Tandem Mass Spectrometry as a Novel Tool for Elucidating Pituitary–Thyroid Relationships

Jacqueline Jonklaas¹ and Steven J. Soldin^{2,3}

Background: Our objective was to determine the performance of liquid chromatography–tandem mass spectrometry (LC-MS/MS) in documenting both group and individual relationships between thyroid hormone and thyroid-stimulating hormone (TSH) concentrations.

Methods: This was a prospective analysis of 50 euthyroid patients undergoing thyroidectomy. Thyroxine (T₄), triiodothyronine (T₃), free T₄ (FT₄), and TSH levels were documented on two occasions before thyroidectomy. After thyroidectomy, patients were treated with levothyroxine (LT₄) to achieve either a normal or low serum TSH concentration. All laboratory evaluations were repeated twice while patients were taking LT₄. Thyroid hormone concentrations were documented by both immunoassay and LC-MS/MS, and their relationship with TSH was studied both in the entire group and in individual patients pre- and postthyroidectomy.

Results: FT₄ and total T₃ correlated better with the log-transformed TSH when measured by LC-MS/MS. Postthyroidectomy the closest correlation was between log TSH and FT₄ ($r = 0.86$, $p < 0.001$). The next best correlation was between log TSH and total T₃ ($r = 0.71$, $p < 0.001$). When all data points were combined, the slope of the relationship between log TSH and total T₃ was relatively blunted compared with the log TSH–FT₄ slope (slope -0.39 vs. -1.38 ; $p < 0.001$), perhaps suggesting autoregulation of T₃ in response to the altered conditions postthyroidectomy.

Conclusion: LC-MS/MS is an excellent tool for documenting the known physiological phenomenon of a log-linear relationship between TSH and thyroid hormone concentrations. In a group of patients studied pre- and postthyroidectomy, both FT₄ and total T₃ measured by tandem mass spectrometry correlate well with TSH. However, T₃ correlates slightly less well and has a relatively blunted relationship with the log-transformed TSH. These paired data suggest that in LT₄-replaced patients T₃ concentrations are held stable in the face of fluctuating T₄ concentrations.

Introduction

LIQUID CHROMATOGRAPHY–TANDEM MASS SPECTROMETRY (LC-MS/MS) is a novel technique that quantifies the amount of analyte in a sample by determining the retention time and mass–charge ratio of parent and fragmentation ions of the analyte. This method has already been shown to be a precise tool for the measurement of serum testosterone (1,2), adrenal and gonadal steroids (3,4), and 25-hydroxyvitamin D concentrations (5,6). When properly validated, these assays are not only highly accurate and sensitive, but also have the capability of measuring hormones in relatively low concentrations (2,7,8). We have recently described the use of this assay to measure thyroid hormones (9–12). LC-MS/MS is more specific than immunoassays for measurement of thyroid

hormones, allowing for the accurate and precise quantification of these analytes.

Detection of thyroid failure or autonomous thyroid functioning depends on accurate measurement of thyroid analytes. Thyroid-stimulating hormone (TSH) assays are accurate and reproducible across various laboratories (13). However, measurement of thyroid hormones by direct analog immunoassay can be inaccurate (14–19). This is particularly true when conditions such as extremes of protein binding or interfering heterophilic antibodies are present. LC-MS/MS may perform better in such circumstances (9,11), and certainly, in the case of free thyroxine (FT₄), it seems to agree better with the gold-standard equilibrium dialysis assay (10).

The thyroid axis is an exquisitely sensitive negative feedback system. Small changes in thyroid hormone concentrations

¹Division of Endocrinology; ²Bioanalytic Core Laboratory, General Clinical Research Center; Georgetown University Medical Center, Washington, District of Columbia.

³Department of Laboratory Medicine, Children's National Medical Center, Washington, District of Columbia.

result in exponential changes in TSH levels (20), thus rendering measurement of serum TSH an extremely sensitive screening tool for detection of hypothyroidism or hyperthyroidism (13). The altered TSH concentration defends thyroid homeostasis. Both T_4 and triiodothyronine (T_3) appear to be important for feedback at the pituitary level (13,21), although opinion differs as to the relative contribution from the two hormones (22). T_3 may be the predominant regulator in conditions of autonomous hyperactivity due to nodular thyroid disease (23). The set point of the pituitary-thyroid axis seems to be individualized (24,25). It also appears to be affected by factors such as nutritional status (26–28), illness (29), and age (30). Some details of the pathways involved in mediating this very responsive system are beginning to emerge. Specifically, hypothalamic thyrotropin-releasing hormone maintains thyroid hormone homeostasis by regulating TSH secretion from the thyrotropes (31). Thyroid hormones feedback on thyrotropin-releasing hormone neurons by altering their messenger RNA expression (32,33). In addition, type II deiodinase appears to be involved in determining the set point of the hypothalamic-pituitary-thyroid axis (34).

Although the thyroid axis has been extensively studied, there are no studies examining this axis initially in a patient with an intact thyroid and then also in the same patient rendered athyreotic. This study involves studying the same patient under conditions of endogenous thyroid functioning and subsequently under conditions of exogenous provision of thyroid hormone. While elaborating such pituitary-thyroid relationships, it was also appealing to utilize LC-MS/MS to measure the thyroid hormone concentrations.

Methods

Study hypothesis

The primary objective of the parent study was to determine whether LT_4 replacement produced lower serum T_3 concentrations in individual patients than they had maintained during their period of endogenous thyroid functioning before thyroidectomy. This study, which showed that serum T_3 levels were adequately maintained during LT_4 replacement, has previously been published (35). This analysis did not, however, examine the log-linear relationship between TSH and FT_4 or T_3 in the study group or in individual patients. An analysis of the same database examining preoperative analytes according to the patient's subsequent histologic diagnosis has also been published (36).

This present study contained two cohorts of participants. Patients with benign thyroid disease were started on replacement levothyroxine (LT_4) after thyroidectomy, and their doses were adjusted to keep their serum TSH in the normal range; others were started on suppression therapy after thyroidectomy because of a diagnosis of thyroid cancer, and their LT_4 doses were adjusted to achieve a TSH below the normal range. Therefore, at study end, patients had a range of serum TSH values, and the primary hypothesis of this substudy was that there would be an inverse relationship between each of the measured hormones and the log-transformed TSH. It was also hypothesized that LC-MS/MS would provide a more accurate representation of this relationship. The null hypothesis was that thyroid hormone levels had no correlation with TSH levels.

Study overview

The study was approved by the Georgetown University Institutional Review Board and conducted in the General Clinical Research Center (GCRC). Written informed consent was obtained, and participants' medications were then reviewed at the initial study visit. A medical history was obtained. Parameters documented during the physical examination included weight, blood pressure, heart rate, thyroid examination, and neurological examination. Participants had thyroid profiles drawn on two separate occasions before their thyroidectomy. Two additional thyroid profiles were obtained while patients were taking LT_4 therapy after their thyroidectomy. The study was conducted over a 42-month period.

Study subjects

Subjects aged 18–65 years were recruited from patients referred to the Department of Otolaryngology-Head and Neck Surgery for total or near-total thyroidectomy. Patients undergoing a lobectomy alone were not recruited. Preoperative diagnoses were goiter, nodular thyroid disease, suspected thyroid cancer, or known thyroid cancer. Patients who had a coexistent diagnosis of hyperthyroidism or hypothyroidism were excluded from the study. Patients were required to have been on a stable dose of any medication known to affect thyroid hormone protein binding. Pregnant or lactating patients were not recruited. Patients with chronic, serious diseases such as cardiac, pulmonary, and renal disease, or who required steroid therapy were not eligible for study participation. Patients whose first or second preoperative thyroid profiles were abnormal were excluded from the study.

Thyroid profiles

Blood tests were obtained on two occasions before thyroidectomy and on two occasions after thyroidectomy. Phlebotomy was performed between 8 and 10.30 AM with the patient in a fasting state. LT_4 administration was delayed until after phlebotomy for the postoperative profiles to obtain trough levels of thyroid hormones (37). Each thyroid profile consisted of a serum TSH, total T_4 , FT_4 , and total T_3 . Two separate pre- and postthyroidectomy profiles were included for each patient to minimize the effect of day-to-day fluctuations in thyroid hormone concentrations and TSH levels.

All thyroid profiles were drawn in the GCRC. Most participants had their thyroid function assessed both by a clinical laboratory (Quest Diagnostics, Madison, NJ; LabCorp, Burlington, NC; or Georgetown University Laboratories, Washington, DC) and by the GCRC core laboratory. Clinical laboratory data were used to make LT_4 dosage adjustments, but only the GCRC laboratory results were used in this analysis. The Dade Dimension RxL Clinical Chemistry Analyzer (Siemens, Wilmington, DE) was used for the T_4 immunoassay. This was an emit homogeneous immunoassay with a sensitivity of 0.5 mcg/dL, and a precision of < 7.4% at all concentrations tested, and was calibrated for the range 0.5–24 mcg/dL. The manufacturer's reference range was 4.7–13.3 mcg/dL. The Dade Dimension RxL Clinical Chemistry Analyzer (Siemens, Wilmington, DE) was used for the direct analog FT_4 immunoassay. This was a heterogeneous immunoassay with a sensitivity of 0.2 ng/dL, and a precision

of < 13% at all concentrations tested, and was calibrated for the range 0–6.5 ng/dL. The manufacturer's reference range was 0.9–1.6 ng/dL. T_3 was assayed using the DPC Immulite 2000 (Siemens, Los Angeles, CA). This chemiluminescent immunoassay has a sensitivity of 19 ng/dL, and a precision of <11% at all concentrations tested, and was calibrated for the range 40–600 ng/dL. The manufacturer's reference range for this assay was 84–172 ng/dL. TSH was also measured using the Dade Dimension RxL Clinical Chemistry Analyzer. This was a colorimetric immunoassay with a sensitivity of 0.01 mIU/L, and a precision of <6.2% at all concentrations tested, and was calibrated for the range 0.01–50 mIU/L. The manufacturer's reference range for this third generation TSH assay was 0.34–4.82 mIU/L.

An aliquot of each blood sample was analyzed in the GCRC Bioanalytic Core Laboratory for T_4 , FT_4 , and T_3 measured by LC-MS/MS. Details of the LC-MS/MS assays are fully described in recent publications (9,11,12). Samples were thawed at room temperature, and for FT_4 400 μ L aliquots were filtered through Centrifree YM-30 ultrafiltration devices (30,000 MW cut-off; Millipore, Bedford, MA) by centrifugation, employing the Eppendorf temperature-controlled centrifuge (model no. 5702 R; Eppendorf AG, Hamburg, Germany) and using a fixed angle rotor at 2900 rpm at a temperature of 25°C for 30 minutes. This ultrafiltration process replaced the dialysis step of the classic equilibrium dialysis method. Briefly, the LC-MS-MS procedure is based on an online extraction/cleaning of the injected samples with subsequent introduction into the mass spectrometer by using a built-in Valco switching valve. One hundred to 400 μ L of the treated plasma sample or plasma ultrafiltrate was injected onto the Supelco LC-18-DB (3.3 cm $\times$ 3.0 mm; 3.0 μ m particle size) chromatographic column equipped with a Supelco Discovery C-18 (3.0 mm) guard column, where it underwent cleaning with 20% (volume/volume) methanol in 5 mM ammonium acetate pH 4.0 at a flow rate of 0.8 mL/min. After 4 min of cleaning, the switching valve was activated, the column was flushed with a water/methanol gradient at a flow rate of 0.6 mL/min, and the samples were introduced into the mass spectrometer. The gradient parameters and T_4 , T_3 , and FT_4 chromatograms are

shown in recent publications (9,11,12). The between-day and within-day precision was assessed at three different concentrations, and yielded coefficients of variation $\leq 5\%$ for T_4 , $\leq 9\%$ for T_3 , and $\leq 7.0\%$ for FT_4 . The reference interval for FT_4 measured by LC-MS/MS was 0.8–2.1 ng/dL.

Thyroid surgery and LT_4 administration

Total or near-total thyroidectomy was performed at Georgetown University Hospital by an experienced Head and Neck Surgeon. The histologic diagnosis of each patient was recorded postoperatively. After their thyroidectomy, study participants were all prescribed a name brand of LT_4 . The particular brand was noted, and adherence to the branded product was verified throughout the study. However, not all subjects were taking the same brand. Participants were not permitted to take any T_3 -containing products. The only exception was thyroid cancer patients who were temporarily placed on liothyronine as part of their preparation for radioiodine scanning and treatment. Patients with thyroid cancer who received liothyronine had been off this therapy for at least 6 weeks before their first postoperative thyroid profile. Postoperative LT_4 dosage was determined by the patient's pathologic diagnosis. Patients with benign disease were initially placed on 1.7 μ g/kg/day LT_4 with the goal of achieving a TSH level in the lower two-thirds of the normal range. Patients with thyroid cancer were placed on 2.2 μ g/kg/day LT_4 with the goal of achieving a subnormal or suppressed TSH level. LT_4 dosage adjustments were made between the two postoperative (third and fourth) thyroid profiles to achieve these goals.

Statistical analysis

Statistical support was provided by the GCRC biostatistical staff. The relationship between the log-transformed TSH values and each associated thyroid hormone concentration was tested by multiple regression analysis. The relationship was examined for three hormones (T_4 , FT_4 , and T_3) measured by two different assay methods (immunoassay and LC-MS/MS). The relationship between these analytes before surgery was compared to their relationship after surgery. A

TABLE 1. CORRELATION OF THYROID HORMONE LEVELS WITH LOG-TRANSFORMED TSH

Thyroid hormone	Assay	Number of data points	Condition (pre- or postthyroidectomy)	Correlation of thyroid hormone values with log-TSH [<i>r</i> (p-value)]	Difference compared with prethyroidectomy relationship (p-value)
FT_4	LC/MS/MS	65	Postthyroidectomy	−0.86 (<0.001)	<0.001
T_3	LC/MS/MS	100	Postthyroidectomy	−0.71 (<0.001)	<0.001
FT_4	Immunoassay	100	Postthyroidectomy	−0.58 (<0.001)	<0.001
T_3	Immunoassay	100	Postthyroidectomy	−0.47 (<0.001)	<0.001
T_4	LC/MS/MS	100	Postthyroidectomy	−0.44 (<0.001)	<0.001
T_4	Immunoassay	100	Postthyroidectomy	−0.42 (0.03)	<0.001
T_3	LC/MS/MS	100	Prethyroidectomy	−0.29 (0.037)	n/a
T_3	Immunoassay	100	Prethyroidectomy	−0.24 (0.07)	n/a
FT_4	LC/MS/MS	56	Prethyroidectomy	−0.10 (NS)	n/a
FT_4	Immunoassay	100	Prethyroidectomy	−0.08 (NS)	n/a
T_4	LC/MS/MS	100	Prethyroidectomy	0.08 (NS)	n/a
T_4	Immunoassay	100	Prethyroidectomy	0.07 (NS)	n/a

TSH, thyroid-stimulating hormone; FT_4 , free thyroxine; T_3 , triiodothyronine; LC/MS/MS, liquid chromatography–tandem mass spectrometry; NS, not significant; n/a, not applicable.

statistically significant difference in the TSH–thyroid hormone slopes was tested by assessing the interaction between surgery status (prethyroidectomy vs. postthyroidectomy) and hormone levels in multiple regression analysis. The relationship between thyroid hormone concentrations and log TSH was also displayed in graphic form for the group and for each individual patient for the two thyroid hormone measurements that had the greatest degree of correlation with the log-transformed TSH during the postthyroidectomy period. A statistically significant difference in the individual and group TSH–thyroid hormone slopes was tested by assessing the interaction between status (group vs. individual) and hormone levels in multiple regression analysis.

Results

Two hundred thyroid profiles or data points were analyzed for T_4 measured by immunoassay and LC-MS/MS, FT_4

measured by immunoassay, and T_3 measured by immunoassay and LC-MS/MS. One hundred of these profiles were prethyroidectomy, and 100 were postthyroidectomy. However, for FT_4 measured by LC-MS/MS there were 56 prethyroidectomy data points and 65 postthyroidectomy data points. There was inadequate remaining specimen to assay for FT_4 by LC-MS/MS to provide the remaining 79 data points.

There did not appear to be significant relationships between any of the thyroid hormones and their associated TSH concentrations for the whole patient group during their period of endogenous thyroid functioning, with the exception of T_3 measured by LC-MS/MS (Table 1). This held true regardless of whether the thyroid hormones were measured by immunoassay or LC-MS/MS. These prethyroidectomy data points are seen as filled or open squares in Figures 1A and B and 2A and B for FT_4 and T_3 , respectively. The filled squares represent patients with benign disease; the open squares represent patients with thyroid cancer.

FIG. 1. (A, B) Free thyroxine (FT_4) measured by immunoassay and liquid chromatography–tandem mass spectrometry (LC-MS/MS), respectively, plotted against log thyroid-stimulating hormone (TSH). Filled squares represent prethyroidectomy data points, benign disease; open squares represent prethyroidectomy data points, thyroid cancer. Filled triangles represent postthyroidectomy data points, benign disease; open triangles represent postthyroidectomy data points, thyroid cancer. (C) Agreement between FT_4 measured by immunoassay versus tandem mass spectrometry. Filled squares represent prethyroidectomy data points; circles represent postthyroidectomy data points.

FIG. 2. (A, B) Triiodothyronine (T_3) measured by immunoassay and liquid chromatography–tandem mass spectrometry (LC-MS/MS), respectively, plotted against log thyroid-stimulating hormone (TSH). Filled squares represent prethyroidectomy data points, benign disease; open squares represent prethyroidectomy data points, thyroid cancer. Filled triangles represent postthyroidectomy data points, benign disease; open triangles represent postthyroidectomy data points, thyroid cancer. (C) Agreement between T_3 measured by immunoassay versus tandem mass spectrometry. Filled squares represent prethyroidectomy data points; circles represent postthyroidectomy data points.

These two analytes were selected for graphic display because they were the analytes that had the greatest correlation with log-transformed TSH in the postthyroidectomy period.

The postthyroidectomy data points are also shown in Figures 1A and B and 2A and B for FT_4 and T_3 , respectively. The filled triangles represent patients with benign disease; the open triangles represent patients with thyroid cancer. In contrast to the prethyroidectomy situation, there were significant inverse relationships between T_4 and log TSH after surgery ($r = -0.42$, $p = 0.03$), FT_4 and log TSH after surgery ($r = -0.58$, $p < 0.001$), and T_3 and log TSH after surgery ($r = -0.47$, $p < 0.001$) when the thyroid hormones were measured by immunoassay (Table 1; Figs. 1A and 2A). Similarly, significant inverse relationships were seen with measurements made by LC-MS/MS after thyroidectomy (Table 1; Figs. 1B and 2B). These significant correlations were $r = -0.44$ for T_4 and log TSH ($p < 0.001$), $r = -0.86$ for FT_4 and log TSH

($p < 0.001$), and $r = -0.71$ for T_3 and log TSH ($p < 0.001$). Moreover, in the case of FT_4 and T_3 there was a more robust relationship between the thyroid hormone concentration and the log TSH when LC-MS/MS was used to measure the thyroid hormone concentrations. These postthyroidectomy correlations are displayed in order of decreasing significance in Table 1.

The interactions between each of the measured hormones (T_4 by immunoassay, T_4 by LC-MS/MS, FT_4 by immunoassay, FT_4 by LC-MS/MS, T_3 by immunoassay, and T_3 by LC-MS/MS) and the log-transformed TSH differed according to whether the surgery status was before surgery (endogenous thyroid function with narrower range of TSH values) or after surgery (exogenous thyroid hormones provided by LT_4 with wider range of TSH values during LT_4 adjustment). In each case the regression slopes were significantly different according to whether the patients were prethyroidectomy or

postthyroidectomy. For T_4 by immunoassay, $t = -4.32$ and $p < 0.001$; for T_4 by LC-MS/MS, $t = -4.17$ and $p < 0.001$; for FT_4 by immunoassay, $t = -4.88$ and $p < 0.001$; for FT_4 by LC-MS/MS, $t = -7.52$ and $p < 0.001$; for T_3 by immunoassay, $t = -4.24$ and $p < 0.001$; and for T_3 by LCMSMS, $t = -6.86$ and $p < 0.001$ (see Table 1). Thus, in each case the before and after thyroidectomy regression slopes were statistically different from one another.

The correlation between FT_4 measured by LC-MS/MS and immunoassay was good at 0.80. The correlation between T_3 measured by LC-MS/MS and immunoassay was less good at 0.59. These correlations are shown in graphic form in Figures 1C and 2C, respectively. The line indicated on the graph illustrates perfect agreement between the assays. For FT_4 there is generally more disagreement at the lower and higher ends of the assay range, with LC-MS/MS reading lower at the lower end and higher at the higher end. In contrast, for T_3 the LC-MS/MS appears to read somewhat higher across the entire range.

Figure 3 shows all the available data points for FT_4 and T_3 measured by LC-MS/MS with each individual patient depicted as a separate line. When all these prethyroidectomy and postthyroidectomy points were combined, the relationship between FT_4 and log TSH (Fig. 3A) correlated more closely than did the relationship between T_3 and log TSH (Fig. 3B), with correlation coefficients of -0.77 and -0.58 , respectively ($p < 0.01$). Moreover, the slopes of these two relationships were significantly different ($p < 0.001$). The slope for the log TSH- FT_4 relationship was -1.38 , whereas the normalized slope for the log TSH- T_3 relationship was -0.39 . (The slope for T_3 was normalized to that of FT_4 by accounting for the relative excursions of the two analytes.) Thus, the T_3 slope was relatively blunted compared with the FT_4 slope. When thyroid hormone-log TSH relationships were analyzed for individual patients versus the entire group, there was a stronger within-patient relationship compared to the entire group relationship ($p < 0.001$).

Discussion

These data illustrate the already well-documented inverse log-linear relationship between TSH and thyroid hormones (13,20). This relationship is generally seen for all the thyroid hormones measured in this particular small group of 50 patients. The relationship between the thyroid hormones and log TSH is strongest for FT_4 , and also stronger for T_3 than for T_4 . Further, it is notable that for FT_4 and T_3 , there is a closer relationship when the hormone is measured by LC-MS/MS. There was no significant difference in the case of T_4 . Thus, the correlation coefficient is greatest for FT_4 measured by LC-MS/MS and next greatest for T_3 measured by LC-MS/MS, followed by FT_4 and then T_3 measured by immunoassay. It is possible that the closer relationship between serum TSH and each of the thyroid hormones measured by LC-MS/MS reflects the greater precision of LC-MS/MS. Perhaps there is a greater ability of LC-MS/MS to detect subtle changes in thyroid hormones due to the greater accuracy of LC-MS/MS. It may also be a reflection of the greater interassay variability of the various immunoassay laboratories employed. The within-day and between-day coefficients of variation are less than 7% and 9% for FT_4 and T_3 measured by LC-MS/MS, respectively.

FIG. 3. (A) Free thyroxine (FT_4) levels plotted against log thyroid-stimulating hormone (TSH). Each line is an individual patient with pre- and postthyroidectomy data points all on the same line. (B) Triiodothyronine (T_3) levels plotted against log TSH. Each line is an individual patient with pre- and postthyroidectomy data points all on the same line.

The thyroid hormone-TSH relationships demonstrated in this study prethyroidectomy as compared with postthyroidectomy illustrate the relatively narrow TSH range seen in euthyroid individuals, compared with the wider TSH range seen postthyroidectomy when some patients had abnormal TSH values secondary to their LT_4 therapy. Before, thyroidectomy data points appeared to be distributed along the X-axis. There was little distribution along the Y-axis, and essentially no slope, as all these patients were euthyroid and had TSH values within a narrow range. In the postthyroidectomy condition, when exogenous LT_4 therapy may have been inadequate or excessive, a greater range of serum TSH concentrations, including low and high TSH values, was demonstrated. Thus, the relationship assumed the steeper slope usually associated with thyroid hormone-TSH rela-

tionships (13,20), as the altered thyroid hormone concentrations impacted the pituitary and drove TSH stimulation or inhibition.

This shift from absence of a slope to the classic negative log-linear slope presumably reflected the different conditions that were present pre- and postthyroidectomy. Prethyroidectomy all individuals were euthyroid, and the narrow distribution of data along the Y-axis reflected the TSH values that were characteristic of the pituitary-thyroid set points of each of the individual 50 patients (mean TSH was 1.12 ± 0.43 mIU/L). However, after thyroidectomy, the data points assumed the more typical slope usually associated with thyroid hormone-TSH relationships. In this situation the effect of the LT_4 therapy producing inadequate or excessive thyroid hormone concentrations for that particular patient's set point stimulated or reduced the TSH output of the thyrotropes, with a greater range of TSH values thus being seen (mean TSH 2.12 ± 3.3 mIU/L). It is also possible that during LT_4 administration the pituitary responsiveness was altered compared with the period of endogenous thyroid functioning due to T_4 peaks associated with LT_4 therapy.

In conclusion, our data show that the most robust thyroid hormone log-TSH relationship is the relationship between FT_4 and TSH. The T_3 and TSH relationship is the next strongest relationship. This appears to be consistent with a body of evidence, perhaps originating with the study of Fish *et al.* (22), and subsequently shown by many others, including the present authors (35), that illustrate the importance of T_3 in regulating serum TSH. When examining the paired pre- and postthyroidectomy data shown in Figure 3, it can be seen that the TSH- T_3 relationship is relatively blunted compared with the TSH- FT_4 relationship. The slope of the relationship is -1.38 for FT_4 compared with a normalized slope of -0.38 for T_3 . It has previously been shown by Lum *et al.* (38) that T_4 -to- T_3 conversion is a regulated step, such that T_3 levels are held relatively stable in the face of fluctuating T_4 levels. It appears that Figure 3 is illustrating this phenomenon. When the individual patient data were examined, it was found that within-patient T_3 values remain more similar than within-patient FT_4 values across a range of TSH concentrations. This would be explained by autoregulation of T_3 .

In addition, our data also show that pituitary-thyroid relationships have better correlation coefficients when LC-MS/MS is used to document the thyroid hormone concentrations. We believe that this was likely the case because LC-MS/MS, being a more accurate assay than the immunoassay, provides a more genuine reflection of the endogenous thyroid hormone milieu. When measurements of FT_4 by LC-MS/MS and immunoassay are compared (Fig. 1C), it can be seen that the LC-MS/MS determinations tend to be lower at the lower end of the range and higher at the higher end of the range, providing a wider range of FT_4 values. Based on our previous studies showing an excellent agreement between LC-MS/MS and equilibrium dialysis ($r = 0.90-0.97$), we would propose that the LC-MS/MS readings are the more accurate. When examining the agreement between T_3 measured by LC-MS/MS and immunoassay (Fig. 2C), the LC-MS/MS determinations generally tended to be higher.

Some laboratories already utilize LC-MS/MS to measure the concentrations of hormones and other small molecules.

However, there are still barriers remaining to its wider adoption. For example, comparisons among various LC-MS/MS assays may still be lacking, and additional testing is required to establish reference intervals for the relevant hormones. However, many other advances have been made in LC-MS/MS technology. These assays can now be accomplished with extremely small sample sizes. For example, the serum sample volume required for FT_4 and FT_3 measurement is 400 μ L. In addition, cost is becoming less prohibitive.

Good clinical decisions in endocrinology are best made when they are supported by assays providing sensitive and specific assessment of prevailing hormone concentrations. Maybe our data, which we believe provide an accurate representation of the functioning of the pituitary-thyroid axis, constitute another piece of evidence that can be used to support the position that LC-MS/MS should, in the future, become the preferred assay for documenting the concentrations of many low molecular weight hormones, including thyroid hormones.

Acknowledgments

Jacqueline Jonklaas is supported by the National Center for Research Resources (NCRR) Grant K23 RR16524. Steven J. Soldin is partially supported by the NIH GCRC Grant M01-RR-020359 and by Applied Biosystems/Sciex. This project was conducted through the General Clinical Research Center at Georgetown University and supported by Grant M01-RR-020359 from the NCRR, a component of the National Institutes of Health (NIH). The authors gratefully acknowledge the dedication of the GCRC nursing staff and the generosity of the study participants, without which this study could not have been completed.

Disclosure Statement

The contents of this work are solely the responsibility of the authors and do not necessarily represent the official views of NCRR or NIH. No competing financial interests exist.

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Address reprint requests to:
Jacqueline Jonklaas, M.D., Ph.D.
Division of Endocrinology and Metabolism
Georgetown University Hospital
Suite 232, Building D, 4000 Reservoir Road, NW
Washington, DC 20007
E-mail: jj@bc.georgetown.edu