Physiological Replacement of T₃ Improves Left Ventricular Function in an Animal Model of Myocardial Infarction-Induced Congestive Heart Failure

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Running Title: Henderson: T₃ replacement augments LV function in CHF

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Background: Patients with congestive heart failure (CHF) often have low serum triiodothyronine (T3) concentrations. In a rodent model of myocardial infarction-induced CHF and low serum T3, we hypothesized that replacing T3 to euthyroid levels would improve left ventricular (LV) function without producing untoward signs of thyrotoxicosis. Methods and Results: Adult male Sprague-Dawley rats were subjected to left anterior descending coronary artery ligation (MI). One week post-MI, LV fractional shortening was significantly reduced to 22±1% in CHF animals vs. 38±1% for sham-operated controls (P<0.001). Serum T3 concentration was also significantly reduced (80±3 vs. 103±6ng/dL; P<0.001), in CHF animals vs. Shams. At 9wks post-MI, systolic function (+dP/dt max) was significantly attenuated in CHF animals (4773±259 vs. 6310±267mmHg/sec; P<0.001) as well as diastolic function measured by half time to relaxation (15.9±1.2 vs. 11.1±0.3msec; P<0.001). α-Myosin heavy chain (MHC) expression was also significantly reduced by 77% (P<0.001), and β-MHC expression was increased by 21%. Continuous T3 replacement was initiated 1wk post-MI with osmotic mini-pumps (6μg/kg/day), which returned serum T3 concentrations to levels similar to Sham controls while resting conscious heart rate, arterial blood pressure and the incidence of arrhythmias were not different. At 9wks, systolic function was significantly improved by T3 replacement (6279±347mmHg/sec; P<0.05) and a trend toward improved diastolic function (12.3±0.6msec) was noted. T3 replacement in CHF animals also significantly increased α- and reduced β-MHC expression, (P<0.05). Conclusions: These data indicate that T3 replacement to euthyroid levels improves systolic function and tends to improve diastolic function, potentially through changes in myocardial gene expression.

Key Words: Myocardial infarction, heart failure, thyroid
INTRODUCTION

Thyroid hormone plays a significant role in maintaining cardiovascular function by contributing to myocardial gene expression, contractility, and blood pressure. In cardiomyocytes, triiodothyronine (T<sub>3</sub>) binds to thyroid hormone nuclear receptors which activate response elements within the promoter region of various genes. A change in serum T<sub>3</sub> concentration induces rapid changes in the rate of gene transcription in cardiomyocytes. T<sub>3</sub> activates gene transcription for α-myosin heavy chain (α-MHC), sarcoplasmic reticulum Ca<sup>2+</sup>-ATPase, Na<sup>+</sup>/K<sup>+</sup>-ATPase, β<sub>1</sub>-adrenergic receptor, atrial natriuretic hormone, and voltage-gated potassium channels. A fall in T<sub>3</sub> concentration reverses these effects and promotes the expression of β-myosin heavy chain (β-MHC), phospholamban, thyroid hormone receptor α<sub>1</sub>, and Na<sup>+</sup>/Ca<sup>2+</sup> exchanger.

Thyroid hormone metabolism is altered in patients with congestive heart failure (CHF) and frequently leads to low serum T<sub>3</sub> concentrations. The reduction in serum T<sub>3</sub> has been correlated with poor left ventricular (LV) function in a rodent model and serves as a strong predictor of short-term outcome in CHF patients. It follows that T<sub>3</sub> replacement could reverse these outcomes as recent reports have demonstrated a hemodynamic benefit for such therapy. However, T<sub>3</sub> supplementation has the potential to cause adverse side effects, such as an increase in metabolic rate, systemic oxygen demand, heart rate, and arrhythmias.

There are several studies on the effects of thyroid hormone supplementation in animal models of CHF. These prior reports have studied both thyroxine (T<sub>4</sub>) and T<sub>3</sub> administration; however recent investigations have demonstrated that in the setting of CHF, T<sub>4</sub> would not be expected to be optimally effective due to reduced peripheral conversion of the prohormone T<sub>4</sub> to the active hormone T<sub>3</sub>. Only one prior report has given replacement doses...
of T3 in a physiologic replacement protocol to avoid the potential adverse effects of excess T3 treatment. The specific objectives of the present study were to: 1) continuously administer a replacement dose of T3 over an 8wk time period to animals with LV dysfunction and low T3 syndrome, 2) analyze the effects of T3 replacement on serum T3 and T4 concentrations, as well as serial measurements of LV structure and function by echocardiography, 3) identify, in real-time, changes in heart rate, blood pressure, ECG, and temperature indicative of thyrotoxicosis in conscious animals, using implanted radiotelemetry transmitters, 4) directly assess LV function by cardiac catheterization, and 5) measure changes in myocardial α-and β-MHC mRNA levels to correlate changes in gene expression with physiological outcomes. Data are presented to indicate that T3 replacement to euthyroid levels improves systolic function and tends to improve diastolic function, potentially through changes in myocardial gene expression.

METHODS

All animal protocols were approved by the Loyola University Chicago Institutional Animal Care and Use Committee. LV structure and function were assessed by transthoracic M-mode and 2D echocardiography (Acuson Sequoia C256, 15L8 transducer (15MHz), Siemens Medical Solutions, PA) in 55 adult male Sprague Dawley rats (294±2.3g) under isoflurane anesthesia (4%). Echocardiography and their corresponding hemodynamic measurements were made by a single, “blinded” echocardiographer. MI surgery: Forty-four animals underwent coronary artery ligation. Due to the severity of the infarct, nine animals did not recover. Detailed methods are presented in the on-line supplement. Sham operations on eleven animals were identical, except the suture around the coronary artery was not ligated. LV structure and function were assessed with echocardiography 1wk post MI-surgery and the degree of LV
dysfunction independently graded on a scale of 1 to 5 by two of the investigators (AMS, KKH). Animals were divided into groups with equal levels of LV dysfunction. In our hands, this MI procedure produces a large anterior wall MI and segmental LV dysfunction, leading to pressure and volume overload and subsequent left ventricular remodeling of the non-infarcted myocardium.\textsuperscript{24,25}

**T\textsubscript{3} delivery:** One week post-MI, osmotic mini-pumps (Model #2004, Alzet, CA) were implanted subcutaneously at the nape of the neck to continuously deliver saline diluent in Sham animals (N = 11) or MI-Saline control animals (N = 15); and T\textsubscript{3} at 3\textmu g/kg/day (N = 6), 6\textmu g/kg/day (N = 10), or 60\textmu g/kg/day (N = 4). Osmotic pumps were replaced at the 4\textsuperscript{th} week following echocardiography procedures.

**Radiotelemetry:** In a subset of animals (N = 9), radiotelemeters (Model C50-PXT, Data Sciences International, St. Paul, MN) were used to monitor blood pressure, ECG, body temperature, and physical activity. For these studies, there were 3 groups of MI animals (3 animals/group) receiving Saline, 6\textmu gT\textsubscript{3}/kg/day or 60\textmu gT\textsubscript{3}/kg/day. Importantly, the level of LV dysfunction was identical in each group. Telemetry data were collected once a week for three hours, during the light (resting/sleeping) cycle of the day.

**Serum T\textsubscript{3} and T\textsubscript{4} concentrations and LV structure and function:** At 1, 3, 5, 7 and 9wks post-MI surgery, rats were anesthetized with isoflurane (3-5\%) and blood samples (1mL) were obtained from the tail vein or the retro-orbital venous plexus. Blood was kept on ice until centrifuged at 16,000g for 15min at 4\textdegree C. Serum samples were collected to measure T\textsubscript{3} and T\textsubscript{4}
concentrations using a chemiluminescence assay (Roche Elecsys). After the blood draw, surface ECG leads were attached to monitor heart rate and arrhythmias, and M-mode and 2D echocardiographic images were obtained in the parasternal long and short axes. At the conclusion of the study (9wks), left ventricular function was directly assessed in each animal. A 2-French Millar pressure-volume catheter (SPR-869, Houston, TX) was advanced from the right carotid artery into the LV and baseline measurements of pressure were recorded. An abdominal incision was made to allow occlusion of the supra-renal vena cava to examine left ventricular end systolic and diastolic pressure-volume relationships. The left jugular vein was then catheterized to administer a dobutamine bolus (20μg/kg) to examine changes in myocardial function following β1-adrenergic stimulation.

**Measurements of α and β MHC gene expression:** Six age- and weight-matched animals were used as a control group for CHF animals receiving saline or T3 at 6 or 60μg/kg/day. Total RNA was extracted from frozen LV samples using the guanidinium thiocyanate method, as previously described26-28. Detailed methods are presented in the on-line supplement.

**Statistical Analysis:** Data are presented as means±SEM. Groups were compared using a t-test, 1-way ANOVA, or 2-way RM ANOVA (Holm-Sidak post-hoc) as appropriate. Because serial blood samples were not always available for the same animal, serum T3 and T4 data were not analyzed with repeated measures. Instead, these data were analyzed at each time point. Some data were used for two comparisons. To account for the repeated analysis of these data sets, the significant value required for a t-test was reduced to P=0.025 and the significant value required for multiple comparisons following an ANOVA was reduced to P<0.01. Elsewhere, P-values
≤0.05 were considered significant. Data were analyzed using Sigma Stat version 3.1 (Systat Software, CA).

Statement of Responsibility: All authors had full access to the data and take responsibility for its integrity. All authors have read and agree to the manuscript as written.

RESULTS

Coronary artery ligation produces significant LV chamber dilatation and LV dysfunction. M-mode and 2D echocardiography were used to serially assess LV structure and function before and following sham surgery or coronary artery ligation. There were no significant differences in body weight, heart rate or LV function in the animals prior to designating them for Sham or MI surgery (data not shown). The effect of surgery on coronary artery ligation on LV remodeling and function 1wk after surgery was examined by comparing pre-surgical baseline, 1wk Sham, and 1wk MI values. Infarct surgery caused significant LV remodeling and attenuated LV function within 1wk after surgery, as compared to sham-operated controls animals (Figure 1, Table 1). LV anterior wall thickness was significantly reduced during systole and diastole. Additionally, LV volumes during systole and diastole were significantly increased, reflective of impaired contractile function and dilated cardiomyopathy. LV systolic function, as assessed by fractional shortening, and ejection fraction, was also significantly reduced in CHF animals. There were no significant effects of sham surgery on LV structure or function. The degree of LV dysfunction and remodeling were then ranked on a scale of 1 to 5, and animals were equally divided into different T₃ treatment groups based upon their degree of LV dysfunction.
Administration of T3 reverses the low T3 syndrome in CHF rats following coronary artery ligation. To examine the effect of surgery or coronary artery ligation on serum T4 and T3 concentrations, pre-surgical baseline values were compared to 1wk Sham and 1wk MI animals. As seen in Figure 2A, 1wk after surgery, serum T4 concentrations tended to be lower in Sham (P=0.05) and MI animals (P=0.05). As seen in Figure 2B, 1wk after surgery serum T3 concentrations were significantly reduced by MI surgery vs. baseline values (P=0.012) and 1wk Sham controls (P<0.001). Sham surgery (Sham vs. baseline), did not significantly change serum T3 concentrations (P=0.141). CHF animals were then assigned to receive saline, 3, 6, or 60μgT3/kg/day delivered by osmotic minipump, and serum T4 and T3 levels were serially measured every 2 wks thereafter. CHF animals receiving saline, had significantly lower T3 concentrations than Sham-operated controls at 3 and 5wks post surgery (Figure 2A/2B). CHF rats receiving increasing concentrations of T3 showed a significant dose-dependent decrease in serum T4 concentration throughout the treatment period (Figure 2A). This confirmed the delivery of T3 to the animals and reflects the negative pituitary feedback response to supplemental T3.29 In contrast, the administration of T3 dose dependently increased serum T3 levels in CHF rats (Figure 2B). Importantly, a dose of 6μgT3/kg/day restored T3 levels to those observed in Sham-operated controls (Figure 2B). Administration of 60μgT3/kg/day increased serum T3 concentrations to levels significantly above those observed in either Sham-operated or saline-treated CHF rats.

Effects of T3 administration on hemodynamic variables in conscious animals.
Radiotelemetry was used to assess the hemodynamic effects of T3 administration on CHF rats in order to ascertain whether constant infusion of T3 at 6, or 60μg/kg/day produced any evidence of
cardiovascular side effects or thyrotoxicosis. As seen in Figure 3A, there was no significant effect of T₃ supplementation on resting/sleeping heart rate (P=0.713) at any time point of the study. Resting ECGs were also recorded, and although occasional periods of 2° AV block were noted in all groups, there was no consistent increase in this arrhythmia with increasing doses of T₃. T₃ supplementation tended to increase body temperature at each time point in the study (Figure 3B), but these values were not significantly different (P=0.596). Systolic blood pressure was also not significantly altered by T₃ supplementation (P=0.502; Figure 3C). There was a trend for the highest concentration of T₃ (60μg/kg/day) to lower diastolic blood pressure (Figure 3D), in keeping with the known effect of T₃ to reduce systemic vascular resistance, but this was not significant (P=0.135). There was no correlation between individual or group T₃ concentrations and heart rate, body temperature, mean arterial blood pressure, systolic or diastolic blood pressure (Supplementary Data, Figure 1). This is probably due to the relatively small changes in T₃ concentrations vs. the thyrotoxic doses used in previous studies. Because of the small sample size in the telemetry data, a power analysis was used to calculate the number of animals required to demonstrate that the replacement dose of 6μgT₃/kg/day increases resting heart rate in conscious animals. Using the average difference in heart rate and average standard deviation between the MI-Saline and MI-6μg/kg/day animals over the 7wks of readings, the power analysis suggested that ~50 animals/group are needed to demonstrate a significant increase in heart rate of ~10 beats/minute. This is somewhat expected as the experiments were designed to deliver a replacement dose of T₃ with minimal effects on heart rate, blood pressure or body temperature. These data suggest that T₃ administration at 6μg/kg/day was sufficient to restore serum T₃ levels without producing overt signs of thyrotoxicosis.
**T₃ infusion does not prevent LV remodeling.** A two-way RM ANOVA was used to determine if MI-surgery altered LV structure and function over time; and whether T₃ supplementation attenuated LV remodeling or improved LV function. Heart rate was measured in all animals under anesthesia during echocardiography (Figure 4A) and was similar to conscious heart rate data (Figures 3A and 4A). Compared to MI-Saline animals, heart rate measured under anesthesia was elevated in animals receiving 6μgT₃/kg/day at the 9th week and in animals receiving 60μgT₃/kg/day at the 3rd and 9th week. Single lead ECG recordings measured under isoflurane anesthesia indicated no differences in PR interval between any groups, but we observed a widened QRS interval in MI animals as compared to sham-operated control animals over the 9wk study. This intraventricular conduction delay was not influenced by T₃ supplementation.

Echocardiography at all time points (1, 3, 5, 7, and 9wks), demonstrated that the anterior wall was significantly thinner in diastole and systole in MI-Saline hearts vs. Sham controls (Figure 4B and 4C). Overall, posterior wall thickness was similar in MI-Saline and Sham controls throughout the study (Figure 4D and 4E). Administration of T₃ at any dose, overall, did not alter anterior or posterior wall thickness during systole or diastole, suggesting that T₃ replacement in rats with CHF does not alter LV structure or mass.

LV chamber volumes during diastole and systole were significantly greater in MI-Saline vs. Sham controls, (Figure 5A and 5B). Accordingly, LV fractional shortening and ejection fraction were significantly attenuated in MI-Saline vs. Sham controls (Figure 5C and 5D). Importantly, left ventricular volume in systole and diastole increased over time in MI-Saline animals (Figure 5A and 5B). In regard to T₃ infusion, there was no effect of any dose on LV chamber volumes during systole or diastole, or on fractional shortening or ejection fraction.
**T₃ administration increases LV contractility in CHF rats.** At 9 weeks, rats were anesthetized and LV contractility and relaxation were measured with a high-fidelity pressure-volume catheter. As seen in Figure 6A, LV contractility (+dP/dt max) and relaxation rate (half-time to relaxation (τ), Figure 6B) were significantly (P<0.001) attenuated in MI animals vs. Sham controls (4773±259 vs. 6310±267 mmHg/sec, and 15.87±1.23 vs. 11.11±0.25 msec respectively). Contractility was significantly improved by administration of both 6μg and 60μgT₃/kg/day (P<0.05). A similar trend was observed for measurements of τ, but the improvement in diastolic function did not achieve statistical significance (P=0.10). In addition, there were no further significant differences in cardiac function under conditions of reduced preload (suprarenal venous occlusion) or β₁-adrenergic stimulation (dobutamine 20μg/kg) between MI-Saline and T₃ treated groups (data not shown).

**Myocardial gene expression:** To determine if the physiologic changes that occur after MI and in response to thyroid hormone are mediated, at least in part, by transcriptional changes, we measured the expression of both myosin heavy chain genes. Expression of the cardiac specific α-MHC gene was significantly (P<0.001) attenuated in MI-Saline animals (to 23±5% of controls) and dose dependently increased with T₃ replacement at 6 and 60μg/kg/day to 42±4% and 150±14% of controls (P<0.05), respectively (Figure 7A). β-MHC expression was significantly reduced by T₃ replacement at 6 and 60μg/kg/day to 71±12% and 73±21% of controls (P<0.05), respectively (Figure 7B).
DISCUSSION

The present studies were designed to test the hypothesis that physiologic replacement of T3 to myocardial infarction-induced CHF animals would improve or restore left ventricular contractile performance. The major finding of the present study is T3 replacement initiated one week after a MI, after the majority of left ventricular remodeling has taken place, can improve left ventricular contractility. It is well known that hypothyroid patients have low cardiac output, increased systemic vascular resistance and impaired LV contractility and relaxation\textsuperscript{30,31}. These effects are due to thyroid hormone mediated changes in systemic vascular resistance as well as direct effects on myocyte gene expression\textsuperscript{1,32}. Therefore, the chronic decreases in T3 concentrations in heart failure patients may further contribute to the development of pathological hypertrophy post-MI\textsuperscript{33,34}.

**T3 Replacement:** Proof of concept studies by Hamilton et al.\textsuperscript{16} and recent studies by Pingitore et al.\textsuperscript{18} have shown that physiologic T3 replacement can produce hemodynamic benefits. Hamilton et al. had previously shown that short-term administration of T3 could be safely infused into patients with advanced heart disease to reduce afterload and increase cardiac output. Multiple studies of T3 infusion to both adults and children undergoing cardiac surgery have shown that correcting low T3 levels improves cardiovascular function\textsuperscript{35-38}. Additionally, administration of T3 has also been shown to decrease plasma noradrenaline, aldosterone, and natriuretic peptide concentrations, which are frequently elevated in heart failure patients\textsuperscript{18}. Most importantly, all human studies to date have confirmed the safety and lack of untoward side effects associated with the replacement of T3 to euthyroid levels\textsuperscript{35-39}. 
**MI animal model:** In the current studies, the level of LV dysfunction induced by coronary artery ligation was assessed 1wk post-MI surgery to appropriately divide animals into experimental groups. In MI-Saline animals, LV function measured by serial echocardiography and LV catheterization at 9wks was significantly attenuated throughout the study vs. Sham-operated controls. Serum T4 and T3 levels were significantly lower one week post MI (P<0.05) and remained significantly lower for at least 5 weeks. These data are similar to the previous observation by Olivares et al. who conducted a time course study of thyroid function after a myocardial infarction in a rodent model40. This group noted that post-MI serum T3 levels were significantly depressed and the ejection fraction was reduced by 50%.

The mechanism for the low T3 syndrome in heart failure patients is currently under investigation. Low T3 levels are associated with increased production of pro-inflammatory cytokines and/or increased clearance of T3. Interleukins 1 and 6 were shown to competitively inhibit co-activators for type 1 deiodinase transcription 41, an enzyme which converts T4 to T3 but also converts T4 and T3 to inactive isomers. Increased clearance of T3 may also be attributed to increased type III deiodinase (D3) expression or activity which is increased in heart failure animal models40,42,43. D3 reduces T3 concentrations by preventing its production (converting T4 to inactive T3), and increasing its degradation (converting T3 to di-iodothyronine). Simonides et al., recently demonstrated that D3 expression can be activated by hypoxia inducible factor (HIF-1)42. During the early compensatory stages of a myocardial infarction, HIF-1 expression is increased in human hearts44, and is also expressed in the non-ischemic mechanically stressed myocardium in MI-rodent models45. If HIF-1 levels subside over time, D3 expression would be attenuated. Such an effect would prevent T4 and T3 degradation and may explain the gradual increase in serum T4 and T3 concentrations in MI-Saline animals in this study.
**T3 replacement and LV function:** For these studies, T3 replacement was initiated 1wk post-MI, after significant LV remodeling had taken place. Recently, Chen et al., demonstrated that intraperitoneal injection of T3 at 14μg/kg/day shortly after MI surgery and continued for 3 days, reduced cardiac myocyte apoptosis along the border zone of the infarct in rats. Similarly, Pantos et al. demonstrated that immediate T4 supplementation, after a MI in a rodent model, improves cardiac function and prevents cardiac remodeling. In both studies, heart rates and T3 concentrations were significantly elevated suggesting that animals were thyrotoxic. It remains to be determined whether immediate T3 “replacement” can attenuate the early stages of LV remodeling and improve LV function. In T3 replacement animals, LVEDV continued to increase over the 9wk study. While T3 replacement did not prevent long term LV remodeling, it improved LV contractility and relaxation in the remaining viable myocardium. These data suggest that the activation of T3 dependent signaling pathways at the onset of an MI, may promote angiogenesis and cell survival and reduce the morbidity and mortality associated with congestive heart failure.

**Gene expression:** In the euthyroid rat ventricle, α-MHC is the predominant MHC isoform. The α-MHC gene is positively regulated by T3, while the β-MHC gene is negatively regulated. In the hypothyroid rat heart, as well as after MI and in heart failure, β-MHC expression predominates and α-MHC expression is reduced. The effect of heart failure on α- and β-MHC expression and the reversal with T3 supplementation have been previously reported. Similar to a prior study from our laboratory, we measured a significant reduction in α-MHC expression in our post-MI animals. In the current study we found that 6μgT3/kg/day significantly decreased β-MHC
expression as well as increased α-MHC expression. These data reflect the relative sensitivity of the cardiac MHC genes to changes in serum T₃ concentrations.

CONCLUSION

Overall, these studies suggest that long-term T₃ replacement to euthyroid levels initiated one week post-MI significantly improves left ventricular function primarily through an increase in contractility. This effect may be linked to changes in α- and β-MHC expression. Left ventricular chamber size and remodeling were not reversed by T₃ replacement when initiated after significant LV remodeling has taken place. It remains to be determined whether LV remodeling can be reversed in heart failure patients by the immediate administration of T₃ and whether this can be accomplished by returning myocardial T₃ concentrations to euthyroid levels.

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DISCLOSURES

Drs. Klein, Danzi, Henderson and Samarel have served as consultants to King Pharmaceuticals. Dr. Klein has served as a consultant to Roche Pharmaceuticals.
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FIGURE LEGENDS

Figure 1: Experimental timeline and 1wk post-surgery echocardiography: Serum T₃ and T₄, and LV structure and function were assessed at 0, 1, 3, 5, 7, and 9wks. MI or Sham surgeries were performed at 0wk. Animals were then divided into groups with equal levels of LV dysfunction, and osmotic pumps with saline or T₃ were implanted at 1wk. Osmotic pumps were replaced at 5wks. At 9wks the LV was catheterized to assess LV function, animals were then sacrificed, and tissue collected for analysis. Long axis and M-Mode echocardiograms for a Sham and MI animal, 1wk post-surgery, are shown below.

Figure 2: Serum T₄ and T₃ concentrations: Measures before (Baseline) and 1wk post surgery in Sham and MI animals. Saline or T₃ supplementation at 3, 6, and 60μg/kg/day in MI animals began after week #1, and serum T₄ and T₃ concentrations were then measured at 3, 5, 7 and 9 weeks. A. Serum T₄ concentrations. B. Serum T₃ concentrations. Significant differences: (@)P<0.025 vs. Baseline (t-test); (*)P<0.025 vs. Sham (t-test); (#)P<0.05 vs. MI-Saline (1-way ANOVA).

Figure 3: Serial radio-telemetry in vivo measures: A. Heart rate, B. Core body temperature, C. Systolic blood pressure, and D. Diastolic blood pressure. Data analyzed with 2-way RM ANOVA, no significant effect of T3 dose was found.

Figure 4: Serial heart rate and LV wall thickness measured by echocardiography: A. Anesthetized heart rate. B/C. Anterior wall (AW) thickness in end diastole (ED) and end systole (ES), respectively. D/E. Posterior wall (PW) thickness in ED and ES, respectively.
Significant differences: (*)P<0.025 vs. Sham (2-way RM ANOVA); (#)P<0.05 vs. MI-Saline (2-way RM ANOVA).

Figure 5: Serial LV dimensions and function measured by echocardiography: A/B. Left ventricular end diastolic volume (EDV) and systolic volume (ESV), respectively. C/D. Left ventricular fractional shortening and ejection fraction, respectively. Significant differences: (*)P<0.025 vs. Sham (2-way RM ANOVA). This analysis also demonstrated that EDV and ESV in MI-Saline animals increased over time (1wk vs. 3, 5, 7, 9wk; and 3wk vs. 5, 7, 9wk; were significantly different, P<0.05).

Figure 6: Left ventricular systolic and diastolic function at 9wks: A. Systolic function (+dP/dtmax). B. Diastolic function (half-time to relaxation). Significant differences: (*)P<0.025 vs. Sham (t-test); (#)P<0.05 vs. MI-Saline (1-way ANOVA). Sham (N = 11), MI-Saline (N = 15), MI-3μgT₃/kg/day (N = 6), MI-6μgT₃/kg/day (N = 10), MI-60μgT₃/kg/day (N = 4).

Figure 7: α- and β-MHC expression at 9wks: A. α-MHC expression as a % of control. B. β-MHC expression as a % of control. Significant differences: (*)P<0.025 vs. Sham (t-test); (#)P<0.05 vs. MI-Saline (1-way ANOVA). Control (N = 6), MI-Saline (N = 15), MI-3μgT₃/kg/day (N = 6), MI-6μgT₃/kg/day (N = 10), MI-60μgT₃/kg/day (N = 4).
**TABLE 1:** Effect of MI surgery on left ventricular structure and function as measured by echocardiography 1wk post-MI.

<table>
<thead>
<tr>
<th></th>
<th>Baseline (n = 55)</th>
<th>1wk Post-Sham (n = 11)</th>
<th>1wk Post-MI (n = 35)</th>
<th>P-value: Base vs. MI</th>
<th>P-value: Sham vs. MI</th>
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<tr>
<td>Body Wt. (g)</td>
<td>294.2±2.3</td>
<td>290.7±3.6</td>
<td>294.2±3.7</td>
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<td>Heart Rate (bpm)</td>
<td>365.1±3.8</td>
<td>379.6±8.9</td>
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<td>LV AW, d (mm)</td>
<td>1.43±0.02</td>
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<td>LV PW, d (mm)</td>
<td>1.47±0.3</td>
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<td>LV AW, s (mm)</td>
<td>2.37±0.04</td>
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<td>LV PW, s (mm)</td>
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<td>LV, s (mm)</td>
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<td>6.70±0.19</td>
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<td>LV, d (mm)</td>
<td>7.36±0.07</td>
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<tr>
<td>LV EDV (μL)</td>
<td>369.2±6.7</td>
<td>376.2±13.7</td>
<td>504.1±18.2</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>LV ESV (μL)</td>
<td>142.8±5.4</td>
<td>144.8±12.3</td>
<td>311.9±17.7</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>LV SV (μL)</td>
<td>226.4±4.8</td>
<td>231.4±8.9</td>
<td>192.3±9.5</td>
<td>&lt;0.001</td>
<td>0.033</td>
</tr>
<tr>
<td>LV CO (ml/min)</td>
<td>82.7±2.0</td>
<td>87.7±3.6</td>
<td>73.3±3.7</td>
<td>0.016</td>
<td>0.043</td>
</tr>
<tr>
<td>LV FS, %</td>
<td>37.8±0.9</td>
<td>37.9±1.7</td>
<td>22.0±1.3</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>LV EF, %</td>
<td>61.6±1.1</td>
<td>61.9±2.1</td>
<td>39.0±2.0</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

Data are means±SEM. LV = Left ventricle, d = diastole, s = systole, mm = millimeters, AW = Anterior wall thickness, PW = Posterior wall thickness, EDV = End diastolic volume, ESV = End systolic volume, SV = Stroke volume, CO = Cardiac output, FS% = Fractional shortening, EF% = Ejection fraction. Significant differences: (@) vs. Baseline, (*) vs. 1wk Sham, P<0.025 (t-tests).
FIGURE 1

1wk post-Sham or MI Surgery, Long Axis and M-Mode

Sham

MI
FIGURE 2

A.

Baseline 1wk 3wk 5wk 7wk 9wk

T4 [μg/dL]

0 1 2 3 4 5

B.

Baseline: N = 55
Sham: N = 11
MI-Saline: N = 15
MI-3ug/kg: N = 6
MI-6ug/kg: N = 10
MI-60ug/kg: N = 4

Baseline 1wk 3wk 5wk 7wk 9wk

T3 [ng/dL]

0 25 50 75 100 125 150 175 200
FIGURE 3

A. 

Beats/min

<table>
<thead>
<tr>
<th>MI-Saline: N = 3</th>
<th>MI-6µg/kg: N = 3</th>
<th>MI-60µg/kg: N = 3</th>
</tr>
</thead>
<tbody>
<tr>
<td>2wk</td>
<td>3wk</td>
<td>4wk</td>
</tr>
</tbody>
</table>

B. 

Temperature (°C)

<table>
<thead>
<tr>
<th>MI-Saline: N = 3</th>
<th>MI-6µg/kg: N = 3</th>
<th>MI-60µg/kg: N = 3</th>
</tr>
</thead>
<tbody>
<tr>
<td>2wk</td>
<td>3wk</td>
<td>4wk</td>
</tr>
</tbody>
</table>

C. 

Systolic Pressure (mmHg)

<table>
<thead>
<tr>
<th>MI-Saline: N = 3</th>
<th>MI-6µg/kg: N = 3</th>
<th>MI-60µg/kg: N = 3</th>
</tr>
</thead>
<tbody>
<tr>
<td>2wk</td>
<td>3wk</td>
<td>4wk</td>
</tr>
</tbody>
</table>

D. 

Diastolic Pressure (mmHg)

<table>
<thead>
<tr>
<th>MI-Saline: N = 3</th>
<th>MI-6µg/kg: N = 3</th>
<th>MI-60µg/kg: N = 3</th>
</tr>
</thead>
<tbody>
<tr>
<td>2wk</td>
<td>3wk</td>
<td>4wk</td>
</tr>
</tbody>
</table>
**FIGURE 4**

A. 

Beats/min

Sham: N = 11  
MI-Saline: N = 15  
MI-3µg/kg: N = 6  
MI-6µg/kg: N = 10  
MI-60µg/kg: N = 4

B.

AW Thickness ED (mm x 10)

C.

AW Thickness ES (mm x 10)

D.

PW Thickness ED (mm x 10)

E.

PW Thickness ES (mm x 10)

1wk 3wk 5wk 7wk 9wk
A. 

![Graph showing mmHg/sec for different groups.]

B. 

![Graph showing msec for different groups.]

FIGURE 6
A. Expression of alpha-MHC mRNA (% control)

B. Expression of beta-MHC mRNA (% control)
Physiological Replacement of T₃ Improves Left Ventricular Function in an Animal Model of Myocardial Infarction-Induced Congestive Heart Failure
Kyle K. Henderson, Sara Danzi, Jennifer T. Paul, Greg Leya, Irwin Klein and Allen M. Samarel

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SUPPLEMENTAL MATERIAL:

METHODS:
Coronary Artery Ligation: Animals were anesthetized with isoflurane (3-5%), intubated with a 14 gauge catheter and mechanically ventilated (tidal volume 1mL/kg, 80 RPM, 1cm H2O PEEP). Ampicillin (150mg/kg, IM) and lidocaine (6mg/kg, IM) were administered to reduce infection and ventricular arrhythmias respectively. Body temperature was maintained by a heated surgical table. A left thoracotomy was performed between the 5th and 6th ribs to expose the heart, the pericardium was opened and the left anterior descending (LAD) coronary artery was ligated between the pulmonary outflow tract and the left atrium with 6-0 silk suture passed through the superficial layers of the myocardium. The lungs were re-inflated by momentarily occluding the outflow of the ventilator. The chest incision was closed in layers with 3-0 chromic suture (ribs and subcutaneous tissue) and 3-0 silk sutures for the skin. After surgery, animals were given ampicillin (150mg/kg, IM) and buprenorphine (0.05mg/kg, IM) to reduce infection rate and post-surgical pain, respectively.

Radiotelemetry Implantation: Radiotelemeters were implanted into the peritoneal cavity and ECG leads were subcutaneously routed to the right chest area and left of the xyphoid process. The arterial blood pressure catheter was routed to the left hind limb and the femoral artery was catheterized.

α and β MHC gene expression: Fifty µg of total RNA was treated with DNase I and subjected to the RNeasy miniprotocol for RNA cleanup (Qiagen, Valencia, CA). RT-PCR for α- and β-MHC was performed with 2ng of total RNA using primers specific for α- or β-MHC mRNA. Reverse primers for each gene annealed to sequences at the 3'-untranslated end of the respective mRNAs [α-MHC5892R (5'-GTGGAATAGCAACAGCGAGGC-3'; GenBank accession no.AH002207) and β-MHC5869R (5'-CTCCAGGTCTCAGGGCTTCAC-3'; GenBank accession no. X16291)], a region that differs between α- and β-MHC. PCR of mRNA was accomplished using the same mRNA reverse primers described above and forward primers: α-MHC5593F (5'-CTACCAGACAGAGGAAGACAAG-3’) and β-MHC5579F (5’-GACAGGAAGAACCTACTGCG-3’). PCR products were run on a 2% agarose gel with ethidium bromide and quantitated by densitometry using Bio-Rad Quantity 4.2.2 software. All RT reactions were done in duplicate.

RESULTS:
Physical parameters: At the conclusion of the study, MI-Saline animals had significantly lower body weights vs. Sham controls (396.6±5.3 vs. 418.8±7.2g; P = 0.018) and increased heart weights (1.52±0.05 vs. 1.35±0.03g; P = 0.018). Accordingly, HW/BW ratios were significantly increased in MI-Saline animals vs. Sham controls (3.22±0.08 vs. 3.85±0.15; P = 0.003). There was no effect of T3 (at any dose) on heart weight or heart/body wt ratio.
A. Heart Rate vs. T3 Concentration

Y-int: 320
Slope: 0.04
r²: 6.88e-3

B. Temperature vs. T3 Concentration

Y-int: 38.4
Slope: 3.67
r²: 2.12e-6

C. MAP vs. T3 Concentration

Y-int: 113
Slope: -0.08
r²: 0.063

D. Systolic Blood Pressure vs. T3 Concentration

Y-int: 122
Slope: -0.03
r²: 0.013

E. Diastolic Blood Pressure vs. T3 Concentration

Y-int: 103
Slope: -0.10
r²: 0.073
Figure 1: T₃ concentrations and hemodynamic variables: Within this experimental design, there were no significant correlations between T₃ concentrations and A. Heart Rate. B. Body Temperature. C. Mean Arterial Pressure (MAP). D/E. Systolic and Diastolic Blood Pressure, respectively.