Published in final edited form as:

Endocr Pract. 2014 August ; 20(8): 797–807. doi:10.4158/EP13453.OR.

3,3'- DIODOTHYRONINE CONCENTRATIONS IN HOSPITALIZED OR THYROIDECTOMIZED PATIENTS: RESULTS FROM A PILOT STUDY@

Jacqueline Jonklaas, MD PhD¹, Anpalakan Sathasivam, MD^{1,2}, Hong Wang, MD MS³, David Finigan, MD¹, Offie P. Soldin, PhD^{1,4}, Kenneth D. Burman, MD², and Steven J. Soldin, PhD^{1,5}

¹Division of Endocrinology, Georgetown University, Washington DC ²Section of Endocrinology, Medstar Washington Hospital Center ³Medstar Health Research Institute, Hyattsville, Maryland ⁴Department of Oncology ⁵Department of Laboratory Medicine, National Institutes of Health

Abstract

Objective—To determine if various medical conditions affect the serum concentrations of 3,3'-diiodothyronine (3,3'-T2).

Methods—100 patients, recruited from a group of inpatient and outpatients with a diverse range of medical conditions, donated a single blood sample that was assayed for thyroid hormone derivatives using liquid-chromatography tandem mass spectrometry. The associations between 3,3'-T2 concentrations and physiologic data and medical conditions were assessed.

Results—Higher quartiles of 3,3'-T2 concentrations (quartile 1: 2.01-7.48, quartile 2: 7.74-12.4, quartile 3: 12.5-17, quartile 4: 17.9-45.8 pg/ml) were associated with decreasing occurrence of critical illness (58%, 11%, 0%, 8%,), stroke (29%, 7.7%, 4%, 0%), critical care unit hospitalization (75%, 39%, 8.3 %, 12%), and inpatient status (83%, 42%, 8%, 12%), all p values <0.001. The same quartiles were associated with increasing frequency of thyroidectomy (4%, 12%, 17%, 60%). In multivariate analyses, after adjustment for age and gender, inpatient status was associated with decreasing concentrations of 3,3'-T2 (46% decrease for inpatients with 95% CI 32-57%, p value <0.0001). Thyroidectomy was associated with increasing concentrations of 3,3'-T2 (29% increase (CI 0.5-66%, p value 0.049).

Conclusion—We demonstrate associations between inpatient status and reduced 3,3'-T2 concentrations. This appears to be a global change associated with illness, rather than an association with specific medical conditions. We newly report higher 3,3'-T2 concentrations in athyreotic outpatients receiving TSH suppression therapy. This demonstrates that there is

@Data presented in part at the 95th Annual Meeting of the Endocrine Society in San Francisco, CA

© 2013 AACE.

Correspondence address: Jacqueline Jonklaas, Division of Endocrinology, Georgetown University, Suite 230, Bldg. D, 4000 Reservoir Road, NW, Washington, DC 20007. jonklaaj@georgetown.edu.

Conflict of interest: JJ, AS, HW, DF, OS, and SJS have no relevant disclosures.

production of 3,3'-T2 from levothyroxine in extrathyroidal tissues. Conversion of T4 to 3,3'-T2 via both T3 and rT3 pathways may prevent excessive T3 concentrations in such patients.

Keywords

3,3'-diiodothyronine; tandem mass spectrometry; illness; thyroidectomy

Introduction

Iodothyronines include thyroxine (T4), triiodothyronine (T3), reverse triiodothyronine (rT3), 3,5- diiodothyronine, and 3,3'-diiodothyronine (3,3'-T2). T4 and T3 have well-established roles and exert the classic effects of thyroid hormones (1). Although an uncontrolled study of 3,5-diiodothyronine administration to humans for 4 weeks by an unspecified route was associated with increased metabolic rate and reduced body weight (2), no specific role of 3,3'-T2 in humans has been demonstrated (3). Animal studies, however, suggest that the 3,5-diiodothyronine and 3,3'-T2 increase metabolic rate (3, 4), by acting at the mitochondrial level to increase hepatic cytochrome oxidase activity (5).

3,3'-T2 has been detected in human serum by radioimmunoassay (6) and has also recently been detected by MS (7). When measured by radioimmunoassay, its concentration generally parallels the concentration of other iodothyronines such as T4 and T3. Specifically with regard to thyroid conditions, it decreases with the hypothyroid state, is normal during thyroid hormone replacement, and increases with the hyperthyroid state (6, 8-13). Several studies have documented altered levels during non-thyroidal medical conditions. Variable concentrations have been reported in patients with cirrhosis (9, 14). In one study, patients with myocardial infarction, malignancies, and uremia, were found to have low levels of 3,3'-T2 (9). In another study its concentrations were low in patients with brain injury, normal in patients with sepsis, and elevated in those with either liver disease or brain tumors, compared with healthy controls (12). Individuals with a diagnosis of anorexia nervosa have lower than normal levels of 3,3'-T2 (10), as do those who undergo a period of calorie restriction (15). Serum 3,3'-T2 concentrations have also been noted to decrease with advancing age (10, 16).

We recently developed a MS method to measure T4, T3, rT3, and 3,3'-T2 simultaneously in blood samples (17). We have previously reported reference intervals for 3,3'-T2 in healthy subjects (7). We now use this methodology to document these analytes in a group of patients with a variety of medical conditions.

This was a pilot study designed to examine the concentrations of thyroid hormone derivatives in inpatients and outpatients with a variety of medical conditions. Our primary hypothesis was that various serious illnesses or simply having an inpatient status would be associated with lower 3,3'-T2 levels than were seen in outpatients. A secondary hypothesis was that thyroid disorders would be associated with altered 3,3'-T2 concentrations.

Methods

Patient eligibility and recruitment

One hundred patients were recruited for this pilot study. Patients were recruited from the Medstar outpatient clinics and inpatient services at Georgetown University and Washington Hospital Center between January and June 2011. Individuals of all ages with any medical diagnoses were eligible to be included in order to capture conditions that could potentially affect thyroid hormone derivative concentrations. All patients, or their authorized representatives, signed a written informed consent form. Patients donated a single blood sample. The status of each patient as an outpatient or inpatient, along with the specific unit in which the patient was hospitalized, was recorded. Information on demographic factors, medical history, and medication use were collected by a combination of chart review and personal interview. Height, weight, body mass index (BMI), systolic blood pressure and diastolic blood pressure were abstracted from a current physical examination.

Assay methodology

All samples were stored at -80°C and analyzed in one batch at the completion of the study. The concentrations of the thyroid hormone derivatives (T4, T3, rT3, and 3,3'-T2) were measured by MS, as previously described (7, 17, 18), with methods similar to those used for free thyroid hormones also (19-22). The method for measuring 3,3'-T2 involves a step to separate it chromatographically from 3,5-diiothyronine (17).

The reference interval for TSH was 0.40-4.00 mIU/L. Reference intervals for each of the thyroid analytes are 3,3'-T2 6.7-23 pg/mL; T3 74-168 ng/dL; rT3 7.7-23.1 ng/dL; T4 4.2-10.9 mcg/dL. These latter reference intervals were obtained using samples from 130 healthy females and 130 healthy males within the age range of 20-60 years (7). The 2.5th and 97.5th percentiles were calculated using 3 different methods, including the Percentile, the Gaussian and the Hoffmann approaches. The reference intervals for T4, T3 and rT3 were similar to those found in the literature. The reference interval for 3,3'-T2 was new and not previously known. The narrowest reference intervals were found when employing the Hoffmann approach. Nevertheless the reference intervals obtained using these 3 approaches agreed fairly well.

Statistical Analysis

Descriptive statistics of physiologic data (age, height, weight, body mass index (BMI), systolic blood pressure (SBP), diastolic body pressure (DBP), gender, and race) and medical data (glycosylated hemoglobin (HgbA1C), glucose, creatinine, inpatient/outpatient status, inpatient unit, thyroid cancer, thyroidectomy, levothyroxine (LT4) therapy, hyperthyroidism, Hashimoto's hypothyroidism, hypertension, taking antihypertensive medications, diabetes, taking oral diabetes medications, taking insulin, critically ill, and stroke) were calculated according to the inpatient or outpatient status and the quartile of thyroid hormone analyte concentrations. Other medications being taken by patients were also documented, but were not included in the analysis as there were many medications, mostly being taken by only a small subset of patients. Data are reported as mean $\pm$ standard deviation or median (1st quartile, 3rd quartile) or frequency (proportion). T-test or analysis of variance (ANOVA)

was used to compare the means and Wilcoxon rank-sum test was used to compare the distribution. Chi-square test was used to compare the proportions among the groups. Thyroid hormone analyte concentrations were analyzed both as categorical variables within quartiles and continuous variables. Log-transformation was applied to variables that were highly skewed in the analyses as continuous variables.

In order to determine which characteristics were important predictors of thyroid analyte concentrations, the association between potential physiologic or medical factors and the levels of the thyroid hormone analytes was examined with use of a logistic regression model or linear regression model with adjustment for age and gender. Variables with p values ≥ 0.2 in the univariate model were included in the multivariate model. P values < 0.05 were considered statistically significant. In order to describe the relationship between variables, in addition to reporting each parameter estimate as a coefficient, estimates were also back log-transformed. The back-log transformed parameter estimate was then reported as a percentage change in the dependent variable for a one unit increase in the continuous variables, or as compared with the referent group for categorical variables. Data were analyzed using SAS version 9.1 (SAS Institute, Cary, NC). Two-sided P values < 0.05 were considered statistically significant.

Results

Population characteristics

Of the 100 study patients, 63 were outpatients and the remaining 37 were inpatients (see table 1). Outpatients were younger ($p=0.015$) and had higher diastolic blood pressure ($p=0.002$) than inpatients (see table 1a). Other characteristics such as height, weight, BMI, and systolic blood pressure were similar for the two groups. Ninety five percent of the inpatients were from critical care units, and were fairly equally divided between the medical intensive care unit, the cardiac care unit, and the neurologic intensive care unit (35%, 33%, and 27% respectively) (see table 1b). Sixty seven percent of the outpatients and 49% of the inpatients were of female gender ($p=0.077$). There were no significant differences in the prevalence of diabetes and hypertension between the inpatients and outpatients (see table 1c). The prevalence of thyroid diseases was higher in the outpatients, compared to the inpatients. Most outpatients with diagnoses of thyroid cancer were taking levothyroxine and had normal or low serum TSH values. With a few exceptions the patients with Hashimoto's hypothyroidism were a subset of the patients diagnosed with thyroid cancer. The following physiologic data and medical conditions had either few or no correlations with the thyroid derivative means, medians, or quartiles, and, for simplicity, their analysis is not reported further: race, BMI, systolic blood pressure, HgbA1C, glucose, creatinine, hyperthyroidism, taking antihypertensive medications, taking oral diabetes medications, and taking insulin.

Thyroid derivative means or medians

The mean or median analyte concentrations documented in the various medical conditions are shown in table 2 after adjustment for age and gender. The concentrations of 3,3'-T₂, T₃, and T₄ were higher in patients with thyroid cancer, patients who had undergone thyroidectomy, and those who were taking LT₄, than patients without. Patients with these

conditions also had lower TSH concentrations than those without. Patients who were either critically ill, had sustained a stroke, were hospitalized in a critical care unit, or were inpatients had lower 3,3'-T₂, T₃, and T₄ concentrations than patients without. Those who were critically ill, hospitalized in the critical care unit, or were inpatients also had higher rT₃ concentrations than those without each of these conditions.

Thyroid derivative concentrations as quartiles

The quartiles used to divide the thyroid derivative concentrations are shown in table 3. These quartiles and categories were then used to examine the effect of physiologic and medical data.

Correlation of thyroid hormone derivative concentrations with physiologic data

Using the thyroid analyte concentrations categorized into the quartiles shown in table 3, the following analytes and physiologic data were significantly associated (see table 4a for changes with quartiles, direction of changes, and p values). 3,3'-T₂ concentration was negatively associated with age, and positively associated with DBP. T₃ concentration was negatively associated with age and height, positively associated with DBP, and positively associated with female gender. T₄ concentration was negatively associated with age, height, but positively associated with female gender. TSH concentration was negatively associated with DBP, and positively associated with Caucasian race. rT₃ concentration was not associated with any physiological variable.

Correlation of thyroid hormone derivatives concentrations with medical data

Using the thyroid analyte concentrations categorized into quartiles, the following medical data and analytes were significantly associated (see table 4b). Decreasing 3,3'-T₂, T₃, and T₄ concentrations were associated with a diagnosis of stroke. Decreasing 3,3'-T₂, T₃, and T₄, but increasing rT₃ concentrations were associated with being in a critical care unit. Similarly, decreasing 3,3'-T₂, T₃, and T₄, but increasing rT₃ concentrations were associated with having a critical illness.

Increasing 3,3'-T₂ and T₄, but decreasing TSH concentrations were associated with both a diagnosis of thyroid cancer and having had a thyroidectomy. Increasing quartiles of T₃ concentration were associated with an increasing percentage of a thyroid cancer diagnosis. No other condition was positively associated with increasing quartiles of T₃ concentration. Increasing 3,3'-T₂ and T₄, but decreasing TSH concentrations were associated with both taking LT₄ and having a diagnosis of hypothyroidism.

Multivariate Analysis

In a multivariate model, the potential independent predictors for the concentration of each thyroid analyte that remained significant after adjustment for age and gender are shown in table 5 and figure 1. (All analytes with p values ≥ 0.2 were log-transformed for this analysis.)

As shown in figure 1 in which estimates are grouped by conditions, older age was associated with decreased concentrations of T₄, T₃, and 3,3'-T₂. Male gender was associated with decreased concentration of T₃. Being critically ill was associated with decreased

concentrations of T3. Being hospitalized in the critical care unit was associated with decreased T3 concentration, but increased concentrations of rT3. Inpatient status was associated with decreased concentrations of T4 and 3,3'-T2. Having had a thyroidectomy was associated with increased concentrations of T4 and 3,3'-T2.

Using back log-transformed data the percentage change in each dependent variable for a unit increase in each continuous variable, or compared with the referent group for categorical variables, is also shown in table 5. As can be seen, for example, 3,3'-T2 concentration was increased by 29% (CI 0.5-65.7%) in athyreotic thyroid hormone-replaced patients and was decreased by 46.2% (CI 32.4-57.2%) in inpatients.

Discussion

These analyses demonstrate the profiles of a panel of thyroid hormone derivatives in patients with various physiologic characteristics and medical conditions with either inpatient or outpatient status at the time of blood sampling.

Multivariate analyses showed a very modest negative effect of age on the concentrations of 3,3'-T2, T3, and T4 concentrations. Other studies of thyroid hormone changes with age from the literature show mixed results, with a decrease in 3,3'-T2 and T3 with age being the most consistent findings. Serum 3,3'-T2 concentrations were seen to decrease with advancing age in 2 studies (10, 16). T3 appears to decrease and rT3 increases with age in general (23), and in the very elderly (24). T4 was shown to decrease significantly with age in the National Health and Nutrition Examination III Survey (25). However, T4 increased with age in a population of elderly men (23).

However, the greatest reductions in thyroid hormone derivatives were associated with being critically ill, being hospitalized in a critical care unit, and having an inpatient status. Both critical illness and being in a critical care unit were associated with decreased T3 concentration. Effects specific to one of these two conditions only were the following: reduced T4 and increased rT3 associated with being hospitalized in a critical care unit. Simply being an inpatient was associated with reduced concentrations of T4, and 3,3'-T2.

While reductions in T3 and increases in rT3 during illness are well-established, the findings with regard to 3,3'-T2 of are less well documented or novel. Prior studies of 3,3'-T2 showed that its levels were differentially affected by various illnesses, but were low in patients with myocardial infarction, malignancies, and uremia, (9), low in patients with brain injury (12), and low during starvation (15) or in individuals with a diagnosis of anorexia nervosa (10). In our analysis 3,3'-T2 concentrations were significantly reduced in individuals who had suffered a stroke in univariate analysis. This could not be confirmed in multivariate analyses, presumably due to the fact that the number of patients who had suffered a stroke was a small subset of all the inpatients. As a novel finding, we observed that 3,3'-T2 concentrations were decreased in patients with an inpatient status, perhaps suggesting a non-specific effect of illness in general or poor nutritional status to reduce 3,3'-T2 concentrations. The observation that 3,3'-T2 concentrations are low in several illnesses that are also associated with increased rT3 and low T3 is interesting. The fact that despite high

rT3 levels, high levels of 3,3'-T2 are not being observed, perhaps suggests that while T3 deiodination is occurring, sufficient type 3 deiodinase activity must be present to prevent accumulation of 3,3'-T2.

Most of our patients who were taking thyroid hormone were from the outpatient group, rather than the inpatient group. They were also mostly athyreotic following thyroidectomy, had a diagnosis of thyroid cancer, and were being managed to maintain low serum TSH values. This was demonstrated by the finding that thyroidectomy was associated with high T4 values and low TSH values. We also documented increased concentrations of 3,3'-T2 in these patients. Prior studies have shown that hypothyroidism and hyperthyroidism are associated with lower (6, 8-12) and higher (6, 8-12) than normal concentrations of 3,3'-T2 respectively. Treatment of hypothyroid patients with T3 was observed to increase their 3,3'-T2 levels (6, 8, 10, 11, 13), as was treatment with T4 (11) and rT3 also (8).

Although the treatment of hypothyroid patients with either T4 or T3 has been shown to increase their serum 3,3'-T2 levels, to our knowledge only one study, published in abstract form only has demonstrated this finding of restored 3,3'-T2 levels in athyreotic patients specifically (26). This study therefore confirms the prior abstract finding that 3,3'-T2 is being produced by peripheral conversion from LT4 in extrathyroidal tissues.

3,3'-T2 is produced from T3 by the action of type 1 and 3 deiodinases. 3,3'-T2 is also produced from rT3 by the action of type 1 and 2 deiodinases. As T3 levels were not also increased above the normal range in our LT4-replaced patients, and neither were rT3 concentrations, this might suggest that the higher than normal levels of T4 are being converted to both T3 and rT3 in similar amounts, such that neither of their individual concentrations are elevated, but that the breakdown of both these thyroid derivatives combines to produce higher than normal concentration of 3,3'-T2. LT4-treated patients have been documented to have higher T4 levels than euthyroid patients not receiving treatment and also frequently have lower than normal T3 levels (27-30). Dual use of these two pathways may be a mechanism for preventing excessive T3 levels in those with high T4 levels. This could, paradoxically, also limit the ability to normalize T3 levels in LT4-treated patients.

Our study has several limitations. Our hospitalized patients, as is typical in most inpatient groups, were being administered several different medications. Being given antihypertensive medications, oral diabetes medications, or insulin was not significantly associated with the concentration of any thyroid derivatives. Other medications were being administered to too small a subset of the population to permit meaningful analysis. However, we cannot rule out that use of multiple medications may be contributing to the changes in thyroid hormone derivatives in inpatients. Additionally, because this was a pilot study designed to capture conditions affecting thyroid analytes, our participants had a wide variety of characteristics. Although multivariate analyses identified independent predictors of thyroid analyte concentrations, some of these predictors were associated with each other (e.g. thyroidectomy and thyroid cancer) and others had minimal overlap (e.g. thyroidectomy and inpatient status).

In summary, this pilot study provided preliminary data that supported our primary and secondary hypotheses. We found low 3,3'-T2 concentrations in participants with an inpatient status. The current role of 3,3'-T2 in humans is unknown and explanations for its decreased concentrations in the inpatient situation, and whether it is an adaptive or maladaptive change, can be examined in future larger studies conducted after confirmation of these findings. We also found that 3,3'-T2 levels tended to be high, and in many cases above the normal reference interval, in LT4-replaced athyreotic patients, perhaps shedding light on why such patients may have high T4 levels, but not high T3 levels that exceed the normal reference interval.

Acknowledgments

This project has been funded in part with Federal funds (UL1TR000101 previously UL1RR031975) from the National Center for Advancing Translational Sciences (NCATS), National Institutes of Health, through the Clinical and Translational Science Awards Program (CTSA), a trademark of DHHS, part of the Roadmap Initiative, "Re-Engineering the Clinical Research Enterprise". Statistical analyses were provided by the Design, Biostatistics, and Population Studies component of the Georgetown-Howard Universities Center for Clinical and Translational Science.

KDB has or has recently had research grants from Pfizer, Eisai, Amgen, Astra Zeneca, and IPE Inc. He is Deputy Editor of the Endocrine Society journal, the Journal of Clinical Endocrinology and Metabolism. He serves on the Food and Drug Administration Endocrine Advisory Committee as an ad hoc member.

Abbreviations

3,3'-T2	3,3'-diiodothyronine
ANOVA	Analysis of variance
BMI	Body mass index
CI	Confidence interval
DBP	Diastolic blood pressure
HgbA1C	Glycosylated hemoglobin
LT4	Levothyroxine
MS	Liquid chromatography tandem mass spectrometry
Q	Quartile
rT3	Reverse triiodothyronine
SBP	Systolic blood pressure
STD	Standard deviation
T3	triiodothyronine
T4	thyroxine
TSH	Thyroid stimulating hormone

References

1. Piehl S, Hoefig CS, Scanlan TS, Kohrle J. Thyronamines--past, present, and future. *Endocr Rev.* 2011; 32:64–80. [PubMed: 20880963]
2. Antonelli A, Fallahi P, Ferrari SM, et al. 3,5-diiodo-L-thyronine increases resting metabolic rate and reduces body weight without undesirable side effects. *J Biol Regul Homeost Agents.* 2011; 25:655–660. [PubMed: 22217997]
3. Moreno M, de Lange P, Lombardi A, Silvestri E, Lanni A, Goglia F. Metabolic effects of thyroid hormone derivatives. *Thyroid.* 2008; 18:239–253. [PubMed: 18279024]
4. Moreno M, Lombardi A, Beneduce L, et al. Are the effects of T3 on resting metabolic rate in euthyroid rats entirely caused by T3 itself? *Endocrinology.* 2002; 143:504–510. [PubMed: 11796504]
5. Lanni A, Moreno M, Cioffi M, Goglia F. Effect of 3,3'-diiodothyronine and 3,5-diiodothyronine on rat liver oxidative capacity. *Mol Cell Endocrinol.* 1992; 86:143–148. [PubMed: 1324858]
6. Burman KD, Strum D, Dimond RC, et al. A radioimmunoassay for 3,3'-L-diiodothyronine (3,3'T2). *J Clin Endocrinol Metab.* 1977; 45:339–352. [PubMed: 407243]
7. Soldin, S.; Nguyen, L.; Sun, K.; Greenan, G.; Hanak, M.; Soldin, O. Adult reference intervals for 3-iodothyronamine, thyroxine, triiodothyronine, reverse T3, and 3,3'-diiodothyronine measured by isotope dilution HPLC tandem mass spectrometry in human serum. Abstract E-68; American Association of Clinical Chemists Annual Meeting; 2011;
8. Wu SY, Chopra IJ, Nakamura Y, Solomon DH, Bennett LR. A radioimmunoassay for measurement of 3,3'-L-diiodothyronine (T2). *J Clin Endocrinol Metab.* 1976; 43:682–685. [PubMed: 956351]
9. Faber J, Kirkegaard C, Lumholtz IB, Siersbaek-Nielsen K, Friis T. Measurements of serum 3',5'-diiodothyronine and 3,3'-diiodothyronine concentrations in normal subjects and in patients with thyroid and nonthyroid disease: studies of 3',5'-diiodothyronine metabolism. *J Clin Endocrinol Metab.* 1979; 48:611–617. [PubMed: 429507]
10. Burger A, Sakoloff C. Serum 3,3'-L-diiodothyronine, a direct radioimmunoassay in human serum: method and clinical results. *J Clin Endocrinol Metab.* 1977; 45:384–391. [PubMed: 903393]
11. Gavin LA, Hammond ME, Castle JN, Cavalieri RR. 3,3'-Diiodothyronine production, a major pathway of peripheral iodothyronine metabolism in man. *J Clin Invest.* 1978; 61:1276–1285. [PubMed: 659591]
12. Pinna G, Hiedra L, Meinhold H, et al. 3,3'-Diiodothyronine concentrations in the sera of patients with nonthyroidal illnesses and brain tumors and of healthy subjects during acute stress. *J Clin Endocrinol Metab.* 1998; 83:3071–3077. [PubMed: 9745405]
13. Nishikawa M, Inada M, Naito K, et al. Serum concentrations of 3, 3'-diiodothyronine, 3', 5'-diiodothyronine, and 3, 5-diiodothyronine in altered thyroid states. *Endocrinol Jpn.* 1983; 30:167–172. [PubMed: 6641638]
14. Faber J, Thomsen HF, Lumholtz IB, Kirkegaard C, Siersbaek-Nielsen K, Friis T. Kinetic studies of thyroxine, 3,5,3'-triiodothyronine, 3,3,5'-triiodothyronine, 3',5'-diiodothyronine, 3,3'-diiodothyronine, and 3'-monoiodothyronine in patients with liver cirrhosis. *J Clin Endocrinol Metab.* 1981; 53:978–984. [PubMed: 7287881]
15. Jaedig S, Faber J. The effect of starvation and refeeding with oral versus intravenous glucose on serum 3,5-,3,3'-and 3'-5'-diiodothyronine and 3'-monoiodothyronine. *Acta Endocrinol (Copenh).* 1982; 100:388–392. [PubMed: 7113605]
16. Nishikawa M, Inada M, Naito K, et al. Age-related changes of serum 3,3'-diiodothyronine, 3',5'-diiodothyronine, and 3,5-diiodothyronine concentrations in man. *J Clin Endocrinol Metab.* 1981; 52:517–522. [PubMed: 7462404]
17. Nguyen L, Gu J, Soldin O, Soldin S. Development and validation of an isotope dilution tandem mass spectrometry method for the simultaneous quantification of 3-iodothyronine, thyroxine, triiodothyronine, reverse T3 and 3,3'-diiodo-L-thyronine in human serum. *Clin Chem.* 2011; 57:A82. Abstract B-99.
18. Soukhova N, Soldin OP, Soldin SJ. Isotope dilution tandem mass spectrometric method for T4/T3. *Clin Chim Acta.* 2004; 343:185–190. [PubMed: 15115693]

19. van Deventer HE, Mendu DR, Remaley AT, Soldin SJ. Inverse log-linear relationship between thyroid-stimulating hormone and free thyroxine measured by direct analog immunoassay and tandem mass spectrometry. *Clin Chem*. 2011; 57:122–127. [PubMed: 21097676]
20. Soldin SJ, Soukhova N, Janicic N, Jonklaas J, Soldin OP. The measurement of free thyroxine by isotope dilution tandem mass spectrometry. *Clin Chim Acta*.. 2005; 358:113–118. [PubMed: 16018881]
21. Gu J, Soldin OP, Soldin SJ. Simultaneous quantification of free triiodothyronine and free thyroxine by isotope dilution tandem mass spectrometry. *Clin Biochem*. 2007; 40:1386–1391. [PubMed: 17936741]
22. Soldin OP, Jang M, Guo T, Soldin SJ. Pediatric reference intervals for free thyroxine and free triiodothyronine. *Thyroid*. 2009; 19:699–702. [PubMed: 19583487]
23. van den Beld AW, Visser TJ, Feelders RA, Grobbee DE, Lamberts SW. Thyroid hormone concentrations, disease, physical function, and mortality in elderly men. *J Clin Endocrinol Metab*.. 2005; 90:6403–6409. [PubMed: 16174720]
24. Mariotti S, Barbesino G, Caturegli P, et al. Complex alteration of thyroid function in healthy centenarians. *J Clin Endocrinol Metab*. 1993; 77:1130–1134. [PubMed: 8077303]
25. Hollowell JG, Staehling NW, Flanders WD, et al. Serum TSH, T(4), and thyroid antibodies in the United States population (1988 to 1994): National Health and Nutrition Examination Survey (NHANES III). *J Clin Endocrinol Metab*. 2002; 87:489–499. [PubMed: 11836274]
26. Burman KD, Dimond RC, McGuire RA, Earll JM, Strum D, Wartofsky L. The effect of varying serum T4 concentrations on extrathyroidal production of T3, reverse T3, and 3,3'-diiodothyronine. *Clin Res*. 1976; 24:270A.
27. Jonklaas J, Davidson B, Bhagat S, Soldin SJ. Triiodothyronine levels in athyreotic individuals during levothyroxine therapy. *JAMA*. 2008; 299:769–777. [PubMed: 18285588]
28. Gullo D, Latina A, Frasca F, Le Moli R, Pellegriti G, Vigneri R. Levothyroxine monotherapy cannot guarantee euthyroidism in all athyreotic patients. *PLoS One*. 2011; 6:e22552. [PubMed: 21829633]
29. Ito M, Miyauchi A, Morita S, et al. TSH-suppressive doses of levothyroxine are required to achieve preoperative native serum triiodothyronine levels in patients who have undergone total thyroidectomy. *Eur J Endocrinol*. 2012; 167:373–378. [PubMed: 22711760]
30. Alevizaki M, Mantzou E, Cimponeriu AT, Alevizaki CC, Koutras DA. TSH may not be a good marker for adequate thyroid hormone replacement therapy. *Wien Klin Wochenschr*. 2005; 117:636–640. [PubMed: 16416346]

Figure 1.

Multivariate model showing the percentage changes in analytes associated with either physiologic data or medical conditions, grouped according to data or conditions (T2 = 3,3'-T2)

Multivariate model. Percentage is the percentage change in dependent variables for one unit increase for continuous variable (age) or as compared with reference group for categorical variables

Table 1

Characteristics of the population studied according to inpatient versus outpatient status

Table 1a	Inpatients (n = 37)		Outpatients (n=63)		Difference
Continuous Variables	Mean	SD	Mean	SD	P value
Age (yrs)	56.7	15.8	48.8	15.1	0.015
Height (cms)	167	11.9	165	9.25	0.353
Weight (kg)	81.9	19.5	84.6	20.1	0.514
BMI (kg/m ²)	28.2	6.6	30.6	8.0	0.138
SBP (mm Hg)	128	26	129	21	0.834
DBP (mm Hg)	69	15	78	11	0.002
HgbA1C for diabetics (%)	7.3	3.1	8.3	2.2	0.370
Serum Glucose mg/dL	144	67	128	78	0.494
Serum Creatinine (mg/dL)	1.16	0.68	1.11	0.99	0.904

Table 1b	Inpatients (n=37)	Outpatients (n=63)	Difference
Categorical variables	Percentage	Percentage	P value
Race			0.385
<i>Caucasian</i>	43%	33%	
<i>African American</i>	49%	52%	
<i>Hispanic/ Asian</i>	8%	14%	
Critical Care Unit			n/a
<i>Intensive care unit</i>	35%	n/a	
<i>Cardiac care unit</i>	33%	n/a	
<i>Stroke care unit</i>	27%	n/a	
<i>No (Medical floor)</i>	5%	n/a	

Table 1c	Inpatients (n=37)		Outpatients (n=63)		Difference
Categorical variables	Yes	No	Yes	No	P value
Male Gender	51%	49%	33%	67%	0.077
Thyroid cancer	3%	97%	40%	60%	<0.0001
Thyroidectomy	3%	97%	35%	65%	0.0002
Taking LT4	3%	97%	37%	63%	0.0001
Hyperthyroidism	0%	100%	10%	90%	0.082
Hashimoto's Hypothyroidism	3%	97%	18%	82%	0.05
Hypertension	54%	38%	49%	51%	0.185
Antihypertensive medications	51%	49%	44%	56%	0.427
Diabetes	27%	73%	38%	62%	0.614
Oral agents for diabetes	5%	95%	22%	78%	0.045
Insulin	30%	70%	22%	78%	0.910
Critically ill	51%	49%	n/a	n/a	n/a
Stroke	22%	88%	n/a	n/a	n/a

Table 2

Thyroid derivative analyte concentration according to the medical condition (only medical conditions with significant differences in analyte concentrations are reported).

Medical condition	Analyte	Analyte concentration in patients with medical condition (mean or median)	Analyte concentration in patients without medical condition (mean or median)	P value
Thyroid cancer (n= 26 with, 74 without)	3,3'-T2	18.4 (12.5-22.3)	11.0 (6.8-15.0)	0.0004
	T3	86.1 ± 18.7	69.6 ± 36.3	0.0091
	rT3	13.6 (11.0-18.4)	17.2 (11.3-23.4)	0.0552
	T4	9.9 ± 2.5	7.6 ± 2.7	0.0004
	TSH	0.54 (0.05-1.21)	1.30 (0.68-2.43)	0.0116
Thyroidectomy (n= 23 with, 77 without)	3,3'-T2	18.9 (13.6-23.6)	11.1 (6.9-15.0)	0.0002
	T3	85.4 ± 19.8	70.5 ± 35.9	0.0364
	T4	10.0 ± 2.6	7.6 ± 2.7	0.0008
	TSH	0.33 (0.05-0.76)	1.37 (0.69-2.42)	0.0072
Taking LT4 (n= 24 with, 76 without)	3,3'-T2	18.4 (12.4-23.0)	11.2 (7.0-15.3)	0.0014
	T3	85.0 ± 20.6	70.4 ± 35.9	0.0371
	rT3	13.8 (10.8-19.1)	16.9 (11.3-24.1)	0.0702
	T4	9.9 ± 2.8	7.6 ± 2.7	0.0017
	TSH	0.51 (0.05-0.80)	1.22 (0.67-2.41)	0.0282
Critically ill (n= 19 with, 81 without)	3,3'-T2	6.4 (4.0-9.1)	14.0 (10.7-18.9)	0.0003
	T3	33.4 ± 29.4	84.7 ± 25.5	<.0001
	rT3	25.0 (13.9-56.2)	13.9 (10.9-19.3)	0.0039
	T4	5.6 ± 2.9	8.8 ± 2.5	0.0004
Stroke (n=10 with, 90 without)	3,3'-T2	6.4 (4.4-7.8)	13.3 (9.4-18.8)	0.0009
	T3	47.8 ± 13.7	77.3 ± 33.5	<.0001
	T4	6.7 ± 1.1	8.4 ± 2.9	0.0017
Critical care unit (n= 35 with, 65 without)	3,3'-T2	7.0 (5.2-10.7)	14.9 (12.0-20.7)	<.0001
	T3	42.6 ± 26.6	91.6 ± 21.9	<.0001
	rT3	22.1 (15.4-35.6)	13.1 (10.7-18.4)	0.0002
	T4	6.5 ± 2.7	9.1 ± 2.5	0.0003
Inpatient (n=37 with, 63 without)	3,3'-T2	7.0 (4.8-10.2)	15.0 (12.0-20.8)	<.0001
	T3	45.4 ± 31.9	90.6 ± 20.5	<.0001
	rT3	21.3 (15.4-30.5)	13.1 (10.6-18.4)	0.0002
	T4	6.6 ± 2.9	9.1 ± 2.5	0.0008

Data are mean ± STD or median (1st quartile, 3rd quartile)

*ANOVA test adjusted for age and gender (log-transformation was applied to variables that were highly skewed)

Table 3

Breakdown of thyroid analytes into quartiles

Analyte	Quartile 1	Quartile 2	Quartile 3	Quartile 4
3,3'-T2				
Concentration range (pg/mL)	2.51-7.48	7.74-12.4	12.5-17.8	17.9-45.8
Number	24	26	24	25
rT3				
Concentration range (ng/dL)	4.84-11.0	11.2-15.4	15.5-22.2	22.4-109.0
Number	24	26	25	25
T3				
Concentration range (ng/dL)	5.66-45.3	46.1-78.0	78.1-92.1	92.5-166.0
Number	25	25	26	24
T4				
Concentration range (mcg/dL)	1.73-6.18	6.36-7.80	7.85-10.3	10.5-14.1
Number	25	25	26	24
TSH				
Concentration range (mIU/L)	0-0.45	0.48-0.98	0.99-2.16	2.23-29.3
Number	25	25	25	25

Reference intervals are as follows: 3,3'-T2 6.7-23 pg/mL; rT3 7.7-23.1 ng/dL; T3 74-168 ng/dL; T4 4.2-10.9 mcg/dL; TSH 0.40-4.00 mIU/L

Table 4a

Quantification of physiologic data according to the quartiles of thyroid derivative analytes

Analyte	Physiologic parameter	Quartiles				Parameter Increases or decreases from Q1 to Q4	P value*
		Q1	Q2	Q3	Q4		
3,3'-T2	Age (years)	63.4±15.8	47.3±15.7	50.3±13.7	47.1±12.7	decreases	0.0007
	DBP (mmHg)	68.5±15.8	75.9±11.5	78.4±12.6	75.7±12.2	increases	0.046
T3	Age	62.2±14.6	51.2±15.1	48.1±14.2	45.5±14.7	decreases	0.0001
	Height	68.2±4.2	65.3±4.5	66.6±3.2	64.9±4.0	decreases	0.031
	DBP	67±16.56	74±10.1	80±10.8	77±13.1	increases	0.0027
	Male Gender (%)	64	32	46	17	decreases	0.0043
T4	Age (years)	61.4±13.9	46.6±16.8	53.8±13.5	44.8±13.8	decreases	0.0017
	Height (inches)	67.6±4.3	66.9±3.7	66.4±3.9	64.3±4.1	decreases	0.0065
	DBP (mmHg)	68±15.2	77±12.8	76±10.7	77±13.7	increases	0.042
	Male Gender (%)	60	48	39	12	decreases	0.0007
TSH	DBP	76±11.6	80±14.6	76±12.6	68±13.4	decreases	0.029

Data are mean ± STD or percentage

Data only shown for significant relationships Q = quartile

* Analysis based on analyte divided into quartiles t-test or ANOVA

Table 4b

The prevalence of medical conditions according to the quartiles of the thyroid derivative analytes

Analyte	Medical condition	Percentage with condition within Quartile [@]				Percentage with characteristic increases or decreases with increasing quartile	P value*
		Q1	Q2	Q3	Q4		
3,3'-T2	Thyroid cancer	4	19	21	60	increases	<0.0001
	Thyroidectomy	4	12	17	60	increases	<0.0001
	Taking LT4	8	15	17	56	increases	0.0002
	Hypothyroidism	4	8	4	28	increases	0.016
	Critically Ill	58	11	0	8	decreases	<0.0001
	Stroke	29	8	4	0	decreases	0.001
	Critical Care Unit	75	39	8	12	decreases	<0.0001
	Inpatient	83	42	8	12	decreases	<0.0001
rT3	Critically ill	8	11	12	48	increases	0.0009
	Critical Care Unit	17	19	36	64	increases	0.0002
	Inpatient	17	23	44	64	increases	0.0002
T3	Thyroid Cancer	4	20	54	25	increases	0.012
	Critically ill	72	4	0	4	decreases	<0.0001
	Stroke	20	20	0	0	decreases	0.0031
	Critical Care Unit	92	36	0	8	decreases	<0.0001
	Inpatient	100	36	0	12	decreases	<0.0001
T4	Thyroid cancer	12	8	35	50	increases	0.0004
	Thyroidectomy	12	4	27	50	increases	0.0004
	Taking LT4	12	4	31	50	increases	0.0003
	Hypothyroidism	8	0	8	29	increases	0.014
	Critically ill	48	20	8	4	decreases	<0.0001
	Stroke	16	16	8	0	decreases	0.039
	Critical Care Unit	64	40	19	13	decreases	<0.0001
	Inpatient	72	40	19	17	decreases	<0.0001
TSH	Thyroid cancer	52	28	16	8	decreases	0.0003
	Thyroidectomy	52	28	4	8	decreases	<0.0001
	Taking LT4	52	24	8	12	decreases	0.0004
	Hypothyroidism	24	12	0	8	decreases	0.033

Data only shown for significant relationships Analysis based on analyte divided into quartiles Q = quartile

[@] percentage of patients within that analyte quartile that have the particular medical condition (e.g. for Q1 3,3'-T2 4% of patients have thyroid cancer and 96% of patients do not have thyroid cancer)

* Chi-square test was used

Table 5

Significant predictors of thyroid derivative analytes

Parameter	Parameter estimate as a coefficient	Standard Error for coefficient	Parameter estimate as a percentage change*	95% CI for percentage change	P
Log 3,3'-T2 MS					
Age	−0.011	0.0033	−1.1	−0.5 – −1.7	0.0017
Thyroidectomy	0.2548	0.1276	29.0	0.5 – 65.7	0.049
Inpatient	−0. 6198	0.1167	−46.2	−32.4 – −57.2	<0.0001
Log rT3 MS					
Critical Care Unit	0.5756	0.1234	77.8	39.6 – 126.5	<0.0001
Log T3 MS					
Age	−0.0084	0.0023	−0.8	−0.4 – −1.3	0.0006
Male Gender	−0.2372	0.0748	−21.1	−8.7 – −31.9	0.0021
Critically ill	−0.6793	0.1184	−49.3	−36.1 – −59.8	<0.0001
Critical Care Unit	−0.4039	0.0998	−33.2	−18.8 – −45.1	0.0001
Log T4 MS					
Age	−0.0073	0.0021	−0.7	−0.3 – −1.1	0.0011
Thyroidectomy	0.2577	0.0879	29.4	8.9 – 53.7	0.0042
Inpatient	−0.1825	0.0771	−16.7	−3.1 – −28.4	0.0201
Log TSH					
Thyroid cancer	−2.2982	0.5062	−90.0	−72.9 – −96.3	<0.0001

* Values are back log-transformed. Parameter estimate as a percentage is the percentage change in dependent variables for one unit increase for continuous variable (age) or as compared with reference group for categorical variables. For example, a one-year increase in age would result in a 1.1% decrease in 3, 3-T2, and an inpatient status was associated with a 46% decrease in 3, 3-T2.