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

Kyle K. Henderson, PhD; Sara Danzi, PhD; Jennifer T. Paul, Sc.B.; Greg Leya; Irwin Klein, MD; Allen M. Samarel, MD

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 function without producing untoward signs of thyrotoxicosis.

Methods and Results—Adult male Sprague-Dawley rats were subjected to left anterior descending coronary artery ligation (myocardial infarction). One week post-myocardial infarction, left ventricular fractional shortening was significantly reduced to 22±1% in CHF animals versus 38±1% for sham-operated controls (P<0.001). Serum T3 concentration was also significantly reduced (80±3 versus 103±6 ng/dL; P<0.001), in CHF animals versus Shams. At 9 weeks post-myocardial infarction, systolic function (+dP/dt max) was significantly attenuated in CHF animals (4773±259 versus 6310±267 mm Hg/s; P<0.001) as well as diastolic function measured by half time to relaxation (15.9±1.2 versus 11.1±0.3 ms; P<0.001), α-myosin heavy chain expression was also significantly reduced by 77% (P<0.001), and β-myosin heavy chain expression was increased by 21%. Continuous T3 replacement was initiated 1 week post-myocardial infarction with osmotic mini-pumps (6 µg/kg/d), 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 9 weeks, systolic function was significantly improved by T3 replacement (6279±347 mm Hg/s; P<0.05) and a trend toward improved diastolic function (12.3±0.6 ms) 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. (Circ Heart Fail. 2009;2:243-252.)

Key Words: myocardial infarction • heart failure • thyroid • low T3 syndrome

Thyroid hormone plays a significant role in maintaining cardiovascular function by contributing to myocardial gene expression, contractility, and blood pressure.1-3 In cardiomyocytes, triiodothyronine (T3) binds to thyroid hormone nuclear receptors that activate response elements within the promoter region of various genes. A change in serum T3 concentration induces rapid changes in the rate of gene transcription in cardiomyocytes. T3 activates gene transcription for α-myosin heavy chain (α-MHC), sarcoplasmic reticulum Ca2+-ATPase, Na+/-K+-ATPase, β1-adrenergic receptor, atrial natriuretic hormone, and voltage-gated potassium channels.4-10 A fall in T3 concentration reverses these effects and promotes the expression of β-myosin heavy chain (β-MHC), phospholamban, thyroid hormone receptor α1, and Na⁺/Ca²⁺ exchanger.9-11

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Thyroid hormone metabolism is altered in patients with congestive heart failure (CHF) and frequently leads to low serum T3 concentrations. The reduction in serum T3 has been correlated with poor left ventricular (LV) function in a rodent model14 and serves as a strong predictor of short-term outcome in CHF patients.15 It follows that T3 replacement could reverse these outcomes as recent reports have demonstrated a hemodynamic benefit for such therapy.16-18 However, T3 supplementation has the potential to cause adverse side effects, such as an increase in metabolic rate, systemic oxygen demand, heart rate, and arrhythmias.2

There are several studies on the effects of thyroid hormone supplementation in animal models of CHF.11,19,20 These prior reports have studied both thyroxine (T4) and T3 administration; however recent investigations have demonstrated that in the setting of CHF, T4 would not be expected to be optimally effective due to reduced peripheral conversion of the prohormone T4 to the active hormone T3.19-21 Only 1 prior report has given replacement doses of T3 in a physiological replacement protocol to avoid the potential adverse effects of excess...
The specific objectives of the present study were to (1) continuously administer a replacement dose of T3 over an 8 weeks 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 (15 MHz), Siemens Medical Solutions, Pa) in 55 adult male Sprague-Dawley rats (294±2.3 g) under isoflurane anesthesia (4%). Echocardiography and their corresponding hemodynamic measurements were made by a single, “blinded” echocardiographer. Myocardial infarction (MI) surgery: 44 animals underwent coronary artery ligation. Because of the severity of the infarct, 9 animals did not recover. Detailed methods are presented in the online supplement. Sham operations on 11 animals were identical, except the suture around the coronary artery was not ligated. LV structure and function were assessed with echocardiography 1 week post-MI–surgery, and the degree of LV dysfunction independently graded on a scale of 1 to 5 by 2 of the investigators (A.M.S., K.K.H.). 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 LV remodeling of the noninfarcted myocardium.

**T3 Delivery**

One week post-MI, osmotic mini-pumps (Model No. 2004, Alzet, Calif) 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 T₃ at 3 μg/kg/d (N=6), 6 μg/kg/d (N=10), or 60 μg/kg/d (N=4). Osmotic pumps were replaced at the 4th week following echocardiography procedures.

Radiotelemetry
In a subset of animals (N=9), radiotelemeters (Model C50-PXT, Data Sciences International, St. Paul, Minn) 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 μgT₃/kg/d or 60 μgT₃/kg/d. Importantly, the level of LV dysfunction was identical in each group. Telemetry data were collected once a week for 3 weeks, during the light (resting/sleeping) cycle of the day.

Serum T₃ and T₄ Concentrations and LV Structure and Function
At 1, 3, 5, 7, and 9 weeks post-MI surgery, rats were anesthetized with isoflurane (3% to 5%) and blood samples (1 mL) were obtained from the tail vein or the retro-oral venous plexus. Blood was kept on ice until centrifuged at 16 000g for 15 minutes at 4°C. Serum samples were collected to measure T₃ and T₄ 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 echocardiography were used to serially monitor heart function and to assess the degree of LV remodeling. LV function was directly assessed in each animal. A 2-F Millar pressure-volume catheter (SPR-869, Houston, Tex) 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 suprarenal vena cava to examine LV end systolic and diastolic pressure-volume relationships. The left jugular vein was then catheterized to administer a dobutamine bolus (20 μg/kg) to examine changes in myocardial function after 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 60 μg/kg/d. Total RNA was extracted from frozen LV samples using the guanidinium thiocyanate method, as previously described.26–28 Detailed methods are presented in the online supplement.

Table. Effect of MI Surgery on Left Ventricular Structure and Function as Measured by Echocardiography 1 Week Post-MI

<table>
<thead>
<tr>
<th>Body wt, g</th>
<th>Baseline (n=55)</th>
<th>1 Week Post-Sham (n=11)</th>
<th>1 Week Post-MI (n=35)</th>
<th>P Value: Base vs MI</th>
<th>P Value: Sham vs MI</th>
</tr>
</thead>
<tbody>
<tr>
<td>Heart rate, bpm</td>
<td>365.1±3.8</td>
<td>379.6±8.9</td>
<td>381.1±4.3*</td>
<td>0.008</td>
<td>0.865</td>
</tr>
<tr>
<td>LV AW, d, mm</td>
<td>1.43±0.02</td>
<td>1.42±0.06</td>
<td>1.01±0.05†</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
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<tr>
<td>LV PW, d, mm</td>
<td>1.47±0.3</td>
<td>1.46±0.05</td>
<td>1.48±0.08</td>
<td>0.949</td>
<td>0.871</td>
</tr>
<tr>
<td>LV AW, s, mm</td>
<td>2.37±0.04</td>
<td>2.35±0.07</td>
<td>1.47±0.11‡</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>LV PW, s, mm</td>
<td>2.40±0.05</td>
<td>2.43±0.10</td>
<td>2.17±0.09*</td>
<td>0.011</td>
<td>0.113</td>
</tr>
<tr>
<td>LV, s, mm</td>
<td>4.59±0.09</td>
<td>4.63±0.18</td>
<td>6.70±0.19‡</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
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<tr>
<td>LV, d, mm</td>
<td>7.36±0.07</td>
<td>7.44±0.13</td>
<td>8.56±0.15*</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>LV EDV, μL</td>
<td>369.2±6.7</td>
<td>376±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>142.5±5.4</td>
<td>144±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. Significant differences: (†) vs Baseline, (‡) vs 1 week Sham, P<0.025 (t-tests).
LV indicates left ventricular; d, diastole; s, systole; 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.

Statistical Analysis
Data are presented as means±SEM. Groups were compared using a t test, 1-way analysis of variance (ANOVA), or 2-way repeated-measures (RM) ANOVA (Holm-Sidak posthoc) 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 2 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, Calif).

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 after sham surgery or coronary artery ligation. There were no significant differences in body weight, heart rate, or LV function in the animals before designating them for Sham or MI surgery (data not shown). The effect of surgery on coronary artery ligation on LV remodeling and function 1 week after surgery was examined by comparing presurgical baseline, 1 week Sham, and 1 week MI values. Infarct surgery caused significant LV remodeling and attenuated LV function within 1 week after surgery, as compared with sham-operated controls animals (Figure 1, Table). 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...
Saline or T3 supplementation at 3, 6, and 60 (baseline) and 1 week postsurgery in Sham and MI animals. MI animals (0.05). As seen in Figure 2B, 1 week after surgery, saline and MI-6 ug/kg/d restored T3 levels to those observed in Sham-operated controls (Figure 2B). Administration of 60 µgT3/kg/d 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 to ascertain whether constant infusion of T3 at 6, or 60 µg/kg/d 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 (60 µg/kg/d) 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 (Supplemental Figure I). This is probably due to the relatively small changes in T3 concentrations versus the thyrotoxic doses used in previous studies. Because of the small sample size in the telemetery data, a power analysis was used to calculate the number of animals required to demonstrate that the replacement dose of 6 µgT3/kg/d increases resting heart rate in conscious animals. Using the average difference in heart rate and average standard deviation between the MI-saline and MI-6 µg/kg/d animals over the 7 weeks of readings, the power analysis suggested that ~50 animals/group are needed to demonstrate a significant increase in heart rate of ~10 bpm. 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 6 µg/kg/d was sufficient to restore serum T3 levels without producing overt signs of thyrotoxicosis.

T3 Infusion Does Not Prevent LV Remodeling

A 2-way RM ANOVA was used to determine whether MI-surgery altered LV structure and function over time; and

Administration of T3 Reverses the low T3 Syndrome in CHF Rats After Coronary Artery Ligation

To examine the effect of surgery or coronary artery ligation on serum T4 and T3 concentrations, presurgical baseline values were compared with 1 week Sham and 1 week MI animals. As seen in Figure 2A, 1 week after surgery, serum T3 concentrations tended to be lower in Sham (P=0.05) and MI animals (P=0.05). As seen in Figure 2B, 1 week after surgery serum T3 concentrations were significantly reduced by MI surgery versus baseline values (P=0.012) and 1 week Sham controls (P<0.001). Sham surgery (Sham versus baseline), did not significantly change serum T3 concentrations (P=0.141). CHF animals were then assigned to receive saline, 3, 6, or 60 µgT3/kg/d delivered by osmotic minipump, and serum T4 and T3 levels were serially measured every 2 weeks thereafter. CHF animals receiving saline had significantly lower T3 concentrations than Sham-operated controls at 3 and 5 weeks postsurgery (Figure 2A and 2B). CHF rats receiving increasing concentrations of T3 showed a significant dose-dependent decrease in serum T3 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. In contrast, the administration of T3 dose dependently increased serum T3 levels in CHF rats (Figure 2B). Importantly, a dose of 6 µgT3/kg/d restored T3 levels to significantly above those observed in either Sham-operated or saline-treated CHF rats.

Figure 2. Serum T4 and T3 concentrations: Measures before (baseline) and 1 week postsurgery in Sham and MI animals. Saline or T3 supplementation at 3, 6, and 60 µg/kg/d in MI animals began after week no. 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 versus baseline (t test); †P<0.025 versus Sham (t test); and ‡P<0.05 versus MI-saline (1-way ANOVA).

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 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 (60 µg/kg/d) 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 (Supplemental Figure I). This is probably due to the relatively small changes in T3 concentrations versus the thyrotoxic doses used in previous studies. Because of the small sample size in the telemetery data, a power analysis was used to calculate the number of animals required to demonstrate that the replacement dose of 6 µgT3/kg/d increases resting heart rate in conscious animals. Using the average difference in heart rate and average standard deviation between the MI-saline and MI-6 µg/kg/d animals over the 7 weeks of readings, the power analysis suggested that ~50 animals/group are needed to demonstrate a significant increase in heart rate of ~10 bpm. 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 6 µg/kg/d was sufficient to restore serum T3 levels without producing overt signs of thyrotoxicosis.

T3 Infusion Does Not Prevent LV Remodeling

A 2-way RM ANOVA was used to determine whether 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 with MI-saline animals, heart rate measured under anesthesia was elevated in animals receiving 6 µg/kg/d at the 9th week and in animals receiving 60 µg/kg/d 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 with sham-operated control animals over the 9 weeks study. This intraventricular conduction delay was not influenced by T₃ supplementation.

Echocardiography at all time points (1, 3, 5, 7, and 9 weeks), demonstrated that the anterior wall was significantly thinner in diastole and systole in MI-saline hearts versus 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 versus Sham controls (Figure 5A and 5B). Accordingly, LV fractional shortening and ejection fraction were significantly attenuated in MI-saline versus Sham controls (Figure 5C and 5D). Importantly, LV 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 versus Sham controls (4773±259 versus 6310±267 mm Hg/s, and 15.87±1.23 versus 11.11±0.25 ms, respectively). Contractility was significantly improved by administration of both 6 µg and 60 µgT₃/kg/d (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 20 µg/kg) between MI-saline and T₃-treated groups (data not shown).

**Myocardial Gene Expression**

To determine whether the physiological 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 \(23\pm5\%\) of controls) and dose dependently increased with T3 replacement at 6 and 60 \(\mu g/kg/d\) to \(42\pm4\%\) and \(150\pm14\%\) of controls \((P<0.05)\), respectively (Figure 7A). Beta-MHC expression was significantly reduced by T3 replacement at 6 and 60 \(\mu g/kg/d\) to \(71\pm12\%\) and \(73\pm21\%\) of controls \((P<0.05)\), respectively (Figure 7B).

**Discussion**

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

**T3 Replacement**

Proof of concept studies by Hamilton et al\(^{16}\) and recent studies by Pingitore et al\(^{18}\) have shown that physiological 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

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**Figure 4.** Serial heart rate and LV wall thickness measured by echocardiography: anesthetized heart rate (A). B and C, AW thickness in ED and ES, respectively. D and E, PW thickness in ED and ES, respectively. Significant differences: *\(P<0.025\) versus Sham (2-way RM ANOVA); #\(P<0.05\) versus MI-saline (2-way RM ANOVA). AW indicates anterior wall; ED, end diastole; ES, end systole; PW, posterior wall.
undergoing cardiac surgery have shown that correcting low T₃ levels improves cardiovascular function. 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. 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.

**MI Animal Model**

In the current study, the level of LV dysfunction induced by coronary artery ligation was assessed 1 week post-MI surgery to appropriately divide animals into experimental groups. In MI-saline animals, LV function measured by serial echocardiography and LV catheterization at 9 weeks was significantly attenuated throughout the study versus Sham-operated controls. Serum T₄ and T₃ levels were significantly lower 1 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 MI in a rodent model. 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 proinflammatory cytokines and/or increased clearance of T₃. Interleukins 1 and 6 were shown to competitively inhibit coactivators for type 1 deiodinase transcription, 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 models. D3 reduces T₃ concentrations by preventing its production (converting T₄ to inactive T₃), and increasing its degradation (converting T₃ to diiodothyronine). Simonides et al recently demonstrated that D3 expression can be activated by hypoxia inducible factor-1. During the early compensatory stages of a MI, hypoxia inducible factor-1 expression is increased in human hearts, and is also expressed in the nonischemic mechanically stressed myocardium in MI-rodent models. If hypoxia inducible factor-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 1 week post-MI, after significant LV remodeling had taken place. Recently, Chen et al, demonstrated that intraperitoneal injection of T₃ at 14 μg/kg/d 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 attenu-
improves LV function primarily through an increase in contractility. This effect may be linked to changes in α- and β-MHC expression. LV 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.

Acknowledgments
The authors acknowledge the expert technical assistance of John Barakat, for his echocardiography measurements.

Sources of Funding
These studies were supported by a grant from King Pharmaceutical Co, the Dr Ralph and Marian Falk Trust for Medical Research, and the Barry and Marilyn Rubinstein Charitable Trust.

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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CLINICAL PERSPECTIVE

Multiple studies have demonstrated that ≈30% of patients with congestive heart failure have low triiodothyronine (T₃) levels with the reduction in serum T₃ concentration proportional to the severity of heart disease. The low T₃ concentrations alter genomic and nongenomic mechanisms resulting in: reductions in left ventricular contractility, cardiac output, shifts in α- and β-myosin heavy chains, and sarcoplasmic reticulum Ca²⁺ ATPase expression. Importantly, reduced serum T₃ concentration is a strong predictor of all-cause and cardiovascular mortality. It follows that T₃ replacement in these patients might improve cardiovascular function. Previous animal studies have demonstrated that supraphysiologic T₃ doses given immediately after an infarction prevent left ventricular remodeling. Unfortunately, these doses may cause untoward side-effects such as increased myocardial oxygen consumption, and cardiac arrhythmias. The present study demonstrates that replacement of T₃ to euthyroid levels 1 week post-myocardial infarction significantly improves left ventricular function through an increase in contractility. This effect may be linked to changes in α- and β-myosin heavy chain expression. It remains to be determined whether immediate euthyroid T₃ replacement can prevent left ventricular remodeling. Overall, these studies suggest that returning patients to the euthyroid state may improve cardiovascular function secondary to T₃ mediated changes in gene expression and cell signaling.
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_Circ Heart Fail._ 2009;2:243-252; originally published online March 25, 2009;
doi: 10.1161/CIRCHEARTFAILURE.108.810747

_Circulation: Heart Failure_ is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
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Print ISSN: 1941-3289. Online ISSN: 1941-3297

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Data Supplement (unedited) at:
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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 xiphoid 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'-GTGGGATAGCAACAGCGAGGC-3'; GenBank accession no.AH002207) 28] 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'-GACAGGAAGAAGACCTACTGCG-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-intercept: 320
Slope: 0.04
r²: 0.063

B. Temperature vs. T3 Concentration

Y-intercept: 38.4
Slope: 3.67
r²: 2.12e-6

C. MAP vs. T3 Concentration

Y-intercept: 113
Slope: -0.08
r²: 0.063

D. Systolic Blood Pressure vs. T3 Concentration

Y-intercept: 122
Slope: -0.03
r²: 0.013

E. Diastolic Blood Pressure vs. T3 Concentration

Y-intercept: 103
Slope: -0.10
r²: 0.073
Figure 1: T$_3$ concentrations and hemodynamic variables: Within this experimental design, there were no significant correlations between T$_3$ concentrations and A. Heart Rate. B. Body Temperature. C. Mean Arterial Pressure (MAP). D/E. Systolic and Diastolic Blood Pressure, respectively.