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Reversible alterations in myocardial gene expression in a young man with dilated cardiomyopathy and hypothyroidism

(myocardium/heart failure)

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ABSTRACT **Thyroid hormone effects on myocardial gene expression have been well defined in animal models, but their relationship to the pathogenesis of cardiac dysfunction in hypothyroid humans has been uncertain. We evaluated a profoundly hypothyroid young man with dilated cardiomyopathy. Before and during 9 months of thyroxine therapy, serial assessment of myocardial performance documented substantial improvements in the left ventricular ejection fraction (16–37%), left ventricular end-diastolic diameter (7.8–5.9 cm), and cardiac index (1.4–2.7 liters·min^{-1}·m^{-2}). Steady-state levels of mRNAs encoding selected cardiac proteins were measured in biopsy samples obtained before and after thyroxine replacement. In comparison with myocardium from nonfailing control hearts, this patient's pretreatment α-myosin heavy-chain mRNA level was substantially lower, the atrial natriuretic factor mRNA level was markedly elevated, and the phospholamban mRNA level was decreased. All of these derangements were reversed 9 months after restoration of euthyroidism. These observations in an unusual patient with profound myxedema and cardiac dilatation permit correlation between the reversible changes in myocardial function and steady-state mRNA levels in a cardiomyopathy. They suggest that alterations in gene expression in the dilated myopathic heart may be correctable when a treatable cause is identified.**

Most patients with dilated cardiomyopathy have a poor long-term prognosis, with progressive myocardial dysfunction. Rarely, a metabolic etiology responsive to specific therapy is identified. For example, previous reports have described reversal of the hemodynamic and morphologic abnormalities of cardiomyopathy with treatment of pheochromocytoma (1), hypocalcemia (2, 3), and carnitine deficiency (4, 5). The "myxedema heart" was first described by Zondek (6), who noted a dilated cardiac silhouette, slow indolent heart action, and low electrocardiographic voltage, which were all corrected by thyroid hormone therapy. Although invasive (7) and noninvasive (8) studies have since confirmed that thyroid hormone deficiency is associated with a reversible decrease in myocardial contractility, it has remained controversial whether hypothyroidism alone can cause a dilated cardiomyopathy and clinical heart failure. Furthermore, although effects of thyroid hormones on myocardial myosin isoenzyme expression (9, 10), β-adrenergic receptor number (11), sarcoplasmic reticulum calcium exchange (12), and guanine nucleotide-binding regulatory proteins (13) have all been described in experimental animals, the fundamental pathogenesis of myocardial dysfunction caused by thyroid hormone deficiency in humans has remained uncertain.

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We report a young man with a dilated cardiomyopathy and profound hypothyroidism. During 9 months of thyroid hormone replacement therapy, serial clinical observations and noninvasive cardiovascular function tests documented substantial improvement in myocardial performance. Quantitation of steady-state mRNA levels in endomyocardial biopsy specimens before and after treatment has provided insight into the molecular pathogenesis of heart failure in hypothyroidism and, more generally, into the reversibility of the alterations in steady-state levels of mRNA that accompany dilated cardiomyopathy.

MATERIALS AND METHODS

Studies were performed with the patient's informed consent in accordance with institutional human studies guidelines. Serum thyroxine and thyrotropin concentrations were quantified by radioimmunoassay (Diagnostics Products, Los Angeles) and chemiluminescent immunoradiometric assay (London Diagnostics, Eden Prairie, MN), respectively. The free thyroxine index was calculated as the product of the serum thyroxine concentration and triiodothyronine (T_3) resin uptake. Creatine phosphokinase was determined by a standard colorimetric technique. Serial determinations of the pulse-wave arrival time (QK_d) (14), metabolic stress test (15), and echocardiographic (16) and radionuclide gated blood pool scan (17) assessments of cardiovascular functions were performed as described. Endomyocardial biopsy samples were obtained by a standard technique (18), placed in sterile polyethylene tubes (17 × 100 mm), immediately frozen in liquid nitrogen, and stored at −70°C.

Steady-state levels of mRNA in the endomyocardial biopsy samples were measured by PCR as described (19, 20) (see Table 2). Total RNA was isolated by a modification of the acid guanidinium thiocyanate/phenol/chloroform extraction (RNAzol B; Cinna/Biotex, Friendswood, TX), and total RNA concentration was assessed spectrophotometrically (Beckman DU-65). First-strand cDNA was then synthesized by reverse transcription of 1 μg of the total RNA isolated from the endomyocardial biopsy sample using oligo(dT) primers (Boehringer Mannheim). The resulting cDNA was amplified in a TempCycler (Coy Laboratory Products, Ann Arbor, MI) with *Thermus aquaticus* DNA polymerase (Perkin-Elmer/Cetus) in the presence of specific oligonucleotide primers complementary to selected regions of the mammalian gene encoding the protein of interest (20) (see Table 2). The 3′ primer of each primer pair was end-labeled with

Abbreviations: ANF, atrial netriuretic factor; IU, international unit(s).

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$[\gamma\text{-}^{32}P]ATP$ by using T4 polynucleotide kinase (Pharmacia LKB) so that each synthesized DNA strand would be radiolabeled. To ensure that the amplification products arose from the appropriate genes, the identity of the PCR product for each primer pair was verified by sequence analysis after asymmetric amplification.

To quantify the amount of PCR product, a known quantity of control RNA containing base pair sequences complementary to those used to amplify the selected cDNA was added to 1 μg of total RNA isolated from the endomyocardial biopsy sample before reverse transcription reaction and PCR. The control RNA was produced by *in vitro* transcription of a synthetic DNA template having a T7 promoter at its 5′ end, an adenine tract at its 3′ end, and an internal base pair sequence. The amplification product of the control cDNA synthesized from this template ≈72 base pairs (bp). As the amplification products of the control cDNA and sample DNA were substantially different in size, they could be separated by agarose gel electrophoresis (Fig. 1). Amplification products were visualized with indirect UV irradiation and cut out from the gel, and radioactivity in the bands of interest was determined by Cerenkov counting. Amplification curves were constructed by removing 10 μl of each PCR mixture during successive cycles of amplification and plotting radioactivity in the excised bands against amplification cycles. Alternatively, standard curves were obtained by including various concentrations of control RNA and total RNA in each PCR and plotting radioactivity against concentrations of the control RNA. For each primer pair, the amount of control RNA in the reverse transcription reaction was adjusted such that the ratios of sample RNA (1 μg) and control RNA would allow for colinearity of the sample RNA and control RNA products in both the amplification and standard curves. The amount of mRNA in the endomyocardial biopsy sample could, therefore, be assessed in relation to the amount of product from the control RNA.

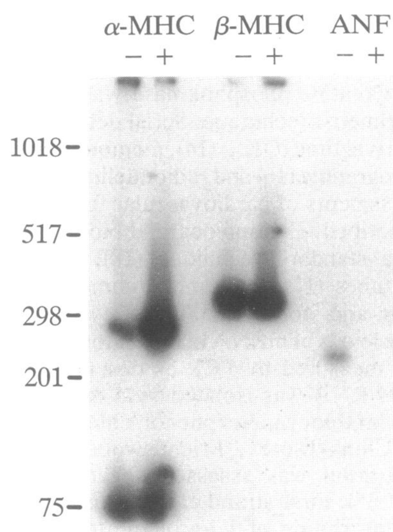

FIG. 1. Autoradiogram demonstrating PCR products from endomyocardial biopsies of a patient with hypothyroidism obtained before (lanes −) and after (lanes +) 10 months of thyroid replacement therapy. One gram of total RNA was reverse transcribed and 1/40th of the resulting cDNA was amplified for 26 cycles with PCR in the presence of trace-labeled oligonucleotide primers for the various cDNAs of interest. In this experiment, performed for illustrative purposes, appropriate concentrations of control RNAs were present only in the reverse transcription reactions for α-myosin heavy-chain amplification. After electrophoretic separation of sample and control (72 bp) amplification products, the agarose gel was dried and exposed to radiographic film. Sizes (bp) of the expected products are indicated on the left. MHC, myosin heavy chain; ANF, atrial natriuretic factor.

RESULTS

A 22-year-old man was transferred to The Johns Hopkins Hospital with the complaint of pleuritic chest pain of 4 days duration, superimposed on a several-month history of progressive fatigue and exertional dyspnea. Initial evaluation 3 days before at another hospital had revealed cardiomegaly (cardiothoracic ratio, 20.5:30.5) and pulmonary vascular congestion on chest x-ray. Biventricular dilatation with global hypokinesis and a moderate posterior pericardial effusion were visualized by echocardiography. Laboratory studies there included a markedly elevated serum creatine kinase concentration, 9902 international units (IU)/liter (normal, 35–374 IU/liter), in association with a less-elevated creatine kinase MB fraction, 38 IU/liter (normal, 1–15 IU/liter); an undetectable level of serum thyroxine, <2.6 nmol/liter (0.2 μg/dl); and elevated serum thyrotropin, >60 milliunits/liter. In retrospect, the patient had experienced slowly progressive lethargy, cold intolerance, hoarseness, and body hair loss over the preceding 5 years. An anemia had been diagnosed 5 years previously and was treated with a brief course of oral iron therapy. There was no history of hepatic, rheumatologic, or other endocrine disease. The patient consumed only 12–24 ounces of beer per week. There was no history of a recent viral syndrome. His mother had been briefly treated for an unknown thyroid disorder in adolescence, but there was no other family history of endocrine or cardiac disease.

On physical examination, the patient was an ill-appearing, lethargic male appearing younger than his chronological age, with sparse secondary sexual hair. He was 168 cm tall and weighed 72.3 kg, with a blood pressure of 86/70 mmHg, a regular pulse of 100 beats/min, and a respiratory rate of 16 per min. He had sallow cool skin, diffuse nonpitting subcutaneous edema, a hoarse voice, and no palpable thyroid tissue. Mild diffuse rales were present on chest auscultation. The cardiac examination was remarkable for a barely palpable but laterally displaced point of maximal impulse, normal first and second beat sounds (S_1 and S_2), an S_4 and S_3, and grade 3/6 holosystolic murmur radiating to the left axilla. A nontender liver edge was palpated 2 cm below the right costal margin; no dependent edema was present. The relaxation phase of his deep tendon reflexes were markedly delayed.

Admission laboratory studies confirmed the presence of severe hypothyroidism with undetectable serum thyroxine, <1.3 nmol/liter (0.1 μg/dl); T_3 resin uptake, 27% (normal 25–35%); and serum thyrotropin concentration, 794 milliunits/liter (normal, 0.5–4.5 milliunits/liter). Serum antimicrosomal thyroid antibodies were not detected. The electrocardiogram showed normal sinus rhythm with normal voltage and no evidence of acute ischemia or infarction. The results of pretreatment noninvasive cardiovascular function tests are summarized in Table 1. On the second day of hospitalization, a right ventricular endomyocardial biopsy was performed, with tissue submitted for histology, electron microscopy, and immediate preservation in liquid nitrogen for subsequent quantitative mRNA analyses. Histopathological examination revealed no specific abnormality; electron microscopy showed nonspecific increases in morphologically normal mitochondria and glycogen as well as prominent interstitial fat globules.

Therapy was initiated with oral levothyroxine sodium at 0.1 mg/day, which was subsequently increased in 0.025-mg increments at 4- to 8-week intervals until the serum thyrotropin concentration was normal (Table 1). The patient was readmitted for clinical evaluations, thyroid function determinations, and noninvasive cardiovascular function testing at frequent intervals up to 314 days of therapy. After 2 weeks of

Table 1. Thyroid function, chemical, and cardiovascular function parameters during treatment of hypothyroidism with levothyroxine sodium

	Thyroxine dose, μg/day (day of treatment)							
	0 (0)	100 (7)	100 (16)	100 (30)	125 (58)	150 (86)	150 (129)	175 (265)
Free thyroxine index	<0.03	0.6	1.2	1.7	2.2	2.2	1.6	1.6
Serum thyrotropin, milliunits/liter	794	614	196	72.6	32.3	0.43	0.38	1.2
Serum cholesterol, mg/dl	211	181	149	137	160	197	176	178
Creatine kinase, IU/liter	4197	4615	1488	181	58	57	52	—
QK_d, msec	424	392	356	338	348	302	282	268
LV ejection fraction, %	16	—	—	—	21	—	36	37
Peak $\dot{V}o_{2max}$, ml·kg·min^{-1}	14.2	—	—	—	16.8	—	32.9	33.1
LV end-diastolic dimension, cm	7.8	—	—	—	—	—	—	5.9
LV end-systolic dimension, cm	6.6	—	—	—	—	—	—	4.5
Cardiac index, liter·min·m^{-2}	1.4	—	—	—	—	—	—	2.7

Normal ranges: free thyroxine index, 1.25–4.2; serum thyrotropin concentration, 0.5–4.5 milliunits/liter; serum cholesterol concentration, 0–199 mg/dl; creatine kinase, 0–175 IU/liter. Peak $\dot{V}o_{2max}$, maximum oxygen consumption obtained during exercise testing, LV, left ventricular.

treatment, the patient experienced less severe fatigue and myalgias but has persistent exertional dyspnea. On day 20 of the therapy, the patient was prematurely readmitted to hospital with a 2-day history of progressive dyspnea and right upper quadrant abdominal pain. New findings included scleral icterus and tender hepatomegaly with hepatojugular venous reflux, which were present without serological or radiological evidence of primary hepatobiliary disease. After addition of furosemide at 40 mg/day and spironolactone at 25 mg/day, symptoms resolved over 4 days and liver function test abnormalities diminished. When reevaluated after 31 days of treatment, the patient had no dyspnea while walking slowly 2 miles each day, but on examination had persistent jugular venous distention, an S_3, and both mitral and tricuspid regurgitant murmurs. The serum creatine kinase concentration, which had steadily declined since the initiation of levothyroxine therapy, was then restored to normal, 181 IU/liter. On day 52 of therapy, the patient was readmitted with recrudescence of his dyspnea and right upper quadrant pain. Digoxin treatment at 0.125 mg/day was initiated and diuretic doses were increased with subsequent resolution of all symptoms and signs of heart failure over 4 days. On day 168 of therapy, digoxin and diuretics were discontinued with no recurrence of cardiopulmonary symptoms.

A comprehensive reassessment was performed after 265 days of therapy, when the patient was taking only levothyroxine (0.175 mg/day). He was completely asymptomatic, performing well in his university, and working part-time delivering pizza. The patient appeared fit and clinically euthyroid. Results of follow-up thyroid and cardiovascular function testing are summarized in Table 1.

DISCUSSION

The clinical presentation and course in this patient strongly suggest that the etiology of his heart muscle disease was hypothyroidism. Myocardial morphology was normal. The patient was young and had a normal blood pressure, normal serum cholesterol concentration, and no other risk factors for atherosclerosis. Furthermore, therapy with levothyroxine resulted in a gradual substantial improvement in the patient's ventricular ejection fraction, functional capacity, and pulse-wave arrival time, all of which persisted after discontinuation of diuretic and digoxin therapy. However, the possibility that partial restoration of myocardial function in this case might also be attributable to spontaneous recovery or a secondary effect of thyroid hormone therapy cannot be excluded. Nonetheless, improvements in the patient's clinical, thyroid, and noninvasive cardiovascular function parameters were accompanied by an 11-fold increase in ventricular α-myosin heavy-chain mRNA levels and a doubling of phospholamban mRNA levels (Table 2). ANF mRNA was abundant before treatment but could not be detected after 10 months of thyroid hormone therapy. In contrast, serial measurements demonstrated no change in the steady-state levels of myocardial β-myosin heavy-chain, β_2-adrenergic receptor, and β-actin mRNAs.

Thyroid hormones exert their cellular actions by regulating expression of a set of mRNAs encoding specific cellular proteins (23). In myocardium, thyroid hormones regulate expression of myosin heavy-chain isoforms, increasing the level of α-myosin heavy-chain mRNA up to 6-fold in hypothyroid rats, whereas expression of β-myosin heavy-chain mRNA is reduced by one-third (9). These changes confer higher myocardial ATP hydrolytic and contractile rates. The increase in steady-state levels of α-myosin heavy-chain mRNA observed in this patient was consistent with these previously studied thyroid hormone effects in animals. It seems likely that this change is accounted for by thyroid hormone action since changes in α-myosin heavy-chain mRNA levels have not been observed in failing human heart

Table 2. Steady-state levels of mRNAs in endomyocardial biopsy samples

Transcript	Pretreatment hypothyroidism	Posttreatment euthyroidism	Nonfailing heart controls (*n*)
α-Myosin heavy chain	3.5×10^6	3.9×10^6	$41 \pm 16 \times 10^6$ (7)
β-Myosin heavy chain	1.5×10^9	1.2×10^9	$0.9 \pm 0.2 \times 10^9$ (7)
ANF	6.0×10^7	Undetectable	Undetectable (8)
Phospholamban	5.1×10^9	1.0×10^9	$0.8 \pm 0.1 \times 10^9$ (8)
β_2-Adrenergic receptor	1.7×10^7	1.9×10^7	$1.5 \pm 0.04 \times 10^7$ (8)
β-Actin	1.2×10^8	1.7×10^8	$1.2 \pm 0.1 \times 10^8$

Values are mean ± SEM. Undetectable indicates $<2.8 \times 10^6$. Values are expressed as molecules of mRNA per μg of total RNA. Each sample was assayed twice. Oligonucleotide sequences of 5′ and 3′ primers, respectively, for PCR amplification have been described (20). Primer pairs were as follows: for α-myosin heavy chain (21), 5′-ATCAAGGAGCTCACCTACCAG-3′ and 3′-CACTCCTCATCGTGCATTTTC-5′; for β_2-adrenergic receptor (22), 5′-ACTGCTATGCCAATGAGACC-3′ and 3′-AGGTTATCCTGGATCACATG-5′.

(24). The patient's posttreatment steady-state level of α-myosin heavy-chain mRNA was similar to those in nonfailing human hearts obtained from organ donors whose hearts could not be used for transplantation ($4.1 \times 10^7 \pm 1.6 \times 10^7$ molecules per μg of total RNA; $n = 7$). In contrast, thyroid hormone replacement produced no change in the β-myosin heavy-chain mRNA level. Before and after levothyroxine therapy, the quantity of β-myosin heavy-chain mRNA in this patient's myocardium was similar to levels previously reported in nonfailing human heart (20). Since mRNA encoding the β-myosin heavy-chain isoform was present in a substantially higher concentration than α-myosin heavy-chain mRNA, improvement in this patient's myocardial function may not be attributable solely to enhanced α-myosin heavy-chain gene expression and myosin ATPase activity alone.

The pattern of myocardial gene expression characteristic of heart failure was altered with thyroid hormone-mediated recovery of left ventricular performance. Increased steady-state ANF mRNA levels and decreased phospholamban mRNA levels in patients with idiopathic dilated cardiomyopathy have previously been reported (20, 25). The substantial pretreatment level of ANF mRNA in this patient's myocardium was consistent with these observations. With improvement in intrinsic cardiac function after 10 months of levothyroxine therapy, ANF mRNA was no longer detectable. Furthermore, there was a 2-fold increase in the phospholamban mRNA level, with posttreatment levels comparable to those previously reported in nonfailing human heart (20). These changes in ANF and phospholamban mRNAs were temporally related to a substantial improvement in cardiac function as assessed by three independent noninvasive techniques. It is doubtful that nonhormonal medical therapy affected the results of the present study, since endomyocardial biopsies were obtained before initiating and 146 days after terminating pharmacologic therapy. Furthermore, it is unlikely that thyroid hormone *per se* was responsible for the observed changes in expression of these genes. In animal models, hypothyroidism has not been associated with significant changes in expression of either the phospholamban (26) or ANF (27) genes, whereas experimental hyperthyroidism actually decreases myocardial phospholamban gene expression and increases myocardial ANF mRNA levels. These previously reported effects are opposite those observed with reversal of this patient's dilated cardiomyopathy. Therefore, the changes in phospholamban and ANF mRNA levels seem most attributable to reversal of heart failure, rather than to direct thyroid hormone effects. Although we cannot exclude the alternative possibility that thyroid hormone regulates myocardial gene expression in a unique manner in humans, these data appear to demonstrate that in humans the abnormal steady-state levels of mRNA associated with congestive heart failure are reversible.

The decrease in steady-state phospholamban mRNA levels in this patient were less than those previously reported in patients with congestive heart failure (20). The absence of medical therapy in this case at the time of endomyocardial biopsy may account for this difference. The physiological significance of diminished phospholamban mRNA levels in this setting remains unclear. However, if associated with a decrease in gene products, they may contribute to the decreased diastolic relaxation that is characteristic of dilated cardiomyopathy.

Quantitative PCR is a valuable tool for assessing steady-state levels of mRNA in the intact human heart. In previous studies, mRNA levels were measured in relatively large (i.e., 1 g) tissue samples obtained at the time of either cardiac transplantation or organ donation (28, 29). However, interpretation of these studies has been difficult because (*i*) tissue was obtained at advanced stages in the disease process, (*ii*) patients were receiving multiple pharmacologic agents, and (*iii*) sequential measurements in the same heart were not possible. By including an internal RNA control in the reverse transcription and subsequent PCRs, we have been able to quantify the absolute amount of mRNA present in small endomyocardial biopsy samples. This approach facilitates acquisition of tissue from patients with milder disease and without multiple drug therapy as well as evaluation of serial myocardial samples. The finding that both β-skeletal actin and β_2-adrenergic receptor mRNAs were similar in the biopsies obtained before and after thyroid hormone replacement suggests that the differences in α-myosin heavy-chain, phospholamban, and ANF mRNA levels could not be accounted for by mRNA degradation.

This report of an unusual hypothyroid patient with dilated cardiomyopathy may provide insight into the fundamental pathogenesis of myocardial dysfunction in human hypothyroidism. Furthermore, these serial studies of steady-state myocardial mRNA levels elucidate the molecular changes that accompany restoration of near normal contractile function in a reversible cardiomyopathy. This patient's clinical course, laboratory, hemodynamic, and pathological findings suggest that significant ventricular hypokinesis and dilatation can be caused by thyroid hormone deficiency without other myocardial disease. Application of a quantitative PCR technique has shown that some, but not all, of the abnormalities in gene regulation previously reported in hypothyroid animals have also been observed in a hypothyroid man with cardiomyopathy. Moreover, these studies illustrate that altered mRNA levels in a dilated myopathic heart can be reversible, with improvement in cardiac function.

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