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Low Thyroid Function Leads to Cardiac Atrophy With Chamber Dilatation, Impaired Myocardial Blood Flow, Loss of Arterioles, and Severe Systolic Dysfunction

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Background—Although thyroid dysfunction has been linked to heart failure, it is not clear whether hypothyroidism alone can cause heart failure.

Methods and Results—Hypothyroidism was induced in adult rats by treatment with 0.025% propylthiouracil (PTU) for 6 weeks (PTU-S) and 1 year (PTU-L). Echocardiographic measurements, left ventricular (LV) hemodynamics, isolated myocyte length (KOH method), myocardial blood flow (fluorescent microspheres), arteriolar morphometry, and gene expression (Western blot) were determined. Heart weight, heart rate, LV systolic blood pressure, LV ejection fraction, LV fractional shortening, and systolic wall thickness were reduced in PTU-S and PTU-L rats. LV internal diameter in systole increased by 40% in PTU-S and 86% in PTU-L. LV internal dimension in diastole was increased in PTU-S and PTU-L rats, but only PTU-L rats showed a significant increase in myocyte length due to series sarcomere addition. Resting and maximum (adenosine) myocardial blood flow were reduced in both PTU-S and PTU-L rats. Impaired blood flow was due to a large reduction in arteriolar length density and small arterioles in PTU-S and PTU-L ($P < 0.05$ or greater for all of the above comparisons). Expression of sarcoplasmic/endoplasmic reticulum Ca^{2+} -ATPase (SERCA)-2a and α -myosin heavy chain were reduced in hypothyroidism, whereas phospholamban and β -myosin heavy chain were increased.

Conclusions—Hypothyroidism led to severe, progressive systolic dysfunction and increased chamber diameter/wall thickness ratio despite a reduction in cardiac mass. Chamber dilatation in PTU-L rats was due to series sarcomere addition, typical of heart failure. Hypothyroidism resulted in impaired myocardial blood flow due to a dramatic loss of arterioles. Thus, we have identified 2 important new mechanisms by which low thyroid function may lead to heart failure. (*Circulation*. 2005;112:3122-3130.)

Key Words: heart failure ■ hormones ■ myocytes ■ pathology ■ remodeling

The cardiovascular system is one of the most important targets on which thyroid hormones act.^{1,2} It has long been recognized that low thyroid function has profound effects on the cardiovascular system, such as impaired cardiac contractility, decreased cardiac output, increased systemic vascular resistance, reduced chronotropy, and cardiac atrophy.³⁻⁷ Growing evidence also suggests that thyroid dysfunction is an independent risk factor for the progression of heart disease to heart failure (HF) and might represent a determining factor directly implicated in the evolution and prognosis of these patients.⁸⁻¹³

In general, hypothyroidism is known to cause a decrease in ventricular pressure and contractility, unloading of the heart, and cardiomyocyte atrophy.^{1,7,14} Hypothyroidism leads to decreased cardiac efficiency in that work is more severely

depressed than oxidative metabolism.¹⁵ This condition has dramatic negative effects on contractile and calcium handling proteins.^{16,17} Because chronic hypothyroidism has been readily diagnosed and treated for many decades, reports in recent years have been limited largely to case studies.¹⁸ In older studies, however, symptoms of HF, including cardiac dilatation, were reported in patients with chronic hypothyroidism.^{19,20} To this day, it is not clear whether severe, chronic hypothyroidism can eventually lead to overt HF. This is not an easy question to answer, because hypothyroidism leads to cardiovascular changes that clearly overlap with HF (eg, reduced cardiac output and contractility and increased chamber diameter/wall thickness). Additionally, changes produced by cardiac unloading may be difficult to differentiate from alterations due to HF.

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Several potential cellular mechanisms by which chronic low thyroid function may contribute to HF have been identified. Hypothyroidism may lead to (1) altered blood lipids and accelerated atherosclerosis, (2) stimulation of myocardial fibrosis, (3) vasoconstriction, and (4) induction of a gene program resembling that of pathological hypertrophy. The purpose of the present study was to provide comprehensive temporal characterization of pathophysiological changes in the heart due to hypothyroidism. Results have identified 2 new cellular mechanisms by which low thyroid function may promote or actually lead to HF.

Methods

Animal Model

Hypothyroidism was induced in 6-month-old female Sprague-Dawley rats (Harlan, Indianapolis, IN) by the addition of PTU (0.025% 6-*n*-propyl-2-thiouracil, Sigma-Aldrich) to drinking water. The effects of short-term (6 weeks, PTU-S) and long-term treatment (1 year, PTU-L) were examined. All procedures were approved by the University of South Dakota Animal Care and Use Committee and followed institutional guidelines for treatment of animals.

Experimental Design

All animals were maintained in the same environment, including the same temperature and humidity and free access to food and water. In the terminal experiment, serum thyroid hormone levels, echocardiography, hemodynamics, myocardial blood flow, tissue histology, tissue morphometry, isolated myocyte length, arteriolar quantitation, and Western blots were collected from each animal group.

Serum Levels of Total Triiodothyronine, Total Thyroxine, and Thyroid-Stimulating Hormone

Blood samples were separated into serum aliquots and frozen. Triiodothyronine (T3), thyroxine (T4), and thyroid-stimulating hormone (TSH) levels were measured with a solid-phase competitive ELISA kit according to the manufacturer's protocol. The T3 kit (human kit) was obtained from Bio-Quant, the T4 kit (human kit) from Diagnostic Systems Laboratories, Inc, and the TSH kit (rat kit) from Amersham Biosciences.

Echocardiography

After body weight was obtained and the chest had been shaved, each animal was placed on an isothermal pad. Anesthesia was obtained by 1.5% isoflurane gas. M-mode images were obtained from the short axis of the left ventricle (LV) at the level of the papillary muscles with a VisualSonics Vevo 660 high-resolution imaging system with a 25-MHz RMV-710 transducer.

Myocardial Blood Flow and Hemodynamics

One day after echocardiography measurements, rats were anesthetized with an intraperitoneal injection of ketamine (30 mg/kg) and xylazine (5 mg/kg), and the right carotid artery was isolated and cannulated for assessment of hemodynamics and myocardial blood flow with a fluid-filled catheter. Fluorescent microspheres were injected into the LV via the right carotid artery. Two different colors of fluorescent microspheres with nonoverlapping emission spectra (Molecular Probes; diameter $15 \pm 0.3 \mu\text{m}$; 200 000/color per animal; blue for resting and orange for maximum blood flow after adenosine injection) were injected into the LV. The left carotid artery was used for withdrawing a reference blood sample from the descending aorta at a rate of 1 mL/min into a heparinized syringe (starting 5 seconds before injection of microspheres and lasting for 60 seconds). The left jugular vein was used for injection of adenosine ($300 \mu\text{g} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$ over 10 seconds) to induce maximum myocardial blood flow, which was confirmed by a significant drop in LV systolic blood pressure and heart rate (>20%) immediately after injection. Fluorescence was extracted from digested tissue and a reference blood sample with Raab's method.²¹ Fluorescence intensity was measured with a luminescence spectrometer (model LS55B, Perkin-Elmer) at the optimal excitation/emission wavelength pair of each dye (blue, 365 and 415 nm; orange, 520 and 560 nm) with excitation and emission slit widths of 4 nm in scanning mode. Myocardial blood flow was subsequently calculated as outlined in the Molecular Probes product manual.

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Myocyte Isolation and Morphology

Potassium hydroxide (KOH) was used to prepare isolated myocytes from formalin-fixed myocardium as described previously.²² Cell length and sarcomere length were measured by image analysis. Cell length, defined as the longest length parallel to the longitudinal axis, was measured in the first 50 undamaged single myocytes encountered in each preparation with a 10 \times objective lens. Sarcomere length was measured in the first 10 undamaged single myocytes encountered in each preparation with a 40 \times objective lens. Mean myocyte length from each animal was normalized to resting, unloaded sarcomere length (1.90 μm) to correct for differences due to contractile phase.

Quantitation of Arterioles

Frozen, formalin-fixed tissues were sectioned at 4 μm , placed on glass slides, and stained with α -smooth muscle actin and laminin (Sigma).²³ Arterioles were defined as vessels between 5 and 50 μm in diameter that had at least 1 layer of smooth muscle. Minor diameter was used to define mean arteriolar diameter. Data were collected from 20 randomly selected fields from each animal at 20 \times magnification. The following raw data were collected from each field: major and minor arteriolar diameter, arteriolar number, and points on myocytes. Arteriolar data were referenced to myocyte area rather than tissue area. This eliminated any errors due to shrinkage or separation artifacts and related arterioles to viable, at-risk myocytes. Arteriolar length density (L_v , average length of arterioles per unit myocyte volume) was calculated on the basis of the following formula developed for the analysis of arterioles arranged in any orientation:^{24,25} $L_v \text{ (mm/mm}^3\text{)} = \sum(a/b) \times M$, where *a* and *b* are the maximum and minimum external arteriolar diameters, respectively, and *M* is the area of myocytes in the reference area.

Western Blot for Expression of Thyroid Response Genes

Tissue samples from the apex region in each rat were powdered in liquid nitrogen and RIPA buffer with protease cocktail inhibitor (EMD Biosciences Inc), 1 mmol/L PMSF, and 1 mmol/L sodium orthovanadate. Each sample was incubated at 4°C for 15 minutes and sonicated to completely homogenize the tissue. Cell lysates were centrifuged at 14 000 rpm for 15 minutes. The supernatant was collected, separated into aliquots, and stored at -80°C until time of use. Protein concentrations of cell lysates were determined by a BCA protein assay. Samples were then mixed with Laemmli buffer that contained 5% β -mercaptoethanol and were evenly loaded onto SDS-PAGE gels. Protein was transferred to PVDF membranes. The membranes were blotted with sarcoplasmic/endoplasmic reticulum Ca²⁺-ATPase (SERCA)-2a (Santa Cruz Biotechnology), phospholamban (Upstate), phospho-phospholamban (P-PLB, Upstate), and α -actinin antibody (Chemicon); α -myosin heavy chain (α -MHC) and β -myosin heavy chain (β -MHC) were detected with antibodies produced by hybridomas purchased from ATCC (Manassas, VA). The antigen-antibody complexes were visualized by the appropriate secondary antibodies and chemiluminescence system (Pierce) and then quantified with a Versadoc Imaging System model 3000 (Bio-Rad Laboratories, Inc).

Statistical Analyses

Data were analyzed with Student's *t* test for comparisons between age-matched animals. $P < 0.05$ was selected to denote statistical significance. Data are presented as mean \pm SD.

TABLE 1. Physical and Hemodynamic Data

| Groups | n | Body Weight, g | Heart Weight, mg | HW/BW, mg/g | Heart Rate, bpm | LVSP, mm Hg | LVEDP, mm Hg |
|----------|---|----------------|------------------|-------------|-----------------|-------------|--------------|
| Control | 8 | 280±18 | 884±55 | 3.16±0.2 | 339±25 | 133±13 | 9±3 |
| PTU-S | 7 | 308±27 | 742±42 | 2.42±0.2 | 233±26 | 111±13 | 7±3 |
| % Change | | ↑ 10% | ↓ 16% | ↓ 23% | ↓ 31% | ↓ 17% | |
| <i>P</i> | | 0.034 | 0.01 | 0.01 | 0.01 | 0.01 | NS |
| Control | 7 | 322±28 | 1064±121 | 3.35±0.2 | 337±44 | 137±23 | 7±4 |
| PTU-L | 9 | 216±26 | 873±79 | 3.85±0.3 | 196±15 | 102±9 | 9±3 |
| % Change | | ↓ 33% | ↓ 18% | | ↓ 42% | ↓ 25% | |
| <i>P</i> | | 0.001 | 0.002 | NS | 0.001 | 0.001 | NS |

HW/BW indicates heart weight/body weight; LVSP, LV systolic pressure; and LVEDP, LV end-diastolic pressure.

Results

Physical Data

There was a significant weight gain in the 6-week treatment group and a significant weight loss in the 1-year treatment group (Table 1). Although body weight was not monitored during the treatment period, 1-year PTU-treated animals appeared to be reasonably healthy until they developed cachexia near the end of treatment. Indeed, the 1-year end point was selected on the basis of the apparent rapid decline in general health of the PTU-treated animals that occurred around that time. There were no noticeable differences in water consumption between treated and untreated animals. There were only 2 deaths in the study, both in the 1-year PTU treatment group. Compared with age-matched controls, both PTU treatment groups had a significant reduction in heart weight (Table 1).

Serum Levels of T3, T4, and TSH

Compared with age-matched controls, serum T3 levels were reduced ($P<0.01$) in both PTU-S rats (0.88 ± 0.2 versus 1.38 ± 0.2 ng/mL; 36% reduction) and PTU-L rats (0.82 ± 0.2 versus 1.15 ± 0.2 ng/mL; 29% reduction). Compared with age-matched controls, T4 levels were significantly reduced in PTU-S rats (4.44 ± 1.30 versus 7.28 ± 1.91 μg/dL; 39% reduction; $P<0.01$). T4 levels were reduced by 33% with PTU treatment for 1 year, but this did not reach statistical significance (4.71 ± 1.23 versus 7.03 ± 3.2 μg/dL; $P<0.14$). Although TSH levels were elevated in both treatment groups, changes did not reach statistical significance (27.1 ± 9.3 versus 20.1 ± 9.3 pg/mL in 6-week study, 35% increase,

$P=0.17$; 25.21 ± 6.6 versus 18.0 ± 8.8 pg/mL in 1-year study, 40% increase, $P=0.11$).

Echocardiography

Echocardiographic data are shown in Table 2. In both PTU treatment groups, LV systolic and diastolic chamber diameters were increased. LV posterior wall thickness in systole was significantly reduced in both PTU treatment groups and tended to be reduced in diastole ($P=NS$). LV ejection fraction and fractional shortening were also reduced in both PTU treatment groups. Representative examples of M-mode echocardiography tracings are shown in Figure 1.

Hemodynamics

Hemodynamic alterations are summarized in Table 1. Compared with the control groups, both treated groups had a significant decline in heart rate and LV systolic pressure but not in LV end-diastolic pressure.

Myocyte Length

Although there were no significant changes in myocyte length in the PTU-S group (132 ± 3 μm) versus control (128 ± 3 μm), there was an 18% increase in PTU-L rats (154 ± 9 μm) versus controls (130 ± 9 μm, $P<0.01$).

Myocardial Blood Flow and Arteriolar Morphometry

Changes in myocardial blood flow and arteriolar morphometry are summarized in Figure 2. Compared with age-matched controls, resting myocardial blood flow was reduced 43%, and adenosine-induced maximum myocardial blood flow was

TABLE 2. Echocardiographic Data

| Groups | n | LVIDd, mm | LVIDs, mm | AWTd, mm | AWTs, mm | PWTd, mm | PWTs, mm | LVEF, % | FS, % |
|----------|---|-----------|-----------|----------|----------|----------|----------|---------|--------|
| Control | 8 | 6.1±0.4 | 3.2±0.5 | 1.9±0.3 | 3.0±0.4 | 2.3±0.3 | 2.9±0.4 | 77.7±8 | 47.9±8 |
| PTU-S | 7 | 7.0±0.5 | 4.5±0.7 | 1.8±0.4 | 2.8±0.4 | 1.9±0.6 | 2.3±0.5 | 64.1±9 | 36.4±7 |
| % Change | | ↑ 15% | ↑ 40% | NS | NS | NS | ↓ 21% | ↓ 17% | ↓ 23% |
| <i>P</i> | | 0.01 | 0.01 | 0.79 | 0.49 | 0.2 | 0.03 | 0.012 | 0.014 |
| Control | 7 | 6.7±0.5 | 2.9±0.5 | 2.0±0.5 | 3.6±0.7 | 2.3±0.8 | 3.7±0.7 | 86±6 | 56±6 |
| PTU-L | 9 | 7.6±0.9 | 5.4±1.1 | 1.8±0.4 | 2.7±0.4 | 2.0±0.4 | 2.8±0.7 | 55±11 | 30±8 |
| % Change | | ↑ 13% | ↑ 86% | NS | ↓ 25% | NS | ↓ 24% | ↓ 36% | ↓ 46% |
| <i>P</i> | | 0.039 | 0.001 | 0.258 | 0.007 | 0.364 | 0.026 | 0.001 | 0.001 |

LVIDd indicates LV internal diameter in diastole; LVIDs, LV internal diameter in systole; AWTd, anterior wall thickness in diastole; AWTs, anterior wall thickness in systole; PWTd, posterior wall thickness in diastole; PWTs, posterior wall thickness in systole; LVEF, LV ejection fraction; and FS, fractional shortening.

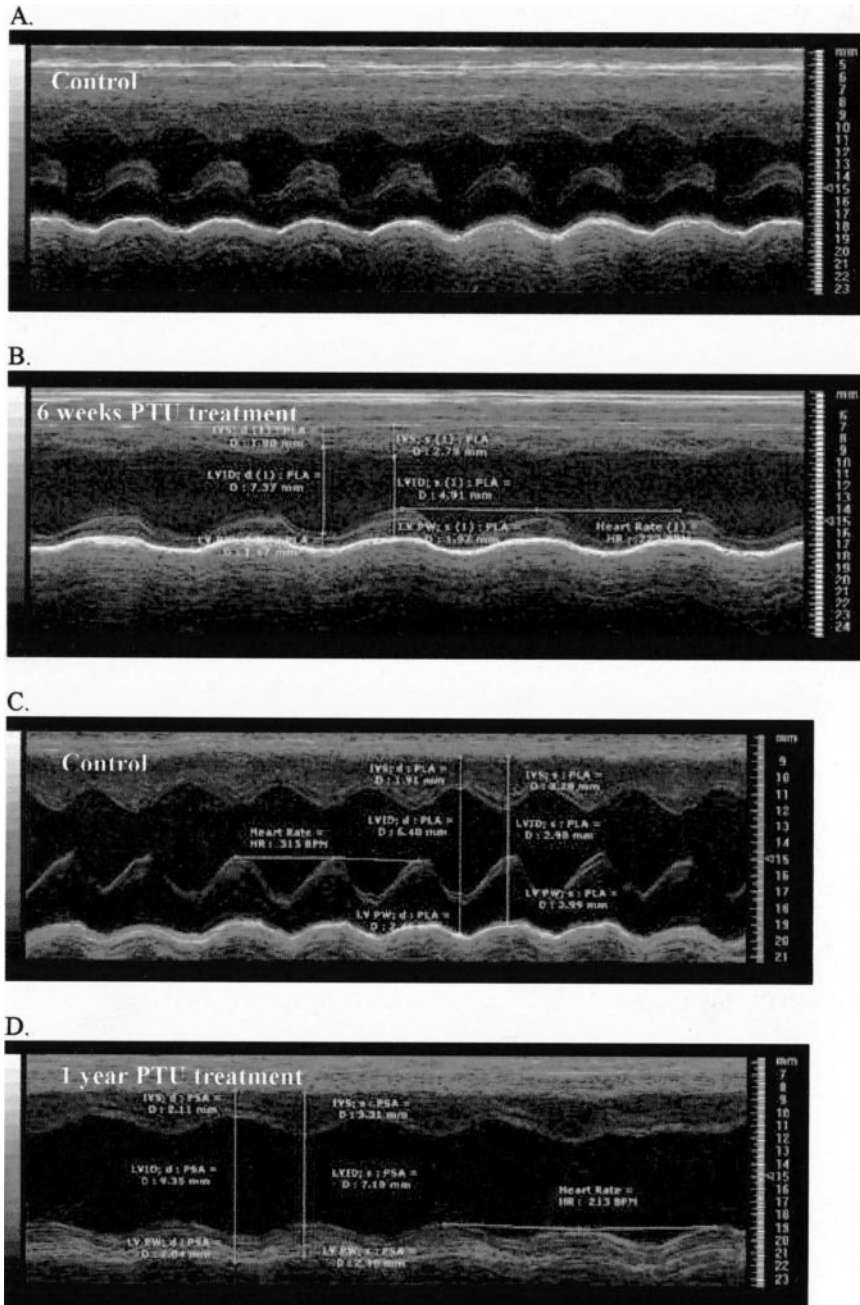


Figure 1. Echocardiography data. M-mode echocardiographic tracing from untreated 7-month-old control (A), age-matched rat treated for 6 weeks with PTU (B), 1.5-year-old control (C), and rat treated with PTU from 6 to 18 months of age (D).

reduced 37% in PTU-S rats (Figure 2A). In PTU-S rats, arteriolar length density was reduced by 41%, and arteriolar number per unit area was reduced by 37% (Figure 2B). The reduction in arteriolar number was due to loss of the smallest arterioles (Figure 2C). In PTU-L, resting myocardial blood flow was reduced 39%, and maximum blood flow was reduced 48% (Figure 2D) compared with age-matched controls. Arteriolar length density was reduced 46%, and arteriolar number per unit area was reduced by 53% in PTU-L rats (Figure 2E). The reduction in arterioles in PTU-L rats was also primarily due to loss of arterioles in the 5- to 15- μ m size range; however, there was also a significant reduction in arterioles in the 15- to 20- μ m and 20- to 30- μ m size ranges (Figure 2F).

Changes in Gene Expression

In the long-term study only, Western blotting was used to analyze the expression of known thyroid responsive genes that are important for regulation of contractility (Figure 3). In the blots shown, each lane was loaded with extract from separate animals, and densitometry was performed on 5 animals per group. Compared with controls, expression levels of α -MHC, SERCA2a, and monomeric phospho-PLB were significantly decreased. Expression levels of β -MHC, pentameric phospholamban, and monomeric phospholamban were significantly increased.

Discussion

Hypothyroidism can produce changes that resemble HF in many respects. For instance, LV function and cardiac output

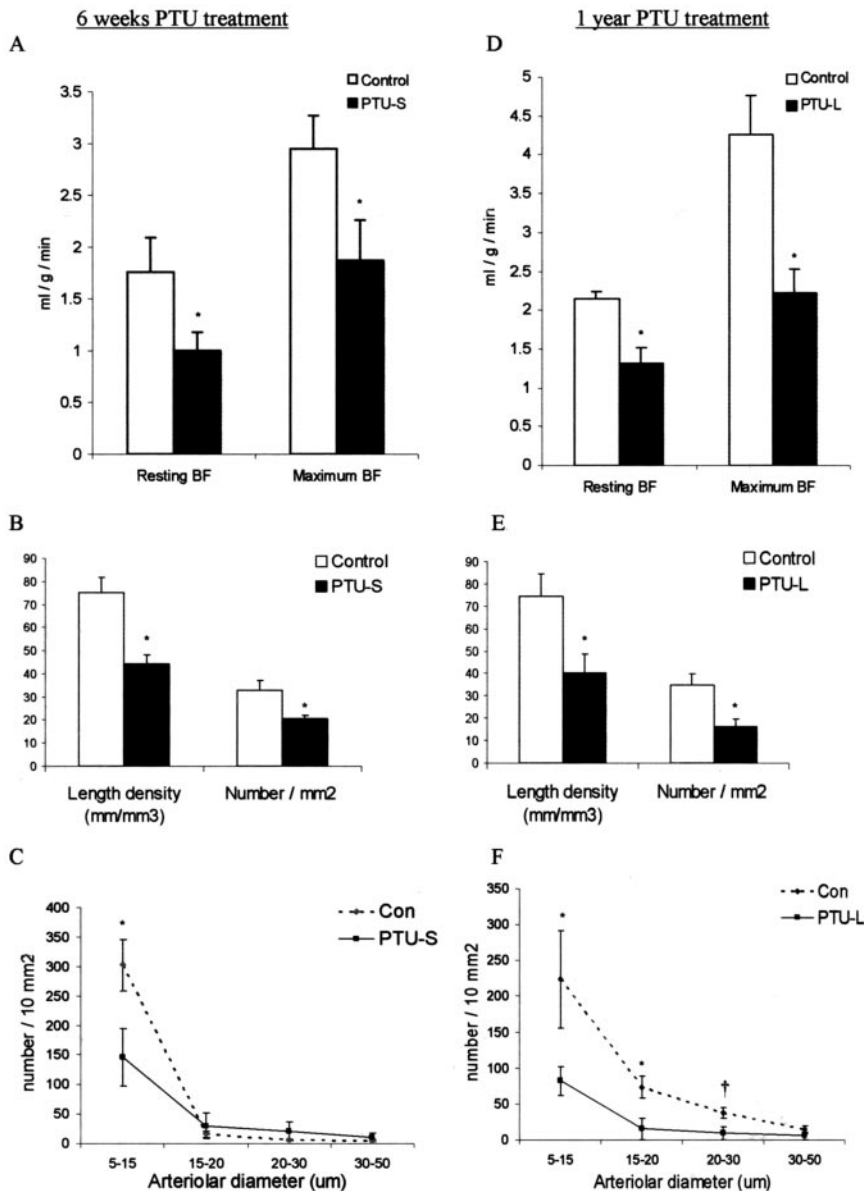


Figure 2. Changes in myocardial blood flow and arterioles. Six-week PTU treatment: resting and maximum myocardial blood flow (A); arteriolar length density and number (B); size distribution of arterioles (C). One-year PTU treatment: resting and maximum myocardial blood flow (D); arteriolar length density and number (E); size distribution of arterioles (F). * $P < 0.01$ vs control; † $P < 0.05$ vs control.

are reduced.^{7,14} Clinical studies have characterized hypothyroid patients as having dilated, thin-walled ventricles.^{19,20} Changes in cardiac gene expression also resemble those found in various heart diseases leading to HF.^{26,27} In the present study, we have again confirmed changes in contractile and calcium handling proteins typical of hypothyroidism. However, many of the changes induced by hypothyroidism may simply relate to cardiac unloading and associated atrophy. In the present study, structural and functional changes in the heart due to short- and long-term hypothyroidism were examined. The aim was to provide evidence that will lead to better characterization of this disorder and the potential consequences on cardiac structural remodeling. Because there is considerable evidence that low thyroid function increases mortality in patients with HF, it is important to determine whether this condition can actually cause HF or should simply be considered another risk factor. We report for the first time that chronic hypothyroidism can eventually lead to

chamber dilatation from series addition of sarcomeres despite a reduction in cardiac mass. This cellular change is a recognized component of HF.^{28,29} We also found that hypothyroidism led to a dramatic reduction in myocardial arterioles. This microcirculatory change occurred early, because the magnitude of arteriolar loss was similar after only 6 weeks of PTU treatment.

The effects of hypothyroidism on the heart are rather difficult to characterize with regard to HF. Typically, HF is preceded by a period of stress (eg, hypertension or ischemia) and a hypertrophic growth response that may at first be compensatory but eventually may evolve to chamber dilatation and pump dysfunction. It has been clearly demonstrated in progression to HF that increased chamber diameter/wall thickness ratio during the decompensated phase is reflected by a similar increase in myocyte length/width ratio.^{28,30,31} Typically in progression to HF, this occurs by myocyte lengthening without a change in myocyte cross-sectional area

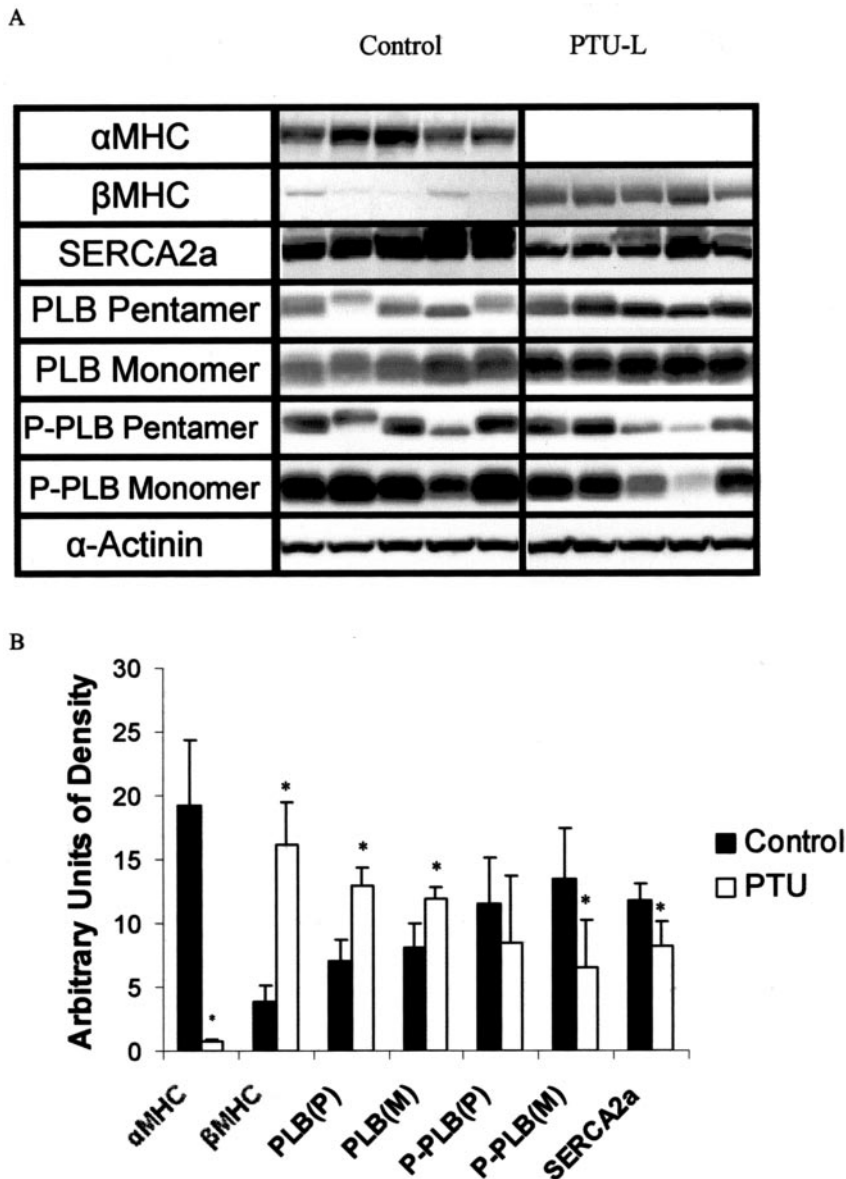


Figure 3. Changes in gene expression. Western blots from 1-year treatment group (A). Quantitative summary of gene expression (B). PLB-Pentamer indicates phospholamban pentamer; PLB-Monomer, phospholamban monomer; P-PLB Pentamer, phosphorylated phospholamban pentamer; and P-PLB Monomer, phosphorylated phospholamban monomer. * $P < 0.01$ vs control.

during the transition phase.^{30,32} We have reported a similar increase in myocyte length/width ratio in rats treated with PTU for 4 weeks, but, in this case, it was due to atrophy of myocyte cross-sectional area without a change in myocyte length.⁷ Thus, the myocyte shape change differed from that which occurs in more common types of HF. Our data from the present study show that hypothyroidism of 6 weeks' duration led to reduced heart weight and unloading, with no change in myocyte length. Although myocyte cross-sectional area was not determined in the present study, we know from our previous work cited above⁷ that early loss of cardiac mass was likely due to a reduction in myocyte cross-sectional area only. Echocardiographic measures for wall thickness in the present study appear to confirm this cellular change, because all 4 parameters showed a reduction (although only 1 measure reached statistical significance). Hypothyroidism of 1-year duration eventually led to chamber dilatation and myocyte lengthening from series addition of sarcomeres. Indeed, this may be the most compelling evidence that hypothyroidism

can eventually lead to overt failure distinct from unloading, because series addition of sarcomeres is a hallmark feature of HF. The reduction in cardiac mass (likely due to reduced work), coupled with cell lengthening in 1-year PTU-treated rats, suggests a further reduction in myocyte cross-sectional area. Although this was not confirmed by cross-sectional area measurements, 2 of 4 echocardiographic parameters for wall thickness were reduced significantly compared with control values. Because whole tissue was needed for myocardial blood flow and histology, we could not use our standard collagenase method for myocyte isolation and comprehensive cell size measurements. The KOH method to isolate myocytes from whole tissue yields reliable values for myocyte length but leads to considerable shrinkage in myocyte cross-sectional area.³³ It is worth noting here that cardiac atrophy from unloading, such as with LV assist device support, leads to series removal, rather than addition, of sarcomeres.³⁴

It appears that an increase in end-diastolic pressure (pre-load) is an important stimulus for progressive chamber

dilatation in HF. Indeed, we have observed a very rapid and dramatic increase in series sarcomeres in rats when a pronounced increase in end-diastolic pressure is triggered by a large infarction.³² A much slower rate of series sarcomere formation was observed in spontaneously hypertensive HF rats progressing to failure, in which it is more difficult to detect significant changes in end-diastolic pressure.^{22,30} In the present experiment, there were no changes in end-diastolic pressure after 6 weeks or 1 year of PTU treatment, which suggests that preload was not changed. However, end-diastolic diameter was increased at 6 weeks, which suggests that a diastolic stretch stimulus was already present but had not yet resulted in significant series addition of sarcomeres. We have shown previously that reduced cardiac output in rats treated with PTU for 4 weeks was due to reduction in both heart rate and stroke volume.⁷ With longer duration of hypothyroidism, however, it appears that subtle and difficult-to-measure changes in preload may provide sufficient stimulus to induce myocyte lengthening. Little is known about specific signaling pathways that may induce series addition of sarcomeres, but several molecules have been implicated.^{35–37} Additionally, we cannot exclude the possibility that an unidentified signaling stimulus may induce cell lengthening independent of a mechanical trigger.

It was anticipated that coronary blood flow would be impaired in hypothyroid rats because thyroid hormones are known to affect the vasoreactive properties of vessels.^{38,39} We expected to observe a reduction in resting blood flow and a larger than normal increase in flow with adenosine due to excessive vasoconstriction. Instead, both resting and maximum blood flow were significantly impaired. Morphometric data confirmed that alterations in blood flow were likely due to loss of arterioles (rarefaction). Because our original observations were in the animals treated with PTU for 1 year, we decided to add a shorter, 6-week treatment group to provide some temporal understanding of the arteriolar changes. We found that a similar impairment of blood flow and associated loss of arterioles was already present at that time. Consequently, this appears to be a relatively early event. To the best of our knowledge, this is the first time that such a change has been observed in adults with hypothyroidism. However, Heron and Rakusan²⁵ observed a reduction in arteriolar number in neonatal hypothyroid rats. In that case, the reduction in arterioles was likely due to an attenuation of growth rather than an actual loss. We have recently observed an identical abnormal coronary blood flow pattern in dilated cardiomyopathic hamsters.⁴⁰ Hormone assays in those BIO-T02 hamsters indicated the presence of subclinical hypothyroidism (normal T3, elevated TSH). Although arteriolar morphometry was not performed in those animals, it is likely that a similar loss of arterioles occurred. At this time, one can only speculate on the potential mechanism of arteriolar loss. However, rarefaction of myocardial arterioles has been observed recently in endothelial NO synthase (eNOS) knockout mice,⁴¹ and NO availability and eNOS are known to be reduced in hypothyroidism.^{42,43} Consequently, reduced eNOS in hypothyroidism may play an important role in arteriolar rarefaction in this disorder. This possibility merits further investigation. It is also worth noting that upregulation of

eNOS is a strong candidate for thyroid hormone-induced coronary angiogenesis.^{42,44}

It should be appreciated that PTU affects many aspects of thyroid hormone metabolism, including iodination, coupling, and deiodination of T4.^{45–47} There is also a possibility that PTU may have a direct toxic effect on coronary arterioles. Case reports indicate that PTU can, on rare occasions, induce pulmonary vasculitis, granulocytopenia, and hepatic damage.^{48–50} We believe that such a change was unlikely in PTU-treated rats in the present study, because livers and lungs appeared normal by gross inspection. Additionally, we could find no published evidence of PTU cardiac toxicity in rats or other species. Nonetheless, it would be worthwhile to confirm that arteriolar loss occurs with hypothyroidism induced by other means. Should loss of myocardial arterioles be induced by PTU toxicity, this would raise another important clinical concern, because this is a commonly used drug to treat hyperthyroidism.

PTU-induced changes in TSH, T3, and T4 suggest the presence of rather mild hypothyroidism in the animals examined in the present study. If true, our findings are of even greater potential clinical relevance, because borderline thyroid dysfunction is more likely to go untreated. The cardiovascular changes in both PTU-treated groups, however, suggest severe hypothyroidism. We were unable to find commercially available ELISA kits specifically developed for assessment of T3 and T4 in rats. The kits used here were developed for use in humans and use human proteins in standardization curves. Although absolute values for thyroid hormone levels may not be directly comparable to values reported in humans, we have confirmed that these T3 and T4 kits are able to detect induced hypothyroidism (present study) and hyperthyroidism⁵¹ in rodent models. TSH changes did not reach statistical significance with the rat kit used in the present study. Another TSH kit developed for humans provided virtually identical results from these samples (data not included). Both kits indicated increased mean TSH levels in excess of 30% in short- and long-term PTU-treated animals. Largely because of considerable variability in individual data points, results were not significantly different with either kit.

Growing evidence suggests a strong link between low thyroid function and worsening outcome in patients with heart disease.^{5,11,13} In the present study, we have identified 2 new cellular mechanisms (eg, myocyte lengthening and arteriolar loss) by which low thyroid function may adversely affect cardiac remodeling and accelerate progression to HF. The dramatic loss of arterioles induced by hypothyroidism raises a number of important questions. What level of thyroid dysfunction is necessary to produce such a change? Does this also occur in subclinical hypothyroidism? How does reduced thyroid function trigger arteriolar loss? Does this occur in humans? If so, what is the clinical impact on mortality and progression of disease? Clearly, more work is needed to better understand the consequences of low thyroid function in heart disease, because improved patient outcome is a realistic possibility.

Summary

Patients with low thyroid function (eg, borderline or overt hypothyroidism) and heart disease are at increased risk for

HF and early death. Whether low thyroid function is simply a risk factor or can actually cause HF is uncertain. To examine potential cellular mechanisms by which this disorder may adversely affect the heart, we examined rats 6 weeks and 1 year after inducing low thyroid function with a commonly used antithyroid drug (PTU). Hearts from rats with low thyroid function were dilated and had severe functional impairment at both time points. Two new cellular mechanisms by which low thyroid function may lead to HF were discovered: (1) PTU-treated rats had a dramatic impairment in cardiac blood flow due to loss of arterioles, and (2) heart dilatation was due to elongation of contracting muscle cells, a specific change typical of HF. The extent of impaired coronary blood flow and arteriolar loss was the same at both time points, which suggests that these changes can occur rather quickly. Results from the present study indicate that low thyroid function alone has the potential to cause HF. Although similar cardiac changes may occur in humans with low thyroid function, this must be confirmed. It will also be important to define what level of low thyroid function produces these changes. Clearly, more research is needed in humans to determine whether specific populations of heart patients will benefit from thyroid hormone treatment.

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