Serum thyroid hormone levels may not accurately reflect thyroid tissue levels and cardiac function in mild hypothyroidism

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Liu Y, Redetzke RA, Said S, Pottala JV, Morreale de Escobar G, Gerdes AM. Serum thyroid hormone levels may not accurately reflect thyroid tissue levels and cardiac function in mild hypothyroidism. Am J Physiol Heart Circ Physiol 294:H2137–H2143, 2008. First published February 29, 2008; doi:10.1152/ajpheart.01379.2007.—The link between thyroid dysfunction and cardiovascular diseases has been recognized for more than 100 years. Although overt hypothyroidism leads to impaired cardiac function and possibly heart failure, the cardiovascular consequences of borderline low thyroid function are not clear. Establishment of a suitable animal model would be helpful. In this study, we characterized a rat model to study the relationship between cardiovascular function and graded levels of thyroid activity. We used rats with surgical thyroidectomy and subcutaneous implantation of slow release pellets with three different T4 doses for 3 wk. In terminal experiments, cardiac function was evaluated by echocardiograms and hemodynamics. Myocardial arteriolar density was also quantified morphometrically. Thyroid hormone levels in serum and heart tissue were determined by RIA assays. Thyroidectomy alone led to cardiac atrophy, severe cardiac dysfunction, and a dramatic loss of arterioles. The low T4 dose normalized serum T3 and T4 levels, but cardiac tissue T3 and T4 remained below normal. Low-dose T4 failed to prevent cardiac atrophy or restore cardiac function and arteriolar density to normal values. All cardiac function parameters and myocardial arteriolar density were normalized with the middle dose of T4, whereas the high dose produced hyperthyroidism. Our results show that thyroid hormones are important regulators of cardiac function and myocardial arteriolar density. This animal model will be useful in studying the pathophysiological consequences of mild thyroid dysfunction. Results also suggest that cardiac function may provide valuable supplemental information in proper diagnosis of mild thyroid conditions.

thyroidectomy; thyroxine; myocardial arterioles

THE CARDIOVASCULAR SYSTEM has long been recognized as one of the most important targets of thyroid hormones (THs). Low thyroid function has been linked to a variety of heart diseases, including myocardial infarction (8), coronary atherosclerosis (4), and congestive heart failure (3). Our previous study demonstrated that hypothyroidism from 1-yr treatment with propylthiouracil (PTU) led to cardiac atrophy, chamber dilatation, impaired myocardial blood flow, and a 45% reduction in myocardial arterioles (26). However, a clinical report suggested that PTU can induce vasculitis (21). Consequently, it is possible that the arteriolar loss may have been due to drug toxicity rather than hypothyroidism. To address this issue, we investigated the effects of surgical thyroidectomy on myocardial arteriolar density.

Although overt hypothyroidism leads to impaired cardiac function and possibly heart failure, the cardiovascular consequences of borderline low thyroid function (e.g., subclinical hypothyroidism, sick euthyroid syndrome, etc.) are not clear. A growing body of clinical evidence suggests that these conditions lead to increased cardiovascular risk and mortality (24, 30). Although reports on the incidence of borderline low thyroid function vary widely, one study suggested a 4–10% prevalence of subclinical hypothyroidism in the general population and an incidence of ~20% in women over age 60 (18). When considering the number of individuals at risk, it is surprising that only one animal study to date has examined the effects of subclinical hypothyroidism and heart disease (12). In that study, we demonstrated dramatic improvement in cardiac function and remodeling in cardiomyopathic hamsters treated with TH. It is clear that a major impediment to research in this area is the lack of a suitable animal model to systematically study the cardiovascular consequences of graded levels of thyroid function. In particular, it would be most useful to examine cardiac risk at various levels of thyroid function between euthyroidism and hypothyroidism. In this study, we have characterized a rat model that will enable such studies to be conducted safely and effectively. Results suggest that cardiac functional measures may provide valuable supplemental information in treatment decisions regarding mild thyroid dysfunction.

MATERIALS AND METHODS

Animal model and study design. Rats used in this study were purchased from Charles River Laboratories (Wilmington, MA). Fourteen 6-wk-old male Sprague-Dawley rats served as controls, and 35 age- and sex-matched thyroidectomized Sprague-Dawley rats were divided into different experimental groups as follows: eight each in placebo, 1.65-mg, and 3.3-mg groups; and 11 in an 8.25-mg group. Subcutaneous placebo and pellets with various doses [1.65, 3.3, and 8.25 mg of thyroxine (T4)] were prepared in 60-day release rate pellets; Innovative Research of America (IRA), Sarasota, FL] were implanted in the neck 1 wk after surgery. All animals were exposed to a 12:12-h light-dark cycle and given standard rat chow and water ad libitum. After 3 wk of T4 treatment, echocardiographic and hemodynamic measurements were collected. Changes in arteriolar density were quantified morphometrically. Serum was collected for total triodo-

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thyronine (T3) and T4 assays. Additionally, myocardial T3 and T4 were determined from animals in each group. All procedures in this study were approved by the University of South Dakota Animal Care and Use Committee and followed institutional guidelines for animals.

**Echocardiography and hemodynamics.** In terminal experiments, echocardiographic and hemodynamic data were collected. Rats were anesthetized with 1.5% isoflurane. After the chest was shaved, each animal was placed on an isothermal pad that was maintained at ~40°C with echocardiographic gel applied to the left hemithorax.

M-mode images were obtained from the short axis of the left ventricle (LV) at the level of the papillary muscle and used to measure left ventricular internal dimensions and posterior/anterior wall thickness (PWT/AWT). A VisualSonic Vevo 660 high-resolution imaging system with a 25-MHz RMV-710 transducer (Toronto, ON, Canada) was used in this study.

After the echocardiograms were completed, the right carotid artery was dissected, isolated, and cannulated with a Millar (Houston, TX) ultraminiature pressure transducer catheter. This pressure catheter was inserted into the right carotid artery and advanced through the aorta into the LV. When the pressure was stable, measurements were recorded and processed electronically by a Digi-Med Heart Performance Analyzer (Micro-Med, Louisville, KY). Left ventricular systolic pressure, heart rate (HR), maximal rate of pressure rise (dP/dT), and maximal rate of pressure decline (NdP/dT) were collected (11, 17).

**Quantification of arterioles.** A transverse slice midway between the base and apex of the LV (~3 mm thickness) was collected and fixed in 10% neutral buffered formalin. Frozen formalin-fixed tissue specimens were sectioned at 5 μm by cryostat (Leica CM1800 Cryostat; North Central Instruments, Polytron, MN) at ~20°C. Mouse anti-α-smooth muscle actin Cy3-conjugated monoclonal antibody (C6198; Sigma, St. Louis, MO) was used to label arterioles. Data were collected from approximately 25 randomly selected fields from each animal at ×20 magnification. On the basis of the minor diameter, arterioles between 5 and 30 μm with at least one layer of smooth muscle were used to quantify numerical density (ND) and length density (LD) in each animal. Arteriolar data were referenced to the whole muscle rather than tissue section area to eliminate errors due to tissue shrinkage or separation artifacts. Additionally, myocytes account for ~95% of the tissue solid mass (10) and nearly all of the tissue oxygen needs. Arteriolar ND was calculated from average number of arterioles per unit myocyte area. Arteriolar LD (average length of arteriolar unit myocyte volume) was calculated based on the following formula: LD (mm/mm3) = Σ (a/b)/M, where a and b are the maximum and minimum external arteriolar diameters, respectively, and M is the weight of the tissue sample that was to be extracted.

**Determinations of T3 and T4 in serum and rat heart tissue.** Serum T3 and T4 were measured with specific and highly sensitive RIAs as described previously (19, 20). In brief, methanol was added to the frozen tissue sample and homogenized. Tracer amounts of [125I]T3 and [131I]T4 were added to the homogenate. Chloroform-methanol (2:1) was added to extract endogenous and added iodothyronines. The iodothyronines were then back extracted into an aqueous phase and purified through resin columns. Iodothyronines were then eluted and evaporated to dryness. RIA buffer was added and measured by highly sensitive RIAs to detect T3 and T4 levels. Concentrations were calculated using the amount of T3 and T4 found in the RIAs, the individual recovery of the [125I]T3 and [131I]T4 added to each sample, and the weight of the tissue sample that was to be extracted.

**Statistical analyses.** To model weight gain, the mean response profile was examined over time by treatment groups with the use of locally weighted regression and scatter plot smoothing, which suggested that a piecewise general linear model would fit well. Akaike’s information criterion was used to compare models with different time point breaks, and joining the line segments together at 5 days produced the best fit. An unstructured covariance matrix was implemented for the repeated measurements. Temperature was measured as often as weight. However, due to severely skewed raw responses, a longitudinal model was not appropriate. Instead, the change from baseline to final measurement prior to the rats being killed (15 or 16 days) was modeled in a good-fitting simple linear regression.

To model the nonlinear responses of ND and LD, different transformations were conducted on the dosage level. Modeling dosage fits quadratically as well as categorically by Akaike’s information criterion method. The dosage level was mean centered to avoid multicollinearity issues in the polynomial terms.

One-way ANOVA models were implemented for all responses not addressed above. Left ventricular systolic pressure was log transformed for analysis. If homogeneity or normality were not satisfactory even after considering natural log transformations, i.e., heart rate, serum T3 and T4, heart T4, AWT in diastole (AWTd), and PWT in diastole (PWTd), a nonparametric one-way ANOVA (Kruskal-Wallis) was performed on the ranked measurements.

**RESULTS**

**Physical data.** One week after surgery, rats were supplemented with the T4 doses indicated in MATERIALS AND METHODS. Body weight (BW) and body temperature were monitored every 3 or 4 days and reported in Fig. 1. A and B. Body temperature was significantly reduced in the placebo group, normalized in the low-dose (1.65 mg) and middle-dose (3.3 mg) groups, and significantly increased in the 8.25-mg group. Placebo rats failed to gain weight, whereas weight gain was slightly but significantly attenuated in the 1.65-mg group (Fig. 1C). Rats treated with the 3.3-mg pellets gained weight normally, whereas 8.25-mg T4 treatment significantly increased BW. Upon arrival to our facility, surgery rats had slightly but significantly higher BW than controls before treatments started despite being age matched (Fig. 1B). However, there was no significant difference in weight 5 days after T4 treatment. Rats with placebo and low-dose (1.65 mg) treatments showed a significant heart atrophy compared with the controls, whereas high-dose (8.25 mg) T4 treatment led to a significant hypertrophy (Fig. 1D). The 3.3-mg dose normalized both heart weight (HW) and BW, whereas the 8.25-mg dose resulted in a significantly higher HW/BW (Fig. 1E).

**Echocardiography and hemodynamics.** LV function and cardiac dimensions, collected by echocardiography in terminal experiments, are reported in Fig. 2. LV fractional shortening was reduced in the placebo and 1.65-mg groups and normalized in the 3.3- and 8.25-mg groups compared with the control group. In general, changes in systolic and diastolic chamber dimensions and wall thickness confirmed cardiac atrophy and severe systolic dysfunction in the placebo group, with limited restoration of these parameters in the 1.65-mg group and normalization in the 3.3-mg and 8.25-mg groups.

**Hemodynamic data** (shown in Fig. 3) confirmed hypothyroidism in the placebo group with a 35% reduction in HR, a 33% reduction in dP/dT, and a 33% reduction in NdP/dT compared with controls. Each of these parameters remained significantly reduced to a similar extent in the 1.65-mg group.
while being normalized in the 3.3-mg group. Hemodynamic measurements from the 8.25-mg group suggested borderline hyperthyroidism with significant increases in HR, left ventricular systolic pressure, and NdP/dT.

Myocardial arterioles. Arteriolar morphometry is summarized in Fig. 4. Arteriolar LD in the placebo group was reduced by 34%, and a similar reduction in ND was also observed (34%, data not shown) compared with the control group. This change was due exclusively to arteriolar loss in the smaller, 5- to 15-μm diameter size range. Larger size of arterioles ranging from 15 to 30 μm was not affected. The 1.65-mg dose resulted in partial restoration of arterioles in the myocardium; however, arteriolar LD in the 1.65-, 3.3-, and 8.25-mg groups was not different from controls.

TH levels in serum and heart tissue. THs in whole serum and rat heart tissue were detected by RIA. As shown in Fig. 5, serum T4 was significantly decreased in the placebo group, whereas serum T3 in placebos was lower, but not significantly.

Fig. 1. Physical changes from all animals included in the study. A: changes in body temperature. Dashed lines are 95% confidence bands for the linear regression lines. [ΔTemp(C) = −1.35 + 0.40 × Dose(mg); r² = 0.88]. B: changes in body weight over time shown with locally weighted regression and scatter plot smoothing [dotted line, placebo (P); short-dashed line, 1.65 mg; medium-dashed line, 3.3 mg; long-dashed line, 8.25 mg; solid line, control (C)]. C: body weight gain rate from day 5 to day 22 after treatment. *Dunnett adjustment for 95% confidence intervals of treatment minus control group; 0.00 (mg) T4 indicated P group. D: heart weight. E: heart weight/body weight ratio (HW/BW). Thyroidectomized rats treated with 3 different doses of T4 are labeled as 1.65, 3.3, and 8.25 (in mg). Values are means ± SE. **P < 0.01 vs. control; *P < 0.05 vs. control.

Fig. 2. Echocardiographic data. Values are means ± SE. **P < 0.01 vs. control; *P < 0.05 vs. control. LVFS, left ventricle fractional shortening; AWTd, anterior wall thickness in diastole; AWTs, anterior wall thickness in systole; PWTd, posterior wall thickness in diastole; PWTs, posterior wall thickness in systole; LVIDd, LV internal dimension in diastole.
Both serum T3 and T4 in the high-dose treatment group (8.25 mg) were increased compared with the control group, suggesting moderate hyperthyroidism. Changes in cardiac T3 and T4 levels generally paralleled those observed in serum. There was a decrease in placebo and 1.65-mg T4-treated rats and a significant increase in the higher-dose-treated (8.25 mg) group but no significant differences in 3.3-mg T4-treated rats.

DISCUSSION

The results of this study confirmed that surgical thyroidec-
tomy led to significant myocardial loss of arterioles, as previ-
ously reported to occur in rats treated with PTU (26). Conse-
quently, this change clearly resulted from hypothyroidism
rather than a specific toxic effect of PTU. Dose-related changes
in arterioles also suggest that THs are a powerful regulator of
vascular density, particularly in low-thyroid conditions. An-
other goal of the current study was to establish and characterize
a rat model allowing consistent and reproducible adjustment of
TH levels across the range of thyroid function. Results con-
firmed that subcutaneous implantation of slow-release pellets
containing T4 in thyroidectomized rats enables investigation of
graded levels of TH function, including mild hypo- and hyper-
thyroidism.

Many studies have examined hyperthyroidism and hypo-
thyroidism (15, 26, 32). The purpose of this study, however, was
quite different. Our intention was to demonstrate the feasibility
of using an animal model to study borderline or mild thyroid
dysfunction safely and effectively for the first time. In the
current experiments, we were able to show that thyroid func-
tion can be controlled in a graded manner using slow pellet
release of T4 in thyroidectomized rats. These excellent results
were achieved without the use of radioactive iodine, thus
markedly improving the safety of the animal model. Nearly
20% of women over age 60 and 4–10% of the general popu-
lation have subclinical hypothyroidism (18). Although the
importance of treating subclinical hypothyroidism remains
debatable, clinical epidemiological studies have shown that
this condition may be an independent risk factor for coronary
heart disease (30) and is also associated with an increased risk
of chronic heart failure in older adults when serum thyrotropin
(TSH) level is 7.0 mIU/l or higher (24). To our knowledge, no
basic research related to this important topic has been con-
ducted, likely due to the lack of an established animal model.

Data collected from echocardiography, hemodynamics, and
arteriolar morphometry from placebo rats with surgical thy-
roidectomy were very similar to previously reported values
from rats treated with PTU (26). Placebo rats developed
cardiac atrophy, severe systolic dysfunction, and a dramatic
loss of arterioles. We found the 1.65-mg T4 dose most inter-
esting because of the disconnection between serum hormone
levels and cardiac indicators of thyroid function. Although
serum T3 and T4 were normalized, cardiac T3 and T4 were
significantly below normal and cardiac function remained im-
paired in this group. All measured cardiac parameters were
normalized with the 3.3-mg dose. However, serum hormone
levels were above normal, again suggesting that a similar disconnection between serum hormones and cardiac function can occur in mild hyperthyroidism. This is not surprising considering the known difficulties in proper diagnosis of mild thyroid dysfunction. Our results also confirm previous work by Escobar-Morreale et al. (7) showing elevated serum T4 levels with a T4 dose that normalized TH in most organs. Indeed, there is a clinical controversy regarding the concept that euthyroidism may be best obtained with combination T3 plus T4 treatment. The general consensus, however, suggests that combination treatment is not substantiated by available information related to risk and/or benefit at this time. Results from the current experiment suggest that a T4 dose between 1.65 and 3.3 mg in rats of this size will likely normalize serum and cardiovascular parameters. Indeed, a recent experiment in male rats that were 1 mo older showed that a 2.7-mg pellet resulted in normal serum T3, T4, and cardiac function (Gerdes AM, unpublished observation).

Clinical diagnosis and treatment of mild thyroid dysfunction is not an exact science. Many factors can also complicate the situation. There is a rather wide range in the currently accepted range for normal values in serum TH and TSH. Seasonal and daily fluctuations contribute to within-individual variations, whereas genetic variability may be considerable in normal individuals (2). Additionally, clinicians rarely know what normal hormone values were for a typical patient who develops thyroid dysfunction. If a given individual was high normal to begin with, low-normal hormone values may represent overt hypothyroidism for that particular patient. Noninvasive cardiac echo data may prove helpful. For instance, if an individual with mild hypothyroidism has bradycardia, increased LV systolic dimension, and impaired ejection fraction with no other apparent indications for heart disease, one might be more comfortable with initiating treatment. Clearly, more clinical studies are needed to confirm that such an approach will lead to improved outcome.

It seems logical that cardiac functional measures would provide a more reliable indicator of TH function at the tissue level than would serum hormone levels. Cardiac function represents the end output of TH action on the heart. At the tissue level, many changes can occur downstream of serum hormone levels. There may be alterations in hormone membrane transporters, activation or deactivation of hormones by intracellular deiodinases, changes in thyroid nuclear receptors, posttranslational modification of gene products, etc.

TH is well known as a regulator of vascular remodeling in the heart. TH can induce cardiac hypertrophy as well as capillary angiogenesis, which has been proven in many animal models such as rat, pig, and rabbit (6, 27, 31). Tomanek and colleagues (28, 29) have shown that TH, as well as its analog 3,5-diiodothyropropionic acid, can promote coronary angiogenesis in physiological hypertrophy and after myocardial infarction. However, the mechanism of myocardial arteriolar loss in hypothyroidism is not clear. A similar reduction in arterioles, known as rarefaction, occurs in hypertension (5, 14). Reduced arteriolar density was also observed in the current experiment. However, hypertension-associated rarefaction is a completely different phenomenon than observed here in hypothyroidism. With arteriolar rarefaction from hypertension, hypertrophying cardiac myocytes push vessels further apart, resulting in reduced vascular density. In the case of hypothyroidism, there is actually atrophy of myocyte cross-sectional area associated with cardiac atrophy (16). In this case, one might expect an increase in vascular density. However, metabolic needs are significantly reduced in hypothyroidism so that fewer vessels may be needed. Rate pressure product (RPP = HR × systolic blood pressure) is an index of myocardial oxygen consumption. RPP was lower in placebos than in controls, suggesting that reduced metabolic needs may play an important role. It is for this reason that we suspect arteriolar loss in hypothyroidism is likely related to disuse; arterioles are simply not needed and die off. Since hypothyroidism leads to arteriolar vasoconstriction (13, 23), it is possible that sustained vasoconstriction leads to arteriolar loss from either hypoxia or apoptosis. Vascular endothelial growth factor and nitric oxide

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**Fig. 5.** Triiodothyronine (T3) and throxine (T4) levels in serum and heart tissue. A: serum T4 levels. B: serum T3 levels. C: rat heart tissue T4 levels. D: rat heart tissue T3 levels. Values are means ± SE. **P < 0.01 vs. control; *P < 0.05 vs. control.
may also play a role. Reduced vascular endothelial growth factor and nitric oxide, known to occur in hypothyroidism, have been implicated in microvascular rarefaction in metabolic syndrome (9).

Whether arteriolar loss occurs in other important vascular beds during low-thyroid states is unknown but could be of considerable clinical consequence. For instance, it is possible that this condition leads to neurovascular loss when one considers the neurological consequences of low thyroid function (25). The model characterized here should be of considerable use to study organ and systemic consequences of thyroid dysfunction and borderline thyroid dysfunction.

The surgical thyroidectomy with T4 replacement model described here is modified from a similar model used by Escobar-Morreale et al. (7). In their experiments, surgical thyroidectomy was followed by 131I to ablate thyroid remnants. Escobar-Morreale et al. (7). In their experiments, surgical thyroidectomy was followed by 131I to ablate thyroid remnants. 

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It should be noted that we have observed batch-related inconsistencies in TH activity of the manufactured pellets used in this experiment. When graded doses were manufactured at the same time, as in the current experiment, an excellent dose-related response was obtained in serum hormone levels and thyroid functional changes. This has been confirmed in another recent experiment in addition to the current report. However, when new delivery dose rates were ordered based on the current experimental results, we observed a batch-related discrepancy in hormone activity on two occasions. Consequently, one may consider having all doses prepared at the same time or the use of osmotic pumps as an alternative. However, Alzet minipumps are much larger and have more limited maximum time release rates than the slow-release pellets used here (6 wk for Alzet pumps vs. 90 days for IRA pellets).

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REFERENCES


