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Thyroid Hormone Replacement Therapy Attenuates Atrial Remodeling and Reduces Atrial Fibrillation Inducibility in a Rat Myocardial Infarction-Heart Failure Model

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Abstract

Introduction—Heart failure (HF) is associated with increased atrial fibrillation (AF) risk.

Accumulating evidence suggests the presence of myocardial tissue hypothyroidism in HF, which may contribute to HF development. Our recent report demonstrated that hypothyroidism, like hyperthyroidism, leads to increased AF inducibility. This study was designed to investigate the effect of thyroid hormone (TH) replacement therapy on AF arrhythmogenesis in HF.

Methods and Results—Myocardial infarction (MI) was produced in rats by coronary artery ligation. Rats with large MIs (>40%) were randomized into L-thyroxine (T4, n=14) and placebo (n=15) groups 2 weeks after MI. Rats received 3.3 mg T4 (in 60-day release form) or placebo pellets in respective groups for 2 months. Compared with the placebo, T4 treatment improved cardiac function and decreased left ventricular internal diameters as well as left atrial diameter. T4 treatment attenuated atrial effective refractory period prolongation (45 ± 1.5 ms in placebo group vs 37 ± 1.6 ms in T4 group, $P < 0.01$) and reduced AF inducibility (AF/atrial flutter /tachycardia were inducible in 11/15 rats, or 73% in placebo vs 4/14 rats, or 29% in the T4 treated group, $P < 0.05$). Arrhythmia reduction was associated with decreased atrial fibrosis but was not associated with connexin 43 changes.

Conclusion—To our knowledge this is the first study demonstrating that TH replacement therapy in HF attenuates atrial remodeling and reduces AF inducibility post MI-HF. Clinical studies are needed to confirm such benefits in patients.

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Keywords

Atrial fibrillation; Heart failure; Thyroid hormone; Arrhythmogenesis

Introduction

Heart Failure (HF) and atrial fibrillation (AF) are two cardiac diseases of epidemic proportion.^{1, 2} The number of people who are affected by HF and AF are more than 5 million and 2.2 million respectively in the United States.^{3–6} AF has a very complex pathophysiology that depends strongly on underlying cardiovascular diseases, in particular HF. The relationship between HF and AF has been described as “HF begets AF, and AF begets HF”.^{7, 8} AF prevalence increases with the severity of HF. Risk of AF reaches about 50% in patients with New York Heart Association functional class IV HF.³ On the other hand, development of AF in HF patients is one of the leading causes of clinical deterioration.⁹ Although the causative relationship between the two conditions has not been fully determined; their coexistence can be explained by many risk factors that are shared amongst them.

Accumulating evidence has shown the presence of myocardial tissue hypothyroidism (low myocardial T3) in various cardiac diseases,^{10, 11} which may contribute to HF development.^{11, 12} Importantly, thyroid hormone (TH) replacement therapy has been shown to improve left ventricular function and structural remodeling in HF.^{11, 13–16} However, TH replacement therapy has not been adopted clinically in HF treatment. One of the major concerns in applying TH therapy in HF is that TH treatment might increase cardiac arrhythmias. The reason behind this concern is because hyperthyroidism can lead to increased atrial arrhythmogenesis,⁶ and potential TH overdosing and its associated arrhythmogenesis is a common fear.

Recently we have demonstrated that both hypothyroidism and hyperthyroidism can increase AF arrhythmogenesis.¹⁷ Based on this finding and evidence that myocardial tissue hypothyroidism is a common pathology in HF,¹¹ we hypothesized that myocardial tissue hypothyroidism may contribute to increased AF arrhythmogenesis in HF. Thus, correcting myocardial tissue hypothyroidism with TH replacement therapy may reduce, rather than increase AF risk in HF. Therefore, this study was designed to investigate the effect of TH replacement therapy on atrial remodeling and AF inducibility in a rat myocardial infarction (MI)-HF model.

Methods

This study was approved by the Institutional Animal Care and Use Committee at New York Institute of Technology College of Osteopathic Medicine and is in compliance with the “Guide for the Care and Use of Laboratory Animals” (NIH Publication No. 85-23, Revised 1996).

Animal model and study design

Adult (12 week old) female Sprague–Dawley rats (Harlan Laboratories, Indianapolis, IN) were used in this study. MI was produced by ligation of the left descending coronary artery, as described in our previous reports.^{14, 15} Two weeks after MI surgery, echocardiography was taken to determine MI size in all surviving animals. Based on our own experience and literature reports, a large MI is needed to develop HF and increase AF inducibility.^{18, 19} Thus, we enrolled only rats with large MI (>40% of left ventricular circumference in short axis view) in this study. Eligible rats (about 80%) were randomly assigned into the following 2 groups: MI control group (treated with placebo, n=15) and L-thyroxine (T4) treated group (MI+T4, n=14). Immediately after enrollment T4 pellets (3.3 mg, 60 days sustained release form, purchased from Innovative Research of America, Sarasota, FL) were implanted subcutaneously in MI+T4 group rats, as previously reported.^{15, 17} Placebo pellets were implanted in the MI control rats. The dosages of T4 pellets were chosen based on our previous studies in that the 3.3 mg T4 pellets improved left ventricular function and ventricular remodeling in MI rats.^{14, 15} After 2 months treatment, cardiac chamber dimensions and function were assessed by echocardiography and left ventricular (LV) catheterization. *In vivo* atrial electrophysiology and AF inducibility test using a catheter approach were performed for each animal at the end of the study. Animals were housed in our institutional animal facility and kept on a 12:12-h light-dark cycle and given standard rat chow and water *ad libitum*.

Echocardiographic measurements

As previously described,¹⁷ a GE Vivid 7 Dimension System (GE VINGMED ULTRASOUND A/S, Horten, Norway) coupled with a M12L linear (Matrix) array ultrasound transducer probe (5–13 MHz) was used to acquire echocardiographic data. Briefly, rats were anesthetized with 1.5% isoflurane. After the chest was shaved, the animals were placed on an isothermal pad that was maintained at ~40°C. Two-dimensional echocardiograms were obtained from short-axis and long axis views of the left ventricle (LV). MI size was determined from the short-axis view by measuring the length of the MI as a percentage of the LV circumference. Two-dimensionally targeted M-mode echocardiograms were used to measure the LV dimensions in systole and diastole. The following parameters were measured: anterior wall thickness in end-diastole and systole, left ventricular diastolic and systolic internal diameter, posterior wall thickness in end-diastole and systole, and LV fractional shortening. Left atrial diameter at the aortic valve level was also measured to estimate the atrial size.

Cardiac hemodynamic measurements

LV hemodynamics were obtained by catheterization of the right carotid artery using a SciSense pressure-volume catheter (Transonic Scisense Inc., London, Ontario, Canada), as previously described.¹⁷ Briefly, the right carotid artery was isolated and cannulated with a 1.9F SciSense pressure-volume catheter under isoflurane anesthesia. The tip of the catheter was advanced through the aorta into the left ventricle. The following parameters were measured: heart rate, LV peak systolic pressure (LVSP), LV end-diastolic pressure

(LVEDP), and positive/negative change in pressure over time ($\pm dP/dt$). The data were acquired and analyzed by the LabScribe software (iWorx Systems, Inc., Dover, NH).

Electrophysiology study and AF inducibility test

In vivo cardiac electrophysiology was assessed using a 1.6F octopolar Millar electrophysiology catheter (EPR-802, Millar Instruments, Inc., Houston, Texas), as previously described.¹⁷ Briefly, the catheter was inserted through the right jugular vein and advanced into the right atrium with 8 poles recording atrial electrograms. Standard surface ECG lead II and 3 right atrial electrocardiograms from 3 pairs of electrodes were displayed and recorded using PowerLab data acquisition systems (ADInstruments, Colorado Springs, CO). The purpose of recording 3 atrial electrograms from distal, middle, and proximal pairs was to facilitate determination of atrial capturing and AF pattern.

Regular pacing and standard S1S2 programmed pacing protocols were used to determine sinus node recovery time and atrial effective refractory period (ERP). The atria were paced at 3x threshold at cycle length of 150 ms or 20 ms shorter than the spontaneous sinus cycle length. Atrial ERP was defined as the longest coupling intervals which did not capture the atria.

Burst pacing containing 200 impulses at 50 Hz was used to induce AF. The duration of the subsequent spontaneous arrhythmias after burst pacing was documented. For each animal, the average arrhythmia duration based on 5 such tests was calculated.

AF was defined as irregular, rapid atrial activations with varying electrogram morphology lasting ≥ 0.5 second, as described previously.¹⁷ The atrial rates in AF were typically >1500 bpm in rats. We noticed in some rats that the induced atrial arrhythmias did not conform to the above AF definition. The induced atrial arrhythmias had regular atrial activation and the rates (ranged from 900 bpm to 1300 bpm) were slower than that in AF. These arrhythmias were analogous to atrial flutter or atrial tachycardia in patients. Since currently there is no clear definition available in the literature for what atrial rates constitute atrial flutter or atrial tachycardia, we generally defined these arrhythmias as atrial flutter/atrial tachycardia. In this study, we have combined typical AF and atrial flutter/atrial tachycardia as a single entity of atrial tachyarrhythmias. Because the induced arrhythmias typically lasted only a few seconds in rats, we assumed the induced arrhythmia was “long-lasting” when the induced spontaneous arrhythmia lasted ≥ 5 minutes. As a result the longest possible duration was 300 second (5 minutes) in this study, observed in 1 rat.

Atrial collagen content and connexin (Cx) 43 immunohistochemical staining

After collecting the above data, the left atrial appendage was taken and immersion-fixed with 4% paraformaldehyde and then processed by paraffin embedding. Serial histological sections (6.0- μ m-thick) were cut and processed with Masson's trichrome stain for determination of fibrosis.

A separate group of sections were immunostained with a monoclonal antibody against rat Cx43 (1:500, MAB3068; Millipore, Temecula, CA). The Cx43 antibody was visualized using a goat-anti-mouse Rhodamine Red-X-conjugated antibody (1:200, 115-295-146;

Jackson ImmunoResearch Laboratories, Inc., West Grove, PA). In addition, we utilized a rabbit anti-laminin antibody (1:30; L9393, Sigma, St Louis, MO) that was visualized by a goat anti-rabbit AlexaFluor 488 antibody (1:200; Molecular Probes Inc., Eugene, OR) to outline the profiles of cardiac myocytes. Nuclei were counterstained with DAPI (4', 6-Diamidino-2-phenylindole dihydrochloride).

Stained sections were examined using an Olympus BX53 microscope and high-resolution digital images were captured at x40 magnification with an Olympus DP72 digital camera. Morphometric and stereological analyses of digitized images were performed in a blinded manner using Image-Pro Analyzer 7.0 software (Media Cybernetics, Inc., Bethesda, MD).

The extent of interstitial fibrosis was estimated using Masson-stained sections and was expressed as the percentage of the total area occupied by interstitial tissue and cardiac myocytes. For each appendage, 5 optical fields were examined and the average data was obtained.

The density of Cx43 was estimated using sections immunostained with anti-Cx43 antibody and was expressed as the percentage of the total area occupied by cardiac myocytes in which expression of Cx43 was determined.

Statistical analysis

All data are expressed as mean $\pm$ standard error of the mean (SEM) where appropriate. Comparison of the AF incidence between the 2 groups was performed using Fisher's exact test. Student's group t-test was used for comparison of other parameters. Because the AF duration data were not normally distributed, square root transformation was applied before performing the t-test. A $P < 0.05$ was required for statistical significance.

Results

Effects of T4 treatment on heart weights and body weight

All rats completed the 8 weeks treatment period. There was no mortality in either the control or T4 treated groups. As shown in Table 1, body weight in T4 treated rats was not statistically different from the control rats. Both total heart weight and LV weight in the T4 treated group were slightly higher than that in the control group. However, the heart weight was not significantly different after adjustment for body weight.

Effects of T4 on echocardiographic parameters

MI size, cardiac dimensions and LV function acquired by echocardiography before terminal experiments are reported in Figure 1. The MI size was comparable in the 2 groups. All rats had enlarged LV chamber dimensions and reduced LV fractional shortening (signs of HF). Compared with the MI control rats, T4 treatment reduced systolic and diastolic LV chamber dimensions and increased LV fractional shortening. There was a trend of increased LV wall thickness, but it was not statistically significant. T4 treatment significantly reduced left atrial diameter.

Effects of T4 on LV hemodynamics

LV hemodynamic data are shown in Figure 2. Compared with the MI control rats, T4 treatment increased LV systolic pressure, LV $\pm dp/dt$ and decreased LVEDP, indicating improved systolic and diastolic LV function.

Effects of T4 on cardiac electrophysiology and AF inducibility

Basic cardiac electrophysiology data are shown in Table 2. The heart rate was slightly higher in T4 treated rats compared to MI control rats (Table 2). The T4 treated rats had shorter corrected sinus node recovery time compared with the control rats. In addition, the atrial effective refractory period was significantly shorter in T4 treated rats compared with the control rats. T4 treatment also reduced atrioventricular conduction time.

Figure 3 shows examples of original ECG and atrial electrogram traces in a rat with induced AF (top panel) and in a rat with induced atrial flutter/tachycardia (bottom panel). The atrial electrograms in AF were irregular and with varying morphology, while atrial flutter/tachycardia had regular and slower atrial activation, compared to the AF traces.

Atrial tachyarrhythmias (AF in 8, atrial flutter/tachycardia in 2, and both AF and atrial flutter/tachycardia in 1) were induced in 11/15 rats, or 73%, in the MI control group (Figure 4, top). In contrast, AF was induced in 4/14 rats or 29% of the T4 treated group ($P=0.027$) and no other arrhythmia was induced in this group. The induced AF duration was also significantly shorter in the T4 treated rats (Figure 4, bottom). The duration was 21.0 ± 19.9 s in MI control versus 0.3 ± 0.2 s in T4 treated rats, $P=0.026$.

Effects of T4 on atrial fibrosis and Cx43 density

As shown in Figure 5, T4 treatment significantly decreased left atrial interstitial fibrosis compared with the control group.

Left atrial myocyte Cx43 immunostaining is shown in Figure 6. The Cx43 density was not statistically different between the treated and the control groups.

Discussion

Major findings

This study examined the effects of TH replacement therapy on atrial remodeling and AF arrhythmogenesis in a rat MI-HF model. Besides confirmation of hemodynamic benefits of TH replacement therapy in HF, our results demonstrated that TH treatment attenuated atrial remodeling and decreased AF inducibility in this animal model. This is in contrast to the common fear that TH treatment may increase cardiac arrhythmias in HF. Thus, our data highlights the potential clinical importance of correcting thyroid dysfunction to prevent cardiac arrhythmias and AF in HF. In other words, withholding TH replacement treatment in HF may do harm.

Current concerns and evidence for TH replacement therapy in HF

Current medical practice to avoid TH replacement therapy in HF may have been unduly influenced by results from the 1972 Coronary Drug Project²⁰, which used an excessive dose of “inactive” D-T4 later found to be contaminated with a toxic dose of active L-T4 in an effort to reduce serum cholesterol. The results showed a slight increase in arrhythmias and mortality, which was not surprising. The message from this study should be that toxic doses of TH may lead to increased arrhythmias and mortality rather than promoting fear of restoring normal TH levels in patients with heart diseases. Aside from another phase II clinical trial using the TH analog DITPA, which reported some hemodynamic and metabolic improvements but no signs of symptomatic relief,²¹ many other short-term studies have demonstrated safety and efficacy of TH replacement therapy in HF.¹¹ It is also worth noting that the DITPA trial likely used an excessive dose too, since treated patients had increased heart rate, decreased body weight, and diarrhea.

Accumulating evidence indicates that myocardial hypothyroidism may contribute to HF development and TH replacement therapy has been shown to improve cardiac function in HF. It has been reported that approximately half of cardiac patients may have diagnosable hypothyroidism or borderline low THs.¹⁰ Animal data suggest that heart diseases in general, activate an enzyme (D3 deiodinase, which converts T4 into the inactive metabolite reverse T3 and also degrades T3) that induces cardiac tissue hypothyroidism.^{22, 23} Data from our animal model suggest that blood TH levels often underestimate the extent of low cardiac T3 in HF.¹¹ Consequently, it is likely that low levels of cardiac tissue T3 may be present in a large majority of HF patients. Importantly, we have demonstrated that hypothyroidism itself can lead to HF.^{11, 12} Thus, cardiac tissue hypothyroidism coexists in HF and may contribute to HF development. Consistent with this theory, recent clinical data^{24, 25} found that increased TSH levels (a clinical marker of hypothyroidism) are associated with worse clinical outcome in patients with HF.

By restoring normal TH function, TH replacement therapy in HF may offer dramatic benefits. Data from our group and others have demonstrated that TH replacement therapy can improve cardiac function in HF.^{11, 13–16} Data from the current study further confirmed the hemodynamic benefits with TH replacement therapy in HF (Figures 1 and 2).

TH replacement therapy may attenuate atrial remodeling and reduce AF arrhythmogenesis in HF

Potential arrhythmogenesis is currently a clinical concern with TH replacement therapy in HF. As mentioned before, the 1972 Coronary Drug Project²⁰ showed a slight increase in arrhythmias and mortality with a toxic dose of D-T4/L-T4. It is well known that hyperthyroidism can increase cardiac arrhythmias, especially AF.⁶ HF also increases AF risk.³ Thus, the current conventional clinical belief is to avoid TH treatment in HF for fear that TH therapy might increase cardiac arrhythmogenesis.

While it is well known that hyperthyroidism increases AF, even subclinical hyperthyroidism is associated with increased AF risk.⁶ However, clinical studies suggest hypothyroidism and subclinical hypothyroidism also promote arrhythmias.^{26, 27} Failure to identify and correct

these conditions may actually be contributing to increased arrhythmias in cardiac patients as well. We have recently demonstrated under controlled experimental conditions that both hypothyroidism and hyperthyroidism are pro-arrhythmic.¹⁷ Based on this finding and the evidence that myocardial tissue hypothyroidism is a common pathology in HF,¹¹ we postulated that myocardial tissue hypothyroidism may contribute to increased AF arrhythmogenesis in HF and thus correcting myocardial tissue hypothyroidism with TH replacement therapy may reduce, rather than increase AF risk in HF.

To our knowledge, this is the first study designed to investigate TH replacement therapy on atrial remodeling and AF arrhythmogenesis in HF. Our results have demonstrated that TH replacement therapy attenuated atrial remodeling in HF and reduced atrial tachyarrhythmia inducibility (Figure 4). In line with our finding, there is a clinical report that TH (triiodothyronine, T3) therapy reduced AF incidence after cardiac operations.²⁸

Potential mechanisms underlying TH replacement therapy in reducing AF in HF

Normal TH levels are required in adult life to maintain normal cardiovascular physiology and function, including heart rate, cardiac structure, myocardial contractility and vascular function.^{11, 12, 29–31} As discussed already, there is evidence that myocardial hypothyroidism occurs in HF, which in turn may contribute to HF development.¹¹ Thus, correcting myocardial hypothyroidism may attenuate HF development and its associated complications.

We believe that the attenuated atrial remodeling by TH replacement therapy may be related to overall cardiac function and hemodynamic improvement, as evidenced with increased cardiac contractility and decreased LVEDP. The decreased LVEDP indicates less increase in atrial pressure and thus less atrial dilation, as reflected by the reduced atrial diameter in the TH treated animals (Figure 1).

We found that TH replacement therapy reduced atrial fibrosis. Atrial fibrosis is considered to be a major factor in promoting AF in HF.¹⁹ Since it is known that hypothyroidism promotes cardiac fibrosis,^{32, 33} it is possible that TH treatment may affect atrial fibrosis, independent of hemodynamic status. The reduced fibrosis content could explain decreased atrial tachyarrhythmia inducibility in the treated animals. In addition, it may also explain attenuated atrial ERP prolongation in the TH treated group. However, our data cannot answer whether the reduced AF inducibility in the treated group is due to a direct effect on atrial electrophysiological and structural remodeling or secondary to ventricular functional improvement. These important mechanistic questions deserve further investigation.

We examined Cx43 expression in atrial tissue but did not find significant changes by TH replacement therapy. Cx43 plays an important role in cell-cell coupling. It has been reported that HF results in redistribution of Cx43 toward transverse cell-boundaries (lateralization of connexin 43 expression) in dogs.^{34, 35} We noticed that the myocardial fibers in rat atrial appendage were not well aligned in a longitudinal direction. It was difficult, if not impossible, to quantify the lateral versus end to end pattern of Cx43 distribution in our sections. In this study, we only determined Cx43 density and it was not altered significantly by TH replacement therapy.

Study limitation

The T4 dosage used in this study has been found to improve cardiac function and remodeling in our previous studies.^{14, 15} It was found that the given dosage increased blood T4 level, but T3 was not significantly altered.¹⁵ Nevertheless, the treated group had a slightly higher heart rate. It was possible that the higher heart rate may indicate the dosage was too high for long-term use. However, hyperthyroidism, even subclinical hyperthyroidism, is associated with increased AF risk clinically,⁶ while AF was reduced in the treated animals in this study. Another possible mechanism for increased heart rate is that TH treatment may improve sinus node function, resulting in a higher heart rate. It has been reported that HF impairs sinus node function.³⁶ Our result showed shortened sinus node recovery time in TH treated animals, indicating TH treatment was associated with improved sinus node function.

Since the blood TH levels may not reflect cardiac tissue TH levels in HF, tissue TH levels may be a more precise measure of organ thyroid status. It would be desirable if cardiac tissue TH levels were determined to confirm myocardial hypothyroidism in HF and normalization of myocardial TH levels with T4 replacement therapy. Unfortunately, rat atrial tissue is too small to conduct such measurements. Thus, the optimal dosages of TH remain to be determined.

In this study only female rats were enrolled. Unlike male rats, female rats maintain a relatively stable body mass during altered thyroid states. In addition, females have a much greater incidence of thyroid disorders in the general population. However, the selection of female rats only may affect generalization of the results to male rats. Thus, whether similar effects could be observed in male rats needs further investigation.

It should be noted that normal rats-or even large animals like dogs-are resistant to AF induction. While disease states (e.g., heart failure) can increase AF inducibility, the induced AF duration is relatively short. “Long-last” AF (> 5 minutes) in rats was rare, which was seen only in 1 animal in this study.

We have to acknowledge that this is not a mortality study. Whether the improved cardiac function and reduced atrial tachyarrhythmia are associated with favorable long-term survival remains to be investigated.

Finally due to the nature of animal studies, direct translation of these experimental findings from rodents to patients is not warranted. It may be prudent to confirm the existence of cardiac tissue hypothyroidism in failing human hearts before large clinical trials could be planned.

Conclusion

In contrast to the common clinical concern of increased arrhythmogenesis, our study demonstrated that TH replacement therapy in HF attenuated atrial remodeling and reduced atrial tachyarrhythmia inducibility in a rat MI-HF model. Clinical studies are needed to confirm such beneficial effects in HF patients.

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References

1. Braunwald E. Shattuck lecture--cardiovascular medicine at the turn of the millennium: triumphs, concerns, and opportunities. *The New England journal of medicine*. 1997; 337:1360–9. [PubMed: 9358131]
2. Seiler J, Stevenson WG. Atrial fibrillation in congestive heart failure. *Cardiology in review*. 2010; 18:38–50. [PubMed: 20010337]
3. Feinberg WM, Blackshear JL, Laupacis A, Kronmal R, Hart RG. Prevalence, age distribution, and gender of patients with atrial fibrillation. Analysis and implications. *Archives of internal medicine*. 1995; 155:469–73. [PubMed: 7864703]
4. Go AS, Hylek EM, Phillips KA, Chang Y, Henault LE, Selby JV, Singer DE. Prevalence of diagnosed atrial fibrillation in adults: national implications for rhythm management and stroke prevention: the AnTicoagulation and Risk Factors in Atrial Fibrillation (ATRIA) Study. *JAMA: the journal of the American Medical Association*. 2001; 285:2370–5.
5. Lloyd-Jones D, Adams R, Carnethon M, De Simone G, Ferguson TB, Flegal K, Ford E, Furie K, Go A, Greenlund K, Haase N, Hailpern S, Ho M, Howard V, Kissela B, Kittner S, Lackland D, Lisabeth L, Marelli A, McDermott M, Meigs J, Mozaffarian D, Nichol G, O'Donnell C, Roger V, Rosamond W, Sacco R, Sorlie P, Stafford R, Steinberger J, Thom T, Wasserthiel-Smoller S, Wong N, Wylie-Rosett J, Hong Y. Heart disease and stroke statistics--2009 update: a report from the American Heart Association Statistics Committee and Stroke Statistics Subcommittee. *Circulation*. 2009; 119:480–6. [PubMed: 19171871]
6. Fuster V, Ryden LE, Cannom DS, Crijns HJ, Curtis AB, Ellenbogen KA, Halperin JL, Kay GN, Le Huez JY, Lowe JE, Olsson SB, Prystowsky EN, Tamargo JL, Wann LS, Smith SC Jr, Priori SG, Estes NA 3rd, Ezekowitz MD, Jackman WM, January CT, Page RL, Slotwimer DJ, Stevenson WG, Tracy CM, Jacobs AK, Anderson JL, Albert N, Buller CE, Creager MA, Ettinger SM, Guyton RA, Hochman JS, Kushner FG, Ohman EM, Tarkington LG, Yancy CW. 2011 ACCF/AHA/HRS focused updates incorporated into the ACC/AHA/ESC 2006 guidelines for the management of patients with atrial fibrillation: a report of the American College of Cardiology Foundation/American Heart Association Task Force on practice guidelines. *Circulation*. 2011; 123:e269–367. [PubMed: 21382897]
7. Efremidis M, Pappas L, Sideris A, Filippatos G. Management of atrial fibrillation in patients with heart failure. *Journal of cardiac failure*. 2008; 14:232–7. [PubMed: 18381187]
8. Maisel WH, Stevenson LW. Atrial fibrillation in heart failure: epidemiology, pathophysiology, and rationale for therapy. *The American journal of cardiology*. 2003; 91:2D–8D.
9. Hunt SA, Abraham WT, Chin MH, Feldman AM, Francis GS, Ganiats TG, Jessup M, Konstam MA, Mancini DM, Michl K, Oates JA, Rahko PS, Silver MA, Stevenson LW, Yancy CW, Antman EM, Smith SC Jr, Adams CD, Anderson JL, Faxon DP, Fuster V, Halperin JL, Hiratzka LF, Jacobs AK, Nishimura R, Ornato JP, Page RL, Riegel B. ACC/AHA 2005 Guideline Update for the Diagnosis and Management of Chronic Heart Failure in the Adult: a report of the American College of Cardiology/American Heart Association Task Force on Practice Guidelines (Writing Committee to Update the 2001 Guidelines for the Evaluation and Management of Heart Failure): developed in collaboration with the American College of Chest Physicians and the International Society for Heart and Lung Transplantation: endorsed by the Heart Rhythm Society. *Circulation*. 2005; 112:e154–235. [PubMed: 16160202]
10. Iervasi G, Pingitore A, Landi P, Raciti M, Ripoli A, Scarlattini M, L'Abbate A, Donato L. Low-T3 syndrome: a strong prognostic predictor of death in patients with heart disease. *Circulation*. 2003; 107:708–13. [PubMed: 12578873]

11. Gerdes AM, Iervasi G. Thyroid replacement therapy and heart failure. *Circulation*. 2010; 122:385–93. [PubMed: 20660814]
12. Tang YD, Kuzman JA, Said S, Anderson BE, Wang X, Gerdes AM. Low thyroid function leads to cardiac atrophy with chamber dilatation, impaired myocardial blood flow, loss of arterioles, and severe systolic dysfunction. *Circulation*. 2005; 112:3122–30. [PubMed: 16275864]
13. Henderson KK, Danzi S, Paul JT, Leya G, Klein I, Samarel AM. Physiological replacement of T3 improves left ventricular function in an animal model of myocardial infarction-induced congestive heart failure. *Circulation Heart failure*. 2009; 2:243–52. [PubMed: 19808346]
14. Chen YF, Pottala JV, Weltman NY, Ge X, Savinova OV, Gerdes AM. Regulation of gene expression with thyroid hormone in rats with myocardial infarction. *PloS one*. 2012; 7:e40161. [PubMed: 22870193]
15. Chen YF, Weltman NY, Li X, Youmans S, Krause D, Gerdes AM. Improvement of left ventricular remodeling after myocardial infarction with eight weeks L-thyroxine treatment in rats. *Journal of translational medicine*. 2013; 11:40. [PubMed: 23409791]
16. Pantos C, Mourouzis I, Markakis K, Tsigoulis N, Panagiotou M, Cokkinos DV. Long-term thyroid hormone administration reshapes left ventricular chamber and improves cardiac function after myocardial infarction in rats. *Basic research in cardiology*. 2008; 103:308–18. [PubMed: 18274800]
17. Zhang Y, Dedkov EI, Teplitsky D, Weltman NY, Pol CJ, Rajagopalan V, Lee B, Gerdes AM. Both hypothyroidism and hyperthyroidism increase atrial fibrillation inducibility in rats. *Circulation Arrhythmia and electrophysiology*. 2013; 6:952–9. [PubMed: 24036190]
18. Pfeffer MA, Pfeffer JM, Fishbein MC, Fletcher PJ, Spadaro J, Kloner RA, Braunwald E. Myocardial infarct size and ventricular function in rats. *Circulation research*. 1979; 44:503–12. [PubMed: 428047]
19. Cardin S, Guasch E, Luo X, Naud P, Le Quang K, Shi Y, Tardif JC, Comtois P, Nattel S. Role for MicroRNA-21 in atrial profibrillatory fibrotic remodeling associated with experimental postinfarction heart failure. *Circulation Arrhythmia and electrophysiology*. 2012; 5:1027–35. [PubMed: 22923342]
20. The coronary drug project. Findings leading to further modifications of its protocol with respect to dextrothyroxine. The coronary drug project research group. *JAMA: the journal of the American Medical Association*. 1972; 220:996–1008.
21. Goldman S, McCarren M, Morkin E, Ladenson PW, Edson R, Warren S, Ohm J, Thai H, Churby L, Barnhill J, O'Brien T, Anand I, Warner A, Hattler B, Dunlap M, Erikson J, Shih MC, Lavori P. DITPA (3,5-Diiodothyropropionic Acid), a thyroid hormone analog to treat heart failure: phase II trial veterans affairs cooperative study. *Circulation*. 2009; 119:3093–100. [PubMed: 19506112]
22. Wassen FW, Schiel AE, Kuiper GG, Kaptein E, Bakker O, Visser TJ, Simonides WS. Induction of thyroid hormone-degrading deiodinase in cardiac hypertrophy and failure. *Endocrinology*. 2002; 143:2812–5. [PubMed: 12072417]
23. Pol CJ, Muller A, Zuidwijk MJ, van Deel ED, Kaptein E, Saba A, Marchini M, Zucchi R, Visser TJ, Paulus WJ, Duncker DJ, Simonides WS. Left-ventricular remodeling after myocardial infarction is associated with a cardiomyocyte-specific hypothyroid condition. *Endocrinology*. 2011; 152:669–79. [PubMed: 21159857]
24. Chen S, Shauer A, Zwas DR, Lotan C, Keren A, Gotsman I. The effect of thyroid function on clinical outcome in patients with heart failure. *European journal of heart failure*. 2014; 16:217–26. [PubMed: 24464626]
25. Mitchell JE, Hellkamp AS, Mark DB, Anderson J, Johnson GW, Poole JE, Lee KL, Bardy GH. Thyroid Function in Heart Failure and Impact on Mortality. *JACC Heart failure*. 2013; 1:48–55. [PubMed: 24159562]
26. Tanase DM, Ionescu SD, Ouatu A, Ambarus V, Arsenescu-Georgescu C. Risk assessment in the development of atrial fibrillation at patients with associate thyroid dysfunctions. *Revista medico-chirurgicala a Societatii de Medici si Naturalisti din Iasi*. 2013; 117:623–9. [PubMed: 24502026]
27. Unal O, Erturk E, Ozkan H, Kiyici S, Guclu M, Ersoy C, Yener F, Imamoglu S. Effect of levothyroxine treatment on QT dispersion in patients with subclinical hypothyroidism. *Endocrine*

- practice: official journal of the American College of Endocrinology and the American Association of Clinical Endocrinologists. 2007; 13:711–5.
28. Klemperer JD, Klein IL, Ojamaa K, Helm RE, Gomez M, Isom OW, Krieger KH. Triiodothyronine therapy lowers the incidence of atrial fibrillation after cardiac operations. *The Annals of thoracic surgery*. 1996; 61:1323–7. discussion 1328–9. [PubMed: 8633935]
 29. Klein I, Ojamaa K. Thyroid hormone and the cardiovascular system. *The New England journal of medicine*. 2001; 344:501–9. [PubMed: 11172193]
 30. Fazio S, Palmieri EA, Lombardi G, Biondi B. Effects of thyroid hormone on the cardiovascular system. *Recent progress in hormone research*. 2004; 59:31–50. [PubMed: 14749496]
 31. Danzi S, Klein I. Thyroid hormone and the cardiovascular system. *The Medical clinics of North America*. 2012; 96:257–68. [PubMed: 22443974]
 32. Burstein B, Nattel S. Atrial fibrosis: mechanisms and clinical relevance in atrial fibrillation. *Journal of the American College of Cardiology*. 2008; 51:802–9. [PubMed: 18294563]
 33. Chen WJ, Lin KH, Lee YS. Molecular characterization of myocardial fibrosis during hypothyroidism: evidence for negative regulation of the pro- $\alpha 1(I)$ collagen gene expression by thyroid hormone receptor. *Molecular and cellular endocrinology*. 2000; 162:45–55. [PubMed: 10854697]
 34. Hsieh MH, Lin YJ, Wang HH, Lo LW, Chang SL, Yan YL, Chou TY, Chen SA, Yeh HI. Functional characterization of atrial electrograms in a pacing-induced heart failure model of atrial fibrillation: importance of regional atrial connexin40 remodeling. *Journal of cardiovascular electrophysiology*. 2013; 24:573–82. [PubMed: 23421445]
 35. Burstein B, Comtois P, Michael G, Nishida K, Villeneuve L, Yeh YH, Nattel S. Changes in connexin expression and the atrial fibrillation substrate in congestive heart failure. *Circulation research*. 2009; 105:1213–22. [PubMed: 19875729]
 36. Yanni J, Tellez JO, Maczewski M, Mackiewicz U, Beresewicz A, Billeter R, Dobrzynski H, Boyett MR. Changes in ion channel gene expression underlying heart failure-induced sinoatrial node dysfunction. *Circulation Heart failure*. 2011; 4:496–508. [PubMed: 21565973]

Figure 1.

Echocardiographic parameters. Values are means±SEM. For each parameter, P value is indicated. LVFS, left ventricular fractional shortening; LADd, left atrial diameter in diastole; MI, myocardial infarction; AWTd, left ventricular anterior wall thickness in diastole; AWTs, left ventricular anterior wall thickness in systole; LVDd, left ventricular diameter in diastole; LVDs, left ventricular diameter in systole; PWTd, left ventricular posterior wall thickness in diastole; PWTs, left ventricular posterior wall thickness in systole.

Figure 2.

Left ventricular hemodynamics. Data presentation and P value are indicated as in Figure 1. LVSP, left ventricular systolic pressure; LVEDP, left ventricular end-diastolic pressure; +dp/dt, positive change in pressure over time; -dp/dt, negative change in pressure over time.

Figure 3.

Atrial fibrillation (AF) inducibility test. The original ECG traces show an example of typical AF after the burst pacing. Atrial electrograms (RA1, RA2) demonstrate rapid, irregular atrial activations with varying electrogram morphology (top panel). Note that different atrial electrical activation pattern recorded from high right atrium (RA1) and lower right atrium (RA2) during AF in this animal. The bottom panel shows an example of induced atrial flutter/tachycardia after burst pacing. The atrial activation is regular and slower (compared to AF in top panel). Note that atrial cycle lengths are the same in the RA1 and RA2. RA: right atrial electrogram.

Figure 4. Atrial fibrillation (AF) incidence and duration in the studied groups. Note that a logarithmic scale is used for the AF duration data. Data presentation and P value are indicated as in Figure 1.

Figure 5.

Left atrial fibrosis content. Representative photomicrographs of left atrial histological slides (Masson Trichrome stain) from 1 rat in each group are shown on top. Data presentation and P value are indicated as in Figure 1.

Figure 6.

Left atrial myocytes connexin (Cx) 43 expression. Representative photomicrographs of left atrial myocytes Cx43 immunostaining (red) combined with laminin immunostaining (green) outlining the myocardial tissue from 1 rat in each group are shown on top. Data presentation and P value are indicated as in Figure 1.

Table 1

Body weight, heart weight and ratio of heart weight/body weight

	Body weight(g)	Heart weight(mg)	LV weight (mg)	HW/BW(mg/g)
MI Control	269±3.7	1335±23	896±17	4.98±0.11
MI + T4	278±5.3	1456±50	971±29	5.26±0.17
P value	0.182	0.037	0.039	0.199

Values are means ± SEM. LV indicates left ventricle; HW/BW indicates ratio of heart weight/body weight.

Table 2

Electrophysiological parameters

	Heart rate(bpm)	SNRTc(ms)	AERP(ms)	AVCT(ms)
MI Control	321±6.2	38±1.6	45±1.5	56±1.9
MI + T4	384±9.9	30±1.4	37±1.6	48±1.2
P value	<0.001	0.002	0.001	0.002

Values are means ± SEM. SNRTc indicates corrected sinus node recovery time; AERP, atrial effective refractory period; AVCT, atrioventricular conduction time.