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Myofibril MgATPase Activities and Energy Metabolism in Cardiomyopathic Mice with Diastolic Dysfunction

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Key Words
Myofibril protein · cTnI gene knockout · ATPase activity · Mouse hearts

Abstract
To study the genomic physiology of cardiac myofibril proteins in the heart, we have successfully created a cardiac troponin I (cTnI; a myofibril protein) gene knockout mouse model using gene targeting techniques. The phenotype of the cTnI gene knockout mouse is a cardiomyopathy with diastolic dysfunction resulting in sudden death in neonates. In the present studies, energy metabolism was analyzed in myocardial cells from cTnI-null hearts. Myofibril MgATPase activities were determined in myocardial cells from either wild-type or cTnI mutant mouse hearts. Furthermore, the quantity and quality of the mitochondria in wild-type and cTnI mutant animals were counted and analyzed. Our results demonstrate that damaged relaxation and increased Ca^{2+}-independent force production in cTnI-null hearts is in part related to the increased myofibril MgATPase activities accompanied by an increase in mitochondria quantity and mitochondrial ATPase activities. These data indicate that cardiomyopathies with diastolic dysfunction are different from cardiomyopathies caused by systolic dysfunction. The former involves the damage of cardiac relaxation due to increased MgATPase activities and increased Ca^{2+}-independent force production inside of myofilaments, while the latter involves the damage of systolic contraction due to decreased MgATPase activities and decreased force production.

Introduction

Cardiomyopathies are disorders that primarily affect cardiac cytoarchitecture, including components of the cardiac contractile sarcomere and the intrasarcomeric and extrasarcomeric cytoskeleton [2, 21, 23]. On the grounds of their morphology and pathophysiology, cardiomyopathies may be classified into a number of disorders, namely hypertrophic cardiomyopathy, dilated cardiomyopathy and restrictive cardiomyopathy.

The contractile sarcomeric proteins consist of a highly ordered arrangement of myosin thick filaments, actin thin filaments and associated proteins, such as the troponin-tropomyosin complex. The repeating sarcomere units are arranged in series, resulting in striated myofibrils. Troponin, a contractile protein of the thin filament of striated muscle, consists of three subunits: troponin C, troponin T and troponin I (TnI). TnI is the inhibitory subunit of the troponin complex. It has an important function in the reg-

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ulation of striated muscle contraction [6, 26, 27, 33]. At basal levels of calcium, TnI inhibits actin-myosin cross-bridges. In systole, as calcium binds to the regulatory site of troponin C, the inhibitory action of TnI is released, thereby activating muscle contraction.

TnI mutation has been linked to hypertrophic cardiomyopathy in human patients [13, 18]. The main discovery has been the mutation R145G on the human cardiac TnI (cTnI) gene [3, 5]. However, other mutations on this gene, such as G203S, have also been reported that are related to human hypertrophic cardiomyopathy [3]. It is not clear thus far as to what the signal pathway is between these mutations on the cTnI gene and the development of hypertrophic cardiomyopathy.

It is clinically important to understand TnI function and TnI gene regulation, because selective proteolysis of TnI has been reported to underlie the pathology of stunned myocardium [10, 14, 20]. Moreover, TnI may become depleted in ischemia [29], infarct [24, 31] and possibly failing myocardium [17]. These studies provide strong evidence for the hypothesis of Kusuoka and Marban [15], who first suggested that the stunning is associated with reversible breakdown and replacement of damaged myofilament proteins.

We have generated a cTnI gene knockout mouse model [11]. The phenotype of the animal is cardiac dysfunction resulting in an increase in cardiac stiffness and sudden death in cTnI homozygous mutants. In the present study, we analyzed myofibrillar protein loss, myofibrillar function and mitochondria function using this unique animal model to determine the role of TnI in cardiac function and TnI deficiency-mediated restrictive cardiomyopathy. Our data demonstrate that damaged relaxation and increased Ca^{2+}-independent force production in cTnI-null hearts stimulated myofibril MgATPase activities, accompanied by an increase in mitochondria quantity and ATPase activities. The results indicate that TnI deficiency-mediated cardiomyopathy, characterized as diastolic dysfunction and restrictive cardiomyopathy, is different from hypertrophic cardiomyopathy in that MgATPase activities and force production are increased in the former while they are decreased in the latter.

Materials and Methods

Experimental Animals and Genotype Determination

Heterozygous (+/–) cTnI mutant mice were maintained in the animal care facilities of Florida Atlantic University. By crossing of heterozygous mice, three genotypes of offspring were obtained: wild type (+/+), heterozygous cTnI mutant (+/–) and homozygous cTnI mutant (–/–). Rapid genotype screening of the offspring was determined by PCR. Genomic DNA was isolated from tail biopsies using the Purgene DNA isolation kit (Genta Systems, Minneapolis, Minn., USA). One pair of primers (sense primer 5′ TAGGTGTGAGGACAGAAGGCCG 3′ and antisense primer 5′ CCGTGAAGAGGAAATCACTGATGGTGGTCC 3′) was designed to produce a 630-bp fragment for the wild type. Another pair of primers (sense primer 5′ TAGGTGTGAGGACAGAAGGCCG 3′ and antisense primer 5′ GTGGAATGTGTGCGAGGCCA 3′) was designed to produce a 390-bp fragment for the targeted alleles. Amplified DNA fragments were separated on a 1.5% agarose gel and visualized under UV light after staining with ethidium bromide.

Western Blot Assays

Myofibril proteins were extracted from mouse hearts with known genotypes. Excised hearts were depleted of blood, and ventricular tissue was diced in relaxing solution (2.5 *M* KCl, 0.5 *M* imidazole, 0.1 *M* $MgCl_2$, 0.1 *M* EGTA, 0.25 g of ATP, pH 7.0) and homogenized for 3–5 s using a Polytron homogenizer. The collected myocytes were then incubated in skinning solution (relaxing solution plus 0.3% Triton X-100 and 0.5 mg/ml bovine serum albumin) for 6 min at room temperature. The concentrations of the skinned myofibrils were measured with a bicinchoninic acid kit for protein determination (Sigma, St Louis, Mo., USA). An equal amount of cardiac myofibril proteins (20–30 μg) was loaded and separated on 14% SDS gels before being transferred onto nitrocellulose membranes. Protein loads were standardized by protein concentration measurement before electrophoresis and by quantitative densitometry of Coomassie blue-stained gels as described previously [12].

Blots were then stained with 0.1% Ponceau S solution to visualize protein bands and confirm both consistent protein loading among wells and complete transfer of proteins to blots. An anti-TnI monoclonal antibody (clone 6F9, Advanced ImmunoChemical Inc, Long Beach, Calif., USA), which recognizes both mouse cTnI and slow skeletal TnI (ssTnI), was used at a dilution of 1:10,000. The identities of positive bands were confirmed by isoform-specific TnI monoclonal antibodies. Bound antibody on immunoblots was visualized by enhanced chemiluminescence and relative protein quantities were determined by densitometry [11].

Cardiac Myofibrillar MgATPase Assay

Cardiac myofibrils were prepared as described previously. The isolated myofibrils from 17-day-old mouse hearts were maintained in relaxing solution. MgATPase reaction solution containing various concentrations of calcium was prepared according to the protocol of Fabiato and Fabiato [7] with modifications: 60 m*M* KCl, 30 m*M* MOPS (pH 7.0), 5.2–5.6 m*M* ATP, 7–7.13 m*M* $MgCl_2$, 2.0 m*M* EGTA, 50 μg/ml leupeptin, 1.0 m*M* PMSF and 5.0 m*M* benzamidine. Ca^{2+} concentration was varied from 10^{-9} to 10^{-4} *M*. 50 μl of myofibril solution containing about 150 μg of protein was added to 950 μl of reaction solution. The reaction was performed at 30°C for 10 min and was terminated by adding 0.5 ml of 15% trichloroacetic acid solution. ATPase activities were measured as inorganic phosphate release from ATP, using the malachite green assay [4].

Count of Mitochondria Number in Cardiac Myocytes

The number of cardiac mitochondria was counted under an electron microscope (Philips CM120 transmission electron microscope). Excised hearts were rapidly immersed in PBS containing 2% (v/v) paraformaldehyde and 2% (v/v) glutaraldehyde. Blocks of tissue

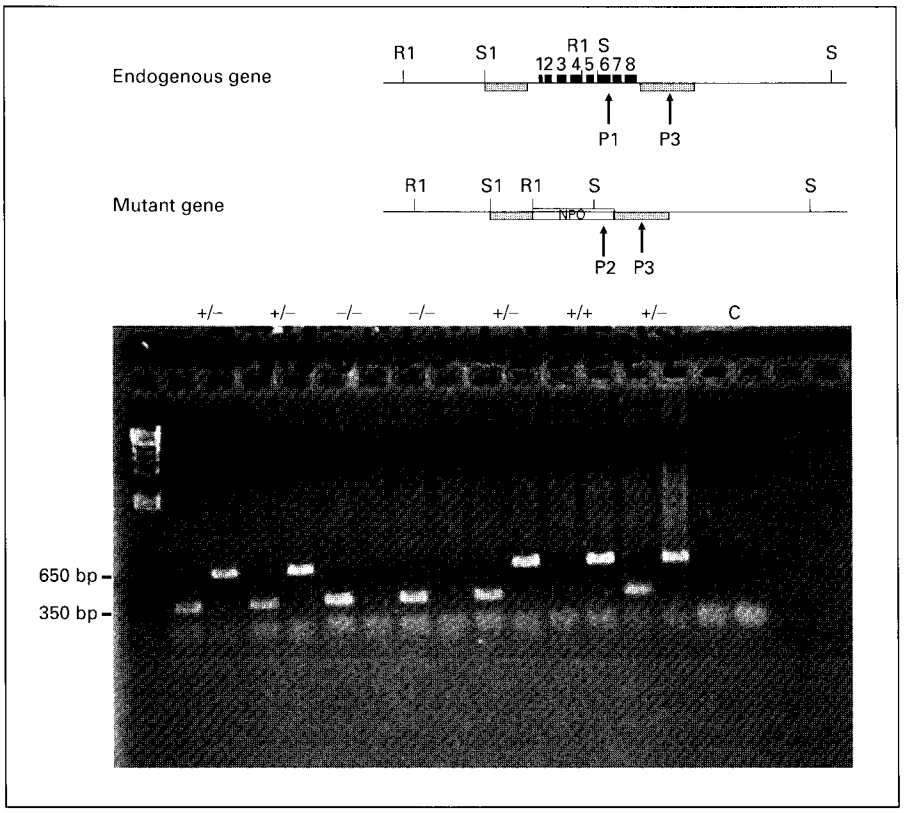

Fig. 1. Genotyping of the experimental animals. By crossing of heterozygous mice, three genotypes of offspring were obtained: wild type (+/+), heterozygous cTnI mutant (+/–) and homozygous cTnI mutant (–/–). Genomic DNA was isolated from tail biopsies. One pair of primers (P1 and P3) was designed to produce a 630-bp fragment for the wild type. Another pair of primers was designed to produce a 390-bp fragment for the mutant alleles. C = Control.

(1 mm^3) were dissected, embedded in resin, sectioned and viewed under an electron microscope. The number of mitochondria was counted in 20 fields either from the samples of wild-type mouse hearts or from the samples of cTnI-null house hearts.

Mitochondrial ATPase Measurement

Cardiac mitochondria were prepared according to the method reported by Sordahl et al. [28]. Generally, cardiac myocytes from 17-day-old mice were gently homogenized with a tissue homogenizer in 10 volumes of ice-cold isolation medium containing 80 m*M* KCl, 10 m*M* EGTA-Tris, pH 7.4, 0.5% bovine serum albumin (Sigma Fraction V) and 10 m*M* HEPES-KOH. The homogenates were centrifuged at 450 *g* for 10 min. The low-speed supernatant was then collected and centrifuged again at 10,000 *g* for 10 min. The top of the pellets obtained after centrifugation at 10,000 *g* was rinsed carefully to remove any light or fluffy material, and resuspended in isolation medium using a glass homogenizer with loose-fitting Teflon pestles. The final protein concentration of the mitochondria preparation was about 40 mg/ml.

For ATPase assays, each sample was sonicated for about 45 s. The reaction mixture contained 50 µmol of Tris-HCl, pH 7.8, 10 µmol of $MgCl_2$ and 50 µl of sonicated mitochondria preparation. The reaction was started by the addition of 10 µmol of ATP in Tris buffer solution. It was performed at 30°C for 5 min and was stopped by adding 1 ml of 10% trichloroacetic acid at the end of the reaction. The ATPase activities were measured as described previously.

Results

Genotyping

By crossing cTnI heterozygous mutant mice (+/–), offspring with three genotypes can be obtained. The genotypes of the experimental animals were determined by PCR using genomic DNA from a tail biopsy. As shown in figure 1, one pair of primers, P1 and P3, was specifically designed to detect a 650-bp fragment in the endogenous gene, and another pair of primers, P2 and P3, produced a 350-bp fragment specifically for the mutant gene. PCR assays clearly revealed the genotype of the experimental animals (fig. 1). According to our previous studies, heterozygous mutants are indistinguishable from their wild-type littermates with respect to both cardiac function and life span. In the present study, we examined MgATPase activities and mitochondria changes only in homozygous mutants and wild-type controls.

Myofibril MgATPase Activities

In our previous studies, we found that more Ca^{2+}-independent force existed in the myofilaments of cTnI-null myocardial cells [11]. In the present study, we investi-

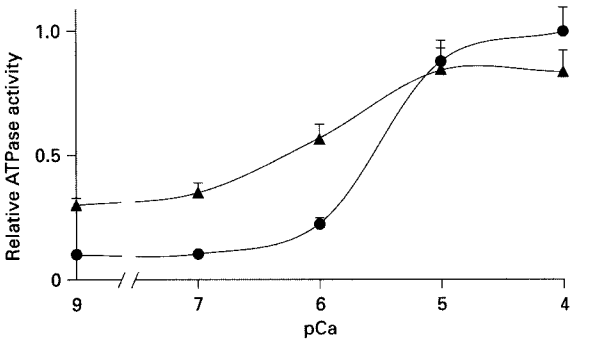

Fig. 2. Myofibril MgATPase activities in various Ca^{2+} concentrations. MgATPase activities were measured in skinned myocardial cells from cTnI-null mice (▲) or wild-type controls (●) as described in Materials and Methods. Data points represent the mean of three separate experiments.

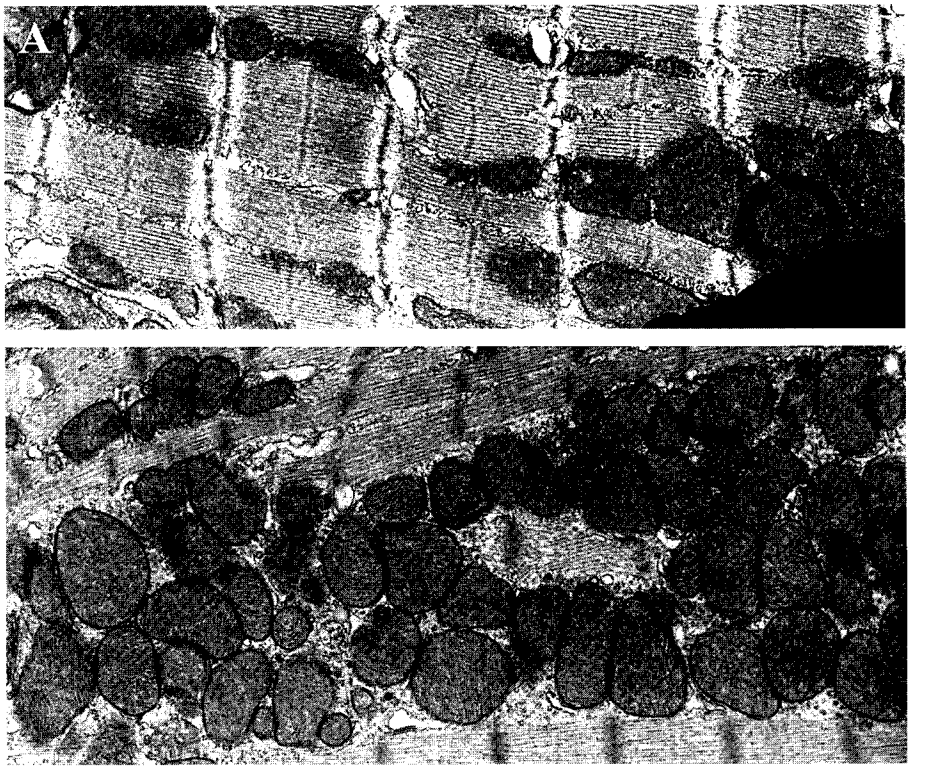

Fig. 3. Electron micrographs of sections from cTnI-null (**B**) or wild-type control (**A**) mouse hearts. The mitochondria number was significantly higher ($p < 0.01$) in 17-day-old cTnI-null mouse hearts (**B**) than in those of their wild-type littermates at the same age (**A**). × 8,200.

gated the myofibril MgATPase activities in cTnI-null myocardial cells. We found that the MgATPase activities were significantly elevated in cTnI-null myocardial cells compared to the wild-type controls, in particular at lower Ca^{2+} concentrations (fig. 2). This means that more ATP is hydrolyzed and consumed in cTnI-null myocardial cells. In contrast, at the higher Ca^{2+} concentrations, the myofibril MgATPase activities decreased in cTnI-null myocardial cells compared to the controls, which is consistent with our previous observation that the active tension in cTnI-null myofibrils is significantly decreased [11].

Cardiac Mitochondria Quantity, ATPase Activities and Cellular ATP Concentration

As it seems that cTnI-null myocardial cells consume more ATP, we further examined the quantity and the quality of the ATP producer, mitochondria, in those cells. We counted the number of mitochondria under an electron microscope. We found that the number of mitochondria in cTnI-null myocardial cells was significantly increased (about 47% more in cTnI-null myocardial cells compared to the control cells, $p < 0.01$) (fig. 3). The ATPase activities in the mitochondria from cTnI-null

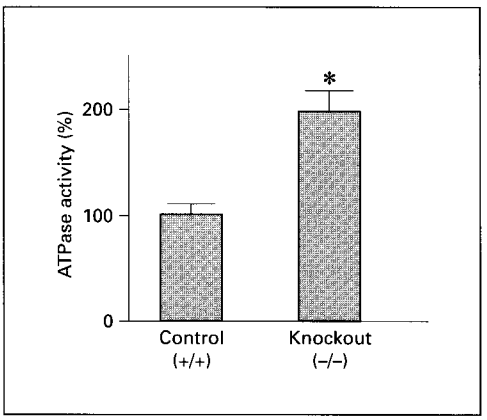

Fig. 4. Mitochondria ATPase activities. ATPase in mouse heart mitochondria was measured as described in Materials and Methods. The ATPase activities were significantly higher in 17-day-old cTnI-null mouse hearts than in wild-type mouse hearts at the same age. The experimental data are expressed as mean ± SE from three separate assays. * $p < 0.05$.

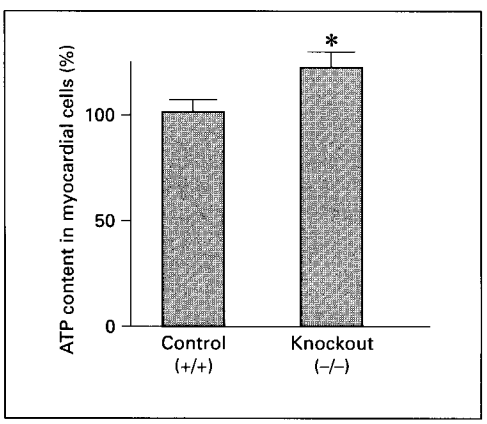

Fig. 5. ATP content measurement in myocardial cells from cTnI-null hearts (–/–) or those of the wild-type controls (+/+). The total ATP content was measured as described in Materials and Methods. The ATP content was significantly elevated in cTnI-null myocardial cells compared to the controls. * $p < 0.05$.

myocardial cells were higher than those of the control cells (fig. 4), and the total ATP content was also higher in cTnI-null myocardial cells compared to the control cells (fig. 5), suggesting that cTnI-null myocardial cells try to produce more ATP to meet an increased ATP demand from the increased force production and ATP consumption.

Discussion

A large number of studies has demonstrated that alterations in the contractile apparatus of cardiac cells contribute to the impairment of heart mechanics, resulting in cardiomyopathies and heart failure [11, 23]. Experimental cardiomyopathies of various types show some common features, i.e. alterations in myofibrillar function, alteration in energy metabolism and alteration in mitochondria function [30]. The first demonstration of a molecular alteration in the contractile system of failing human hearts was made in studies carried out on myofibrils from failing hearts and nonfailing hearts. The myofibrillar ATPase activity in the failing hearts was significantly reduced [1]. In addition, an important question has yet to be answered: Is there a sufficient energy supply in cardiomyopathic hearts? In our cTnI gene knockout mouse model, we have discovered that cTnI homozygous mutant mice die on day 18 after birth when cTnI content is too low to maintain normal cardiac functions and life [11]. The cause of the sudden death seems to be related to the loss of TnI, resulting in an increase in Ca^{2+}-independent force inside the myofilaments. The increased force causes an increase in heart stiffness and damages the relaxation of the heart, which eventually results in a decrease in cardiac output. In the present study, we have demonstrated that the increased force in cardiac myofilaments is in part related to the increase in myofibril MgATPase activity in cTnI-null myocardial cells. The increase in myofibril MgATPase was more evident at lower Ca^{2+} concentrations, i.e. in the conditions when the heart is at diastolic stage (intracellular Ca^{2+} concentration in cardiac cells of a resting heart is about 0.2 μM). This indicates that death arises from the mechanical failure of the TnI-null heart during the loss of TnI. In one possible scenario, the gradual loss of ssTnI, which until day 17 substituted for cTnI, increases the Ca^{2+} sensitivity such that cardiac diastole is no longer possible and cardiac output collapses. In a different scenario, the heart muscle cannot achieve systolic relaxation that is sufficient to support coronary perfusion (blood flow is impeded during systole and occurs primarily during diastole). As a consequence, the heart slowly drifts into ischemic failure. TnI deficiency-caused diastolic dysfunction is different from the cardiomyopathies with systolic dysfunction where the myofibril MgATPase activities and force production decrease, resulting in a failing heart. In an in vitro troponin reconstitution assay, it was shown that C-terminal truncation of cTnI can also increase the Ca^{2+} sensitivity of myofilaments and is less effective in inhibiting ATPase activity at low Ca^{2+} [9]. In

that in vitro study, although only 17 amino acids from the C-terminal of cTnI were truncated, the consequence of the cTnI truncation in vitro was very similar to our experimental results with cTnI depletion as regards myofibril MgATPase activities and force development.

Experimental cardiomyopathies are characterized by an altered energy metabolism. In myopathic hamsters, tissue levels of ATP have been shown to decrease considerably in the heart [32], whereas inorganic phosphate is increased and intracellular pH is decreased [25], thus indicating a metabolic deficiency. The energy demand should be different for normal myofibrils and for myofibrils in a rigor state. The increased force in the direction of normal active muscle contraction observed in cTnI-null myocardial cells would demand an increase in O_2/ATP consumption, which is consistent with our observation of the increase in the numbers of mitochondria and the increase in mitochondria ATPase activities.

One functional measure of the calcium switch in muscle is the rate of ATP hydrolysis by myofilaments (actin-activated myosin or myofibrillar MgATPase activity) [19]. Many experiments have shown that in cardiomyopathic and failing hearts, myofibrillar MgATPase is significantly reduced. The functional consequences of the depressed ATPase activity are not completely clear. However, it is believed that the depressed activity is associated with a decrease in the contractility of the heart muscle [1].

Among the various types of cardiomyopathies, restrictive cardiomyopathy manifests itself as a restricted amount of blood that can fill the heart because the heart is abnormally stiffened due to unknown etiology. A retrospective study of 106 cats was carried out in the Feline Center of the University of Bristol, UK. It revealed that hypertrophic cardiomyopathy is the most common form of cardiomyopathy seen (57.5%), followed by restrictive cardiomyopathy (20.7%), dilated cardiomyopathy (10.4%) and unclassified cardiomyopathy (10.4%) [8].

In humans, in particular in infants, cases of idiopathic restrictive cardiomyopathy have often been reported. Very recently, the clinical features of restrictive cardiomyopathy were described as a marked elevation of left ventricular end-diastolic pressure and profoundly abnormal myocardial relaxation [22]. This patient died suddenly at the age of 13 months [22]. A French medical group also reported a case of restrictive cardiomyopathy that may have been related to myofibrillar myopathy [16]. The cTnI-null mouse model provides us with an excellent tool to investigate the mechanisms underlying cardiomyopathy with diastolic dysfunction.

Acknowledgments

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References

1 Alpert NR, Mulieri LA, Warshaw D. The failing human heart. Cardiovasc Res 54:1–10; 2002.
2 Bonne G, Carrier L, Richard P, Hainque B, Schwartz K. Familial hypertrophic cardiomyopathy: From mutations to functional defects. Circ Res 83:580–593;1998.
3 Burton D, Abdulrazzak H, Knott A, Elliott K, Redwood C, Watkins H, Marston S, Ashley C. Two mutations in troponin I that cause hypertrophic cardiomyopathy have contrasting effects on cardiac muscle contractility. Biochem J 362:443–451;2002.
4 Chan KM, Delfert D, Junger KD. A direct colorimetric assay for Ca^{2+}-stimulated ATPase activity. Anal Biochem 157:375–380;1986.
5 Deng Y, Schmidtmann A, Redlich A, Westerdorf B, Jaquet K, Thieleczek R. Effects of phosphorylation and mutation R145G on human cardiac troponin I function. Biochemistry 40: 14593–14602;2001.
6 El-Saleh SC, Warber KD, Potter JD. The role of tropomyosin-troponin in the regulation of skeletal muscle contraction. J Muscle Res Cell Motil 7:387–404;1986.
7 Fabiato A, Fabiato F. Calculator programs for computing the composition of solutions containing multiple metals and ligands used for experiments in skinned muscle cells. J Physiol (Paris) 75:436–505;1979.
8 Ferasin L, Sturgess CP, Cannon MJ, Caney SM, Gruffydd-Jones TJ, Wotton PR. Feline idiopathic cardiomyopathy: A retrospective study of 106 cats (1994–2001). J Feline Med Surg 5:151–159;2003.
9 Foster DB, Noguchi T, VanBuren P, Murphy AM, Van Eyk JE. C-terminal truncation of cardiac troponin I causes divergent effects on ATPase and force: Implications for the pathophysiology of myocardial stunning. Circ Res 93:917–924;2003.
10 Gao WD, Liu Y, Perez NG, Murphy AM, Marban E. Role of troponin I proteolysis in the pathogenesis of stunned myocardium. Circ Res 80:393–399;1997.
11 Huang XP, Pi Y, Lee KJ, Henkel AS, Gregg RG, Powers PA, Walker JW. Cardiac troponin I gene knockout: A mouse model of myocardial troponin I depletion. Circ Res 84:1–8;1999.
12 Huang XP, Pi Y, Lokuta AJ, Greaser ML, Walker JW. Arachidonic acid stimulates protein kinase C-ε redistribution in heart cells. J Cell Sci 110:1625–1634;1997.
13 Kimura A, Harada H, Park JE, Nishi H, Satoh M, Takahashi M, Hiroi S, Sasaoka T, Ohbuchi N, Nakamura T, Koyanagi T, Hwang TH, Choo JA, Chung KS, Hasegawa A, Nagai R, Okazaki O, Nakamura H, Matsuzaki M, Sakamoto T, Toshima H, Koga Y, Imaizumi T, Sasazuki T. Mutations in the cardiac troponin I gene associated with hypertrophic cardiomyopathy. Nat Genet 16:379–382;1997.

14 Kogler H, Soergel DG, Murphy AM, Marban E. Maintained contractile reserve in a transgenic mouse model of myocardial stunning. Am J Physiol 280:H2623–H2630;2001.
15 Kusuoka H, Marban E. Cellular mechanisms of myocardial stunning. Annu Rev Physiol 54: 243–256;1992.
16 Ligi I, Fraisse A, Chabrol B, Paut O, Bourlon F, Metras D, Bonnet JL, Pellissier JF. Restrictive cardiomyopathy due to myofibrillar myopathy. Arch Pediatr 10:432–435;2003.
17 Missov E, Calzolari C, Pau B. Circulating cardiac troponin I in severe congestive heart failure. Circulation 96:2953–2958;1997.
18 Mogensen J, Klausen IC, Egeblad H. Sudden cardiac death in familial hypertrophic cardiomyopathy is associated with a novel mutation in the troponin I gene. Circulation 100 (suppl):I–618;1999.
19 Murphy AM. Troponin I: In sickness and in health – and normal development. Circ Res 91: 449–450;2002.
20 Murphy H, Kogler H, Georgakopoulos D, McDonough JL, Kass DA, Van Eyk JE, Marban E. Transgenic mouse model of stunned myocardium. Science 287:488–491;2000.
21 Olson TM, Michels VV, Thibodeau SN, Tai YS, Keating MT. Actin mutations in dilated cardiomyopathy, a heritable form of heart failure. Science 280:750–752;1998.
22 Palka P, Lange A, Ward C. A fatal case of idiopathic restrictive cardiomyopathy. Cardiol Young 13:469–471;2003.
23 Redwood CS, Moolman-Smook JC, Watkins H. Properties of mutant contractile proteins that cause hypertrophic cardiomyopathy. Cardiovasc Res 44:20–36;1999.
24 Ricchiuti V, Zhang J, Apple FS. Cardiac troponin I and T alterations in hearts with severe left ventricular remodeling. Clin Chem 43: 990–995;1997.
25 Sievers R, Parmley WW, James T, Wikman-Coffelt J. Energy levels at systole vs. diastole in normal hamsters vs. myopathic hamster hearts. Circ Res 53:759–766;1983.
26 Solaro RJ. Troponin I, stunning, hypertrophy, and failure of the heart. Circ Res 84:122–124; 1999.
27 Solaro RJ, Rarick HM. Troponin and tropomyosin, proteins that switch on and tune in the activity of cardiac myofilaments. Circ Res 83: 471–480;1998.
28 Sordahl LA, Johnson C, Blailock ZR, Schwartz A. The mitochondrion. Methods Pharmacol 1:247–286;1971.
29 Toyo-Oka T, Ross J Jr. Ca^{2+} sensitivity change and troponin loss in cardiac natural actomyosin after coronary occlusion. Am J Physiol 240: H704–H708;1981.
30 Veksler V, Ventura-Clapier R. In situ study of myofibrils, mitochondria and bound creatine kinases in experimental cardiomyopathies. Mol Cell Biochem 133/134:287–298;1994.
31 Westfall MV, Solaro RJ. Alterations in myofibrillar function and protein profiles after complete global ischemia in rat hearts. Circ Res 70: 302–313;1992.
32 Whitmer JT. Energy metabolism and mechanical function in perfused hearts of Syrian hamsters with dilated or hypertrophic cardiomyopathy. J Mol Cell Cardiol 18:307–317;1986.
33 Zot AS, Potter JD. Structural aspects of troponin-tropomyosin regulation of skeletal muscle contraction. Annu Rev Biophys Biophys Chem 16:535–539;1987.