.

Collectively, these data revealed three important points: 1) the frequency-dependent increase in diastolic $[\text{Ca}^{2+}]_{\text{nuc}}$ is much larger than the increase in diastolic $[\text{Ca}^{2+}]_{\text{cyt}}$; 2) it persists with β-adrenergic stimulation; and 3) this effect is even more pronounced in failing myocytes.

**Frequency-dependent activation of nuclear CaMKII and nuclear export of HDAC**

In cardiomyocytes from sham-operated mice stimulated at low frequency (0.5 Hz), there was only weak staining of the autonomously active form of CaMKII, phospho-CaMKII, in the cytoplasm and within the nucleus (Figure 6c). Higher stimulation frequency (5 Hz) increased CaMKII phosphorylation, corresponding to the increase in $[\text{Ca}^{2+}]_{\text{nuc}}$ and $[\text{Ca}^{2+}]_{\text{cyt}}$ under the same pacing conditions, with much larger increases in the nucleus (particularly in the NE), and especially markedly in HF (n=25; Figure 6c). The phospho-CaMKII signals were prevented by KN-93 (1μM) preincubation, confirming the functional basis of the observed signals.

We also expressed the FRET-based biosensor CaMKII activity reporter Camui in control and HF rabbit cardiomyocytes. Camui displayed similar cellular distribution as the endogenous
phospho-CaMKII detected by immunocytochemistry, consistent with known CaMKII localization. Camui signals indicated significantly higher CaMKII activation in the nucleus and cytoplasm of failing cardiomyocytes at rest (n>32; Figure 6d).

Histone-deacetylase 4 (HDAC4) is a transcriptional regulator that is a downstream effector of CaMKII in the nucleus. That is, CaMKII binds to and phosphorylates HDAC4 to drive HDAC4 nuclear export and derepression of hypertrophic transcription. We found that in HF vs. control myocytes, HDAC4 was less nuclear at low frequency stimulation and was more readily driven out by increasing pacing frequency (n>20; Figure 6e) as expected from the frequency-dependent increase in nuclear CaTs and CaMKII activation (Figure 6a-c).

Role of IP₃ in regulation of nucleoplasmic [Ca²⁺] transients

IP₃ is an important regulator of nucleoplasmic CaTs. Inhibition of IP₃Rs (using 2-APB) causes selective decreases in diastolic nucleoplasmic [Ca²⁺]. Furthermore, perinuclear IP₃R expression is augmented in HF (Figures 2 and S4). Figure 7a shows linescan images of CaTs from electrically stimulated cardiomyocytes in the absence or presence of ATII, which is known to cause IP₃ production in cardiomyocytes. In sham-operated mice, application of ATII increased diastolic [Ca²⁺] in both the cytoplasm and nucleus (n=15; Figure 7b and 7c). The increase in [Ca²⁺]ₙuc was much larger than the increase in diastolic [Ca²⁺]ₜₚₒ and this effect was augmented in 7 weeks post-TAC cardiomyocytes. Large increases in diastolic [Ca²⁺] led to significant increases in systolic [Ca²⁺] in both compartments (Figure 7d), while CaT amplitude remained unaltered. ATII raised systolic [Ca²⁺]ₜₚₒ to similar levels in sham vs. TAC cardiomyocytes, while upon ATII application systolic [Ca²⁺]ₙuc was significantly higher in 7 weeks post-TAC cardiomyocytes compared to controls. Application of 2-APB (3 µM) completely blocked the effect of ATII (not shown), consistent with the ATII effects being IP₃-mediated.
Discussion

The present study is the first to provide direct compelling evidence for structural and functional changes of the NE and nucleoplasmic Ca\textsuperscript{2+} handling during cardiac remodeling and HF in mouse and rabbit models of pressure overload and in human hearts. The progressive decrease in NE invagination density and changes in the Ca\textsuperscript{2+}-regulatory protein expression patterns (schematically summarized in Figure 8) were associated with alterations of nucleoplasmic [Ca\textsuperscript{2+}] handling and consequent activation of gene transcription via the nuclear CaMKII-HDAC4 axis in electrically stimulated cardiomyocytes isolated from hypertrophied and failing hearts. Changes in nuclear CaTs occurred before cytoplasmic CaTs were affected, with an onset so early that they may well be involved in the development and progression of hypertrophy and HF.

The NE of numerous cell types (including cardiomyocytes) contains invaginations including deep, branching tubular structures (for review see\textsuperscript{9}). As caffeine experiments (Figure 1c) demonstrated, the NE and its invaginations represent functional Ca\textsuperscript{2+} stores capable of releasing and re-accumulating Ca\textsuperscript{2+}. It is tempting to speculate that – similar to T-tubular sarcolemmal invaginations that are critical for coordinated Ca\textsuperscript{2+} cycling throughout the myocyte – nuclear tubular invaginations may be critical for minute control of nucleoplasmic Ca\textsuperscript{2+} release and removal. Recent work suggested that the NE invaginations might be an artifact caused by NE folding due to the shorter sarcomere length in isolated cells.\textsuperscript{15} However, we also observed NE invaginations in multicellular cardiac tissue (Figure 2a). Furthermore, the fact that sarcomeres in cardiomyocytes rhythmically shorten and lengthen during the contraction-relaxation cycle suggests that NE invaginations might change in number and depth in the beating heart.

There are several important consequences of the NE invaginations penetrating into the
nucleus. In general, they facilitate intranuclear regulation of ions and transcription factors that travel between cytoplasm and nucleoplasm by decreasing the diffusion delay and by increasing membrane surface area, which may be critical for the regulation of gene transcription. The presence of NPCs on the NE invaginations ensures effective nucleo-cytoplasmic ion diffusion and cargo transport in regions that would otherwise be remote from the nuclear periphery. Expression of SERCA pumps on the invaginations enables Ca\(^{2+}\) removal from deep within the nucleus, but since most SERCA2 pumps face the cytoplasmic side, their position with respect to nuclear pores may be important in shaping nucleoplasmic CaT kinetics.

Our working hypothesis, based on NE invaginations and NPC, SERCA2 and RyR2 distribution is the following (Figure 8): Normally the rise in \([\text{Ca}^{2+}]_{\text{nuc}}\) is driven mainly by the RyR2-dependent rise in \([\text{Ca}^{2+}]_{\text{cyto}}\) that occurs during E-C coupling, and Ca\(^{2+}\) diffusion via NPCs (including invaginations) is the cause of slower rise times and peak \([\text{Ca}^{2+}]_{\text{nuc}}\). NE SERCA2 is mainly facing the cytoplasm (where there is also much more SERCA on SR) indicating that most Ca\(^{2+}\) may have to diffuse out of the nucleus to be pumped back into the NE and SR (accounting for the much slower \([\text{Ca}^{2+}]_{\text{nuc}}\) decline). The loss of NE invaginations which occurs early in HF (in mouse, rabbit and human) would reduce NPC and SERCA within the nuclear core and exacerbate the slowing of nuclear CaT transients and elevate diastolic \([\text{Ca}^{2+}]_{\text{nuc}}\) especially at higher heart rates. Indeed, we have directly measured all those effects here, even in the presence of \(\beta\)-adrenergic stimulation. The overall decrease in SERCA functional expression in HF slows \([\text{Ca}^{2+}]_{\text{cyto}}\) decline, which further increases diastolic \([\text{Ca}^{2+}]_{\text{nuc}}\) in HF. The profound increase in diastolic \([\text{Ca}^{2+}]_{\text{nuc}}\) and consequent higher CaMKII activation level that we observed in myocytes from the early TAC group at higher pacing frequencies may be causally involved in the remodeling processes leading to HF, in particular when considering the often elevated heart rates.
of patients with HF. Indeed, the SHIFT trial data demonstrate a beneficial effect of lowering heart rate by ivabradine in patients with advanced systolic heart failure, both in terms of attenuated LV remodeling and less frequent hospitalizations.

The simple working hypothesis above gets complicated somewhat by the IP$_3$R localization that was preferentially (but not exclusively) facing the nucleoplasm. $G_{q}$-coupled receptors ($\alpha$-adrenergic, endothelin and ATII receptors) that induce IP$_3$ production may be more activated during HF progression. Moreover, elevated [Ca$^{2+}$]$_{\text{nuc}}$ would enhance the IP$_3$ sensitivity of IP$_3$Rs and could well synergize with the other factors which elevate [Ca$^{2+}$]$_{\text{nuc}}$ in HF and at high heart rate, as we have shown for ATII. Indeed, IP$_3$R-mediated Ca$^{2+}$ release from the NE can elevate local [Ca$^{2+}$]$_{\text{nuc}}$ independently from [Ca$^{2+}$]$_{\text{cyto}}$. This pathway may be central in cardiac excitation-transcription coupling and, hence, increased IP$_3$R expression was proposed to be important during hypertrophy and HF. In line with this notion, we observed increased perinuclear IP$_3$R expression, and higher diastolic [Ca$^{2+}$]$_{\text{nuc}}$ vs. [Ca$^{2+}$]$_{\text{cyto}}$ in HF cardiomyocytes treated with ATII.

Perinuclear RyR expression was reduced here in HF, which would slow further the rise in [Ca$^{2+}$]$_{\text{nuc}}$ but could also shift perinuclear Ca$^{2+}$ signaling in favor of IP$_3$R-mediated Ca$^{2+}$ release during the progression from hypertrophy to HF. Nuclear factor of activated T-cells (NFAT) is a mediator of calcineurin-dependent nuclear signaling, and ATII and endothelin-1 could activate this system in adult ventricular cardiomyocytes. It was speculated that the perinuclear region could represent a local reserve of NFAT that is poised for shuttling in and out of the nucleus when local [Ca$^{2+}$] is elevated via IP$_3$-mediated Ca$^{2+}$ release. Furthermore, nuclear calcineurin is increased in human HF and required for full transcriptional effects of NFAT. Similarly, in adult ventricular myocytes $\alpha$-adrenergic receptor activation selectively increased nuclear
CaMKIIδ phosphorylation (without altering SR associated CaMKII), an effect attributed to Ca^{2+} mobilized through nuclear IP₃-sensitive stores. Accumulation of IP₃Rs in this region in cardiomyocytes from failing hearts would support the idea of enhanced NFAT and CaMKII signaling and activation of a hypertrophic gene program.

During the past two decades great advances have been made in understanding alterations in cytoplasmic ion homeostasis in cardiomyocytes during the development of hypertrophy and HF. Here, we show for the first time how nucleoplasmic Ca^{2+} homeostasis is altered during this hypertrophy-HF process. We provide evidence for structural and functional alterations of the nucleus and nuclear Ca^{2+} signaling as remodeling progresses. Importantly, the changes were observed not only in animal models of hypertrophy and HF but also in human HF. Our results implicate [Ca^{2+}]_{nuc} as an important determinant of cardiac remodeling which may contribute to the development and progression of hypertrophy and HF. Normalization of nucleoplasmic Ca^{2+} regulation may, therefore, be a novel therapeutic approach for preventing adverse cardiac remodeling.

**Acknowledgments:** We thank Anthony Lai for providing the RyR antibody, Eva-Maria Gutschi and Elisabeth Bock for excellent technical assistance and Kenneth Ginsburg for help with rabbit HF model.

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**Conflict of Interest Disclosures:** None.
References:


13. Erickson JR, Patel R, Ferguson A, Bossuyt J, Bers DM. Fluorescence resonance energy...


24. Taylor CW, Tovey SC. IP(3) receptors: toward understanding their activation. Cold Spring


Figure Legends:

Figure 1. Nuclear envelope remodeling in hypertrophy and heart failure. (a) Original 2D confocal images of Mag-fluo-4 fluorescence of nuclei from sham- and TAC-operated mice isolated 1 and 7 weeks after the intervention (top), non-failing and failing rabbit hearts (middle), and non-failing and failing human hearts (bottom). (b) NE invagination density, calculated as number of invaginations per NE circumference, in cardiomyocytes from sham- and TAC-operated mice, isolated 1 and 7 weeks after the intervention (left, n=90/group), non-failing and failing rabbit hearts (middle, n=30/group) and non-failing and failing human hearts (right, n=20/group). * P<0.05
versus 1 week post-sham mice or non-failing rabbit and human controls; \# P<0.05 versus 1 week post-TAC mice. (c) Original 2D images of Mag-fluo-4 fluorescence of a nucleus from control mice before, during and after caffeine application. (d) Average values of fluorescence recovery after depletion in different regions of the NE and its invaginations from 7 ventricular cardiomyocytes.

**Figure 2.** Nuclear envelope invaginations and localization of Ca\(^{2+}\)-regulating proteins in non-failing and failing cardiomyocytes. (a) Transmission electron micrograph of a nucleus from human left ventricular trabeculae (left), zoom on the NE invaginations (middle and right) and NPC (red arrows and inset). (b) Original 2D images of cardiomyocytes isolated from sham- and TAC-operated mice 7 weeks after the intervention (left) and non-failing and failing human cardiomyocytes (right) following immunostaining for NPC (top), IP\(_3\)R (middle), RyR (middle) and SERCA2a (bottom). Scale bars indicate 5 \(\mu\)m. Transmission electron micrograph of control mouse myocardium stained for (c) RyR, NE and its surrounding (top and middle), and T-tubules (TT) and their surroundings (bottom); (d) IP\(_3\)R, zoom on NE (top) and perinuclear region (bottom) and (e) SERCA2a, zoom on NE (top and middle), distribution between inner and outer nuclear membrane (n=11; bottom, left) and expression level on NE in sham- vs. TAC-operated mouse myocardium (n=6; bottom, right). * P<0.05 versus inner nuclear membrane or 7 week post-sham mice. (c)-(e) Red arrows and insets indicate gold particles. Scale bars indicate 0.2 \(\mu\)m.

**Figure 3.** Characterization of cytoplasmic, nucleoplasmic and subnucleoplasmic CaTs after pressure overload-induced hypertrophy and heart failure in mouse cardiomyocytes. (a) Linescan imaging of cytoplasmic and nucleoplasmic CaTs in a cardiomyocyte. (b) Averaged original recordings of distinct subcellular regions, as indicated in the scheme in (a): nucleus (red) versus
cytoplasm (black) of cardiomyocytes from sham- (left) and from TAC-operated mice isolated 1 (middle) and 7 (right) weeks after intervention. (c) Diastolic [Ca\textsuperscript{2+}], (d) amplitude and (e) kinetic parameters (time to peak (left) and DT\textsubscript{50} (right)) of nucleoplasmic (red) and cytoplasmic (black) CaTs. (b-e) n=15 myocytes/group. * P<0.05 versus 1 week post-sham. (f) Linescan imaging of subnucleoplasmic CaTs in a cardiomyocyte. (g) Averaged original recordings of CaTs from central (green) versus subnucleolemmal (black) regions of ventricular nuclei from sham- (left) and TAC-operated animals isolated 1 (middle) and 7 (right) weeks after intervention. (h) Diastolic [Ca\textsuperscript{2+}], (i) kinetic parameters (time to peak (left), DT\textsubscript{50} (middle) and velocity of spread (right)) of the central (green) and subnucleolemmal (black) CaTs. (g-i) n=10 myocytes/group. * P<0.05 versus 1 week post-sham; # P<0.05 versus subnucleolemmal.

**Figure 4.** Characterization of cytoplasmic and nucleoplasmic CaTs of cardiomyocytes from non-failing, moderately failing and severely failing human hearts. (a) Averaged original recordings of CaTs in nucleus (red) versus cytoplasm (black) of cardiomyocytes from healthy controls (N=6, left), moderately failing (N=2, middle) and end-stage failing human hearts (N=5, right). (b) Diastolic [Ca\textsuperscript{2+}], (c) amplitude and (d) kinetic parameters (time to peak (left) and DT\textsubscript{50} (right)) of nucleoplasmic (red) and cytoplasmic (black) CaTs. (a-d) n=6-10 myocytes/group. * P<0.05 versus non-failing.

**Figure 5.** Correlation between nuclear envelope invaginations and nuclear CaT kinetics in cardiomyocytes from non-failing and failing hearts. (a) Rabbit: Original confocal images of 3 nuclei stained with Mag-fluo-4 (left) and corresponding Rhod-2 recordings of CaTs in the same nuclei (right). (b) Kinetic parameters (time to peak (left) and DT\textsubscript{50} (right)) of the nucleoplasmic
(corrected for cytoplasmic) CaTs as a function of NE invagination density in cardiomyocytes from sham- and TAC-operated mice (top) and non-failing and failing rabbit hearts (bottom). In all groups, there was a significant correlation (with the correlation coefficient r as given in the figure). n=12-14 myocytes/group.

**Figure 6.** Frequency-dependent changes of nucleoplasmic versus cytoplasmic CaTs, phosphorylation of CaMKII and HDAC4 nuclear export in non-failing and failing cardiomyocytes.  
(a) Original recordings of nucleoplasmic (red) versus cytoplasmic (black) CaTs of cardiomyocytes from sham- (top) and TAC-operated mice (1 (middle) and 7 weeks (bottom) after intervention) during increases of stimulation frequency from 0.5 to 5 Hz. (b) Original recordings of nucleoplasmic (red) versus cytoplasmic (black) CaTs of cardiomyocytes from non-failing (top) and failing rabbits (bottom) during increases of stimulation frequency from 0.2 to 1 Hz. (c) Original confocal images (top) of cardiomyocytes from sham- and TAC-operated mice (1 and 7 weeks after intervention) immunostained for phospho-CaMKII following 10 min stimulation at 0.5 (left) or 5 Hz (right). Average phospho-CaMKII fluorescence values (bottom left) and increases in phospho-CaMKII levels calculated as difference to sham (F, bottom right) from 25 cardiomyocytes. * P<0.05 versus 0.5 Hz (left) or 1 week post-TAC group (right); # P<0.05 versus cytoplasm. (d) Original confocal images of CFP and YFP emission signals from non-failing and failing rabbit cardiomyocytes expressing Camui (left) and average data of Camui activation (F_{CFP}/F_{YFP}) in nucleus and cytoplasm of 32 non-failing and 90 failing cardiomyocytes at baseline conditions (right). * P<0.05 versus non-failing. (e) Original confocal images (left) of non-failing rabbit cardiomyocytes immunostained for HDAC4 following 15 min stimulation at 0.2 (top) or 1.5 Hz (bottom) and average values of nucleoplasmic/cytoplasmic HDAC4 fluorescence ratio in 28 non-
failing and 20 failing cardiomyocytes. * P<0.05 versus 0.2 Hz non-failing; # P<0.05 versus 0.2 Hz failing. (c)-(e) Scale bars indicate 20 μm.

**Figure 7.** IP₃-dependent changes of nucleoplasmic vs. cytoplasmic CaTs in non-failing and failing mouse cardiomyocytes. (a) Linescan images of cytoplasmic and nucleoplasmic CaTs in a non-failing (top) and failing (bottom) mouse cardiomyocyte without (Normal Tyrode, NT) or with angiotensin II (+ATII; 100 nM). (b) Averaged original recordings of nucleoplasmic (red) versus cytoplasmic (black) CaTs of cardiomyocytes from sham- (top) and TAC-operated (bottom) mice in the absence (left) or presence (right) of ATII. Average diastolic (c) and systolic (d) [Ca²⁺] (n=15 cardiomyocytes/group). * P<0.05 versus NT; # P<0.05 versus sham ATII.

**Figure 8.** Scheme of perinuclear Ca²⁺ stores and nucleoplasmic Ca²⁺ signaling alterations during cardiac remodeling. Ca²⁺ influx (red arrows) and removal (green arrows) pathways in the nucleus are highly balanced to assure specific regulation of Ca²⁺-dependent signaling and transcription (top). During development of hypertrophy, nuclear structure is altered, leading to a bigger nucleus size and less frequent NE invaginations (bottom). Expression levels of receptors and channels involved in Ca²⁺ homeostasis are altered, with increased perinuclear IP₃R expression and decreased perinuclear expression of RyR and SERCA. These structural and functional changes in NE and perinuclear regions contribute to the alterations of nucleoplasmic Ca²⁺ handling, especially to the increase in diastolic [Ca²⁺]ₙuc. ET-1, endothelin-1; ATII, angiotensin II; PE, phenylepinephrine; ECM, extracellular matrix; PLC, phospholipase C; IP₃, inositol-1,4,5-trisphosphate; IP₃R, IP₃ receptor; RyR, ryanodine receptor; SERCA, sarcoplasmic reticulum-Ca²⁺-ATPase.
Figure 1

(a) Fluorescence images showing the number of invaginations per NE circumference for mouse, rabbit, and human tissues treated with Sham, Sham 7w, TAC 1w, TAC 7w, non-failing, and failing conditions. Scale bars: 5 µm.

(b) Bar graph depicting the mean ± SEM of invaginations per NE circumference for mouse, rabbit, and human tissues treated with Sham, Sham 7w, TAC 1w, TAC 7w, non-failing, and failing conditions. *p < 0.05 compared to Sham.

(c) Photographs showing the effect of caffeine on control tissue at 0s, 5s, and 600s. Scale bar: 10 µm.

(d) Bar graph showing the recovery from depletion of nuclear envelope and invaginations in mouse tissues. n.s. indicates no significant difference.
Figure 3
Figure 4
Figure 5

(a) Images showing the density of NE invaginations at different levels: high, medium, and low. 
(b) Graphs illustrating the relationship between the number of invaginations and various parameters. 

For each graph, the correlation coefficient is indicated: * indicates significance. 

- **Mouse** data: 
  - Sham 7w vs. TAC 7w 
  - Correlation coefficient: $r = -0.640^*$ 
  - Correlation coefficient: $r = -0.518^*$ 

- **Rabbit** data: 
  - Non-failing vs. Failing 
  - Correlation coefficient: $r = -0.687^*$ 
  - Correlation coefficient: $r = -0.452^*$
Figure 6
Figure 7
Figure 8
Early Remodelling of Perinuclear Ca\textsuperscript{2+} Stores and Nucleoplasmic Ca\textsuperscript{2+} Signalling During the Development of Hypertrophy and Heart Failure

Senka Ljubojevic, Snjezana Radulovic, Gerd Leitinger, Simon Sedej, Michael Sacherer, Michael Holzer, Claudia Winkler, Elisabeth Pritz, Tobias Mittler, Albrecht Schmidt, Michael Sereinigg, Paulina Wakula, Spyros Zissimopoulos, Egbert Bisping, Heiner Post, Gunther Marsche, Julie Bossuyt, Donald M. Bers, Jens Kockskämper and Burkert Pieske

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Supplemental Material

Materials and Methods

Cell Preparation

Murine myocyte isolation

The procedure was approved by the local Animal Care and Use Committees according to the Guide for the Care and Use of Laboratory Animals prepared by the U.S. National Academy of Sciences (National Institutes of Health publication No. 85-23, revised 1996). Adult ventricular myocytes were isolated by a liberase-based Langendorff perfusion protocol as described in detail previously. All in vitro experiments were conducted at room temperature (22-24°C).

Human myocyte isolation

The procedure was approved by the Ethical Committee of the Medical University of Graz (ref. No. 20-277 ex08/09) and was carried out in accordance with the Declaration of Helsinki. Myocardial samples were obtained from the explanted failing hearts at the time of transplantation (N=5) and from unused donor hearts (N=8). Heart function was evaluated by transthoracic echocardiography and divided into three groups: non-failing (EF≥55%), moderately failing (55%>EF>35%) and severely failing (EF≤35%). In severely failing group, only patients with dilated cardiomyopathy (DCM) were included in this study. Patient characteristics are summarized in Table S1.

The procedure for cell isolation was modified after Sipido et al. Immediately following the resection, hearts were perfused with Custodiol® cardioplegic solution in the operating room at the time of surgery. Hearts were kept at 4°C in Custodiol® solution supplemented with 2,3-butanedione-monoxime (BDM). A wedge of the left ventricular wall with its perfusing coronary
artery was carefully excised, the artery was cannulated and perfused with Tyrode’s solution (in mM: 130 NaCl, 5.4 KCl, 1.2 MgSO₄, 1.8 CaCl₂, 20 HEPES, 10 taurine, 12.5 glucose, 2 pyruvate and 1 Na-L-lactate; pH 7.2 with NaOH). After cannulation the tissue wedge was perfused at 37°C with a Ca²⁺-free Tyrode’s solution for 10 min followed by enzyme perfusion for 25 min (liberase 0.15 mg/ml, trypsin 0.056 mg/ml, in 0.05 mM CaCl₂ Tyrode’s solution). The tissue was sectioned in slices of 5-7 mm and minced in 0.1 mM CaCl₂ Tyrode’s solution. The preparation was filtered and a small part of the cell yield was fixed for immunocytochemistry. In the remainder the solution was stepwise replaced by Tyrode’s solution containing 1.8 mM CaCl₂. Ca²⁺-tolerant cell yield was 5-20% and it was used for CaT recordings.

*Rabbit myocyte isolation*

Isolation protocol was performed in accordance with the Guide for the Care and Use of Laboratory Animals and approved by the University of California, Davis Institutional Animal Care and Use Committee. Adult rabbit ventricular cardiomyocytes were isolated from New Zealand white rabbits by standard enzymatic dissociation as described previously³.

*Animal disease models*

*Mice hypertrophy and heart failure model*

The surgical procedure was approved by the responsible government agency (ref. No. BMWF-66.010/0062-II/10b/2010).

Eight to eleven weeks old C57BL/6 wild-type mice of 21±1 g (females) and 24±1 g (males) body weight underwent transverse aortic constriction (TAC) surgery as described previously⁴. An age- and weight-matched group of mice underwent sham procedure consisting of aortic exposure
without ligation and served as control. Ventricular cardiomyocytes were isolated 1 and 7 weeks after TAC/sham.

One and six weeks after sham/TAC intervention, transthoracic echocardiography was performed and left ventricular fractional shortening (FS) and left ventricular mass (LV_{mass}) was calculated as previously described.\(^4\)

**Rabbit heart failure model**

HF was induced in New Zealand White rabbits by combined aortic insufficiency and stenosis as previously described.\(^3\) HF progression was assessed by 2D echocardiography and rabbits were studied ~12 months later, when end-systolic dimension exceeded 12 mm.\(^3\) All protocols followed the Guide for the Care and Use of Laboratory Animals and were approved by the University of California, Davis Institutional Animal Care and Use Committee.

**Confocal Ca\(^{2+}\) imaging of nucleoplasmic and cytoplasmic CaTs**

Cells were loaded with the Ca\(^{2+}\)-sensitive fluorescent dye Fluo-4 (Molecular Probes, Leiden, The Netherlands) as described previously.\(^6\) and placed on the stage of an inverted microscope equipped with a Plan Neofluar 40x/1.3 N.A. oil-immersion objective and a Zeiss LSM 510 Meta confocal laser point scanning system (Zeiss, Jena, Germany). Alternatively, an Olympus Fluoview 1000 confocal microscope (Olympus, Center Valley, PA, USA) was used. Excitation and emission wavelengths were 488 nm and >515 nm, respectively. The pinhole was set to 1 Airy unit, resulting in an optical slice thickness of 0.9 μm. The confocal plane was set to the middle (z-axis) of the nuclei, ensuring that only fluorescence originating from the nucleoplasm was collected. A 512 pixel scan line was positioned to include the nucleus, and scanned every 1.27 ms. Consecutive scan lines were stacked over time and visualized as 2D image.
For quantification of nucleoplasmic vs. cytoplasmic CaTs cells were field-stimulated via two platinum electrodes at 1 Hz (mouse) or 0.5 Hz (rabbit and human). To measure the frequency-dependent changes in CaTs, stimulation frequency was gradually increased from 0.5 Hz to 5 Hz (mouse) or from 0.2 Hz to 1.5 Hz (rabbit). Isoprenaline (30 nM) and angiotensin II (ATII, 100 nM) were used to investigate the effects of β-adrenergic stimulation and IP₃ signaling, respectively. Cytoplasmic and nucleoplasmic fluorescent signals were transformed into calibrated [Ca²⁺] using the previously described method.

Isolation of cardiac nuclei from human hearts

Ventricular myocardium from human hearts (N=12) was homogenized (H), filtered, diluted with water (3:1), and layered onto 1.5 volumes of buffer A (in mM: Hepes (pH 7.2) 10; sucrose 320; MgCl₂ 3; disodium pyrophosphate 25; EGTA 5; PMSF 1; leupeptin 0.001; pepstatin 0.001; DTT 1). Following a first centrifugation at 700g for 10 min at 4°C, the pellet (P1) containing the nuclei was suspended in hypotonic buffer A1 (buffer A without disodium pyrophosphate and with only 2.4 mM sucrose). The suspension was centrifuged at 50.000 g for 90 min at 4°C. Afterwards, the pellet (P2) containing the nuclei was suspended in nucleus storage buffer (in mM: Hepes (pH 7.6) 50; sucrose 320; magnesium acetate 5; Na₃EDTA 0.1; EGTA 1; PMSF 0.1; leupeptin 0.001; pepstatin 0.001; DTT 1). Finally, this suspension was spun down at 800g for 10 min, and the final nuclear pellet (N) was suspended in 0.1 ml nucleus storage buffer. Immunoblot analysis of the various fractions (H = whole tissue homogenate; S1 and S2 = supernatant after first and second centrifugation step, respectively; P1 and P2 = pellet after first and second centrifugation step, respectively; N = final purified nuclear fraction) revealed enrichment of nuclear markers (Nup62, Nkx2.5) in P1, P2, and N, and the absence of the cytoplasmic marker GAPDH in these fractions.
(Figure S4a). In addition, microscopic analysis showed intact isolated nuclei in the final nuclear fraction (Figure S4b).

The final nuclear fraction (N) was tested for expression of Ca\(^{2+}\)-regulating proteins (Figure S4c) using standard immunoblot techniques with commercially available antibodies (see section Immunocytochemistry; anti-Nup62 (610497, BD Transduction Laboratories, Oxford, UK) and anti-Nkx2.5 (sc-14033, Santa Cruz Biotechnology, Santa Cruz, CA, USA)). The anti-RyR (1093) antibody used was custom-made ⁷. For quantification, signals were normalized to GAPDH (H) or Ponceau staining (H and N).

**Electron microscopy and immunogold labeling**

Electron microscopic analyses of the NE were performed on human ventricular endocardial trabeculae, prepared as previously described ⁸. The samples were fixed at 4°C over night in 0.1 M sodium cacodylate buffer, pH 7.4, containing 2% glutaraldehyde and 2.5% formaldehyde. The samples were post-fixed for 2h at room temperature in the same buffer additionally containing 2% osmium tetroxide, rinsed and dehydrated in a series of graded ethanol solutions. Using propylene oxide as an intermedium, the samples were embedded in TAAB epoxy resin (TAAB, Aldermaston, UK). 60 nm thick sections were cut on a Leica UCT ultramicrotome (Leica Mikrosysteme Handelsges.m.b.H., Vienna, Austria). The sections were stained in lead citrate and uranyl acetate using a Leica EM AC20 contrasting instrument, and then visualized using a FEI Tecnai G²20 transmission electron microscope (FEI, Eindhoven, Netherlands), as described previously ⁹.

For immunogold labeling, mouse hearts were quickly excised and perfused with PBS. Ventricles were placed in fixing solution (0.1% glutaraldehyde, 2% paraformaldehyde, in 0.1 % sodium-phosphate buffer, pH 7.4) and sliced with a razor blade in cubes sized 1-2 mm\(^3\). After 1-3h, tissue
pieces were rinsed and dehydrated in a series of graded ethanol solutions. Samples were incubated in a mixture of LR White Embedding Medium and 80% ethanol (1:1) for 1h and twice in pure LR White for 1h. Resin was allowed to polymerize for 24h at 50ºC. Ultrathin sections were cut and mounted on nickel grids. Grids were washed in phosphate buffered gelatine (PBG) solution (0.8% BSA in PBS, 0.1% fish gelatine, 0.1M glycin) and incubated in blocking solution (5% normal goat (for goat anti-rabbit IgG gold conjugate) or normal rabbit (for rabbit anti-goat IgG gold conjugate) serum in PBG solution containing 0.05% Tween20). Grids were incubated for 2h with primary anti-IP$_3$R and anti-RyR antibodies as mentioned in Immunocytochemistry section, and a rabbit polyclonal anti-SERCA2a antibody (A010-20, Badrilla, Leeds, UK)). After rinsing, sections were incubated with goat anti-rabbit IgG (10 nm) or rabbit anti-goat IgG (5 nm) gold conjugates from British BioCell International (BBI, Cardiff, UK). Slices were finally washed with PBS and distilled water and left to air dry. Isotype controls with identical staining procedures, but replacing the primary antibodies with negative controls (Rabbit IgG Ab-1, NC-100-P1, Neo Markers, Fremont, CA, USA and Goat IgG-UNLB, 0109-01, Southern Biotech, Birmingham, AL, USA) showed no labeling. Additional negative controls, replacing the primary antibodies with PBS were performed on every experimental day.

Imaging of the sections was performed as described above. For each nucleus, the entire 2D cross section of nuclear envelope was visualized by merging consecutive high magnification images (see Figure S8). For quantitative analyses of SERCA2a staining, the number of gold particles within a narrow band at distances of <50 nm from the midline between the inner and the outer nuclear membrane was assessed.
**Supplemental Tables**

### Supplemental Table 1:

**Basic characteristics of patients who underwent transplantation or heart donors**

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50% 57±6 61±5 50% 72±5 45±0 60% 76±5 45±0 15±2

Data are mean±SD. EF – ejection fraction; Meds – medications; CAT – catecholamine; AB – antibiotics; PP – phenprocoumon; ACEI – ACE inhibitors; BB – β-blockers; D – diuretics; ARB – angiotensin II receptor blocker.

### Supplemental Table 2:

**Left ventricular echocardiographic parameters in control and 1 and 6 weeks after TAC intervention**

<table>
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<td>4.53±0.22*</td>
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<tr>
<td>LVESD (mm)</td>
<td>2.30±0.09</td>
<td>2.38±0.10</td>
<td>3.57±0.38*</td>
</tr>
<tr>
<td>IVS (mm)</td>
<td>0.72±0.02</td>
<td>0.95±0.03*</td>
<td>1.18±0.06*</td>
</tr>
<tr>
<td>PW (mm)</td>
<td>0.65±0.01</td>
<td>0.86±0.04*</td>
<td>1.12±0.06*</td>
</tr>
<tr>
<td>FS (%)</td>
<td>40±2</td>
<td>35±2</td>
<td>18±4*</td>
</tr>
<tr>
<td>EF (%)</td>
<td>70±2</td>
<td>65±3</td>
<td>36±8*</td>
</tr>
<tr>
<td>Rel. WT (mm/mm)</td>
<td>0.36±0.01</td>
<td>0.50±0.02*</td>
<td>0.52±0.03*</td>
</tr>
<tr>
<td>LV mass (mg)</td>
<td>71±3</td>
<td>121±8*</td>
<td>237±18*</td>
</tr>
</tbody>
</table>

50% 76±5 45±0 60% 76±5 45±0 15±2

Data are mean±S.E.M., *P<0.05 vs. Sham 1 week, #P<0.05 vs. TAC 1 week.
### Supplemental Table 3:

Stimulation frequency-dependent changes of CaTs after pressure overload-induced hypertrophy in adult mouse CMs

<table>
<thead>
<tr>
<th>Normal Tyrode</th>
<th>0.5 Hz</th>
<th>1 Hz</th>
<th>2 Hz</th>
<th>5 Hz</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>cytoplasm</td>
<td>nucleus</td>
<td>cytoplasm</td>
<td>nucleus</td>
</tr>
<tr>
<td><strong>Diastolic [Ca²⁺] (nM)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sham 1 week (n=12)</td>
<td>88 ± 5</td>
<td>126 ± 4</td>
<td>112 ± 10</td>
<td>152 ± 10</td>
</tr>
<tr>
<td>TAC 1 week (n=12)</td>
<td>96 ± 6*</td>
<td>151 ± 7*</td>
<td>134 ± 8*†</td>
<td>201 ± 13*†</td>
</tr>
<tr>
<td>TAC 7 weeks (n=10)</td>
<td>118 ± 5*</td>
<td>207 ± 9*</td>
<td>175 ± 9*†</td>
<td>277 ± 12*†</td>
</tr>
<tr>
<td></td>
<td>199 ± 11*</td>
<td>379 ± 9*</td>
<td>309 ± 35*†</td>
<td>508 ± 25*†</td>
</tr>
<tr>
<td><strong>Systolic [Ca²⁺] (nM)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sham 1 week (n=12)</td>
<td>687 ± 50</td>
<td>499 ± 45</td>
<td>711 ± 45</td>
<td>516 ± 35</td>
</tr>
<tr>
<td>TAC 1 week (n=12)</td>
<td>724 ± 46*</td>
<td>539 ± 44*</td>
<td>731 ± 47*</td>
<td>554 ± 36*</td>
</tr>
<tr>
<td>TAC 7 weeks (n=10)</td>
<td>795 ± 56*</td>
<td>639 ± 32*</td>
<td>779 ± 35</td>
<td>617 ± 38*</td>
</tr>
<tr>
<td></td>
<td>868 ± 63*</td>
<td>734 ± 41*</td>
<td>797 ± 29</td>
<td>715 ± 37</td>
</tr>
<tr>
<td><strong>Amplitude (nM)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sham 1 week (n=12)</td>
<td>599 ± 46</td>
<td>373 ± 44</td>
<td>599 ± 49</td>
<td>364 ± 36</td>
</tr>
<tr>
<td>TAC 1 week (n=12)</td>
<td>628 ± 41</td>
<td>388 ± 44</td>
<td>596 ± 52</td>
<td>353 ± 35</td>
</tr>
<tr>
<td>TAC 7 weeks (n=10)</td>
<td>677 ± 54*</td>
<td>432 ± 35*</td>
<td>604 ± 40</td>
<td>330 ± 42†</td>
</tr>
<tr>
<td></td>
<td>669 ± 53</td>
<td>355 ± 42</td>
<td>488 ± 58*†</td>
<td>208 ± 43*†</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Isoprenaline</th>
<th>0.5 Hz</th>
<th>1 Hz</th>
<th>2 Hz</th>
<th>5 Hz</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>cytoplasm</td>
<td>nucleus</td>
<td>cytoplasm</td>
<td>nucleus</td>
</tr>
<tr>
<td><strong>Diastolic [Ca²⁺] (nM)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sham 1 week (n=12)</td>
<td>221 ± 10</td>
<td>232 ± 13</td>
<td>253 ± 20</td>
<td>275 ± 23</td>
</tr>
<tr>
<td>TAC 1 week (n=12)</td>
<td>222 ± 10</td>
<td>251 ± 18</td>
<td>270 ± 20</td>
<td>364 ± 24*†</td>
</tr>
<tr>
<td>TAC 7 weeks (n=10)</td>
<td>223 ± 10</td>
<td>292 ± 19*</td>
<td>300 ± 22*†</td>
<td>508 ± 26*†</td>
</tr>
<tr>
<td></td>
<td>225 ± 10</td>
<td>375 ± 24*</td>
<td>499 ± 19*†</td>
<td>726 ± 28*†</td>
</tr>
<tr>
<td><strong>Systolic [Ca²⁺] (nM)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sham 1 week (n=12)</td>
<td>1742 ± 26</td>
<td>1556 ± 36</td>
<td>1527 ± 75†</td>
<td>1187 ± 91†</td>
</tr>
<tr>
<td>TAC 1 week (n=12)</td>
<td>1743 ± 29</td>
<td>1551 ± 41</td>
<td>1533 ± 82†</td>
<td>1207 ± 98†</td>
</tr>
<tr>
<td>TAC 7 weeks (n=10)</td>
<td>1750 ± 28</td>
<td>1568 ± 43</td>
<td>1541 ± 87†</td>
<td>1225 ± 112†</td>
</tr>
<tr>
<td></td>
<td>1754 ± 28</td>
<td>1577 ± 53</td>
<td>1518 ± 94†</td>
<td>1252 ± 125†</td>
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<tr>
<td><strong>Amplitude (nM)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sham 1 week (n=12)</td>
<td>1521 ± 32</td>
<td>1325 ± 36</td>
<td>1273 ± 67†</td>
<td>912 ± 82†</td>
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<tr>
<td>TAC 1 week (n=12)</td>
<td>1522 ± 35</td>
<td>1300 ± 43</td>
<td>1263 ± 71†</td>
<td>843 ± 83†</td>
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<tr>
<td>TAC 7 weeks (n=10)</td>
<td>1527 ± 32</td>
<td>1277 ± 43</td>
<td>1242 ± 75†</td>
<td>717 ± 111*†</td>
</tr>
<tr>
<td></td>
<td>1529 ± 33</td>
<td>1202 ± 49*</td>
<td>1018 ± 81*†</td>
<td>526 ± 127*†</td>
</tr>
</tbody>
</table>

Data are mean±S.E.M., *P<0.05 vs. previous frequency, †P<0.05 vs. Sham 1 week.
**Supplemental Figures**

**Supplemental Figure 1.** Characterisation of pressure overload-induced hypertrophy in adult WT mice. (a) LVEDD (dilation) in conjunction with increased LV thickness (hypertrophy), measured as the sum of intraventricular septum thickness and posterior wall thickness for sham (N=16) and TAC-operated mice (after 1 week N=14 and after 6 weeks N=5). Heart weight normalized to left tibia length (N=5-6 per group) or body weight (N=6-10 per group) are shown in (b) and (c), respectively. Asterisks indicate P<0.05 versus 1 week sham controls.
Supplemental Figure 2. Nucleus size and number of tubular structures after pressure overload-induced hypertrophy in mouse cardiomyocytes and in cardiomyocytes from non-failing and failing rabbit and human hearts. Nuclear length (left), width (middle) and number of tubular invaginations per nucleus (right) from mouse (top), rabbit (middle) and human (bottom) hearts. Data from a total of 90 myocytes per group for mouse, 30 myocytes per group for rabbit and 20 myocytes per group for human. Asterisks indicate P<0.05 versus 1 week sham or non-failing controls.
Supplemental Figure 3. Expression and localization of RyR after pressure overload-induced hypertrophy in mouse cardiomyocytes. Original 2D confocal images of 2 example cardiomyocytes isolated from sham- (left) and TAC-operated mice (right) 7 weeks after the intervention and double staining with an anti-RyR antibody and DAPI to visualize the nuclei.
Supplemental Figure 4. Quantification of SERCA2a on the nuclear envelope of cardiomyocytes isolated from non-failing and failing murine hearts.

For each nucleus, the entire 2D cross section of the NE was visualized by merging consecutive high magnification images, as illustrated here, allowing counting of gold particles (highlighted as red dots or indicated with red arrows for more comprehensive representation) and measuring NE circumference. Gold particles were counted within a distance of <50 nm from the midline between the inner and the outer nuclear membrane.
Supplemental Figure 5. Expression of Ca\textsuperscript{2+}-regulating proteins in isolated human cardiac nuclei.

(a) Immunoblots of the various fractions of the protocol used for isolation of the nuclei from cardiac tissue. Nucleoporin 62 (Nup62) and Nkx2.5 served as nuclear markers, whereas GAPDH served as cytoplasmic marker. H – tissue homogenate; S1 and 2 – the first and the second supernatant; P1 and 2 – the first and the second pellet; N - the final purified nuclear fraction (N). (b) Original 2D transmitted light (top), Syto-16 fluorescence (bottom) and merged (middle) image of a nucleus from the final purified nuclear fraction. (c) Example immunoblots of heart homogenate (H) and purified nuclear fraction (N) from non-failing and failing human myocardium stained for RyR, IP\textsubscript{3}R and SERCA2a. For quantification, signals were normalized to GAPDH (H) or Ponceau staining (H and N). (d) Change in expression levels of Ca\textsuperscript{2+} release channels, RyR and IP\textsubscript{3}R, and SERCA in the nuclear fractions of left ventricles (red) and left ventricular tissue homogenates (black) from end-stage failing human myocardium as compared to non-failing (dashed line). Asterisks indicate P<0.05 versus non-failing controls.
Supplemental Figure 6. Hypertrophy-induced increase in diastolic [Ca^{2+}] in the nucleus versus cytoplasm of mouse and human ventricular myocytes. Increase in diastolic [Ca^{2+}] calculated as the difference to sham-operated mice or non-failing human hearts. Data from a total of 15 mouse and 6-10 human ventricular myocytes. Asterisks indicate P<0.05 versus 1 week post-TAC or moderately failing group and pound signs indicate P<0.05 versus cytoplasm.
Supplemental Figure 7. Characterisation of subcytoplasmic CaTs after pressure overload-induced hypertrophy in electrically stimulated mouse cardiomyocytes. (a) Line scan confocal imaging of subcytoplasmic CaTs in an electrically stimulated ventricular cardiomyocyte. (b) Averaged CaTs of distinct subcellular regions, as indicated in the schematic representation of the cell in (A): central (blue) versus subsarcolemmal region (black) from Sham-operated animals (left) and TAC-operated animals isolated 1 (middle) and 7 (right) weeks after the intervention. (c) Diastolic [Ca^{2+}] and (d) kinetic parameters (time to peak (left) and DT_{50} (right)) of the central (blue) and subsarcolemmal (black) CaTs. Data in (b) to (d) from a total of 10 mouse ventricular myocytes per group. Asterisks indicate P<0.05 versus 1 week Sham controls and pound signs indicate P<0.05 versus subsarcolemmal.
Supplemental Figure 8. Characterisation of cytoplasmic and nucleoplasmic CaTs of cardiomyocytes isolated from non-failing and failing rabbit hearts.

(a) Averaged original recordings of electrically stimulated CaTs in the nucleus (red) versus cytoplasm (black) of ventricular cardiomyocytes from non-failing (N=3, left) and failing (N=3, right) rabbit hearts. (b) Diastolic [Ca$^{2+}$], (c) amplitude and (d) kinetic parameters (time to peak (left) and DT$_{50}$ (right)) of the nucleoplasmic (red) and cytoplasmic (black) CaTs. Data in (a-d) from a total of 15 myocytes per group. Asterisks indicate P<0.05 versus non-failing group.
References


