Published in final edited form as:
Circ Heart Fail. 2015 January ; 8(1): 119–127. doi:10.1161/CIRCHEARTFAILURE.114.001496.

Metabolic Efficiency Promotes Protection From Pressure Overload in Hearts Expressing Slow Skeletal Troponin I

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Abstract

Background—The failing heart displays increased glycolytic flux that is not matched by a commensurate increase in glucose oxidation. This mismatch induces increased anaplerotic flux and inefficient glucose metabolism. We previously found adult transgenic mouse hearts expressing the fetal troponin I isoform, (ssTnI) to be protected from ischemia by increased glycolysis. In the present study we investigated the metabolic response of adult mouse hearts expressing ssTnI to chronic pressure overload.

Methods and Results—At 2–3 months of age ssTnI mice or their nontransgenic (NTG) littermates underwent aortic constriction (TAC). TAC induced a 25% increase in NTG heart size but only a 7% increase in ssTnI hearts ($P<0.05$). NTG TAC developed diastolic dysfunction (65% increased E/A ratio), while the E/A ratio actually decreased in ssTnI TAC. Isolated perfused hearts from NTG TAC mice showed reduced cardiac function and reduced PCr:ATP (16% reduction), but ssTnI TAC hearts maintained cardiac function and energy charge. Contrasting NTG TAC, ssTnI TAC significantly increased glucose oxidation at the expense of palmitate oxidation, preventing the increase in anaplerosis observed in NTG TAC hearts. Elevated glucose oxidation was mediated by a reduction in PDK4 expression, enabling PDH to compete against anaplerotic enzymes for pyruvate carboxylation.

Conclusions—Expression of a single fetal myofilament protein into adulthood in the ssTnI-TG mouse heart induced downregulation of the gene expression response for PDK to pressure overload. The consequence of elevated pyruvate oxidation in ssTnI during TAC reduced anaplerotic flux, ameliorating inefficiencies in glucose oxidation, with energetic and functional protection against cardiac decompensation.

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Disclosures
None.

Keywords

hypertrophy; enzymes; myocardial metabolism; myofilament protein

In the failing heart there is a reversion to a more fetal metabolic profile, with an increased reliance on glucose metabolism at the expense of fatty acid oxidation (FAO)[1–3]. Although increased glycolytic metabolism can improve efficiency as it relates to oxygen consumption, it is increasingly becoming apparent that the failing heart does not increase the oxidation of glucose in a concordant manner, leading to a mismatch between the rate of pyruvate formation through glycolysis and its entry into the TCA cycle via pyruvate dehydrogenase[1, 4, 5]. A variety of mechanisms have been proposed that link the imbalance between the glycolytic rate and the rate of glucose oxidation to the development of contractile dysfunction in the failing heart[4, 6]. Recent work has identified increased anaplerosis as one such mechanism of inefficient glucose metabolism for oxidative production of NADH in the mitochondria[1, 4, 7]. As now confirmed by other laboratories, this elevated anaplerosis in the pressure overloaded heart diverts pyruvate from entry into the TCA cycle as acetyl CoA, resulting in a significantly lower ATP yield per molecule of glucose metabolized[3, 4, 8–10]. It has been previously shown that reducing anaplerotic flux in the failing heart represents a previously unappreciated means of improving contractile function due to increased energetic availability[3, 4].

There is some evidence to suggest normalizing the specific metabolic profile of the failing heart may not be required, but rather increasing net substrate delivery to the mitochondria should be the priority[3, 4, 11]. Although these goals are not specifically opposing, they are not necessarily identical, which may partially explain why strategies to increase FAO in the failing heart have not always been successful[11, 12], as such an intervention will negatively influence glucose oxidation and potentially exacerbate the uncoupling between glycolysis and glucose oxidation. Conversely, treating the failing heart with dichloroacetate increases the reliance on glucose by increasing glucose oxidation and is associated with increased contractility, despite pushing the failing heart further away from what is accepted as a normal metabolic profile[4]. It must be recognized, however, that simply increasing glucose delivery to the failing heart has not shown success[13], nor has inhibiting FAO to indirectly increase glucose utilization always been fruitful either[14]. Substrates must be both delivered at the necessary level and metabolized in an efficient manner.

It was previously demonstrated that replacement of the adult isoform of cardiac troponin I (cTnI) with the isoform expressed in the fetal heart, slow skeletal troponin I (ssTnI), confers ischemic protection by delaying the decline in myocardial ATP levels[15]. ATP stores were maintained through an upregulation of the glycolytic rate during ischemia while no phenotype was evident at baseline. This model displayed the capacity for a demand accessible increase in glucose utilization during an acute ischemic insult. In the present study we have chosen to investigate the impact of a more prolonged cardiac stress, chronic pressure-overload, to determine what protection may exist in hearts with continued expression of ssTnI in the adult heart. The results once again demonstrate that the substitution of a single myofilament protein can have a significant impact on the metabolic

response of the heart to stress. Specifically hearts from ssTnI mice were able to withstand the chronic stress of pressure-overload by maintaining metabolic efficiency as the increase in anaplerosis was prevented in ssTnI mice. Additionally, it is demonstrated herein that reduced anaplerotic flux prevented the decline in energetic stores that are typically observed in the hypertrophic heart[3, 4].

Methods

Cardiac hypertrophy model

Surgeries to induce chronic pressure-overload via transverse aortic constriction (TAC) were done at 2–3 months of age in male mice expressing either cTnI, the adult isoform of cardiac troponin I (NTG); or ssTnI, the fetal isoform (ssTnI). Mice were bred from heterozygous males and NTG females and the male offspring were randomly selected to undergo TAC or sham surgery. TAC was induced by placement of a titanium metal microclip to constrict the ascending aorta (ID 0.4 mm). The ssTnI model has already been described in detail elsewhere[16, 17]. Mice were maintained on the CD-1 background. Sham surgeries were performed on both NTG and ssTnI mice to serve as controls. At 10 weeks post-surgery, mice were anaesthetized, and *in vivo* cardiac function was determined via echocardiography, as described previously[18]. At 12–13 weeks post-surgery, the hearts were isolated and perfused to assess cardiac metabolism and isolated heart function. All procedures were approved by the University of Illinois at Chicago Animal Care and Use Committee and are in accordance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals (NIH Pub. No. 85-23, Revised 2011).

Isolated Heart Perfusion

12–13 weeks after TAC or sham surgery, hearts were isolated and retrogradely perfused with Krebs buffer (in mM: 0.4 palmitate, 10 glucose, 0.5 lactate). The ratio of palmitate:BSA was 3:1. Hearts were perfused in a 14.1 T NMR magnet. The contribution of ^{13}C palmitate ([4,6,8,10,12,14,16-^{13}C7]palmitate) or ^{13}C glucose ([1, 6-^{13}C2]glucose) to acetyl CoA was used to determine the relative contribution of exogenous LCFA versus glucose to acetyl CoA production by the TCA cycle. The ^{13}C endpoint enrichment of the glutamate pool in acid soluble extracts was quantified via NMR spectroscopy in 5 mm ^{13}C probe (Bruker Instruments, Billerica, MA) [1, 19]. The glutamate pool is in equilibrium with the TCA cycle intermediate alpha-ketoglutarate and therefore enrichment of the glutamate pool can be used to determine the fractional enrichment of [2-^{13}C] acetyl CoA[19]. The relative anaplerotic flux was determined from the ^{13}C labeling of the glutamate pool as described previously[4]. Left ventricular function (developed pressure, heart rate) was monitored by a fluid-filled balloon inserted into the left ventricle. The energetic status of the mice (PCr:ATP) was determined by dynamic mode ^{31}P NMR.

Protein Expression

Protein expression was measured by Western blot in heart tissue using commercially available antibodies (AMP kinase α, 2532S Cell Signaling; β-myosin heavy chain, M8424-. 2ML Sigma Aldrich; calsequestrin, PA1-913 Pierce Thermo Scientific; malic enzyme 1, ab97445 Abcam; phospho-AMP kinase α, 2535S Cell Signaling; pyruvate dehydrogenase,

ab110330 Abcam; phosphorylated pyruvate dehydrogenase, ab92696 Abcam; pyruvate dehydrogenase kinase 4, ab63157 Abcam; pyruvate carboxylase, ab128952 Abcam). Protein concentrations in whole cell lysates were determined by BCA protein assay. Gels were loaded with 10–60 μg of protein to measure protein expression levels. Calsequestrin was used as a loading control in all gels. Intensity of the bands of interest was normalized to the intensity of the loading control and the relative increase in expression over baseline (NTG) was reported. When the comparison of more than one gel was required, each gel was normalized to the baseline samples within that gel.

Statistical Analysis

Differences in the mean within groups (NTG sham vs. NTG TAC or ssTnI sham vs. ssTnI TAC) were determined by student's t test. Differences in the mean across groups were determined by one-way ANOVA followed by Tukey-Kramer post hoc test (Prism 4, Graphpad Software Inc.). Means were said to be significantly different when $p<0.05$. Data is presented as mean±SE.

Results

Cardiac Hypertrophy

Heart weight and heart weight to tibia length, measured in isolated hearts at the end of perfusion, increased 25% in NTG mice 12–13 weeks after transverse aortic constriction (TAC). However, in ssTnI mice, TAC resulted in only a modest increase in both heart weight and heart weight to tibia length, neither one of which were significantly different (Figure 1A&B). Thickening of the left ventricular posterior wall and dilation of the left ventricule during diastole was evident via echocardiography in NTG hearts after TAC, while no significant changes in these parameters where evident in ssTnI hearts after TAC (Figure 1C&D). In agreement with this attenuation of hypertrophic remodeling in ssTnI mice, TAC also failed to upregulate the expression of the fetal isoform of myosin heavy chain, β-myosin heavy chain, which is typically induced in response to chronic pressure-overload[20]. In contrast, there was a dramatic upregulation of β-myosin heavy chain observed in NTG mice 12–13 weeks after TAC (Figure 1E).

ssTnI Expression Prevented the Decline in Contractile Function Following TAC

Although no changes in *in vivo* systolic function were apparent from echocardiography (Supplementary Figure 1), diastolic function was significantly affected (Figure 2) following TAC. In NTG mice, there was a clear development of a restrictive filling pattern, indicated by the increase in the E/A ratio (Figure 2A) due to an increase in the E Wave (Figure 2B). A restrictive filling pattern was confirmed by rapid transmitral deceleration of early filling (Figure 2C). This was contrasted by an inverse change in the E/A ratio in ssTnI hearts following TAC, which actually decreased due to a significant increase in the A wave (Figure 2D). An increase in the A wave was necessitated in order to compensate for the prolonged isovolumic relaxation in ssTnI TAC mice (Figure 2E), a potential consequence of the increased calcium sensitivity of the myofilament inherent in the ssTnI mouse[21]. There were no changes in *in vivo* heart function observed in sham ssTnI mice compared to NTG sham mice.

In isolated perfused hearts, there was a clear reduction in contractile function observed in NTG hearts after TAC (Figure 3A–C) that was absent in ssTnI mice. The decline in rate-pressure product (Figure 3A) and contractility (slower kinetics of contraction (Figure 3B) and relaxation (Figure 3C)) in NTG hearts following the development of hypertrophy was absent in ssTnI hearts.

ssTnI expression induced a greater shift in the metabolic response to TAC

The metabolism of exogenous palmitate and glucose was assessed in isolated hearts at 12–13 weeks post-TAC via ^{13}C end-point enrichment analysis of the glutamate pool. Despite the absence of significant contractile changes, hearts from ssTnI mice displayed a robust metabolic remodeling in response to TAC (Figure 4). The relative contribution of palmitate to acetyl CoA formation was significantly decreased (Figure 4A&B), while the relative contribution of glucose increased (Figure 4C). These results suggest that, in ssTnI mice, TAC caused a decrease in palmitate oxidation and an increase in glucose oxidation. In NTG mice, there was a very modest metabolic response to TAC that did not result in significant changes in the relative contributions of the exogenous substrates examined to acetyl CoA formation (Figure 4B&C). The increase in glucose oxidation was at least partially mediated by a reduction in PDK4 expression (Figure 4D). PDK4 controls PDH activity through phosphorylation, which reduces PDH activity. There was also a reduction in the phosphorylation of PDH in ssTnI mice exposed to TAC (Figure 4E), which is in agreement with a reduction in PDK4 expression. No significant changes in total PDH expression was evident in any of the models (Figure 4F).

Attentuated Anaplerotic Flux Response in ssTnI hearts Following TAC Improves Efficiency of Glucose Oxidation

Since the increase in glucose oxidation observed in ssTnI hearts following TAC is indicative of improved coupling of glycolysis and glucose oxidation, we investigated whether the level of anaplerotic flux into the TAC cycle following TAC was altered by ssTnI expression. As expected from previous studies, TAC induced elevations in anaplerotic activity in NTG littermates[1,3,4,8]. However, by increasing the relative contribution of glucose to acetyl CoA formation in the ssTnI hearts, the TAC-induced increase in anaplerosis was prevented (Figure 5A). Anaplerotic entry of pyruvate into the TCA cycle is controlled by two enzymes within the heart: malic enzyme 1 (ME1) and pyruvate carboxylase (PC)[22]. Both ME1 and PC expression were induced by TAC in NTG mice (Figure 5B&C), while PC expression was not induced in ssTnI mice exposed to TAC. Interestingly, however, ME1 expression was higher both at baseline and after TAC in ssTnI hearts (Figure 5B). These results suggest that the downregulation of PDK4 expression plays a key role in reducing anaplerotic flux in the ssTnI hearts exposed to TAC. The downregulation of PDK4 expression reduces PDH phosphorylation to increase PDH activity, thereby allowing PDH to outcompete ME1 for pyruvate.

Energetic Status is Maintained in ssTnI Hearts Following TAC

Hypertrophic remodeling and heart failure are associated with a decline in energetic stores, specifically the PCr:ATP ratio[1, 2, 9, 23, 24]. As discussed, the increase in anaplerosis in the hypertrophic heart leads to metabolic inefficiencies that may contribute to the decline in

energy stores. Since hearts from ssTnI mice showed evidence of improved energetic efficiency of glucose metabolism through a reduction in anaplerosis, the energetic status of the hearts following TAC was investigated. In agreement with the attenuation of anaplerosis in the ssTnI TAC mice, energetic stores were also maintained, as indicated by the PCr:ATP ratio (Figure 6A&B). The lack of a decline in PCr:ATP was confirmed by a failure of TAC to induce an increase in phosphorylation of the energy sensing AMPK in ssTnI TAC mice (Figure 6C&D). In NTG mice, the decline in the PCr:ATP ratio was associated with an increase in AMPK phosphorylation, indicating energy deprivation in these hearts. In contrast, the increased flux from glucose into acetyl-CoA within the TCA cycle in the TAC ssTnI hearts occurred independently of AMPK activation.

Discussion

This study demonstrated that substitution of a single myofilament protein with the fetal isoform had a significant impact on the response of the heart to chronic stress. Somewhat surprisingly, hearts from ssTnI mice showed a more robust metabolic shift in response to TAC when compared to NTG mice. Despite the observed increase in glucose utilization, ssTnI mice did not recapitulate the traditional fetal metabolic profile that is seen in response to chronic pressure-overload. Although under acute stress, hearts from ssTnI mice respond by increasing their glycolytic rate, under chronic stress, hearts from ssTnI mice are able to increase the oxidation of glucose and thereby limit the rate of anaplerosis while increasing glucose utilization. By preventing the increase in anaplerotic flux, hearts from ssTnI mice were able to be metabolically more efficient and therefore maintain energetic stores during the chronic stress of pressure-overload. This study further demonstrates that, when treating the failing heart, rather than achieving a specific profile of substrate metabolism, energy delivery should be maximized by maximizing both substrate delivery and metabolic efficiency.

The attenuation of anaplerosis in response to TAC in the ssTnI mice was not associated with a normalization of ME1 levels, as might otherwise have been expected[4]. In the ssTnI heart, there was a baseline increase in ME1 expression that was not further increased by TAC. In NTG hearts, the expression of ME1 increased with TAC, mirroring the changes in anaplerosis, and in agreement with previous work[4]. The expression of PC did mirror the changes in anaplerosis within the four different animal models, indicating that its expression may be governing the differences in anaplerosis presented. PC activity is relatively low in the heart compared to ME1, and so caution should be applied in attributing activity to the relative changes in expression[22, 25]. An alternative is that competition between ME1 and PDH (and possibly PC) regulates the relative rates of anaplerosis. The downregulation of PDK expression and the decrease in PDH phosphorylation in ssTnI hearts exposed to TAC permitted PDH to more actively compete for pyruvate. This was similarly shown to occur in an acute intervention model, where dichloroacetate was able to reduce anaplerosis in hypertrophic rat hearts without a reduction in ME1 expression[4]. Dichloroacetate is a non-isoform specific inhibitor of PDK and is therefore able to acutely increase PDH activity and increase the ability of PDH to compete with the anaplerotic enzymes for pyruvate[4, 26]. Overall the results suggest that an elevated expression of ME1 is insufficient alone to result in an increase in anaplerotic flux independently of changes in the coupling between

glycolysis and glucose oxidation. The results also identify the PDH complex as an important lead for pharmacological development of a treatment for the failing heart.

PDK4 expression is not typically increased in the hypertrophic heart[27, 28]. However, increased PDK4 expression is induced following chronic angiotensin-II infusion and plays a significant role in the development of metabolic inefficiency and hypertrophy in this model of heart failure[29]. PDK4 expression is increased by AMPK[30], which may suggest a potential mechanism linking energetic status of the heart and PDK4 expression in the failing heart. AMPK activation leads to an increase in glucose[31] and LCFA uptake[32], however AMPK does not directly increase glucose oxidation but rather favors LCFA oxidation[33], potentially at the expense of glucose oxidation. One possibility is that the upregulation of glucose metabolism in the hypertrophic heart is attenuated by confounding signals originating from AMPK, and that in the absence of AMPK activation, both glycolysis and glucose oxidation would increase in a concordant manner.

A recent study by Kolwicz et al[3] revealed an interesting result, demonstrating that increasing FAO in the failing hearts is also capable of reducing anaplerosis. In their mouse model of acetyl CoA carboxylase 2 knockdown (ACC2KO), they were able to increase myocardial FAO in hearts exposed to TAC, and this was associated with reduced rates of anaplerosis. This speaks to the concept that matching the rate of glycolysis and glucose oxidation could be the key, rather than a necessity to increase glucose oxidation in the failing heart, however this needs to be investigated further as direct comparison of the rates of glycolysis and glucose oxidation was not performed. As has been discussed elsewhere in a recent commentary[34], the study of Kolwicz et al[3] also demonstrated the capacity for metabolic signals to control the cardiac hypertrophic response to stress. At baseline, hearts from the ACC2KO mouse display increased in FAO. When these hearts are exposed to TAC their metabolic profile prevents hypertrophic remodeling without affecting the magnitude of the external stressor. These results led to the suggestion that metabolic signals from the heart are capable of determining myocardial geometry[34]. Hearts expressing ssTnI that are exposed to TAC present with a very different metabolic response, yet both of these models show resistance to hypertrophic remodeling. A commonality between the acetyl CoA carboxylase overexpressing mouse and the ssTnI mouse models when they are exposed to TAC is the maintenance of metabolic efficiency. Although efficiency is often viewed in relation to oxygen consumption, oxygen is not limiting during hypertrophic remodeling as it is during other forms of cardiac stress. Potentially of more significance when the heart is exposed to chronic pressure-overload is the total energetic yield, as the external stress of pressure-overload requires increased cardiac work. As hypertrophic remodeling progresses and the heart transitions into decompensation, insufficient vascularization may limit the availability of oxygen. However, in the initial response to pressure-overload, this may not yet occur. By also limiting anaplerosis, both of these models of ACC2KO and ssTnI expression, improve metabolic efficiency.

Anaplerosis is also symptomatic of a mismatch between the glycolytic rate and the rate of pyruvate oxidation in the mitochondria. An additional mechanism that links hypertrophic remodeling and the mismatch in glucose metabolism is the activation of the hexosamine biosynthetic pathway[35]. Increased formation of O-GlcNAc from fructose-6-phosphate has

been documented in the hypertrophic heart, and O-GlcNAc signaling is an essential component of the hypertrophic response[36]. It can be proposed that by improving coupling between glycolysis and glucose oxidation, the hypertrophic response was partially reduced due to a reduction in the hexosamine biosynthetic pathway.

Linkage between ssTnI expression and metabolic signaling in the intact heart has documented to confer improved ischemic/hypoxic tolerance due to enhanced anaerobic glycolysis as in the fetal heart[15, 37–40]. While the question remains as to how expression of the fetal isoform might result in protection against hypertrophic remodeling through a metabolic response, the current findings indicate a phenotype associated with enhanced glucose oxidation during pathophysiological stress. Of course the other properties associated with ssTnI function and interactions with the myofilament that might suggest other positive effects during pressure overload, such as increased calcium sensitivity [21, 41–43]. Therefore, a combination of this beneficial metabolic response to pathological stress and the separate physical characteristics of the ssTnI isoform and calcium sensitivity may afford the attenuated functional decline o during TAC.

Although ssTnI and cTnI differ at a number of points in their amino acid sequence[44], single amino acid substitutions in cTnI are capable of recapitulating much of the phenotype observed when ssTnI is expressed within the myofilament[45]. Substitution of the alanine at position 164 with a histidine within cTnI causes the myofilament to behave in a similar manner to one in which ssTnI is expressed[45, 46]. Hearts from mice with this substitution, similar to the ssTnI expressing adult heart[15], were resistant to acute ischemic damage[46]. These hearts also show reduced hypertrophic remodeling following permanent left coronary artery ligation, measured at 6 months after surgery[46]. Some of the positive effects of this myofilament modification were attributed to an increase in energetic efficiency, however changes in metabolic pathways had not been investigated.

In summary, replacement of a single myofilament protein with its fetal isoform in the adult heart induced a dramatic change in the response of the heart to chronic pressure-overload. Hearts from ssTnI mice were resistant to hypertrophic remodeling and maintained their energetic stores by attenuating the decline in metabolic efficiency that is inherent in the hypertrophic failing heart (see Figure 7). The results highlight the need to increase our understanding of the interactions between the mitochondria and the myofilament beyond one of energy producer and consumer. Finally, the data supports an emerging concept that increasing energy availability and efficiency of metabolism is potentially more important than returning the metabolic profile of diseased hearts to some baseline ideal.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

Sources of Funding

This work was supported by NIH grants PO1HL062426 (EDL and RJS), R01HL62702 (EDL), and RO1HL22231 (RJS).

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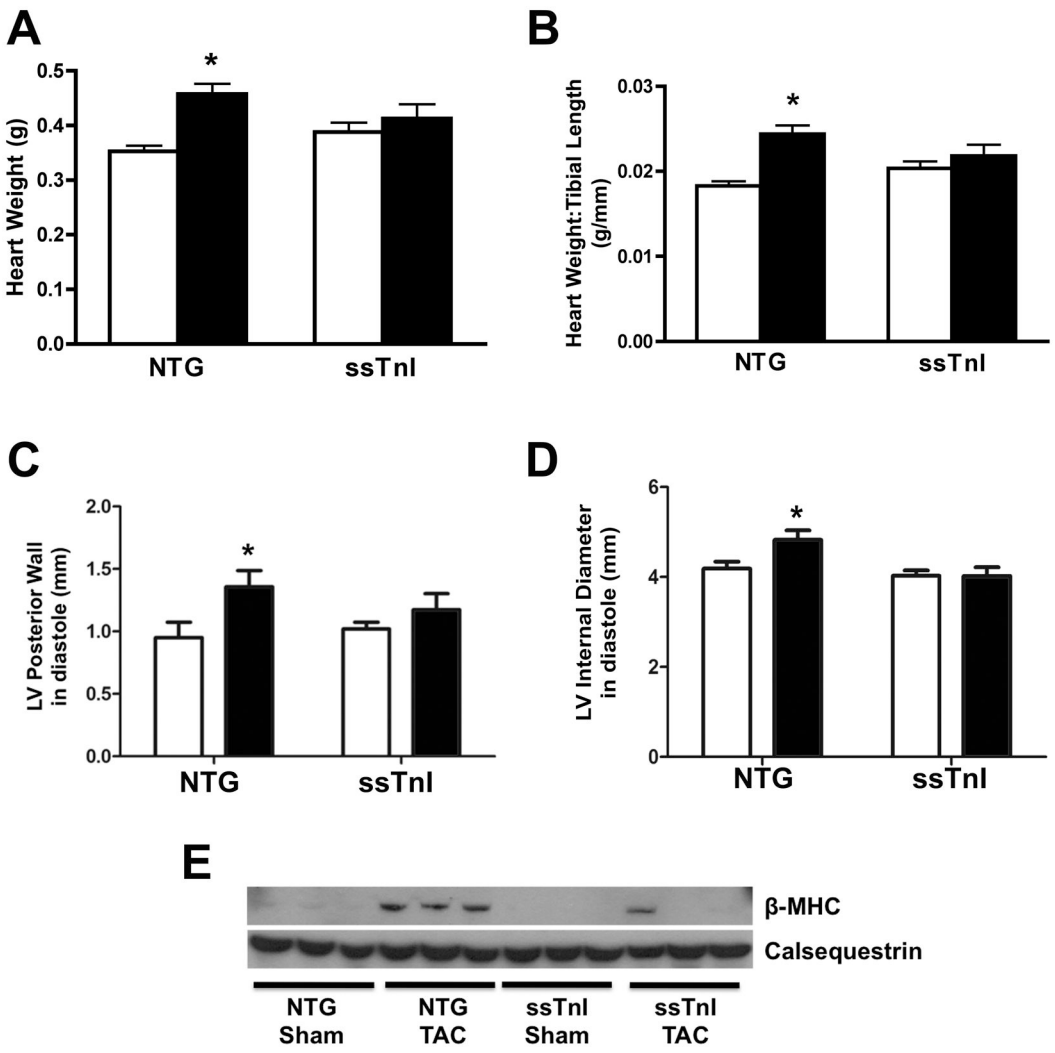

Figure 1. Expression of ssTnI in the adult heart attenuates cardiac hypertrophy

Heart weight (A) and heart weight to tibia length (B) was assessed at the end of isolated heart perfusion (n=10–12). Left ventricular posterior wall thickness (C) and left ventricular internal diameter during diastole (D) were assessed *in vivo* via echocardiography (n=8–10). Sham hearts indicated by open bars; hearts undergoing TAC indicated by solid bars, *p<0.05 vs. NTG Sham. The expression of the fetal contractile protein β-MHC (E) was assessed in frozen heart tissue.

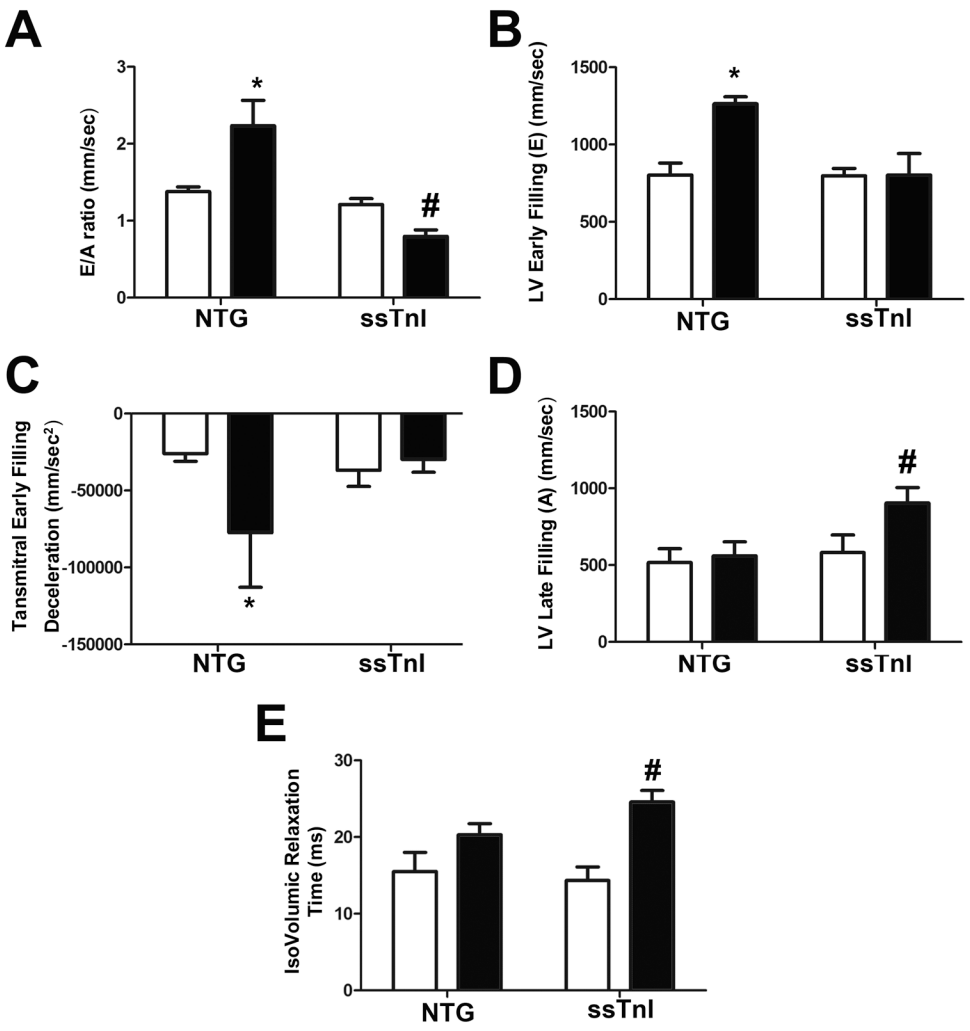

Figure 2. TAC induces diastolic dysfunction in NTG hearts

In vivo heart function was assessed in anesthetized mice 10 weeks after TAC or sham surgery (n=8–10). Data is mean±SE. *p<0.05 vs. NTG Sham, #p<0.05 vs. ssTnI sham. Sham hearts indicated by open bars; hearts undergoing TAC indicated by solid bars.

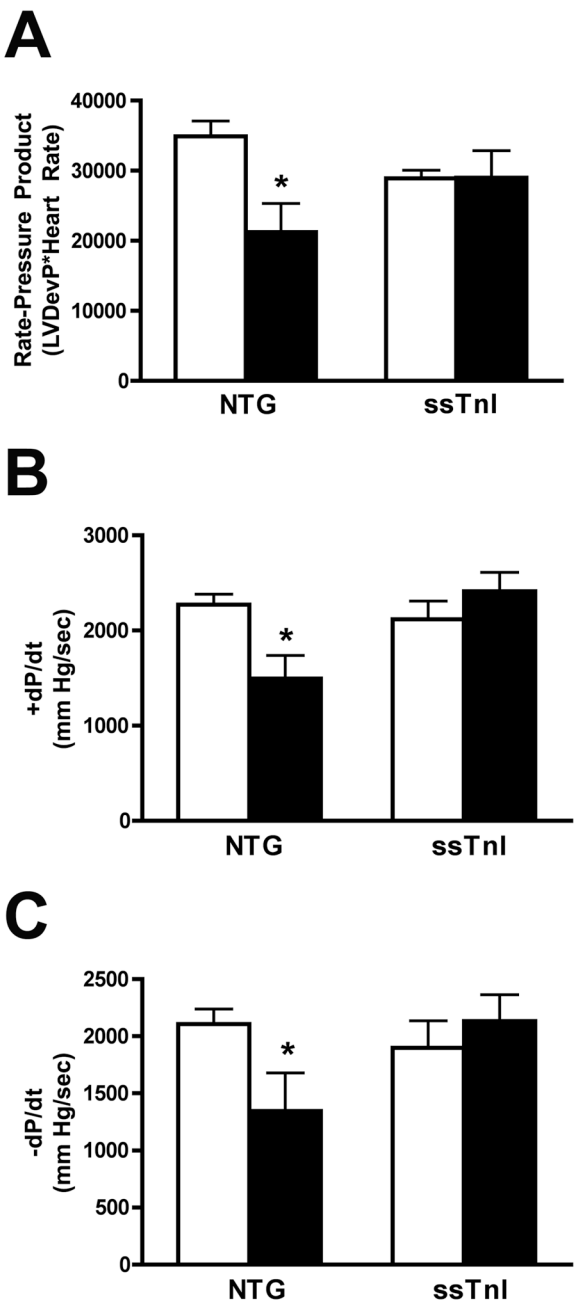

Figure 3. Isolated hearts from ssTnI mice exposed to TAC are protected from the development of contractile dysfunction

Rate-pressure product (A), +dP/dt (B), and −dP/dt (C) were assessed in isolated perfused hearts 13 weeks after TAC or sham surgery. Data is mean±SE, n=10–13. *p<0.05 vs. NTG sham. Sham hearts indicated by open bars; hearts undergoing TAC indicated by solid bars.

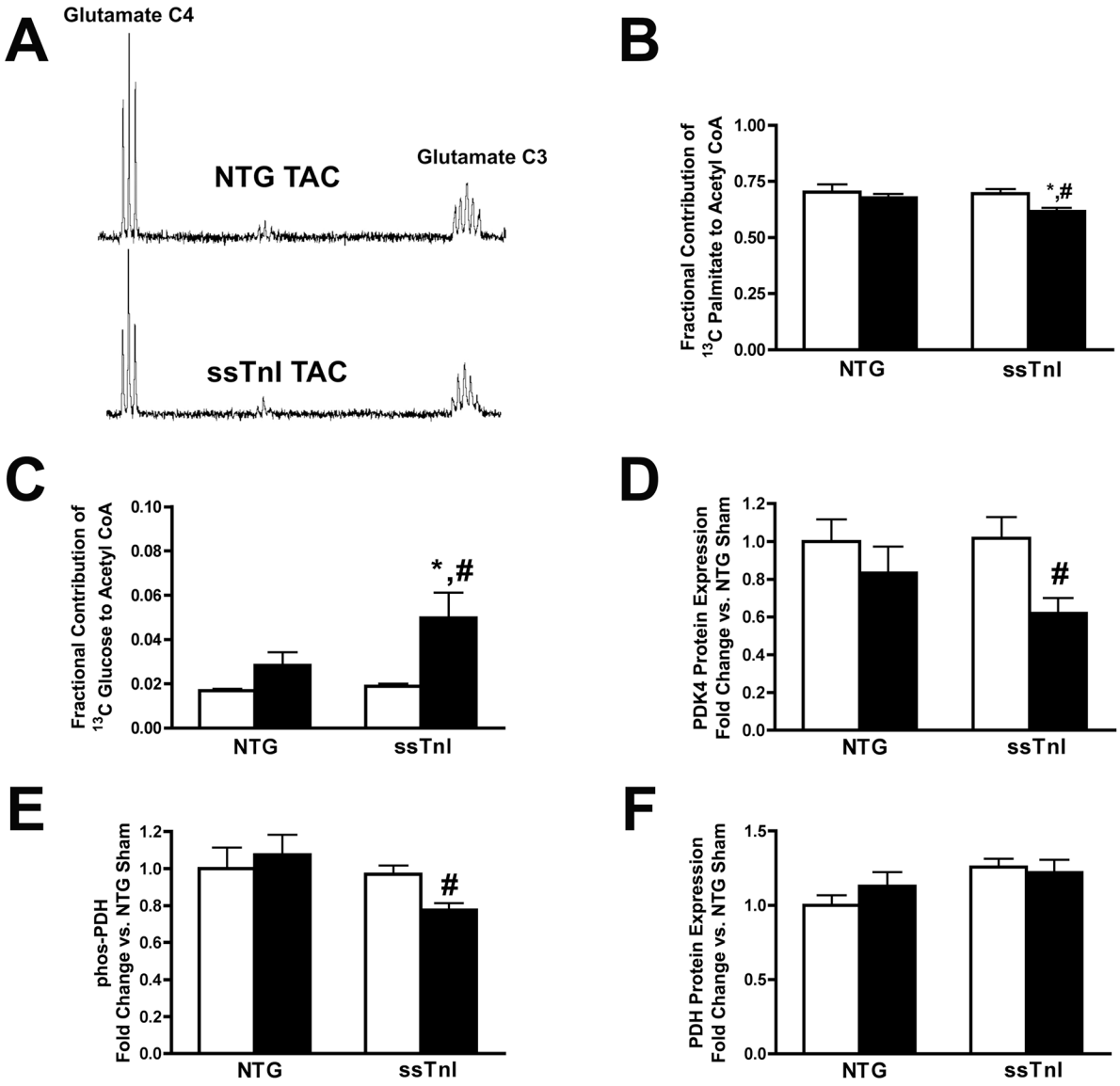

Figure 4. Hearts from ssTnI mice exposed to TAC demonstrate robust metabolic remodeling

Representative spectra showing the end-point enrichment of ^{13}C into the glutamate pool from NTG TAC and ssTnI TAC hearts perfused with ^{13}C palmitate (A). The fractional contribution of palmitate (B) (n=6–9) and glucose (C) (n=6–9) to acetyl CoA production was determined by ^{13}C end–point enrichment of the glutamate in acid soluble extracts. The protein expression of PDK4 was determined (D) (n=3) and shown to be downregulated by TAC in ssTnI mice. The phosphorylation of PDH (phos-PDH) (E) (n=5–6) was downregulated by TAC in ssTnI mice, while PDH expression was not affected (F) (n=5–6). Data is mean±SE. *p<0.05 vs. NTG sham, #p<0.05 vs. ssTnI sham. Sham hearts indicated by open bars; hearts undergoing TAC indicated by solid bars.

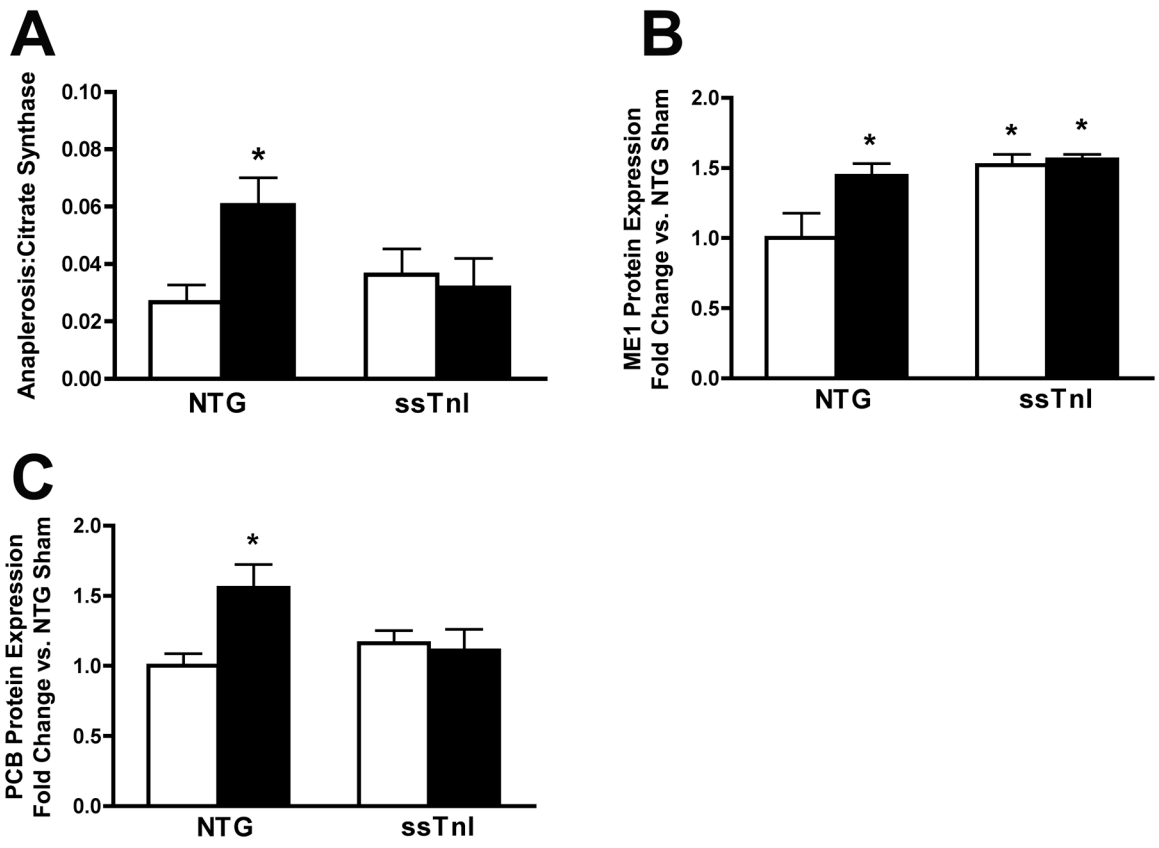

Figure 5. The TAC-induced increase in anaplerosis is prevented in ssTnI hearts

Anaplerotic flux (A) was determined in acid soluble extracts by looking at ^{13}C end-point enrichment of glutamate (n=6–9). The expression of ME-1 (B) and PC (C) was measured. Data is mean±SE. *p<0.05 vs. NTG sham. Sham hearts indicated by open bars; hearts undergoing TAC indicated by solid bars.

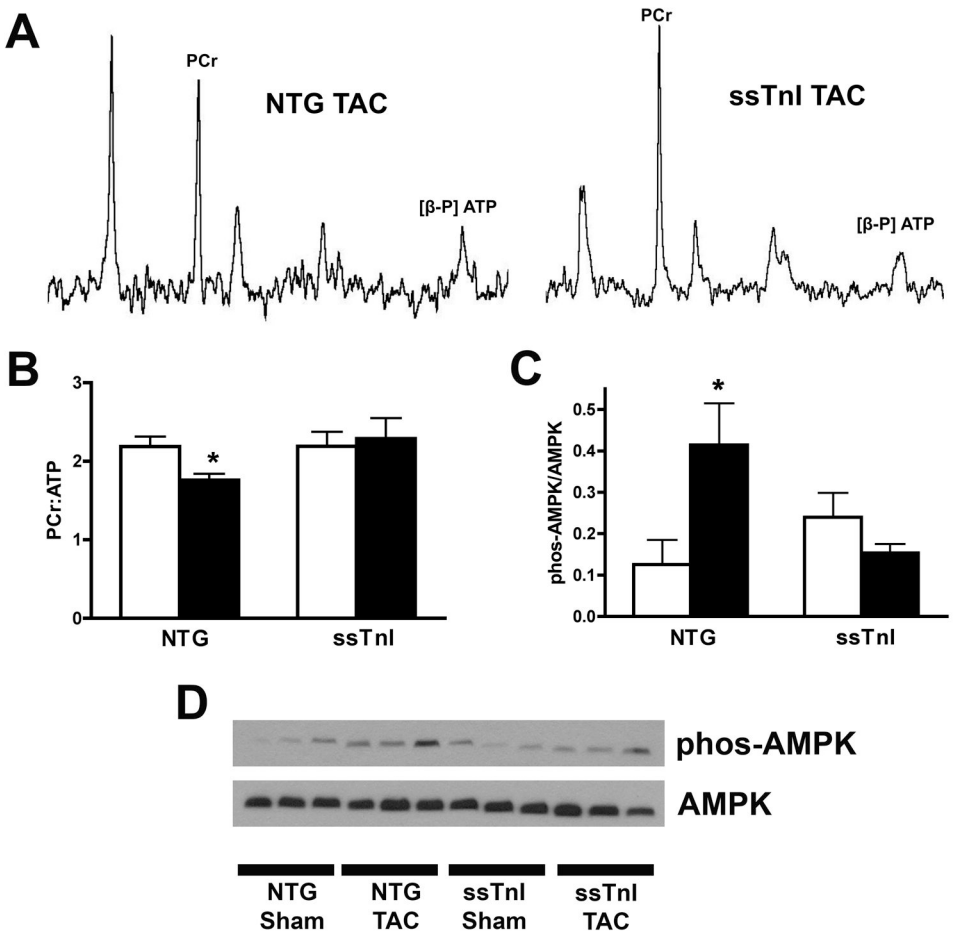

Figure 6. Energetic stores are maintained in ssTnI hearts 13 weeks after TAC

Representative dynamic ^{31}P spectra from intact perfused hearts NTG TAC and ssTnI TAC hearts (A). The PCr:ATP ratio (B) was determined by dynamic mode ^{31}P NMR spectroscopy (n=7–11). The expression of the energetic sensor AMPK and phosphorylated AMPK (phos-AMPK) (C) was determined in frozen heart tissue after isolated perfusion (n=3). The gels showing phos-AMPK and AMPK protein expression are presented (D). Data is mean±SE. *p<0.05 vs. NTG sham. Sham hearts indicated by open bars; hearts undergoing TAC indicated by solid bars.

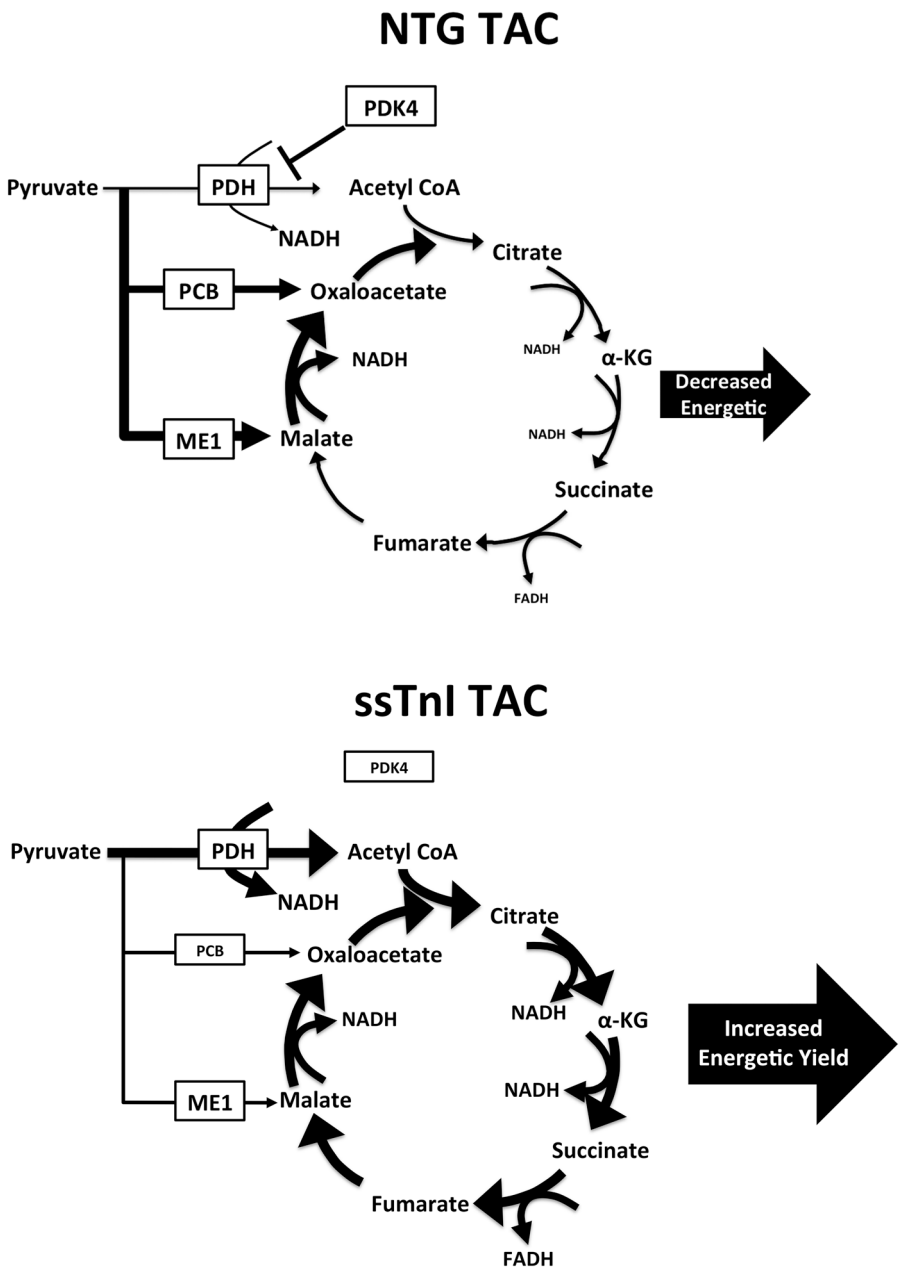

Figure 7. Continued ssTnI expression in the adult heart prevents the upregulation of anaplerotic flux following TAC

In response to the chronic cardiac stress of pressure overload the heart increases flux of pyruvate through the anaplerotic pathways controlled by malic enzyme (ME1) and pruvate carboxylase (PCB). This decreases the overall energetic yield of pyruvate from the TCA cycle by bypassing key energy generating steps within the mitochondria and results in a significant reduction in energetic efficiency (indicated by reduced flux through NADH generating spans of the TCA cycle). In hearts from ssTnI mice, TAC induces an increase in flux through pyruvate dehydrogenase (PDH), through a reduction in the expression of pyruvate dehydrogenase kinase 4 (PDK4). The increase in flux through PDH ensures that

pryuvate passes through all spans of the TCA cycle, resulting in greater yield of reducing equivalents (NADH and FADH) for the electron transport chain.