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Cholesterol is required for maintaining T-tubule integrity and intercellular connections at intercalated discs in cardiomyocytes

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Abstract

Backgrounds—Low serum cholesterol levels are associated with cardiac arrhythmias and poor prognosis in patients with chronic heart failure. However, the underlying mechanisms by which decreases in cholesterol content lead to cardiac dysfunction remain unclear. Multiple studies have implicated damage to cardiac transverse (T)- tubules as a key mediator of excitation-contraction (E-C) coupling dysfunction and heart failure. Since the T-tubule membrane system is enriched in cholesterol, we hypothesized that depletion of membrane cholesterol promotes T-tubule remodeling and E-C coupling dysfunction.

Methods and results—We first examined the impact of membrane cholesterol depletion on T-tubule architecture by treating isolated C57BL/6 murine cardiomyocytes with methyl- β -cyclodextrin (M β CD). T-tubule structural integrity was progressively decreased by M β CD in a concentration- and time-dependent manner. Membrane cholesterol depletion also promoted a severe decrease in the amplitude of Ca²⁺ transients and an increase in Ca²⁺ release dyssynchrony as well as a significant increase in the frequency of spontaneous Ca²⁺ sparks. Reintroduction of cholesterol restored T-tubule integrity and partially restored Ca²⁺ handling properties in acutely-treated myocytes and slowed T-tubule deterioration in response to chronic M β CD exposure. Studies were extended to determine the impact of membrane cholesterol depletion on T-tubule structure in intact hearts. In addition to T-tubule remodeling, Langendorff perfusion of M β CD resulted in rapid and severe disruption of the intercellular connections between cardiomyocytes, in particular at intercalated disc regions in intact hearts.

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Conflict of interest: None.

Conclusions—These data provide the first evidence that cholesterol plays a critical role in maintaining cardiac T-tubule structure as well as the integrity of intercalated discs.

Keywords

Cholesterol; T-tubules; Intercalated discs; Cardiomyocytes; Ca^{2+} handling

1. Introduction

Transverse tubules (T-tubules) are orderly invaginations of the surface plasma membrane into the cell interior that form tight physical couplings with the terminal cisternae of the sarcoplasmic reticulum (SR). Precise communication between the voltage-gated L-type Ca^{2+} channels (LTCCs) located mainly on the T-tubule membrane and Ca^{2+} release ryanodine receptor channels (RyRs) on the SR is essential for instantaneous excitation and synchronous activation of nearly all Ca^{2+} release sites throughout the large ventricular myocytes [1,2]. Severe damage to the morphology of T-tubules in cardiomyocytes leads to abnormal Ca^{2+} handling, contractile dysfunction and the development of heart failure [3,4]. T-tubule remodeling has been documented in failing ventricular myocytes from patients with heart failure as well as multiple animal models of cardiac stress [5–8]. These studies provide clear evidence that maintenance of the T-tubule ultrastructure is paramount for normal cardiac function. Indeed, work from our group has provided compelling evidence that strategies that maintain T-tubule architecture prevent cardiac dysfunction following cardiac stress [9].

Cholesterol is an essential structural component of the plasma membrane in animal cells, with 80–95% of the total cellular cholesterol content distributed to the plasma membrane [10]. Several studies have demonstrated that lower serum cholesterol levels are associated with poor clinical outcomes in patients with heart failure, yet the underlying mechanisms by which low cholesterol contributes to cardiac damage remain poorly defined [11–13]. Since the T-tubule membrane system is enriched in cholesterol [14], we hypothesized that T-tubule integrity is regulated by membrane cholesterol content. Herein we demonstrate that depletion of membrane cholesterol with methyl- β -cyclodextrin (M β CD) promotes T-tubule remodeling and Ca^{2+} handling dysfunction in isolated cardiomyocytes in a time- and concentration-dependent manner. Replenishing membrane cholesterol prevents further damage to T-tubules and Ca^{2+} handling. Unexpectedly, in situ perfusion of M β CD in intact hearts resulted in severe disruption of intercalated discs, in addition to T-tubule damage. These data identify a key role for membrane cholesterol in the maintenance of normal T-tubule architecture, intercalated discs and myocyte contractile function.

2. Methods

2.1. Animal studies and experimental methods

Animal experiments were performed in accordance with the Guide for the Care and Use of Laboratory Animals (NIH publication No. 85-23, revised 1996) and were approved by the Institutional Animal Care and Use Committee at the University of Iowa. All experiments

were performed using male and female C57BL/6J mice (Jackson Laboratories) at 2–3 months of age.

2.2. Adult mouse ventricular myocyte isolation and culture

Adult ventricular cardiomyocytes were isolated from C57BL/6J mice using standard enzymatic methods as described previously [15]. After incubating in 1.0 mM Ca^{2+} Tyrode's solution (in mM: NaCl 137, KCl 5.4, HEPES 10, Glucose 10, MgCl_2 1, NaH_2PO_4 0.33, pH adjusted to 7.4 with NaOH) for 30 min, cell pellets were suspended in minimum essential medium (MEM) with 10% fetal bovine serum (FBS) and plated in dishes containing laminin (10 $\mu\text{g}/\text{ml}$)-precoated glass. Myocytes were cultured in a 5% CO_2 incubator at 37 °C for 2 h, then the medium was changed to FBS-free MEM for experiments. For some studies with extended treatment, myocytes were maintained at 37 °C with 5% CO_2 for 24–48 h.

2.3. Adenoviral-mediated gene expression

Adenoviruses containing Caveolin 3 (Ad-Cav3) or empty Ad5 virus (Ad-empty) were produced by University of Iowa Gene Transfer Vector Core. Freshly isolated cardiomyocytes were infected with Ad-Cav3 or Ad-Empty and experiments performed 48 h after infection.

2.4. M β CD treatment and cholesterol replenishment

Freshly isolated cardiomyocytes were treated with serum-free MEM without or with the cholesterol chelator M β CD (M7439, Sigma) at a concentration of 3 mM for 1–3 h or 100–300 μM for 24–48 h at 37 °C to deplete membrane cholesterol. For cholesterol replenishment, cardiomyocytes were treated with 3 mM M β CD for 1 h or 200 μM M β CD for 24 h, followed by washout and treatment with cholesterol (0.25 mM, final concentration) premixed with 2.5 mM M β CD for an additional 2 h or 24 h, respectively [16]. Washout only without other treatment served as a control.

2.5. T-tubule imaging and analysis

For imaging T-tubules in isolated cardiomyocytes, following treatment T-tubules were stained with Di-8-ANEPPS (10 μM , AAT BioQuest) in Ca^{2+} -free Tyrode solution at room temperature for 30 min. For imaging T-tubules in intact hearts in situ, adult mice were heparinized (100 IU i.p.) and euthanized by pentobarbital (120 mg/kg, i.p.), followed by excision and perfusion of hearts with K-H solution (in mM: NaCl 113, KCl 5.4, NaHCO_3 25, glucose 11, Napyruvate 5, taurine 10, creatine 5, MgCl_2 1.2, NaH_2PO_4 1.2) at room temperature. T-tubules from intact hearts were stained with MM 4–64 (2.5 μM , AAT BioQuest) in Ca^{2+} - free K-H solution via Langendorff perfusion at room temperature for 30 min (baseline), followed by addition of 300 μM M β CD to the perfusate for an additional 40 min. Sequential images were acquired at base- line and 15–45 min after M β CD perfusion as previously described [3, 9]. T-tubule structure was visualized by confocal microscopy (LSM510, Carl Zeiss MicroImaging Inc.) using a 63 $\times$ lens. Quantitative analysis of T-tubule integrity was processed with IDL image analysis program as previously reported [3]. The power value (TT_{power}) reflects the strength of the regularity of T-tubule organization.

2.6. Ca²⁺ imaging and analysis

Cultured cardiomyocytes were loaded with 5 μ M Fluo-4 AM (Invitrogen) in 1.8 mM Ca²⁺ Tyrode solution for 30 min. Cells were then washed with dye-free Tyrode solution for 20 min for de-esterification before imaging. Ca²⁺ images were acquired using a confocal microscope in line-scan mode along the longitudinal axis of myocytes. Steady-state Ca²⁺ transients were measured in Tyrode's solution containing 1.8 mM Ca²⁺ under field stimulation of 1 Hz. Ca²⁺ sparks were recorded ~5 s after field stimulation was halted. Quantitative analysis of Ca²⁺ transients was processed using an in-house program developed in MatLab [17]. Quantitative analysis of Ca²⁺ sparks was processed with IDL image analysis program as previously reported [15].

2.7. Western blotting assay

Protein concentrations were determined using the Pierce BCA assay (Pierce, Thermo Scientific). Whole cell lysates and cytosol and membrane fractions were separated by SDS-PAGE (10% acrylamide) and transferred to PVDF membranes. Primary antibodies that recognize Caveolin-3 (BD Transduction Laboratory, #610420) and GAPDH (Cell Signaling, #2118) were applied overnight at 4 °C. HRP-linked anti-goat IgG (1:5000), anti-mouse (1:5000) or anti-rabbit IgG (1:10,000) were used to visualize bound primary antibodies with the SuperSignal chemi-luminescence substrate (Pierce, Thermo Scientific), and imaged with Fuji life science imaging system (LAS-3000, Fujifilm). The protein bands were quantified using NIH Image J software (version 1.43d).

2.8. Statistics

Data are expressed as mean $\pm$ SE. Student's *t*-test was used for two- group comparison. Statistical analyses were carried out using SPSS V.15.0 (SPSS). *p* values of < 0.05 were considered statistically significant.

3. Results

3.1. Cholesterol depletion with M β CD disrupts T-tubule structural integrity

To determine the impact of cholesterol on T-tubule membrane stability, freshly isolated murine ventricular cardiomyocytes were treated with M β CD and T-tubule structure analyzed by confocal microscopy. As compared to untreated cardiomyocytes, depletion of membrane cholesterol with 3 mM M β CD for 1 h resulted in a decrease in T-tubule integrity that became more prominent at 2 and 3 h (Fig. 1A, B). We next determined the effect of low-dose M β CD and found that chronic exposure of isolated cardiomyocytes to doses as low as 100 μ M M β CD for 24 or 48 h resulted in a significant decrease in TT_{power}, an index of T-tubule integrity (Fig. 1C, D and Supplemental Fig. S1). Similar results were achieved in myocytes acutely treated with 2 mM M β CD for 1–3 h or chronically treated with 300 μ M M β CD for 18 h (Supplemental Fig. S2). Consistent with our previous study [15], we observed progressive T-tubule remodeling with prolonged culture, though addition of M β CD exacerbated this effect (Fig. 1 D). These data suggest that membrane cholesterol is necessary to maintain cardiomyocyte T-tubule structural integrity.

3.2. Cholesterol reintroduction restores impaired T-tubule structure

We next determined if T-tubule remodeling induced by M β CD is due to the loss of membrane cholesterol. M β CD depletes membrane cholesterol by sequestering cholesterol in a hydrophobic pocket in a concentration-dependent manner. Therefore, we utilized cholesterol-saturated M β CD to replenish membrane cholesterol following cholesterol depletion with 3 mM M β CD for 1 h. Whereas washout of M β CD was not sufficient to restore T-tubule integrity in isolated ventricular myocytes, cholesterol replenishment resulted in a dramatic recovery of TT_{power} as compared to cholesterol-depleted myocytes (Fig. 2A, B). In cardiomyocytes that were exposed to low-dose (200 μ M) M β CD for prolonged period (24 h), replenishing membrane cholesterol prevented further T-tubule damage as compared to M β CD wash out control (Fig. 2C, D). These data provide further evidence that membrane cholesterol is required for T-tubule structural integrity in cardiomyocytes.

3.3. Depletion of membrane cholesterol results in E-C coupling dysfunction

Since T-tubules play important role in controlling SR Ca²⁺ release in ventricular cardiomyocytes, we next examined the effect of membrane cholesterol depletion on Ca²⁺ handling properties in isolated cardiomyocytes. Treatment with 3 mM M β CD for 1 h resulted in a significant decrease in the amplitude of Ca²⁺ transients and V_{max}, an increase in the dyssynchronous index and a delay in Ca²⁺ uptake (T₅₀) as compared to control treatment (Fig. 3A, B), in line with T-tubule observations in Fig. 2. Membrane cholesterol replenish for 2 h partially restored these changes induced by cholesterol depletion (Fig. 3A, B). In addition, membrane cholesterol depletion resulted in a pronounced increase in the frequency of spontaneous Ca²⁺ sparks as compared to control myocytes, which was restored by repletion of membrane cholesterol (Fig. 3C, D). M β CD had no effect on other properties of Ca²⁺ sparks, such as amplitude (F/F₀), duration (FDHM), and width (FWHM), suggesting cholesterol has no direct impact on SR membrane ryanodine receptor Ca²⁺ release channels. These findings indicate that cholesterol plays a pivotal role in maintaining T-tubule structure and thus E-C coupling function in isolated myocytes.

To further dissect the impact of M β CD on myocyte E-C coupling function, we examined whether M β CD exerts any acute effects on Ca²⁺ handling properties. Acute perfusion of M β CD for 10 min at either low (300 μ M) or high (3 mM) concentrations had no significant effects on Ca²⁺ transients or Ca²⁺ sparks (Fig. 4). These data further indicate that the above-mentioned observations of M β CD on cardiomyocytes (Fig. 3) are due to its effects on T-tubule integrity.

3.4. Overexpression of Cav3 does not protect against T-tubule damage in response to membrane cholesterol depletion

Based on previous studies in myotubules, it has been suggested that T-tubules and the caveolae protein caveolin-3 (Cav3) share a common mechanism for biogenesis [18–22]. In addition, genetic deficiency of Cav3 is associated with an abnormal T-tubule structure in skeletal muscle [20]. We first established that Cav3 levels in cardiomyocytes do not decrease after prolonged culture (Supplemental Fig. S3). Since others have reported that treatment with M β CD disrupts caveolae and Cav3 organization [23–25], we examined whether overexpression of Cav3 can protect against M β CD-induced T-tubule remodeling. Isolated

cardiomyocytes were infected with an adenovirus containing Cav3 cDNA (Ad-Cav3) for 48 h in the presence of 200 μ M M β CD. Levels of Cav3 were 1.5-fold higher in myocytes infected with Ad-Cav3 as compared to empty vector control (Fig. 5A, averaged data not shown). Unexpectedly, overexpression of Cav3 failed to protect against M β CD- induced T-tubule damage (Fig. 5B, C). Consistent with these findings, myocytes overexpressing Cav3 demonstrated decreased Ca^{2+} amplitude and V_{max} and an increase in the dyssynchronous index following exposure to low-dose M β CD (Fig. 5D), demonstrating that Cav3 overexpression does not prevent E-C coupling dysfunction induced by membrane cholesterol depletion.

3.5. Membrane cholesterol depletion in intact hearts results in pronounced damage to the intercalated discs

We next examined effect of cholesterol depletion on T-tubule ultra- structure in intact hearts using state-of-the-art in situ confocal imaging of Langendorffperfused hearts. Interestingly, gaps were visible at the intercalated discs following perfusion with 300 μ M M β CD for only 15 min (Fig. 6). By 30 min of M β CD perfusion, large gaps had formed between myocytes at the end of the longitudinal axis (Fig. 6). In addition, the membrane staining appears to be blurry with minor disorganization of the T-tubule structure. However, after 40 min of M β CD perfusion, considerable myocyte shrinkage was apparent, with some evidence for cell death. This phenomenon was observed in studies of 4 independent hearts.

4. Discussion

Hypercholesterolemia is a risk factor for coronary artery disease, whereas too little cholesterol is associated with higher morbidity and mortality in patients with chronic heart failure [11,12,26–28]. The mechanism underlying this “reverse epidemiology” [13] remains poorly understood. Herein we examined the impact of lowering membrane cholesterol by using a membrane cholesterol depletion reagent on the integrity of T-tubule membrane system in isolated cardiomyocytes and in intact hearts. We found depletion of membrane cholesterol with M β CD induced pronounced myocyte membrane damage and E-C coupling dysfunction. These effects were partially reversed by replenishing cholesterol, suggesting that cholesterol is critical for T-tubule stability and maintenance and thereby normal myocyte function. Overexpression of Cav3, a protein implicated in T-tubule development, was not sufficient to maintain T-tubule integrity in response to membrane cholesterol depletion. Unexpectedly, in situ Langendorff perfusion of M β CD resulted in the additional effects of disruption at intercellular connections and damage to the intercalated discs, which occurred much faster than damage to the T-tubule architecture. These findings are consistent with clinical observations that patients with extremely low serum cholesterol levels are prone to cardiac arrhythmias. Taken together, our data identify a key role for membrane cholesterol in the preservation of T-tubule integrity, E-C coupling function and the intercellular connections between working cardiomyocytes.

The effects of membrane cholesterol depletion with M β CD on T-tubule system is concentration- and time-dependent [29]. We found that short-term treatment with a high M β CD concentration and long-term treatment with a low concentration both resulted in

obvious T-tubule damage and E-C coupling dysfunction in adult murine myocytes. These detrimental effects could be partially reversed or further damage prevented by reintroduction of membrane cholesterol. Thus, the degree of T-tubule remodeling is related to the concentration and duration of M β CD exposure. Other groups have also examined the effect of M β CD on T-tubule structure and reported no significant difference using 1–2 mM M β CD for 1 h [30,31]. We have also performed experiments under these same conditions (2 mM) and found a minimal yet significant decrease in T-tubule integrity at 1–2 h that became more pronounced at 3 h (Supplemental Fig. S2). It is important to note that the TT_{power} spectrum analysis that we use to quantitate T-tubule structural integrity is able to detect subtle differences that may not be apparent by visual examination [32–34]. The scanning ion conductance microscopy used by Gorelik group images T-tubule openings but not the deep interior regions of the T-tubule membrane [30], which may also miss minor changes in T-tubule ultrastructure. In addition, we observed significant T-tubule remodeling when we performed experiments with prolonged exposure of cultured cardiomyocytes to lower concentrations of M β CD (i.e., 100–300 μ M M β CD for 24–48 h), providing further support for a mechanistic role of membrane cholesterol in stabilization of T-tubule structure.

Several studies have demonstrated that cholesterol depletion with M β CD alters Ca²⁺ handling properties in cardiomyocytes. For example, M β CD decreases the frequency, amplitude, and size of Ca²⁺ sparks in neonatal cardiomyocytes without altering the temporal characteristics (i.e., duration) [23]. On the contrary, we observed a pronounced increase in Ca²⁺ spark frequency upon acute cholesterol depletion, although no changes in other Ca²⁺ spark properties were detected. We also found that cholesterol repletion partially restored Ca²⁺ handling properties, which we speculate may be due to incomplete recovery of resident proteins in the membrane at the 2-h time point. Membrane cholesterol depletion also disrupts L-type Ca²⁺ channel function, including channel activation by β 2 adrenergic signaling [24,25,35,36]. It has been reported that, like L-type Ca²⁺ channels, β 2 adrenergic receptors are localized primarily on the T-tubule membrane, whereas β 1 adrenergic receptors are distributed throughout the plasma membrane [30]. Based on our findings, disruption of membrane cholesterol impairs E-C coupling at least partially through T-tubule alterations.

Perhaps the most surprising finding of this study was the severe disruption of intercellular connections following acute perfusion of M β CD in intact hearts. Specifically, we observed dramatic damage to the intercalated discs after only 15 min of exposure to lower concentrations of M β CD (300 μ M) in intact hearts. These data demonstrate that cholesterol depletion has profound detrimental effects on intercalated discs in addition to T-tubule structural integrity. Intercalated discs are comprised of three types of adhering junctions: fascia adherens junctions, desmosomes, and gap junctions. These mechanical connections allow for propagation of action potentials throughout cardiomyocytes. Limited studies have examined the effect of M β CD on intercalated disc integrity. For example, cholesterol depletion with M β CD alters the subcellular localization of Kv1.5 [37], which is mainly located at the intercalated disc in rat atrial myocytes. Our work herein for the first time demonstrates a fundamental role of cholesterol in stabilizing cell-cell connection at the intercalated discs.

5. Conclusions

In summary, our data provide the first evidence that membrane cholesterol is critical to maintain cardiomyocytes T-tubule structural integrity, Ca^{2+} handling properties and the interconnections between cardiomyocytes. These findings offer a mechanism to explain the clinical associations between low serum cholesterol content and worse clinical outcomes in chronic heart failure patients [11–13]. Thus, caution should be taken when lowering cholesterol levels in patients with established chronic heart failure. Lastly, taken with the mounting evidence that T-tubule remodeling is a driving event in the development of heart failure, this study also advances the field by revealing a new mechanism for T-tubule regulation in cardiomyocytes.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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Appendix A. Supplementary data

Supplementary data to this article can be found online at <http://dx.doi.org/10.1016/j.yjmcc.2016.05.013>.

Fig. 1.

Membrane cholesterol depletion with MβCD results in T-tubule remodeling in freshly isolated or cultured cardiomyocytes. **A.** Representative T-tubule images of primary isolated murine cardiomyocytes treated with 3 mM MβCD for 1, 2, and 3 h and stained with a lipophilic marker MM 4–64. **B.** Mean values of TT_{power} at different time points after MβCD (3 mM) exposure. *n* = 20–25 cells per group. * *p* < 0.05, ** *p* < 0.01 vs. freshly isolated cardiomyocytes. **C.** Representative T-tubule images of cardiomyocytes treated with MβCD at the indicated concentrations for 48 h. **D.** Mean values of TT_{power} at 24 and 48 h after exposure to 100 μM, 200 μM or 300 μM MβCD. *n* = 20–25 cells per group. * *p* < 0.05, vs. control; # *p* < 0.05 vs. 100 μM MβCD; ‡ *p* < 0.05 vs. 200 μM MβCD.

Fig. 2.

Cholesterol replenishment restores impaired T-tubule structure in cardiomyocytes. A. Representative T-tubule images of primary isolated murine cardiomyocytes treated with 3 mM MβCD for 1 h, followed by wash out of MβCD and addition of cholesterol-saturated MβCD to replenish cholesterol for an additional 2 h. Wash out only without replenishing cholesterol serves as a control. B. Mean values of TT_{power}. n = 20–25 cells. * p < 0.05 vs. freshly isolated cardiomyocytes; # p < 0.05 vs. 3 mM MβCD for 1 h; ‡ p < 0.05 vs. MβCD + replenish cholesterol. C. Representative T-tubule images of cardiomyocytes treated with 200 μM MβCD for 24 h, followed by wash out only or wash out and replenishment of cholesterol for an additional 24 h. D. Mean values of TT_{power}. n = 20–25 cells. * p < 0.05 vs. control; # p < 0.05 vs. 200 μM MβCD; ‡ p < 0.05 vs. 200 μM MβCD + replenish cholesterol.

Fig. 3.

Depletion of membrane cholesterol decreases the amplitude of Ca^{2+} transients and increases spontaneous Ca^{2+} sparks. A. Representative cytosolic Ca^{2+} images in freshly isolated cardiomyocytes after treatment with 3 mM M β CD for 1 h, followed by wash out and replenishment of membrane cholesterol for 2 h. B. Average data of the amplitude, $V_{\max}$, dyssynchronous index, and time to 50% decay (T_{50}) of Ca^{2+} transients. n = 15–30 cells per group. C. Representative Ca^{2+} spark images in freshly isolated cardiomyocytes treated as in (A). D. Mean values of the frequency, amplitude, duration (FDHM, full duration at half maximum) and spacial width (FWHM, full width at half maximum) of Ca^{2+} sparks. n = 15–30 cells per group. * $p < 0.05$, ** $p < 0.01$ vs. freshly isolated; # $p < 0.05$, ## $p < 0.01$ vs. M β CD group.

Fig. 4.

MβCD exerts no acute effects on Ca²⁺ handling function in freshly isolated myocytes. Acute perfusion of MβCD (10 min) at both low and high concentrations had no significant effects on field-stimulated Ca²⁺ transients (A, n = 15–20 cells per group), or Ca²⁺ sparks (B, n = 10–15 cells per group).

Fig. 5.

Overexpression of Cav3 is not sufficient to prevent T-tubule damage or E-C coupling dysfunction induced by membrane cholesterol depletion. A. Western blot of Cav3 expression in isolated cardiomyocytes infected with Cav3-containing adenovirus (Ad-Cav3) or empty virus (Ad-Empty). GAPDH, loading control. B. Representative T-tubule images of cardiomyocytes infected with Ad-Empty or Ad-Cav3 and treated with 200 μ M M β CD for 48 h. C. Mean value of TT_{power}. n = 15–20 cells. * p < 0.05, vs. control (untreated). D. Representative cytosolic Ca²⁺ images in isolated cardiomyocytes treated as in (B), and Average data of the amplitude, V_{max}, dyssynchronous index, and time to 50% decay (T₅₀) of Ca²⁺ transients. n = 15–20 cells. * p < 0.05 vs. control; # p < 0.05 vs. Ad-Empty + M β CD.

Fig. 6. Membrane cholesterol depletion in intact hearts severely disrupts the integrity of intercalated discs and induces myocyte shrinkage. Sequential in situ confocal T-tubule images from an intact heart at baseline and after perfusion with 300 μ M M β CD for the indicated times. Arrows denote large gaps that formed at intercalated discs after exposure to M β CD. Also note the blurry appearance of T-tubules at 15 and 30 min after M β CD perfusion. Similar results were obtained following M β CD perfusion of three separate hearts.