


Laboratory Thyroid Function Testing: Do Abnormalities Always Mean Pathology?

Clinical Pediatrics
52(4) 287–296
© The Author(s) 2013
Reprints and permissions:
sagepub.com/journalsPermissions.nav
DOI: 10.1177/0009922813475706
cpj.sagepub.com


Amit Lahoti, MBBS¹ and Graeme R. Frank, MD¹

Introduction

Thyroid function tests (TFTs) are the most frequently ordered endocrine investigations in children and adolescents. Abnormalities in TFTs can help in diagnosis of primary thyroid disorders (i.e. disorders in which the defect is at the thyroid level) as well as secondary or central thyroid disorders (in which defect is at the pituitary level). Hypothyroidism is the most common thyroid disorder in the pediatric (as well as adult) population. Although there is a paucity of population studies on the prevalence of acquired hypothyroidism in children, the few studies that have been done on this subject indicate an increase in the prevalence of clinical hypothyroidism over the years. A study from the United Kingdom found a population prevalence of hypothyroidism in the <22 years age-group of 0.135% for the period of 1993–1995, higher than the 0.04% to 0.06% reported prevalence in prior studies.¹ In the United States, reports based on the National Health and Nutritional Examination Survey (NHANES) during a similar time period (1988 and 1994) as well as more recently (1999–2002) found the prevalence of clinical hypothyroidism to be 0.3%.² This higher prevalence in the United States is likely because the population group studied was ≥ 12 years and the prevalence of hypothyroidism increases with age.

Symptoms of hypothyroidism are relatively nonspecific, diverse and often mild. Many children with symptoms of attention-deficit or hyperactivity, fatigue, obesity, constipation and hair loss have TFTs ordered to rule out hypothyroidism or thyroid hormone resistance as the cause of these symptoms. The majority of these patients, however, have normal results.³

In recent years, there appears to be an increase in the use of TFTs by pediatricians during annual health assessments of healthy children. The frequent thyroid function testing and the relatively low prevalence of true thyroid pathologies has led to a high detection rate of values that are minimally outside given normal ranges. In general, on finding an “abnormal” TFT, the physician informs the parents of the abnormal values and recommends referral to a pediatric endocrinologist. In many cases, however, these values represent nonpathologic normal variants. The aim of this review is to provide

pediatricians with guidelines that will help them distinguish between variants of normal from true thyroid pathology, and in doing so, avoid unnecessary parental anxiety and unwarranted subspecialist referrals.

Thyroid Hormone: Synthesis, Secretion, and Regulation

Iodine is the rate-limiting substrate for synthesis of thyroid hormone and the first step in the synthesis of thyroid hormone is iodide trapping by the thyroid gland. This inorganic iodide is organified by the enzyme thyroid peroxidase (TPO) and incorporated into thyroglobulin (Tg), a large glycoprotein synthesized by thyroid follicular cells, and mono- and diiodotyrosine (MIT and DIT) residues are formed. Subsequent coupling of these iodotyrosines within Tg molecule leads to the formation of the thyroid hormones: triiodothyronine (T3) and thyroxine (T4) that are stored in follicular colloid, the proteinaceous material contained within the thyroid follicular lumen. Under the effect of thyroid stimulating hormone (TSH), there is colloid resorption by the follicular cells and enzymatic degradation by lysosomal enzymes release MIT, DIT, T4, and T3. Whereas T4 and T3 are subsequently released in the circulation, MIT and DIT get deiodinated for recycling of iodine.⁴

Only 20% of the total T3 in bloodstream is secreted by the thyroid gland; the remaining 80% is derived from conversion of T4 by deiodinases, which are enzymes present in peripheral tissues. Most of the thyroid hormone circulating in the bloodstream is bound to carrier proteins: thyroxine-binding globulin (TBG), thyroxine binding prealbumin (TBPA or transthyretin), and albumin. Although TBG is the most important carrier protein for T4, T3 appears to be equally bound to TBG and

¹ Cohen Children's Medical Center of New York, North Shore LJ Health System, New Hyde Park, NY, USA

Corresponding Author:

Amit Lahoti, Division of Pediatric Endocrinology, Cohen Children's Medical Center of New York, North Shore LJ Health System, 1991 Marcus Avenue, Suite M100, Lake Success NY 11042, USA
Email: alahoti@nshs.edu

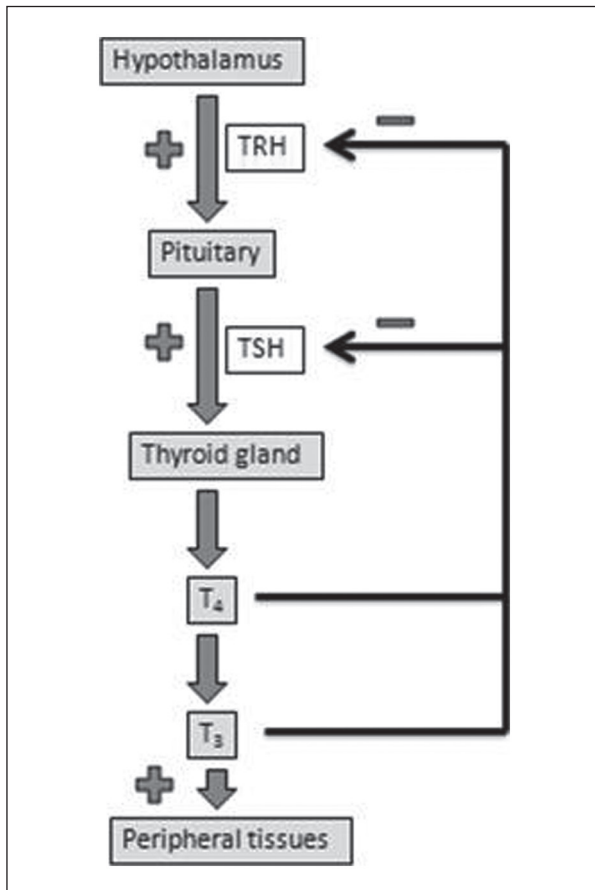


Figure 1. Thyroid hormone secretion is regulated by feedback loops working at the level of pituitary and hypothalamus. Abbreviations: TRH, thyrotropin-releasing hormone; TSH, thyroid-stimulating hormone; T₄, thyroxine; T₃, triiodothyronine.

albumin. These bound hormones are in equilibrium with their free forms and in euthyroid state, free T₄ accounts for <0.1% of the total T₄ and free T₃ accounts for ~0.3% of total T₃.⁵

Thyroid hormone synthesis and secretion is closely regulated by the hypothalamic–pituitary–thyroid axis. Thyrotropin-releasing hormone (TRH) synthesized in the paraventricular nucleus of the hypothalamus travels across the medial eminence to the anterior pituitary through the hypophyseal portal venous system. TRH stimulates secretion of TSH from the pituitary. TSH is released into the systemic blood circulation and transported to the thyroid gland where it stimulates thyroid hormone synthesis and release. Regulatory feedback loops working at the level of pituitary and hypothalamus modulate thyroid hormone secretion (Figure 1).

Measures of Thyroid Function

A number of tests are available to assess thyroid function and these can be broadly divided into 2 main categories:

1. *Thyroid profiling tests* to detect or exclude disturbances in thyroid function. This includes TSH and total and free levels of T₄ and T₃. Additional tests that are often included in “thyroid panels” of commercial laboratories include Tg, TBG, and T₃ uptake (T₃U) or T₃ resin uptake (T₃RU).
2. *Definitive tests* of thyroid to identify the etiology of thyroid disease. These tests mainly include antibodies against various thyroid antigens, which are markers of autoimmune thyroid disease—both hypothyroidism and hyperthyroidism, namely, antithyroglobulin antibody, antithyropoxidase antibody, TSH receptor antibody, and thyroid-stimulating immunoglobulin.

Several comprehensive reviews in the past have discussed abnormalities in these tests, which suggest hyperthyroid or hypothyroid states. This review will focus on “abnormalities” in these lab tests that do not necessarily indicate a pathologic state.

Abnormalities in TSH Levels

Elevated TSH with normal thyroid hormone level in the neonate

Case 1: The newborn screen in a full-term male drawn on the second day of life shows the following results:

Analyte	Result	Reference Range
Thyroxine:	Screen negative	>6 µg/dL
TSH:	37.5 µIU/mL	<20 µIU/mL

The state newborn screening report includes the statement: Possible hypothyroidism. The mother had an uneventful pregnancy, had no history of thyroid disease, and on examination, the infant has no goiter and no clinical features of hypothyroidism.

An elevated TSH level on a newborn screen should alert a practitioner to the possibility of congenital hypothyroidism. However, awareness of the thyroid adaptation in the immediate period after birth is very important for careful interpretation of this “screening” test.

- An abrupt increase in pituitary TSH secretion occurs soon after birth and reaches a peak of about 60 to 80 µIU/mL at 25 to 30 minutes of life. Serum TSH concentration decreases rapidly over the first 24 hours of life, and there-

Table 1. Normal Range for Thyroid Function Tests for Different Age-Groups.

Age	TSH ($\mu\text{IU/mL}$)	T4 ($\mu\text{g/dL}$)	T3 (ng/dL)	Free T4 (ng/dL)
Cord blood	1.0-17.4	7.4-13.0	15-75	0.9-2.2
1-4 days	1.0-39.0	14.0-28.4	100-740	2.2-5.3
2-20 weeks	1.7-9.1	7.2-15.7	105-245	0.9-2.3
5-24 months	0.8-8.2	7.2-15.7	105-269	0.8-1.8
2-7 years	0.7-5.7	6.0-14.2	94-241	1.0-2.1
8-20 years	0.7-5.7	4.7-12.4	80-210	0.8-1.9
21-45 years	0.4-4.2	5.3-10.5	70-204	0.9-2.5

Abbreviations: TSH, thyroid-stimulating hormone; T4, thyroxine; T3, triiodothyronine.

Adapted from Marks and LaFranchi⁶ with kind permission from Springer Science and Business Media.

after more slowly and generally falls to below 10 $\mu\text{IU/mL}$ by 1 month.^{4,6} Therefore, results should be compared with the age-appropriate normal ranges for interpretation (Table 1).

- Newborn screen (NBS) for hypothyroidism may use a primary TSH test or a primary T4 test and this varies from state to state. The New York State hypothyroidism screening program is an example of a T4-based screening program. Total T4 is measured on these specimens and the lowest 10 percent are retested for T4 with additional TSH measurement.⁶
- NBS specimens obtained before 48 hours of life have an increased rate of false positives especially for TSH-based assay. Although T4-based assays tend to be less affected by sampling time, at least a 24-hour wait is advisable before drawing the heel stick specimen to get satisfactory results. With shortening hospital stays this has become increasingly difficult. Hence, the NBS should be drawn after 48 hours of birth (optimal: between 2 and 6 days) or just prior to hospital discharge whichever comes first and results should be interpreted in relation to the postnatal age at time of sampling.⁷

Follow-up on case 1: Patient is evaluated at pediatrician's office on day of life 9 and is feeding well with no jaundice and has regained birth weight. Serum sample is drawn to repeat TFTs and shows

Test	Result	Lab Reference Range
TSH:	8.5 $\mu\text{IU/mL}$	0.27-4.2 $\mu\text{IU/mL}$
Total T4:	9 $\mu\text{g/dL}$	4.6-12 $\mu\text{g/dL}$

Most lab reference ranges are not age adjusted and are generally adult normal ranges. The TSH value obtained from venipuncture on day

9, although above the lab reference range, is within the age adjusted normal range of 1.7 to 9.1 $\mu\text{IU/mL}$ (Table 1). The family is therefore reassured that patient's TFTs are normal for age and no further endocrine follow-up is needed.

Hence, if the first screen is drawn at less than 48 hours of life and the TSH is moderately elevated (20-50 $\mu\text{IU/mL}$), repeat sampling is appropriate, which may be a repeat NBS or serum specimen to a commercial lab based on clinical situation and/or state policies. In general, the commercial laboratories will provide a result within 24 hours, while repeat NBS screens sent to the state lab may take several days.

Elevated TSH with normal thyroid hormone level in young children and adolescents

Case 2: A 9-year-old obese girl has TFTs done at her annual visit, which show the following results:

Test	Result	Lab Reference Range
TSH:	7.5 $\mu\text{IU/mL}$	0.27-4.2 $\mu\text{IU/mL}$
Total T4:	8 $\mu\text{g/dL}$	4.6-12 $\mu\text{g/dL}$

She has no goiter and no family history of thyroid disease.

There are two possible explanations for TSH values that are slightly outside the normal range:

- *Normal variant:* Reference range for any laboratory test result defines the 95% confidence interval, that is, 95% healthy individuals will have their lab result within this range. It follows, therefore, that 5% healthy individuals may have values outside this range, 2.5% of which lie below and 2.5% above this range. Hence, a patient with an isolated minimally elevated TSH may be one of these individuals.
- *Early compensated or subclinical hypothyroidism:* This is defined as an elevated TSH (usually mild, between the upper limit of assay and 9.9 $\mu\text{IU/mL}$ for most children) with normal total and free T4 level. Presence of goiter, positive family history of autoimmune thyroid disease, and positive antithyroid antibodies would make this diagnosis more likely. Repeat TSH with thyroid antibodies in 3 to 6 months will help distinguish between normal variant and early compensated (subclinical hypothyroidism). The presence of

antithyroid antibodies and a rising TSH support the diagnosis of the latter.

Follow-up on case 2: Patient is asked to have repeat TSH and T4 and antithyroid antibodies done after 3 months. Results show the following:

Test	Result	Lab Reference Range
TSH:	6.5 $\mu\text{IU/mL}$	0.27-4.2 $\mu\text{IU/mL}$
Total T4:	9.2 $\mu\text{g/dL}$	4.6-12 $\mu\text{g/dL}$
Antithyroglobulin antibodies:	<20 IU/mL	<20 IU/mL
Antithyroperoxidase antibodies:	<10 IU/mL	<10 IU/mL

Given negative thyroid auto antibodies, the absence of a family history of autoimmune thyroid disease and the normal appearing thyroid gland, this TSH value, which is minimally outside the reference range, likely represents a normal variant and referral for formal thyroid evaluation is not necessary.

Low TSH with normal thyroid hormone level

Case 3: A 14-year-old girl presents with unintentional weight loss. TFTs are done:

Test	Result	Lab Reference Range
TSH:	0.15 $\mu\text{IU/mL}$	0.27-4.2 $\mu\text{IU/mL}$
Total T4:	8.2 $\mu\text{g/dL}$	4.6-12 $\mu\text{g/dL}$
Total T3:	140 ng/dL	80-200 ng/dL

In a child with hyperthyroidism, the thyroid laboratory profile generally shows elevated T3 levels with or without elevated T4 levels and a suppressed TSH level (TSH < 0.1 $\mu\text{IU/mL}$). The third-generation ultrasensitive TSH assays can measure values as low as 0.01 $\mu\text{IU/mL}$ and in overt hyperthyroidism, TSH level is below this level (reported as <0.01 $\mu\text{IU/mL}$).^{4,8} A low but nonsuppressed TSH (ie, a TSH value below the lower limit of normal but not <0.1 $\mu\text{IU/mL}$) together with normal-for-age free and/or total T4 and T3 levels presents following possibilities:

- *Subclinical hyperthyroidism:* TSH is a very sensitive marker of thyroid function and even minor changes in free T4 and/or T3 concentrations have negative feedback on pituitary TSH secretion. Hence small alterations in thyroid function can be detected by changes in TSH level even when T4 and T3 levels are within normal range.

- *Normal variant:* A healthy child who is one of the 2.5% individuals who have a TSH value below the given normal range with no thyroid disease. Repeat TSH in 4 to 6 weeks will help distinguish between normal variant and subclinical hyperthyroidism. Only in the latter will the TSH decline.⁹
- *Drug effect:* Certain drugs can suppress TSH. Examples include glucocorticoids, dopamine agonists, somatostatin analogues (eg, octreotide), rexinoids (type of chemotherapeutic agent), and possibly metformin and carbamazepine/oxcarbamazepine.¹⁰

Follow-up on case 3: Repeat TFTs 6 weeks later showed TSH 0.25 $\mu\text{IU/mL}$, with normal thyroid hormone levels. Additional testing to pursue the etiology of weight loss revealed positive celiac screening and a gastroenterologist confirmed the diagnosis of celiac disease through a duodenal biopsy.

Abnormalities in T4 Levels

Low T4 level with normal TSH

Case 4: The family of a 6-year-old child born in Chicago, Illinois is moving to New York and has transferred her care to a local pediatrician. TFTs done at the initial encounter are as follows:

Analyte	Result	Reference Range
Total T4:	2.8 $\mu\text{g/dL}$	6-14.2 $\mu\text{g/dL}$
TSH:	3.2 $\mu\text{IU/mL}$	0.7-5.7 $\mu\text{IU/mL}$

A low total T4 with a TSH value in the normal range should alert a practitioner to the following possibilities:

- *Central hypothyroidism (CH):* This is characterized by inadequate thyroid hormone production (hence, low total and free T4) due to insufficient stimulation by TSH of an otherwise normal thyroid gland. The TSH level, however, is generally measurable and is frequently in the normal/ low range. The reported prevalence of CH ranges from 1:20 000 to 1:80 000 in the general population and has been reported to be about 1:160 000 among live newborns. It should be noted that newborn screening tests based on first-line TSH measurement will miss CH. The majority of CH cases are sporadic but there are some genetic forms. CH usually occurs as a part of combined pituitary hormone deficiencies and

isolated forms are less common. In an otherwise healthy child who is clinically well, without midline defects (which can be associated with pituitary deficiencies), no history of a central nervous system insult or infection and no evidence of other pituitary hormone deficiencies, central hypothyroidism is very unlikely.¹¹

- *Thyroid-binding globulin (TBG) deficiency:* The more likely diagnosis in this scenario is partial or complete TBG deficiency. This can be hereditary or acquired:
 - (a) *Hereditary TBG deficiency:* The prevalence of hereditary TBG deficiency varies from 1:5000 to 1:12 000 newborn infants. TBG deficiency is an X-linked trait. Both TBG and total T4 levels are very low in affected males and modestly low in carrier females.⁵
 - (b) *Acquired forms of TBG deficiency:* Endocrine diseases such as Cushing syndrome and acromegaly are associated with low TBG levels. Nonendocrine diseases such as nephrotic syndrome are characterized by urinary loss of TBG. Drugs associated with low TBG include glucocorticoids, high-dose androgens; L-asparaginase, danazol, and niacin (presumably decrease TBG production).¹² Neither forms of TBG deficiency require thyroid replacement therapy.
- *Drugs causing low T4 with normal TSH without significantly affecting TBG levels:*
 - (a) Salicylates, high-dose furosemide, certain nonsteroidal anti-inflammatory drugs such as mefenamic acid (inhibit binding of T4 to TBG)
 - (b) Heparin (activates lipoprotein lipase releasing free fatty acids in serum, which inhibits binding of T4 to TBG)
 - (c) Anticonvulsant drugs such as phenytoin and carbamazepine (increase hepatic metabolism of T4 and T3 by cytochrome P450 and displacement of hormones from binding proteins).^{12,13} Although these antiepileptic drugs can give spuriously low free T4 levels, TSH level is normal.

If the history and examination are not suggestive of an underlying systemic or endocrine illness (such as Cushing syndrome or acromegaly) and patient is not taking any drugs implicated in abnormalities of TFTs, the major differentials are central hypothyroidism and hereditary TBG deficiency. The following tests can help differentiate these diagnoses:

- *T3 resin uptake (T3RU):* This test gives an indirect measure of serum TBG. The test is performed

by incubating patient's serum with radiolabeled T3 tracer, which binds to the unoccupied T4-binding sites (mostly TBG). Subsequently an insoluble resin (eg, dextran-coated charcoal) is added which traps the remaining unbound radiolabeled T3. The percentage of resin bound tracer is reported, which varies inversely with the number of available unoccupied T4-binding sites in serum (thus indirectly measuring TBG). The T3RU is therefore increased in TBG deficiency, whereas it is generally low in patients with true hypothyroidism.¹⁴

- *Free T4:* Free T4 levels are normal in TBG deficiency, whereas the level will be low in central hypothyroidism. When differentiating between these 2 entities, free T4 measurement by direct assays (such as equilibrium dialysis) may be preferred over analog assays to minimize interference in the results due to hormone-binding proteins.
- *TBG:* TBG level can be measured by one of the commercially available immunometric assays (immunochemiluminometric assay or ICMA) and are low in hereditary or acquired TBG deficiency.

Follow-up on case 4: In view of a low total T4, additional tests are done along with repeat TSH and total T4:

Test	Result	Age-Appropriate Reference Range
TSH:	2.6 μ U/mL	0.7-5.7 μ U/mL
Total T4:	2.5 μg/dL	6.0-14.2 μ g/dL
Free T4:	1.7 ng/dL	1.0-2.1 ng/dL (by equilibrium dialysis)
T3 uptake:	52%	22.5% to 37.0%

The normal free T4 with elevated T3 uptake in this otherwise healthy child confirms the diagnosis of TBG deficiency. The family is reassured by the primary physician as to the benign nature of the condition and that no further thyroid investigation is warranted. It should be noted that newborns with this condition born in a state that has a TSH-based NBS (such as Illinois) will not be identified on newborn screen, whereas in states that have a T4-based NBS, these newborns will usually have an abnormal NBS and diagnosed early.

Elevated T4 level with nonsuppressed TSH

Case 5: A mother, who states that she was diagnosed with Graves's disease in her late teenage

years, brings in her 17-year-old daughter for thyroid function evaluation. Other than fatigue, her daughter has no symptoms of hyperthyroidism or hypothyroidism and has no goiter. TFTs show the following:

Test	Result	Lab Reference Range
TSH:	2.5 μ IU/mL	0.27-4.2 μ IU/mL
Total T4:	14 μg/dL	4.6-12 μ g/dL

A number of conditions result in elevated total T4 with normal (nonsuppressed) TSH and no signs or symptoms of hyperthyroidism. In most of these situations, free T4 is normal or just slightly elevated. These conditions are collectively called euthyroid hyperthyroxinemia. Although these may represent true pathologies, they are often benign variants, not requiring treatment.

- *TBG excess due to decreased clearance:* Estrogens increase the TBG levels by enhancing the glycosylation and thereby slowing clearance from bloodstream. Although pregnancy is a common hyperestrogenic state and TBG (and total T3 and total T4) levels increase by 25% to 50%, a more common cause in adolescent population is a patient taking oral contraceptives.¹²
- *TBG excess due to increased synthesis in hereditary TBG excess:* This is an X-linked disorder affecting newborns with varying frequencies across continents (1 in 6000 in England to 1 in 40 000 in New York). Affected males can have TBG levels 4 to 5 times normal and carrier females have TBG levels that are intermediate between normal values and the high levels in males.⁴
- *Pathological states:* Certain disease states such as hepatitis (acute or subacute hepatitis even with minimal elevations of serum aminotransferases) and acute intermittent porphyria can also raise TBG.¹²

TBG excess can be diagnosed by

- *T3 resin uptake (T3RU):* As discussed above T3RU varies inversely with TBG levels. Therefore, T3RU is low in patients with TBG excess.
- *Free T4 level:* Theoretically, direct free T4 assay should not be affected by alterations in TBG levels. However, few of the commercial automated assays can provide an accurate free T4 value that is not affected by binding protein abnormalities or low levels of albumin.^{12,15}

Ordering a *free T4 by equilibrium dialysis*, although more expensive than analog assays, generally gives more accurate measurement of the free hormone and is characteristically normal in TBG excess.

Certain conditions without TBG abnormalities can also cause euthyroid hyperthyroxinemia. These include the following:

- *Familial dysalbuminemic hyperthyroxinemia:* This is an autosomal dominant genetic disorder, most often affecting patients with Hispanic background (approximately prevalence of 0.2% in this population). This is caused by a mutation in the ALB (albumin) gene and the variant albumin molecule has an approximately 60-fold increase in affinity for T4 (but not T3). This results in increased total T4 concentration in circulation with a non-suppressed TSH. The unbound fraction of T4 (free T4) remains normal and these patients are clinically euthyroid. In practice, however, most of the analog free T4 assays give a spuriously high result. Equilibrium or symmetric dialysis minimally disturbs the equilibria between T4 and its serum binding proteins and therefore free T4 measurement by this technique are characteristically within normal range.¹⁵ A diagnosis of familial dysalbuminemic hyperthyroxinemia should be considered in an euthyroid patient with the biochemical thyroid profile discussed above and it can be confirmed by albumin genotyping.
- *Generalized resistance to thyroid hormone:* Most of these patients have an autosomal dominant negative mutation in the thyroid hormone receptor beta (THR β) gene, though THR α gene mutations have also now been described. The biochemical profile in patients with this condition also shows a high free and/or total T4 with serum TSH concentrations either normal or above the reference range. The clinical picture in these patients can vary from euthyroidism to hypothyroidism or even hyperthyroidism because THR defect can differentially affect various organs.¹⁴
- *Other causes:* Drugs such as amiodarone, propranolol, iodinated radiographic contrast agents ipodate and iopanoic acid (inhibit extrathyroidal T4 deiodination to T3), acute psychosis (mechanism unknown), high altitude, and amphetamines (probably mediated by a central nervous system mechanism).¹²

In the setting of euthyroid hyperthyroxinemia, TBG excess as a drug effect and an underlying systemic illness should be considered. After these diagnoses have been ruled out, a specialist referral may be considered.

Follow-up on case 5: On further history, patient reported to be taking oral contraceptive pills for the preceding 4 months for irregular menstrual cycles. Since this was a plausible explanation for her elevated T4 level, further testing was not needed and family was reassured that she does not have a thyroid disorder. However, if checked, her T3RU would be low and free T4 within normal range.

Abnormalities in T3 Levels

Elevated T3 level with normal TSH

Case 6: A 5-year-old otherwise healthy clinically euthyroid girl with no goiter and no family history of thyroid disease has TFTs performed as a part of her annual evaluation and the tests show the following results:

Test	Result	Lab Reference Range
TSH:	2.2 μ IU/mL	0.27-4.2 μ IU/mL
Total T4:	10.1 μ g/dL	4.6-12 μ g/dL
Total T3:	235 ng/dL	70-204 ng/dL

In this 5-year-old with a T3 above the reference range and a normal TSH, a number of factors should be considered

- A true elevation of T3 is very unlikely with a non-suppressed TSH because TSH is a very sensitive marker of thyroid function.
- Serum T3 (and T4) levels are significantly higher in prepubertal children compared with healthy adults and levels should therefore be compared to age-appropriate reference ranges for interpretation^{16,17} (Table 1).
- Serum T3 levels steadily decline from around the time of puberty and generally reach adult levels after the age of 17 years.
- Serum T3 is not indicated when evaluating a child for possible hypothyroidism. However, when suspecting hyperthyroidism, total T3 level is more useful than T4 level since in Graves's disease, there is an increase in secretion of T3 by the thyroid as well as increased T4 to T3 conversion peripherally and as a consequence, while the T3 is elevated, the T4 may be in the normal range (*T3 toxicosis*).

Follow-up on case 6: Total T3 level is interpreted as normal when compared to age-appropriate reference range: 94-241 ng/dL (Table 1). No routine follow-up TFT recommended.

Thyroid Autoimmunity

Case 7: A 13-year-old girl with family history of acquired autoimmune hypothyroidism is referred for evaluation and treatment of abnormal TFTs

Test	Result	Lab Reference Range
TSH:	2.0 μ IU/mL	0.27-4.2 μ IU/mL
Total T4:	11.2 μ g/dL	4.6-12 μ g/dL
Antithyroglobulin antibodies:	202 IU/mL	<20 IU/mL
Antithyroperoxidase antibodies:	372 IU/mL	<10 IU/mL

She denies any symptoms of hypothyroidism or hyperthyroidism and does not have a goiter.

Although there are several types of thyroid autoantibodies described, antibodies directed against the thyroglobulin and thyroperoxidase enzyme (antithyroglobulin or TgAb and antithyroperoxidase or TPOAb, respectively) are most prevalent. Before the identification of TPO, antibodies against the thyroid microsomal antigen (located on the exocytic vesicles through which newly synthesized thyroglobulin is transferred to the follicular lumen) were detected in patients with autoimmune thyroid disease. Later assays, showed a significant degree of correlation between antimicrosomal and TPOAb. Most of the newer assays are against TPO, however, antimicrosomal assays are still used in some laboratories and the term can still be found in the laboratory test lists and in the laboratory reports. Their presence is usually associated with Hashimoto's autoimmune thyroiditis and consequent hypothyroidism.

- Hashimoto's thyroiditis (or chronic autoimmune thyroiditis) is the most common cause of nonendemic goiter and hypothyroidism in children older than 6 years. Up to 30% to 40% of patients with this disorder have a family history of thyroid disease and similar to most other thyroid disorders, occurs more often in females. Serum concentrations of TPOAb or TgAb are elevated in more than 95% of patients with Hashimoto's thyroiditis. In the NHANES III data, the prevalence of clinical hypothyroidism

was strongly associated with positive TPOAb but not with positive TgAb. None of the hypothyroid individuals had positive TgAb in the absence of positive TPOAb, thereby making anti-TPO a more sensitive test for autoimmune thyroiditis (although most of the times, these antibodies are ordered together).^{2,4,18,19}

- These antithyroid antibodies are not specific for Hashimoto's thyroiditis and are also found in more than 70% patients with Graves's disease. In addition, 15% to 20% of otherwise healthy subjects with no evidence of thyroid disease may also have antithyroid antibodies (NHANES III).
- While patients with Hashimoto's thyroiditis can develop hypothyroidism, they may also be euthyroid. Although the hypothyroid patients usually have the highest titers of antithyroid antibodies, the titer of antibodies does not always correlate with thyroid function. In most of hypothyroid patients with Hashimoto's thyroiditis TPOAb titers tend to decrease with levothyroxine replacement; however, they do not correlate with thyroid function status and therefore following the titers in these patients is not of much clinical relevance.¹⁹
- Positive antibodies in otherwise healthy individuals are thought to correlate with the presence of foci of lymphocytic infiltration within the thyroid gland. Despite being positive for these antibodies, these individuals may not develop thyroid dysfunction for several decades or their entire life span. Though some of these individuals may have early Hashimoto's thyroiditis (presence of thyroid autoantibodies without hypothyroidism), these are more likely the individuals with a family history of thyroid disease and/or goiter and hence, may be followed with TSH and total T4 measurements every 6 to 12 months. However, in otherwise healthy individuals with isolated positive antithyroid antibodies, especially low titer, without any other risk factors (family history of thyroid disease or goiter) evidence for recommending routine follow-up TFTs is not strong. Hence, until further data becomes available, this subgroup may just be followed clinically.²⁰
- In the past decade, there have been some studies to investigate a possible benefit of prophylactic levothyroxine therapy in euthyroid patients with Hashimoto's thyroiditis. These are mostly animal models or studies done in adult subjects and whereas some of these have found a

decrease in serological and cellular markers of thyroid autoimmunity, others have reported a decrease in size of thyroid gland as determined by ultrasound.^{21,22} However, currently there is no consensus about this proposed benefit and the long term clinical advantage of this approach is not known. Hence, in the absence of strong evidence, euthyroid individuals with isolated TPOAb or TgAb positivity do not need to be treated with levothyroxine.

Follow-up on case 7: TSH repeated 6 months later remains almost unchanged at 2.5 μ IU/mL. She is being followed with annual exams and TSH measurements. Treatment will be considered for enlarging thyroid or increasing TSH.

Thyroglobulin

Case 8: A 10-year-old boy is found to have abnormal thyroglobulin level on a screening thyroid panel:

Test	Result	Lab Reference Range
TSH:	4.1 μ IU/mL	0.27-4.2 μ IU/mL
Total T4:	8.9 μ g/dL	4.6-12 μ g/dL
Thyroglobulin (ICMA):	70 ng/mL	2-35 ng/mL
Antithyroglobulin antibodies:	<20 IU/mL	<20 IU/mL
Antithyroperoxidase antibodies:	<10 IU/mL	<10 IU/mL

He has no family history of thyroid disease and has no goiter.

Thyroglobulin (Tg) undergoes proteolytic cleavage in the thyroid follicular cells to release T4 and T3. About 90% or more of the Tg molecule undergoes degradation by lysosomal enzymes and is recycled. However, a fraction of Tg remains "undigested" and enters the circulation. An elevated Tg level in a child with no preexisting thyroid disease and with normal thyroid hormone levels is of limited diagnostic significance as of itself it cannot rule in or rule out a diagnosis. Hence, routine measurement of Tg levels as a part of "thyroid panels" is not indicated and should only be done in postoperative thyroid cancer monitoring or in evaluation of specific diagnoses like congenital hypothyroidism and congenital goiter.

Follow-up on case 8: This patient's family is reassured that there is no significance to the elevated Tg level in his case.

Summary

Recent studies on the prevalence of thyroid disorders have found that most patients with hypothyroidism have a subclinical disease (i.e. subclinical hypothyroidism - see above) with a prevalence of 3.4% (compared to 0.3% prevalence of clinical hypothyroidism).² The diagnosis in these patients is based on abnormal TFTs in apparently asymptomatic patients. Hence it is important to differentiate the abnormal thyroid function results from variants of normal. The clinical scenarios discussed above highlight that an “abnormal” value reported in thyroid function test results does not necessarily mean pathology. Several of these “abnormal” results are likely variations of normal and not true thyroid pathology.

In summary,

- Recognize that normal ranges provided by laboratory may not always be age appropriate. Hence before interpreting a result as abnormal, compare it with age-appropriate normal range.
- TSH is a very sensitive marker of primary thyroid disease. Therefore, an isolated derangement in T4 and/or T3 without any change in TSH is less likely to represent true thyroid pathology.
- Abnormal lab tests should be interpreted taking the whole picture into account. If symptoms are scant, family history and clinical findings such as goiter may be helpful in making clinical decisions about the significance of the lab results and formulating follow-up plan.
- When considering primary thyroid disease, ordering serum TSH alone or TSH with total T4 should suffice. If these suggest hypothyroidism, TPOAb and TgAb may be added.
- When considering hyperthyroidism in the differential, order serum TSH and total T3 with or without a total T4 (and/or free T4) as the initial tests.

Declaration of Conflicting Interests

The authors declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

Funding

The authors received no financial support for the research, authorship, and/or publication of this article.

References

1. Hunter I, Greene SA, MacDonald TM, Morris AD. Prevalence and aetiology of hypothyroidism in the young. *Arch Dis Child.* 2000;83:207-210.
2. Aoki Y, Belin RM, Clickner R, Jeffries R, Phillips L, Mahaffey KR. Serum TSH and total T4 in the United States population and their association with participant characteristics: National Health and Nutrition Examination Survey (NHANES 1999-2002). *Thyroid : official journal of the American Thyroid Association.* Dec 2007;17(12):1211-1223.
3. Krude H, Gruters A. Diagnostic tests of thyroid function in children and adolescents. In: Ranke MB, Mullis P-E, eds. *Diagnostics of Endocrine Function in Children and Adolescents.* 4th ed. Basel, Switzerland: S. Karger; 2011:85-101.
4. Fisher DA, Grueters A. Thyroid disorders in childhood and adolescence. In: Sperling MA, ed. *Pediatric Endocrinology.* 3rd ed. Philadelphia, PA: Saunders/Elsevier; 2008:227-253.
5. Fisher DA, Grueters A. Disorders of the thyroid in the newborn and infant. In: Sperling MA, ed. *Pediatric Endocrinology.* 3rd ed. Philadelphia, PA: Saunders/Elsevier; 2008:198-226.
6. Marks AG, LaFranchi SH. Assessing thyroid function in infants and children. In: Brent GA, ed. *Thyroid Function Testing.* 1st ed. New York, Springer; 2010:173-185.
7. Kaye CI, Accurso F, La Franchi S, et al. Introduction to the newborn screening fact sheets. *Pediatrics.* 2006;118:1304-1312.
8. Spencer CA, Takeuchi M, Kazarosyan M. Current status and performance goals for serum thyrotropin (TSH) assays. *Clin Chem.* 1996;42:140-145.
9. Pantalone KM, Nasr C. Approach to a low TSH level: patience is a virtue. *Cleve Clin J Med.* 2010;77: 803-811.
10. Haugen BR. Drugs that suppress TSH or cause central hypothyroidism. *Best Pract Res Clin Endocrinol Metab.* 2009;23:793-800.
11. Persani L. Central hypothyroidism: pathogenic, diagnostic, and therapeutic challenges. *J Clin Endocrinol Metab.* 2012;97:3068-3078.
12. Ross DS. Euthyroid hyperthyroxinemia and hypothyroxinemia. In: Basow DS, ed. *UpToDate.* Waltham, MA: Wolters Kluwer Health; 2012.
13. Verrotti A, Laus M, Scardapane A, Franzoni E, Chiarelli F. Thyroid hormones in children with epilepsy during long-term administration of carbamazepine and valproate. *Eur J Endocrinol.* 2009;160:81-86.
14. Ross DS. Laboratory assessment of thyroid function. In: Basow DS ed. *UpToDate.* Waltham, MA: Wolters Kluwer Health; 2012.

15. Cartwright D, O'Shea P, Rajanayagam O, et al. Familial dysalbuminemic hyperthyroxinemia: a persistent diagnostic challenge. *Clin Chem*. 2009;55:1044-1046.
16. Corcoran JM, Eastman CJ, Carter JN, Lazarus L. Circulating thyroid hormone levels in children. *Arch Dis Child*. 1977;52:716-720.
17. Zurakowski D, Di Canzio J, Majzoub JA. Pediatric reference intervals for serum thyroxine, triiodothyronine, thyrotropin, and free thyroxine. *Clin Chem*. 1999;45:1087-1091.
18. Huang SA. Hypothyroidism. In: Lifshitz F, ed. *Pediatric Endocrinology*. Vol 2. 5th ed. New York, NY: Informa Healthcare; 2009:405-413.
19. Marcocci C, Marino M. Thyroid-directed antibodies. In: Braverman LE and Utiger RD, eds. *Werner & Ingbar's The Thyroid: A Fundamental, Clinical Text*. 9th ed. Philadelphia, PA: Lippincott Williams & Wilkins; 2005:360-372.
20. Hutfless S, Matos P, Talor MV, Caturegli P, Rose NR. Significance of prediagnostic thyroid antibodies in women with autoimmune thyroid disease. *J Clin Endocrinol Metab*. 2011;96:E1466-E1471.
21. Aksoy DY, Kerimoglu U, Okur H, et al. Effects of prophylactic thyroid hormone replacement in euthyroid Hashimoto's thyroiditis. *Endocr J*. 2005;52:337-343.
22. Padberg S, Heller K, Usadel KH, Schumm-Draeger PM. One-year prophylactic treatment of euthyroid Hashimoto's thyroiditis patients with levothyroxine: is there a benefit? *Thyroid*. 2001;11:249-255.