To address this issue, a selective exchange procedure was used in which 50% and 70% of the endogenous cTn complex in permeabilized human cardiomyocytes was exchanged with recombinant unphosphorylated human cTn. Cardiomyocytes isolated from healthy donor hearts showed almost saturated phosphorylation levels at the ser23/24 of cTnI. Endogenous phosphorylated cTn of donor cardiomyocytes (pCa_{50}= 5.45 ± 0.03) was exchanged with 0.5 and 1.0 mg/ml unphosphorylated recombinant human cTn (cTn-U), which resulted in an increase in Ca^{2+}-sensitivity (ΔpCa_{50}=0.08). Subsequent incubation of the cells with PKA reversed Ca^{2+}-sensitivity to baseline levels (pCa_{50}= 5.46 ± 0.03).

To study if the effect of PKA-mediated phosphorylation on cTnI ser23/24 depends on phosphorylation of other contractile proteins, failing human cardiac tissue was used in which phosphorylation of cTnI and cMyBP-C is depressed. Cells from failing tissue showed increased Ca^{2+}-sensitivity (pCa_{50} 5.56 ± 0.03) compared to donor cells. Endogenous cTn of failing cardiomyocytes was exchanged with 0.5 and 1.0 mg/ml cTn pre-treated with PKA to fully saturate ser23/24 (cTn-bisP). However, upon exchange with the cTn-bisP complex, Ca^{2+}-sensitivity did not decrease. Subsequent PKA incubation reduced pCa_{50} back to the level observed in donor myocardium. This indicates that the effect of cTnI ser23/24 bis-phosphorylation on Ca^{2+}-sensitivity is dependent on PKA-mediated phosphorylation of other contractile protein(s). Preliminary protein phosphorylation data point towards the involvement of cMyBP-C.

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EM and 3D-Reconstruction of Thin Filaments Reconstituted with Truncated Troponin I Associated with Myocardial Stunning

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Myocardial ''stunning'', a condition resulting from a short period of ischemia followed by reperfusion, can cause cardiac dysfunction, despite the absence of tissue necrosis. Stunning can be associated with rapid proteolytic truncation of the C-terminal 17 acids of TnI (to form ''$TnI_{1\text{-}192}$''). Expression of $TnI_{1\text{-}192}$ in transgenic mice is sufficient to account for the stunning phenotype (Murphy *et al.*, 2000), where for example myofibrils containing $TnI_{1\text{-}192}$ and otherwise normal troponin-tropomyosin display increased Ca^{2+}-sensitivity (Narolska *et al.*, 2006; also Foster *et al.*, 2003). In the current study, electron microscopy and 3D-image reconstruction of thin filaments containing $cTnI_{1\text{-}192}$ and control TnC, TnT and tropomyosin was performed to determine if the truncation causes an imbalance in the tropomyosin distribution between different regulatory states. Negatively stained ''mutant'' filaments showed characteristic periodic troponin projections and tropomyosin strands. Both helical reconstruction and single particle analysis indicated that at low-Ca^{2+} the tropomyosin localized on the inner aspect of the outer domain of actin. As expected, tropomyosin moves to the inner domain of actin in Ca^{2+} (Foster *et al.*, 2003). However, truncated TnI appears to promote an extra transition of tropomyosin from the Ca^{2+}- induced, closed position on actin toward the myosin-induced, open-state position. Here, tropomyosin in myosin-free thin filaments appears biased towards the open-state in the presence of only Ca^{2+}. Cross-correlation of filament segments to models of the blocked-, closed-, and open-states (as in Pirani *et al.*, 2005) confirms this open-state bias, which correlates well with the increase in Ca^{2+}-sensitivity observed in *in vitro* and in fiber assays of function.

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Impact Of N-terminal Truncation Of Cardiac Troponin I On Myofilament Chemo-mechanical Transduction: Implications For The Enhanced Cardiac Function In Hemodynamic Adaptation

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The deletion of N-terminal extension of cardiac troponin I($cTnI_{ND}$) by restricted proteolysis has been recently proposed to be a novel mechanism to regulate cardiac function during hemodynamic adaptation. In vivo and isolated working heart from transgenic mice overexpressing $cTnI_{ND}$ revealed an enhanced rate of relaxation and reduced end diastolic pressure. However, the functional effect of $cTnI_{ND}$ on myofilament properties has not been fully evaluated. Accordingly, we determined the functional effects of $cTnI_{ND}$ on cardiac tension cost(cross-bridge cycling), maximal tension development(F-max) and Ca^{2+}-sensitivity(EC_{50}) using mechanical force- and enzyme-coupled UV absorbance measurements. Wild-type(WT) or $cTnI_{ND}$ containing recombinant troponin(cTn) complexes were exchanged for endogenous cTn in skinned rat cardiac trabeculae. $cTnI_{ND}$ induced a significantly reduction in Fmax and Ca^{2+}-sensitivity but increased cross-bridge cycling rate. In addition, by using steady-state fluorescence measurements, we found that the decreased myofilament Ca^{2+} sensitivity is due to a decrease in Ca^{2+} binding affinity of the regulatory site of cTnC in the thin filament. We conclude that increased cross-bridge cycling rate by $cTnI_{ND}$ may underlie, in part, the modulation of cardiac function and hemodynamic adaptation associated with $cTnI_{ND}$.

Summarized Table

	WT (N=10)	cTnIND (N=8)	p value
Fmax (mN/mm2)	42.9 ± 5.1	17.8 ± 1.7	0.0007*
Hill	4.1 ± 0.7	5.1 ± 0.6	0.2587
EC50 (uM)	2.6 ± 0.2	3.7 ± 0.2	0.0022*
Tension Cost	7.5 ± 0.6	11.4 ± 1.3	0.0140*

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Structural and Proteomic Analysis of the Drosophila Cardiac Tube

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Drosophila melanogaster possess a simple linear heart tube which is an efficient in vivo system for studying basic developmental and physiological processes as well as for investigating potentially conserved pathogenic mechanisms of genetically inherited cardiac disorders. Human cardiomyopathies commonly arise from cytoarchitectural mutations. We previously showed that, as in humans, Drosophila exhibit disparate cardiac responses to depressed or enhanced myosin biomechanical properties. Here, we further characterized the morphological and ultrastructural consequences of altered myosin function on the Drosophila heart. Fluorescent microscopy revealed D45 flies, expressing myosin with depressed ATPase and in vitro sliding properties, show cardiac dilation with relatively normal myofibrillar organization. However, Mhc5 fly hearts, expressing myosin with enhanced molecular properties show centrally located restricted regions, a loss of contractile material and myofibrillar disarray. Moreover, electron microscopy revealed perturbed sarcomeric organization of the cardiomyocytes in both mutants. Mitochondria appeared swollen with apparent increased matrix volume and membranic rupture resulting in a prevalence of vacuolization. These cardiac phenotypes bear similarity to those observed in human cardiomyopathies and imply the existence of conserved pathological responses to altered myosin motor function. To further substantiate the use of the Drosophila heart as a model for investigating developmental, physiological and pathological processes and to identify conserved and potentially unique molecular components, we have undertaken preliminary proteomic analysis of isolated hearts. LC-MS/MS analysis identified ~450 proteins with high confidence. The cardiac proteins derive primarily from the sarcomere, cytoskeleton and the mitochondria. Many of the major cardiac components appear conserved between flies and humans. We ultimately seek to use quantitative proteomic studies to identify how specific lesions of myosin perturb protein networks within the Drosophila heart, and to determine how these perturbations contribute to the pathogenesis of cardiomyopathy.

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In-Solution Proteomic Workflow for Purification of Endogenous Sarcomeric Proteins and Identification of Distinct Charged Variants of Regulatory Light Chain

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The molecular conformation of the myosin motor is modulated by intermolecular interactions with the light chains, C-protein and titin, and governed by post-translational modifications (PTMs). These PTMs are important in regulation of function in ejecting ventricles as mechanisms downstream of Ca^{2+} fluxes at the level of the sarcomere appear to dominate ejection and sustain