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Treatment of subclinical hypothyroidism reverses ischemia and prevents myocyte loss and progressive LV dysfunction in hamsters with dilated cardiomyopathy

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Khalife, Wissam I., Yi-Da Tang, James A. Kuzman, Tracy A. Thomas, Brent E. Anderson, Suleman Said, Patricia Tille, Evelyn H. Schlenker, and A. Martin Gerdes. Treatment of subclinical hypothyroidism reverses ischemia and prevents myocyte loss and progressive LV dysfunction in hamsters with dilated cardiomyopathy. *Am J Physiol Heart Circ Physiol* 289: H2409–H2415, 2005. First published July 15, 2005; doi:10.1152/ajpheart.00483.2005.—Growing evidence suggests that thyroid dysfunction may contribute to progression of cardiac disease to heart failure. We investigated the effects of a therapeutic dose of thyroid hormones (TH) on cardiomyopathic (CM) hamsters from 4 to 6 mo of age. CM hamsters had subclinical hypothyroidism (normal thyroxine, elevated TSH). Left ventricular (LV) function was determined by echocardiography and hemodynamics. Whole tissue pathology and isolated myocyte size and number were assessed. TH treatment prevented the decline in heart rate and rate of LV pressure increase and improved LV ejection fraction. The percentage of fibrosis/necrosis in untreated 4-mo-old CM (4CM; 15.5 ± 2.2%) and 6-mo-old CM (6CM; 21.5 ± 2.4%) hamsters was pronounced and was reversed in treated CM (TCM; 11.9 ± 0.9%) hamsters. Total ventricular myocyte number was the same between 4- and 6-mo-old controls but was reduced by 30% in 4CM and 43% in 6CM hamsters. TH treatment completely prevented further loss of myocytes in TCM hamsters. Compared with age-matched controls, resting and maximum coronary blood flow was impaired in 4CM and 6CM hamsters. Blood flow was completely normalized by TH treatment. We conclude that TH treatment of CM hamsters with subclinical hypothyroidism normalized impaired coronary blood flow, which prevented the decline in LV function and loss of myocytes.

thyroid hormones; remodeling; fibrosis; blood flow

THE EFFECTS OF THYROID HORMONES (TH) on the cardiovascular system have been well studied. It is clear that both hypothyroidism and hyperthyroidism can lead to deleterious changes in cardiovascular function. Decreased TH levels have been reported in a variety of nonthyroidal illnesses (18), including congestive heart failure (12) and myocardial infarction (6). The decrease in TH levels also appears to be related to the severity of heart failure (12). This is not a minor point, because low 3,5,3'-triiodothyronine (T₃) concentrations are a strong, independent predictive marker of poor prognosis in cardiac patients and might represent a determining factor directly implicated in

the evolution and prognosis of these conditions (14). Growing evidence also suggests that subclinical thyroid dysfunction might play an important role in heart failure (10, 21). This is highly relevant from a clinical standpoint because this patient group does not typically receive TH treatment.

Development of heart failure is accompanied by a variety of neuroendocrine changes. Cardiac failure was shown to be associated with both a decline in circulating TH levels (11, 17) and altered cardiac TH signaling, as evidenced by changes in myocardial expression of TH nuclear receptor isoforms (15, 16). The observation that short-term TH administration improves cardiac performance, both in animal models (2, 23) of cardiac dysfunction and in patients (11, 17, 22) suffering from cardiac failure, agrees with this notion. At this time, our understanding of the temporal adaptive response to TH supplementation in heart disease is very limited. Animal studies examining the potential benefits of TH treatment of dilated cardiomyopathy are limited to a single veterinary outpatient study in dogs with dilated cardiomyopathy (31). Consequently, no information is available regarding the pathophysiological consequences of TH treatment in animals with dilated cardiomyopathy.

Our hypothesis is that treatment of low thyroid function in dilated cardiomyopathy will prevent or attenuate progressive pathophysiological alterations leading to heart failure. In the present study, the effects of TH supplementation on the progression of left ventricular (LV) dysfunction and remodeling were examined in the Bio T0-2 (cardiomyopathic, CM) hamster model of dilated cardiomyopathy. Although the related 14.6 strain develops overt hypothyroidism (19), our thyroid assays in Bio T0-2 hamsters suggest that this model has subclinical hypothyroidism. We chose the CM model because it is a widely accepted animal model of dilated cardiomyopathy, displays thyroid dysfunction, and progresses to heart failure more rapidly than the 14.6 strain. The results of our study suggest an important new mechanism, impairment of coronary blood flow, by which low thyroid function may adversely alter the progression of dilated cardiomyopathy to heart failure.

MATERIALS AND METHODS

Animal model. A cardiomyopathic Syrian hamster model was chosen in the present study because it exhibits features resembling

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those of dilated cardiomyopathy in humans (29). It is a reproducible, spontaneously transmitted autosomal recessive, progressive model of cardiac hypertrophy, dilation and failure. The mechanism for the development of heart disease in CM hamsters is a genetic mutation of δ -sarcoglycan (27). Life expectancy is 10–12 mo in the CM hamster (Bio T0-2 strain), as opposed to 2–2½ yr in the Bio F1B hamster. The necrotic phase of myocardial remodeling begins at ~2 mo of age in Bio T0-2 hamsters. In our experiments, we elected to begin treatment at a time when some pathology was present but the animals were not yet sick. Our thoughts were that selection of such a starting point might provide evidence regarding both reversibility and progression of pathological changes. Four-month-old male CM hamsters of the Bio T0-2 strain and four-month-old Bio F1B controls were obtained from Bio Breeders (Watertown, MA). All procedures in this study were approved by the University of South Dakota Animal Care and Use Committee and followed institutional guidelines for animals.

Experimental design. We divided the animals into five groups: 4-mo-old Bio F1B (4C), 4-mo-old Bio T0-2 untreated (4CM), 4-mo-old Bio T0-2 treated with 0.08% desiccated TH [T5146, porcine grade II; contains 0.17% organic iodine Liothyronine 0.14 mg/mg (dry) and levothyroxine 0.57 μ g/mg (dry); Sigma-Aldrich, St. Louis, MO] in ground food until 6 mo of age (i.e., 2-mo treatment; TCM), 6-mo-old Bio T0-2 (6CM), and 6-mo-old Bio F1B (6C). All animals were maintained in the same environment including temperature and humidity and free access to food and water. Oxygen consumption and respiration rate were checked regularly to monitor the effects of the treatment. On the basis of recently completed studies in rats (30), we used a dose of TH that was unlikely to induce hyperthyroidism. In the terminal experiment, echocardiography, hemodynamics, isolated myocyte size, myocyte number, tissue histology, and tissue morphometry were assessed in each animal group. It was necessary to repeat the experiment again to obtain the above data from an adequate number of animals. On the basis of results of the above experiments, the experiment was repeated a third time to collect data for myocardial blood flow.

Measurement of oxygen consumption. Hamsters were placed into a cylindrical Plexiglas chamber for measurement of oxygen consumption as described previously (28). Oxygen consumption was corrected to STPD values and divided by body weight. Hamsters were weighed, placed into the chamber, and exposed to air for 30 min of acclimatization and subsequent determination of oxygen consumption.

Echocardiography and hemodynamics. After body weight was obtained and the chest was shaved, each hamster was placed on an isothermic pad. Anesthesia was obtained by 1.5% isoflurane gas. M-mode images were obtained from the short axis of the LV at the level of the papillary muscles with a Hewlett-Packard Sonos 2000 echo machine with a 7.5-MHz transducer. From this image, LV free wall thickness and internal ventricular diameter during systole and diastole were determined. After echocardiography, the right carotid artery was isolated and cannulated for assessment of hemodynamics as described previously (20).

Myocyte isolation and morphology. After hemodynamic data were collected, heparin (1 ml/100 g, 0.16%) was injected intraperitoneally. Hearts were removed, blotted, weighed, and cannulated through the aorta for perfusion with collagenase for isolation of myocytes as described previously (8). Freshly isolated cardiac myocytes were fixed immediately in 2% glutaraldehyde in 80 mM phosphate buffer for subsequent determination of myocyte length (L ; microscopy), volume (V ; Coulter Channelyzer), and cross-sectional area (CSA) as described previously (8).

Whole heart preparation. Hearts were trimmed and blotted, and ventricular and atrial weights were determined. Hearts were then cannulated and flushed with cold Joklik medium to remove blood. From the middle third of the ventricles, two transverse slices of ~1–2 mm were taken and fixed in 10% formalin ($n = 5$ for TCM and 4 for all other groups). The remaining basal and apical portions of the ventricles were flash frozen.

Histopathology and morphometry. Formalin-fixed transverse sections were stained with hematoxylin and eosin. The percentage of ventricular myocytes and areas of fibronectin replacement were determined morphometrically by point counting as described previously (7, 8). Histological sections were viewed under a microscope with a color video camera, and data were collected from 20 randomly selected fields from each animal.

Ventricular myocyte number (VMN) was calculated from isolated myocyte volume and whole tissue morphometry as described previously but with some modifications (1). This method essentially determines VMN from the quotient of total ventricular myocyte volume and mean ventricular myocyte volume (MV; Coulter Channelyzer values) as follows: $VMN = MVF \times VTV/MV$, where MVF is myocyte volume percentage and VTV is ventricular tissue volume. VTV can be calculated by dividing ventricular weight by the specific gravity for myocardium (1.06). MVF cannot be reliably calculated from routine histological preparations because of tissue shrinkage, separation, and collapsed blood vessels. However, we showed previously (8) with multilevel morphometric sampling of 1- μ m plastic sections of glutaraldehyde perfusion-fixed rodent heart that ~73% of whole ventricle is occupied by myocytes. Because we could accurately identify areas of myocytes and myocyte replacement with fibronecrosis in the hematoxylin and eosin preparations used here, these values were multiplied by 0.73 to determine the ventricular volume percentage of these components. VMN was calculated for each heart used for isolated myocytes with the mean value for MVF as determined above for each animal group. Because regional heart weights may be affected by collagenase perfusion during cell isolation, ventricular weight for these hearts was estimated by multiplying ventricular percentage of total heart weight (mean value from whole heart preparations used) by heart weight.

Serum levels of thyroxine and TSH. Blood samples were separated into serum aliquots and frozen. Thyroxine (T_4) was assessed by solid-phase radioimmunoassay according to the manufacturer's protocol (Diagnostic Products, Los Angeles, CA). TSH levels were determined with the rat TSH Biotrak EIA system (Amersham Biosciences, Piscataway, NJ) according to the manufacturer's protocol.

Myocardial blood flow. A separate set of animals from each group was used to obtain myocardial blood flow measurements. Under ketamine (30 mg/kg) and xylazine (5 mg/kg) anesthesia, fluorescent microspheres were injected into the LV via the right carotid artery with a fluid-filled catheter attached to a transducer. Two different colors of fluorescent microspheres (orange for resting and blue for maximum blood flow after adenosine injection) with nonoverlapping emission spectra (Molecular Probes, Eugene, OR; diameter $15 \pm 0.3 \mu$ m, 100,000 microspheres \cdot color $^{-1} \cdot$ animal $^{-1}$) 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 heparinated syringe (starting 5 s before microsphere injection and lasting for 60 s). The left jugular vein was used for injection of adenosine (300 μ g \cdot kg $^{-1} \cdot$ min $^{-1}$ over 10 s) to induce maximum coronary blood flow, which was confirmed by a significant drop in LV systolic blood pressure and heart rate (>20%) immediately after injection of adenosine. Fluorescence was extracted from digested tissue and reference blood sample by the method of Raab et al. (25). Fluorescence intensity was measured with a luminescence spectrometer (model LS55B, PerkinElmer) at the optimal excitation-emission wavelength pair for 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 product manual (Molecular Probes).

Terminal deoxynucleotide transferase-mediated dUTP nick-end labeling assay. An in situ cell death kit (Roche Diagnostics, Indianapolis, IN) was used to visualize nuclei with extensive DNA damage characteristic of apoptosis. Formalin-fixed tissue slices were cryosectioned at 5 μ m ($n = 4$ for each group). For each experiment, a positive control was produced by incubating sections from controls with

DNase (50 U/ml). For each sample, 10 fields at $\times 40$ ($\sim 1,000$ cells/heart) were analyzed and positive cells were counted. No distinction was made between cell types.

Statistical analyses. All data are presented as means (SD). One-way ANOVA was used to compare data in each group. The Bonferroni test was used to examine statistically significant differences observed with ANOVA. Results were considered significant when $P < 0.05$.

RESULTS

Physical data. Changes in body and heart weight are summarized in Fig. 1. Typical of male rodents, C and CM hamsters gained weight between 4 and 6 mo of age. Weight gain was slightly, but significantly, attenuated in TH-treated CM hamsters. All CM hamsters had significantly lower body mass than age-matched controls. Although there was likely some increase in heart weight in both strains of hamsters because of body mass increase from 4 to 6 mo, there was no effect of TH treatment on heart weight.

Serum T_4 and TSH and oxygen consumption. Serum levels of T_4 were similar for all untreated hamster groups. Mean values for T_4 were significantly elevated in the TCM group vs. 6C and 6CM hamsters (Fig. 2A). TSH was significantly elevated in 6CM vs. 6C hamsters and was reduced toward normal values in TCM hamsters (Fig. 2B). Although TSH levels

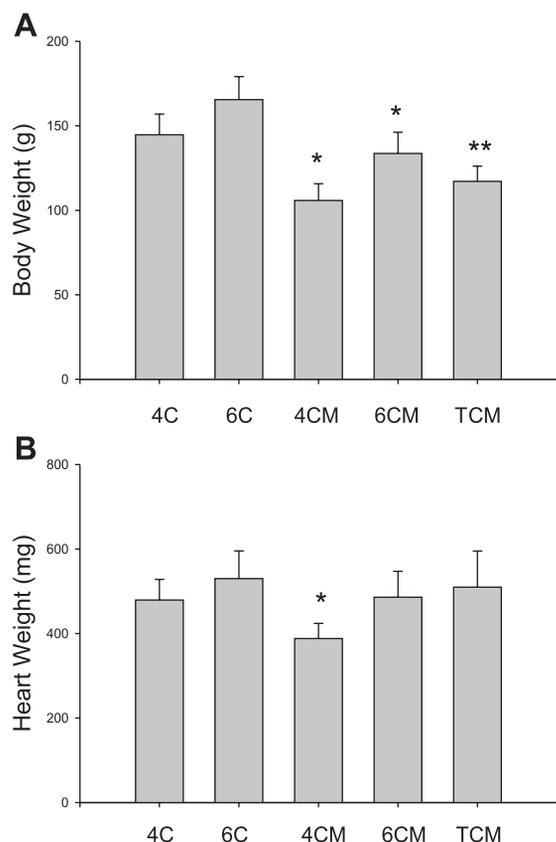


Fig. 1. Changes in body weight (A) and heart weight (B) from all animals included in the study. 4C, 4-mo-old controls ($n = 18$); 6C, 6-mo-old controls ($n = 17$); 4CM, 4-mo-old cardiomyopathic (CM) hamsters ($n = 19$); 6CM, 6-mo-old cardiomyopathic hamsters ($n = 18$); TCM, 6-mo-old CM hamsters treated with thyroid hormones for 2 mo ($n = 20$). * $P < 0.05$ vs. age-matched controls; ** $P < 0.05$ vs. 6CM. All values in Figs. 1–4 are presented as means (SD).

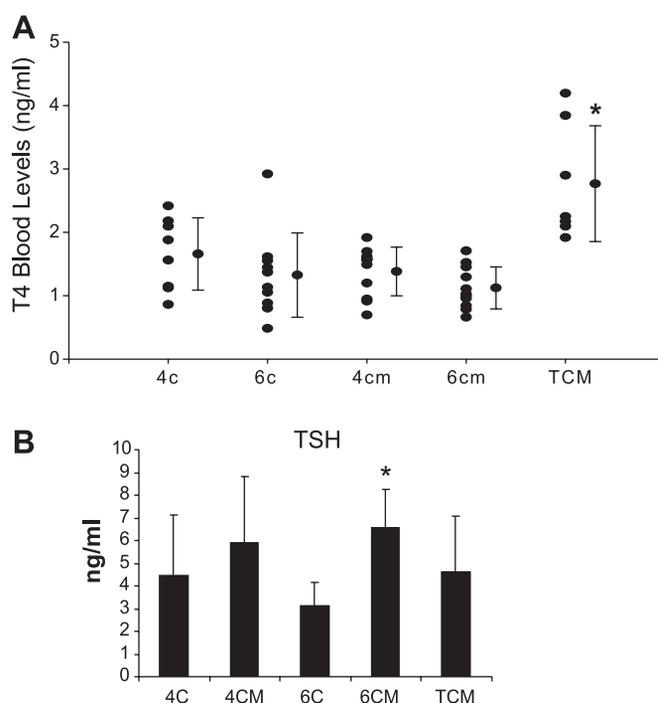


Fig. 2. Serum thyroxine (T_4 ; A) and TSH (B) levels in 4C ($n = 8$), 6C ($n = 10$), 4CM ($n = 11$), 6CM ($n = 11$), and TCM ($n = 7$). * $P < 0.05$ vs. 6C and 6CM.

tended to be higher in 4CM vs. 4C hamsters, this did not reach statistical significance (Fig. 2B). Weight-corrected oxygen consumption declined by 50% ($P < 0.01$) in CM hamsters between 4 and 6 mo of age, and the decline was largely prevented by TH treatment [18% decline, not significant (NS); data not shown].

Echocardiography. Echocardiographic assessment of cardiac function (Table 1) indicated that TH treatment significantly attenuated progressive LV systolic and diastolic chamber dilation. Ejection fraction was significantly improved in TCM vs. 6CM hamsters. CM hamsters at both ages had significantly dilated ventricles, thinner walls, and reduced ejection fractions compared with age-matched controls.

Hemodynamics. Hemodynamic alterations are summarized in Table 2. There was a significant decline in heart rate, LV end-systolic pressure (LVPes), maximum rate of LV pressure increase ($+dP/dt$), and maximum rate of LV pressure reduction ($-dP/dt$) in CM hamsters between 4 and 6 mo of age. TH treatment prevented or attenuated decline in these parameters. For instance, values for TCM hamsters were not significantly different from those for 4CM hamsters for any of these parameters and values for heart rate and $+dP/dt$ in TCM hamsters were significantly different from those in 6CM hamsters. Although group differences did not reach statistical significance, values for $-dP/dt$ and LVPes in TCM hamsters were intermediate between those of 4CM and 6CM hamsters, suggesting that treatment tended to attenuate decline in those hemodynamic indexes.

Histopathology. We did not observe any areas of fibroncrosis in any of the control animals. There were significant areas of myocardial fibroncrosis in 4CM hamsters, which continued to increase with age. TH treatment prevented further increase in ventricular fibroncrosis. There was, in fact, a

Table 1. *Echocardiographic data*

Groups	<i>n</i>	LVIDd, mm	LVIDs, mm	PWTd, mm	PWTs, mm	EF, %
4C	12	3.8 (0.4)	1.9 (0.3)	1.9 (0.2)	2.4 (0.3)	87 (6)
4CM	12	5.2 (0.5)	3.9 (0.5)	1.0 (0.2)	1.1 (0.2)	56 (6)
6C	9	4.5 (0.4)	2.2 (0.4)	1.6 (0.2)	2.2 (0.3)	86 (5)
6CM	15	6.6 (0.7)	5.1 (0.7)	1.1 (0.2)	1.5 (0.3)	49 (7)
TCM	11	5.9 (0.7)	4.1 (0.7)	1.3 (0.4)	2.0 (0.7)	58 (10)
ANOVA		Yes	Yes	Yes	Yes	Yes
Bonferroni		4CM > 4C 6CM > 6C 6CM > TCM	4CM > 4C 6CM > 6C TCM > 6C 6CM > TCM	4C > 4CM 6C > 6CM 4C > TCM	4C > 4CM 6C > 6CM	4C > 4CM 6C > 6CM 6C > TCM TCM > 6CM

Values are means (SD). 4C, 4-mo-old Bio F1B hamsters; 4CM, 4-mo-old cardiomyopathic hamsters (CM) untreated; 6C, 6-mo-old Bio F1B; 6CM, 6-mo-old CM untreated; TCM, 6-mo-old CM treated with thyroid hormones (TH); LVIDd, left ventricular (LV) internal diameter in diastole; LVIDs, LV internal diameter in systole; PWTd, postwall thickness in diastole; PWTs, postwall thickness in systole; EF, ejection fraction; Yes, significantly different among the groups ($P < 0.05$).

tendency for reversal of fibronecrosis with TH in TCM vs. 4CM hamsters. Reduced fibronecrosis in TCM vs. 6CM hamsters was readily apparent by gross observation. Quantitative data for fibronecrosis are shown in Fig. 3A.

Myocyte morphometry. LV isolated myocyte data are shown in Table 3. *V*, *L*, and *CSA* tended to increase in controls as a result of the age-related increase in body mass typical of male rodents (NS). *V*, *CSA*, and *L* increased significantly in both treated and untreated CM hamsters between 4 and 6 mo of age. Although the increase in cellular dimensions between 4 and 6 mo tended to be less in TCM hamsters, there were no significant differences between TCM and 6CM hamsters in *V*, *CSA*, or *L*.

There was no difference in myocyte number between 4- and 6-mo-old controls. 4CM hamsters had 30% fewer myocytes than age-matched controls. There was a further loss of myocytes in CM hamsters between 4 and 6 mo of age. This loss of myocytes was completely prevented by TH treatment (Fig. 3B).

Terminal deoxynucleotide transferase-mediated dUTP nick-end labeling. Control hamsters displayed little or no terminal deoxynucleotide transferase-mediated dUTP nick-end labeling (TUNEL) at 4 or 6 mo of age (range 0–1 positive cells per 1,000). In untreated CM hamsters, there was also very little TUNEL labeling, with a range of 0–2 per 1,000 cells at 4 mo and 0–4 per 1,000 cells at 6 mo. Thyroid treatment had no effect on labeling in CM hamsters (range 1–4 per 1,000 cells).

Myocardial blood flow. Changes in myocardial blood flow are summarized in Fig. 4. Values for resting and maximum myocardial blood flow were significantly reduced in CM hamsters at both 4 and 6 mo of age compared with age-matched controls. TH treatment completely normalized resting and adenosine-induced maximum myocardial blood flow.

DISCUSSION

This is the first laboratory animal study investigating the pathophysiological consequences of TH therapy in an animal model of dilated cardiomyopathy. The principal findings of our study were that resting and maximum myocardial blood flow was reduced in CM hamsters and this was associated with cardiac myocyte loss and increased fibronecrosis. Although cardiac function continued to deteriorate between 4 and 6 mo of age in untreated CM hamsters, this decline was generally prevented or attenuated by TH treatment. Additionally, treatment of CM hamsters with TH restored resting and maximum myocardial blood flow to normal, prevented further loss of myocytes, and reduced the extent of fibronecrosis. Serum levels of T_4 and TSH suggest that CM hamsters had subclinical hypothyroidism and that the selected dose of TH was effective. The results suggest that subclinical hypothyroidism may lead to a reduction in the myocardial blood flow in dilated cardiomyopathy, with profound effects on cardiac structure and function.

 Table 2. *Hemodynamics*

Groups	<i>n</i>	Heart Rate, beats/min	LVPes, mmHg	LVPed, mmHg	+dP/dt, mmHg/s	−dP/dt, mmHg/s
4C	12	464 (38)	158 (12)	7.2 (2.4)	9,075 (1,210)	6,363 (1,607)
4CM	12	478 (66)	125 (9)	9.6 (2.7)	8,040 (1,660)	4,978 (910)
6C	11	432 (70)	129 (14)	5.4 (2.5)	6,511 (908)	4,838 (997)
6CM	11	393 (38)	95 (14)	8.1 (3.1)	5,689 (1,011)	3,423 (537)
TCM	14	477 (37)	111 (14)	8.4 (3.4)	7,432 (1,228)	4,444 (566)
ANOVA		Yes	Yes	Yes	Yes	Yes
Bonferroni		4C > 6CM 4CM > 6CM TCM > 6CM	4C > 6C 4C > 4CM 6C > 6CM 4CM > 6CM	4CM > 6C	4C > 6C 4CM > 6CM TCM > 6CM	4C > 6C 4C > 4CM 4CM > 6CM 4C > TCM

Values are means (SD). LVPes, LV end-systolic pressure; LVPed, LV end-diastolic pressure; +dP/dt, maximum rate of LV pressure increase; −dP/dt, maximum rate of LV pressure reduction; Yes, significantly different among the groups ($P < 0.05$).

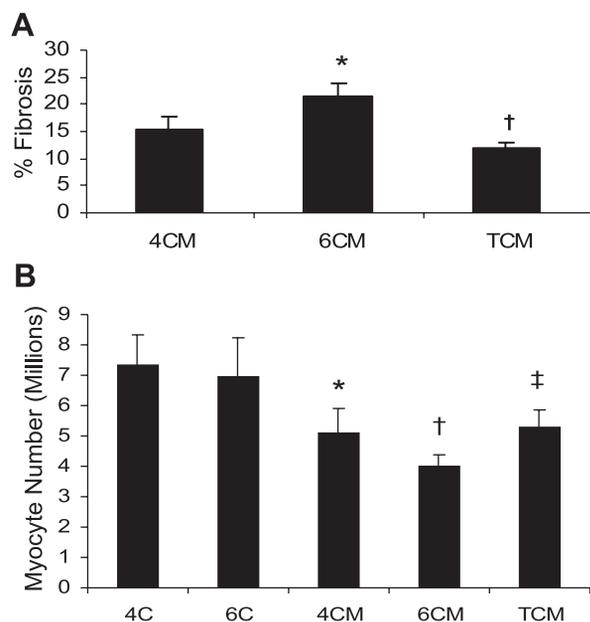


Fig. 3. A: effects of thyroid treatment on myocardial fibrosis; $n = 5$ for TCM and 4 for all other groups. * $P < 0.05$ vs. 4CM; † $P < 0.05$ vs. 6CM. B: effects of thyroid treatment on myocyte number; n as shown in Table 3. * $P < 0.05$ vs. 4C; † $P < 0.05$ vs. 4CM; ‡ $P < 0.05$ vs. 6CM.

After initial experiments demonstrated that TH treatment prevented myocyte loss and tended to reverse areas of fibro-necrosis, we investigated the potential role of myocardial ischemia in these changes. This decision was based on work by Ryoke et al. (26), who concluded that ischemic myocyte loss (oncosis), rather than apoptosis, was the likely explanation for pathological changes found in CM hamsters. Because we did not see an increase in TUNEL labeling in CM hamsters, our findings agree with Ryoke et al. regarding the absence of an apoptotic mechanism. Our fluorescent microsphere measurements confirmed that CM hamsters had a reduction in resting myocardial blood flow. We expected to observe a larger increase in adenosine-induced maximum myocardial blood flow in TH-treated CM hamsters because hypothyroidism is known to increase coronary resistance (9). In fact, the magnitude of the blood flow response to adenosine was similar in all animal groups (approximate doubling). This finding suggests that arterioles were responding normally in treated and un-

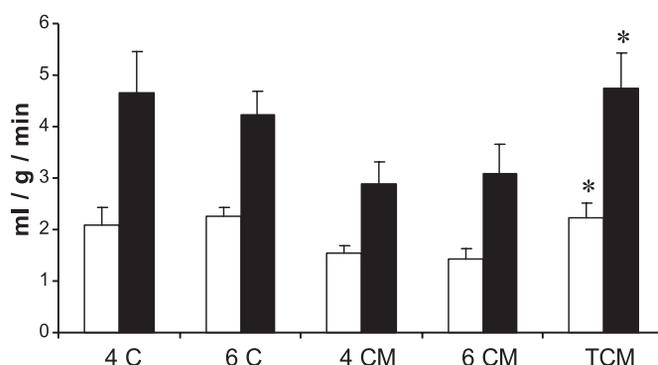


Fig. 4. Effects of thyroid hormone treatment on myocardial blood flow; $n = 6$ for 4C, 6C, and TCM; $n = 7$ for 4CM and 6CM. Open bars, resting blood flow; filled bars, maximum blood flow. Resting and maximum blood flow was significantly reduced in CM hamsters at both 4 and 6 mo ($P < 0.05$). * $P < 0.05$ vs. 4CM and 6CM.

treated CM hamsters. However, the number of arterioles was likely reduced in CM hamsters and restored to normal with TH treatment. Although this appeared to be the case (e.g., arterioles were clearly easier to find in TCM than untreated CM hamsters), this must be confirmed morphometrically in future experiments. Alternatively, the possibility that TH treatment led to an increase in arteriolar diameter cannot be excluded. It should be noted that values for resting myocardial blood flow in Bio T0-2 and Bio F1B hamsters obtained in our experiments are virtually identical to those reported previously by Panchal and Trippodo (24) for these strains.

The effects of TH on coronary microvasculature have been examined by others. Several studies have shown that thyroxine and the TH analog 3,5-diiodothyropropionic acid (DITPA) stimulate angiogenesis and increase baseline and maximum myocardial blood flow (4, 9, 32–34, 38). Another study by Heron and Raksuan (13) showed that neonatal hypothyroidism led to a reduction in myocardial arterioles. Consequently, there is strong evidence that TH can alter the myocardial microcirculation both anatomically and functionally. In this study, we report for the first time that TH dysfunction may have dramatic adverse effects on the myocardial microcirculation in dilated cardiomyopathy. Without quantitative assessment of arteriolar changes, however, the underlying basis of altered coronary blood flow and its reversal by TH in CM hamsters is not certain. Although it appears that subclinical hypothyroidism may have promoted a loss of arterioles in CM hamsters and arteriogenesis with TH treatment, other possibilities should be considered. TH treatment may have led to increased diameters of resistance vessels. Blood flow measurements may also have been affected by aortic perfusion pressure (flow = pressure/resistance). Perfusion pressure was reduced in CM hamsters. Coronary perfusion, which occurs primarily during diastole, may also have been affected by the onset of bradycardia in the 6-mo-old untreated CM group. We suspect that TH treatment promoted new vascular growth based on the above-mentioned studies, but this has yet to be confirmed. The mechanism by which TH exerts a proangiogenic effect is not clear at this time. However, Davis et al. (5) demonstrated that TH-induced angiogenesis is MAP kinase dependent and mediated by FGF. A recent study using DITPA, a thyroxine analog, also showed upregulation of FGF, VEGF, angiotensin, and Tie-2, factors known to stimulate angiogenesis (34).

Table 3. Isolated myocyte morphology

Groups	n	Cell Volume, μm^3	Cell Length, μm	CSA, μm^2
4C	6	44,586 (6,295)	152.7 (8.7)	293 (41)
4CM	7	37,275 (4,262)	148.4 (8.9)	252 (37)
6C	7	51,404 (7,483)	162.6 (9.2)	316 (35)
6CM	6	56,082 (5,842)	176.7 (10.1)	313 (26)
TCM	7	49,660 (2,372)	169.3 (7.8)	293 (9)
ANOVA		Yes	Yes	Yes
Bonferroni		6CM > 4CM	6CM > 4CM	6C > 4CM
		6CM > 4C	TCM > 4CM	6CM > 4CM
		6C > 4CM	6CM > 4CM	
		TCM > 4CM	TCM > 4CM	
			6C > 4CM	

Values are means (SD). CSA, cross-sectional area; Yes, significantly different among the groups ($P < 0.05$).



Blood levels of T₄ in 6CM hamsters were within the normal range, but elevated TSH levels indicated the presence of subclinical hypothyroidism by that time. TSH levels tended to be elevated in 4CM, but this was not yet statistically significant. Because we have observed a significant increase in TSH levels by 5 mo of age in Bio T0-2 hamsters (Gerdes AM, unpublished observations), it appears that thyroid dysfunction was developing by the time treatment was initiated. This also suggests that thyroid dysfunction begins early in this model and is not simply a consequence of developing heart failure. Indeed, results from the current experiments suggest that earlier treatment with TH before the onset of pump dysfunction, fibronecrosis, and myocyte loss may have prevented the pathophysiological changes from occurring.

It could be argued that hyperthyroidism was induced in TCM based on the significant rise in T₄ levels. However, all other evidence suggests that the treatment dose did not produce overt hyperthyroidism. If overt hyperthyroidism were induced by treatment, one would expect TSH levels to be significantly below normal, yet they tended to remain slightly above normal. One should also note that treatment of CM hamsters did not lead to an increase in any hemodynamic or functional parameters compared with values at the beginning of treatment. Treatment did not produce tachycardia, cardiac hypertrophy, myocyte hypertrophy, or increased oxygen consumption. Oxygen consumption, in fact, remained below control levels in TCM hamsters. It should be appreciated that CM hamsters are in a state of declining health and cardiovascular function. The onset of bradycardia, decline in oxygen consumption, and rise in TSH levels observed in untreated 6CM hamsters suggest the emergence of thyroid dysfunction in this model. At this time, we know very little about thyroid dysfunction in heart disease. Optimum dosing is certainly something that should be addressed in future experiments. It seems intuitively obvious that the lowest dose that provides optimum benefits would be most desirable.

Although not measured in our experiments, it is possible that increased activity of specific deiodinases may contribute to hypothyroidism at the myocardial tissue level. Indeed, a recent study by Wassen et al. (35) demonstrated a dramatic increase in the type III deiodinase in ventricular tissue in a model of hypertension and heart failure. This deiodinase converts T₃ and T₄ to inactive metabolites. It is also possible that changes in the transport of TH into myocardial tissues may be affected in heart disease, but little is known about this. Importantly, no data are available on myocardial tissue levels of TH in either animals or humans with heart failure of any etiology. It is likely that serum TH and TSH levels alone provide a limited understanding of the extent of thyroid dysfunction in heart disease. Clearly, more comprehensive investigations of metabolism of TH, their transport into myocardial tissue, and deiodinase activity in heart failure are needed.

Although the Bio F1B strain is commonly used for comparison to CM hamsters, it may not be an ideal control. The Bio F1B hamster is prone to develop atherosclerosis when fed a high-cholesterol diet (36). In our experiments, we also noted a decline in LV function in this strain between 4 and 6 mo of age. Of relevance to this study, however, the Bio F1B hamsters displayed normal ventricular morphology, stable myocyte number, and normal resting and maximum coronary blood flow.

TH are known to have direct effects on cardiac fibroblasts. Cardiac fibroblasts grown in TH-depleted media show increased abundance of mRNA for procollagen and increased nuclear incorporation of thymidine (3). T₄ treatment of cultured cardiac fibroblasts leads to a decrease in procollagen mRNA and induction of protooncogenes (37). In general, TH-induced cardiac hypertrophy is distinguished by a lack of LV cardiac fibrosis (37). In the CM hamster model used here, we believe the key change with thyroid treatment is a reduction in myocyte loss, which leads to a reduction in replacement fibrosis. Direct effects of TH on myocardial collagen, however, cannot be ruled out.

This is the first study to examine the effects of TH treatment on myocyte shape and number in heart failure. Additionally, this is the first time a method of proven reliability was used to assess changes in ventricular myocyte number in heart failure. The consistency of this method is again illustrated by our data showing stable myocyte number in controls between 4 and 6 mo of age despite the presence of a considerable increase in heart mass and myocyte size from physiological growth.

It should be stressed that these data are from an animal model of dilated cardiomyopathy and must be confirmed in humans. It is clear from many human studies, however, that TH dysfunction, including subclinical hypothyroidism, is common in heart failure, is associated with increased mortality, and often goes untreated. Cumulative data from humans and animals show that low thyroid function promotes atherosclerosis, promotes myocardial fibrosis, increases coronary resistance, reduces inotropy, and may also lead to the profound blood flow alterations observed here. If untreated subclinical hypothyroidism in patients also leads to the microvascular changes observed here, the potential for improving outcome in heart failure is obvious.

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