BRITISH JOURNAL OF HOSPITAL MEDICINE

CORE TRAINING FOR DOCTORS

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Thyroid function testing

Introduction

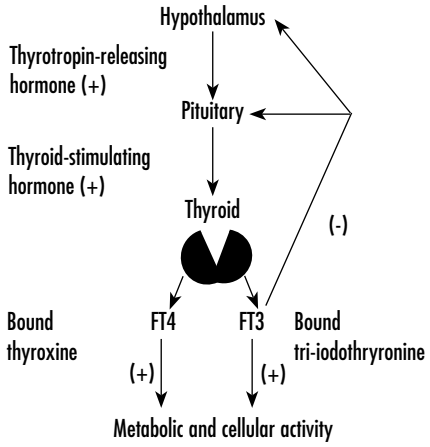
Thyroid disorders are one of the most prevalent medical conditions, so thyroid function tests are a common laboratory request in primary and secondary care. This article provides a guide for the indication and interpretation of thyroid function tests.

The hypothalamic–pituitary–thyroid axis

The hypothalamic–pituitary–thyroid axis is shown in *Figure 1*. Thyroid-stimulating hormone produced in the anterior pituitary gland is the main regulator of thyroid homeostasis.

Thyrotropin-releasing hormone is released from the hypothalamus to stimulate the production and release of thyroid-stimulating hormone from the pituitary.

Figure 1. The hypothalamic–pituitary–thyroid axis. FT3 = free tri-iodothyronine; FT4 = free thyroxine; (+) = stimulates; (-) = inhibits.



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Thyroid-stimulating hormone then stimulates the synthesis and release of thyroxine and tri-iodothyronine from the thyroid gland. Tri-iodothyronine is also produced from 5'-monodeiodination of thyroxine in extra-thyroidal tissues.

Both thyrotropin-releasing hormone and thyroid-stimulating hormone are under negative feedback regulation from free tri-iodothyronine in the circulation. More than 99% of tri-iodothyronine and thyroxine is bound to hormone-binding globulins, but only the free hormones can actively bind nuclear receptors to have a downstream physiological effect.

What laboratory tests are available?

Thyroid-stimulating hormone is usually the initial test in thyroid diagnostics, as it is the most sensitive and specific test for detecting early dysfunction (Ladenson et al, 2000), and for monitoring thyroid hormone replacement (Association of Clinical Biochemistry et al, 2006). A normal serum thyroid-stimulating hormone level can rule out thyroid disease provided there is no evidence of hypothalamic–pituitary disease or non-thyroidal illness syndrome, and the individual is not taking any medication that could affect thyroid test results (Surks et al, 2004; Warner and Beckett, 2010).

Thyroid hormones are measured in parallel with thyroid-stimulating hormone. Measurement of the 'free' hormones is preferred because they are not affected by the affinity and binding of thyroid hormone-binding proteins in plasma. However, the tests can be affected by thyroxine-binding globulin deficiency, severe illness and drugs that interfere with hormone binding, the extent of which depends on the assay being used (Sapin, 2001).

Free thyroxine is the primary secretory product of the normal thyroid gland and is the most common thyroid test used as an adjunct to thyroid-stimulating hormone, and as a monitor of thionamide therapy. Free tri-iodothyronine is usually measured as a second-line test in cases of suspected thyrotoxicosis where free tri-iodothyronine is often elevated earlier and to a

greater extent than free thyroxine. Free tri-iodothyronine should be determined in all patients taking amiodarone and where thyroid-stimulating hormone and free thyroxine results are equivocal (Association of Clinical Biochemistry et al, 2006).

Antibody testing is a second-line test for thyroid dysfunction, the most sensitive being thyroid peroxidase antibodies which are found in a wide range of immunologically-mediated disorders (e.g. Hashimoto's thyroiditis and Graves' disease) (Roos et al, 1996).

Typical thyroid function test serum reference intervals from the authors' laboratory at St James's University Hospital, Leeds are provided in *Table 1*. Reference intervals and 'cut-offs' will differ according to local laboratory methods and should be used with caution and flexibility. In pregnant women, trimester-related reference intervals that are appropriate to the laboratory should be applied (Fantz et al, 1999; Stockigt and Lim, 2009).

How do I interpret abnormalities?

Table 2 provides a simple guide to interpretation of thyroid function tests.

Algorithms suggesting routes of investigation following an abnormal thyroid-stimulating hormone result are shown in *Figures 2* and *3*. If thyroid-stimulating hormone is suppressed, the laboratory should have a cascade system in place to measure free tri-iodothyronine, which can distinguish between hyperthyroidism (high free tri-iodothyronine) and non-thyroidal illness syndrome (low free tri-iodothyronine).

Thyroid function tests should not be used for general population screening, and are not reliable in acutely unwell patients, including those with psychiatric disturbance and clinical depression, since non-thyroidal illness syndrome and drugs that affect the axis are common in such cases (Weetman, 1997). Nevertheless, regular thyroid function tests are recommended in specific patient groups (*Table 3*) (Surks et al, 2004; Smellie et al, 2010).

Table 1. Typical serum reference intervals in non-pregnant adults*

Thyroid-stimulating hormone	0.45–4.5 mU/litre
Thyroid peroxidase antibodies	<100 U/litre
Free thyroxine	9.0–25 pmol/litre
Free tri-iodothyronine	3.5–7.8 pmol/litre

* Currently, a reference interval harmonisation programme is in process in the UK (www.pathologyharmony.co.uk/)

Non-thyroidal illness syndrome

Non-thyroidal illness syndrome, also called 'sick euthyroid syndrome', can be caused by a range of chronic or acute illnesses and can lead to abnormalities in thyroid function tests even though patients are clini-

Figure 2. Routes of investigation when thyroid-stimulating hormone is above the reference interval, assuming a reference interval of 0.45–4.5 mU/litre for thyroid-stimulating hormone. Colour code: red = evidence of hypothyroidism; yellow = evidence of other pathology affecting thyroid function test results; blue = no evidence of thyroid dysfunction but monitoring is required. FT4 = free thyroxine; TSH = thyroid-stimulating hormone; TSHoma = TSH-producing pituitary adenoma. From Col et al (2004); Association of Clinical Biochemistry et al (2006); Vaidya and Pearce (2008).

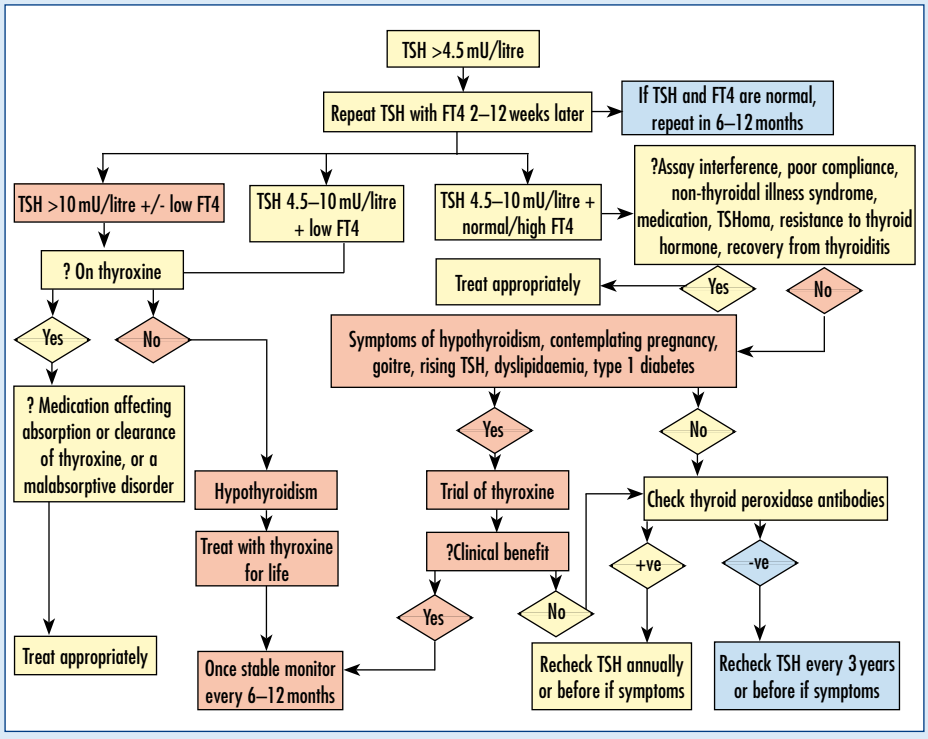

Table 2. Thyroid function test interpretation guide

	Low TSH	Normal TSH	High TSH
Low free thyroxine	Pituitary or hypothalamic hypothyroidism, severe non-thyroidal illness syndrome	Pituitary or hypothalamic hypothyroidism, severe non-thyroidal illness syndrome, late thyroiditis	Primary hypothyroidism (TSH above the reference interval)
Normal free thyroxine	Subclinical hyperthyroidism, thyroxine over-replacement, thyroid autonomy, non-thyroidal illness syndrome	Euthyroid, non-thyroidal illness syndrome (low free tri-iodothyronine)	Subclinical hypothyroidism, end organ resistance, assay interference, poor compliance
High free thyroxine	Primary hyperthyroidism (TSH fully suppressed)	Assay interference, TSH-producing pituitary adenoma, end organ resistance, non-thyroidal illness syndrome, early thyroiditis	End organ resistance, assay interference, poor compliance, TSH-producing pituitary adenoma

TSH = thyroid-stimulating hormone. From Wilson and Curry (2005)

cally euthyroid (Warner and Beckett, 2010). In most cases, thyroid-stimulating hormone will be normal and provides the best guide of thyroid status.

Primary hypothyroidism

Overt primary hypothyroidism is a condition with significant morbidity, but many of the symptoms and signs are non-specific (Todd, 2009). It is indicated by a serum thyroid-stimulating hormone level above the upper limit of the reference interval with a serum free thyroxine level below the reference interval (Surks et al, 2004). Possible causes of primary hypothyroidism are listed in *Table 4* (Vaidya and Pearce, 2008). Patients with hypothyroidism who are taking thyroxine may become hypothyroid if given drugs that decrease thyroxine absorption or increase its clearance (Haugen, 2009).

Figure 3. Routes of investigation when thyroid-stimulating hormone is below the reference interval, assuming a reference interval of 0.45–4.5 mU/litre for thyroid-stimulating hormone. Colour code: red = evidence of thyrotoxicosis; yellow = evidence of other pathology affecting thyroid function test results; blue = no evidence of thyroid dysfunction but monitoring is required. FT3 = free tri-iodothyronine; FT4 = free thyroxine; T3 = tri-iodothyronine; TSH = thyroid-stimulating hormone. From Col et al (2004); Association of Clinical Biochemistry et al (2006); Vaidya and Pearce (2008).

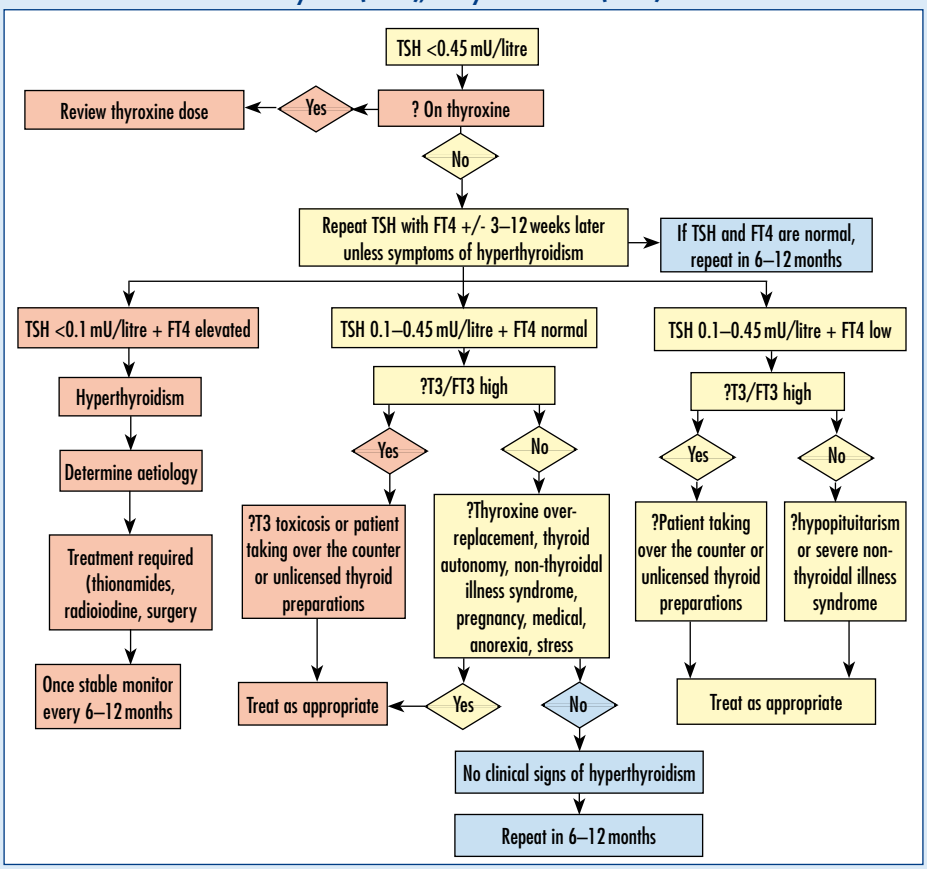

Table 3. Recommended use of thyroid function testing in specific populations

Patients presenting with a suspected goitre, thyroid nodule, atrial fibrillation, osteoporosis, subfertility or dyslipidaemia
Annually in patients with type 1 diabetes and autoimmune Addison's disease, and at diagnosis in all type 2 diabetics
Annually in women with a past history of post-partum thyroiditis
Annually in patients with Down's syndrome or Turner's syndrome
Annually in all patients who have received radioiodine treatment, neck radiotherapy, or who have had a subtotal thyroidectomy
In all patients on amiodarone or lithium therapy according to established guidelines

Secondary hypothyroidism

Secondary hypothyroidism is a biochemical diagnosis where thyroid-stimulating hormone can be low, within or just above the reference interval, combined with a low free thyroxine level (Association of Clinical Biochemistry et al, 2006). A combination of thyroid-stimulating hormone, free thyroxine and free tri-iodothyronine measurement may be required to differentiate secondary hypothyroidism from non-thyroidal illness syndrome, especially in older patients where symptoms are often vague and non-specific.

Referral to an endocrinologist for additional pituitary function tests (prolactin, follicle-stimulating hormone, luteinizing hormone and adrenocorticotropic hormone), cortisol and sex steroid measurement may be required to make a diagnosis of hypopituitarism (Todd, 2009).

Subclinical hypothyroidism

Subclinical disease is the earliest sign of thyroid dysfunction (Col et al, 2004), but clinical symptoms and signs are often mild or absent (Surks et al, 2004). In subclinical disease, thyroid-stimulating hormone is outside its reference interval but free thyroxine and free tri-iodothyronine are within expected intervals (Todd, 2009).

Subclinical hypothyroidism is common in clinical practice, particularly in iodine-replete populations, individuals >55 years old, women and caucasians (Diez and

Table 4. Possible causes of primary hypothyroidism

Autoimmune thyroiditis (Hashimoto's, atrophic)
Thyroiditis (post-partum, subacute, silent)
Post-ablative (radioiodine, surgical)
Drug-induced (e.g. lithium, amiodarone)
Poor compliance with thyroxine therapy or suboptimal treatment
Iodine deficiency
Congenital (thyroid agenesis or dysgenesis, ectopic thyroid remnants, dyshormonogenesis)
Treatment of head and neck cancer (surgery, radiation)

Iglesias, 2004). It is characterized by a thyroid-stimulating hormone concentration above the reference interval but <10 mU/litre and a free thyroxine within the reference interval (Todd, 2009). It should be confirmed by a repeat thyroid function test after 2 weeks to 3 months to exclude transient increases in thyroid-stimulating hormone (Col et al, 2004; Surks et al, 2004). The patient should also be evaluated for signs and symptoms of hypothyroidism, previous treatment of hyperthyroidism, thyroid gland enlargement and evidence of family history of thyroid disease (Surks et al, 2004). Other causes of an elevated thyroid-stimulating hormone level must be excluded, e.g. drug interference, recent adjustments in thyroxine dose, or a transient increase as a result of recovery from non-thyroidal illness syndrome or thyroiditis (Smellie et al, 2010). Approximately 2–5% of cases of subclinical hypothyroidism will progress to overt hypothyroidism per year (Surks et al, 2004).

If on repeat testing the serum thyroid-stimulating hormone level is >10 mU/litre, with a normal or low serum free thyroxine, then treatment with thyroxine is recommended (Association of Clinical Biochemistry et al, 2006). If serum thyroid-stimulating hormone is above the reference interval, but <10 mU/litre, serum thyroid peroxidase antibodies should be measured (*Figure 2*). If the thyroid peroxidase antibody concentration is high, serum thyroid-stimulating hormone should be measured annually, or earlier if symptoms develop. If antibody levels are not raised, repeat measurement of serum thyroid-stimulating hormone every 3 years is sufficient (Association of Clinical Biochemistry et al, 2006).

Thyroxine therapy should always be considered in subclinical hypothyroid patients who are pregnant, have symptoms (despite thyroid-stimulating hormone being <10 mU/litre), or in the presence of a goitre, rising thyroid-stimulating hormone concentration or dyslipidaemia (Surks et al, 2004).

Primary hyperthyroidism

Primary hyperthyroidism is diagnosed by an undetectable serum thyroid-stimulating hormone level with a raised free thyroxine and free tri-iodothyronine (free thyroxine is measured first line) (Association of Clinical Biochemistry et al, 2006). Biochemical confirmation is necessary in all cases. Hyperthyroidism may also occur with normal free thyroxine concentrations but elevated free tri-iodothyronine: this syndrome of 'tri-iodothyronine toxicosis' can be seen following radioiodine treatment and surgery for hyperthyroidism, or can be caused by mild toxic thyroid nodules or early Graves' disease. Free tri-iodothyronine may also be raised in subjects taking porcine-derived thyroid extract (Armour Thyroid; Forest Pharmaceuticals, USA) that can be purchased online by the public, but is not currently licensed in the UK (Vaidya and Pearce, 2008). The relative quantities of thyroxine and tri-iodothyronine differ markedly from the healthy human thyroid and consumption can cause a high serum free tri-iodothyronine, low free thyroxine and a suppressed thyroid-stimulating hormone.

Other causes of a low thyroid-stimulating hormone level should be excluded, such as over-replacement of thyroxine, normal pregnancy, commonly prescribed drugs, stress, non-thyroidal illness syndrome and anorexia nervosa (Col et al, 2004). The patient should be further evaluated to determine the aetiology by radioactive iodine uptake measurement and scanning. If low thyroid-stimulating hormone is the result of thyroiditis this should resolve spontaneously and only requires symptomatic treatment (Surks et al, 2004). Possible causes of primary hyperthyroidism are listed in *Table 5* (Todd, 2009). If serum thyroid-stimulating hormone is within the reference interval, hyperthyroidism can be ruled out, except in the rare instances of a thyroid-stimulating hormone-secreting adenoma and resistance to thyroid hormone action (Beck-Peccoz et al, 2009).

Diagnosis of Graves' disease is usually possible clinically, and measurement of antibodies may not contribute to diagnosis or management (Weetman, 2000). Thyroid-stimulating hormone receptor antibodies are a more specific indicator of Graves' disease than thyroid peroxidase antibodies (Costagliola et al, 1999), and have a role in assessing the risk of neonatal hyperthyroidism in pregnant women with a history of Graves' disease, and the risk of relapse after a course of anti-thyroid drugs (Association of Clinical Biochemistry et al, 2006).

Subclinical hyperthyroidism

Subclinical hyperthyroidism is a biochemical diagnosis, defined as a low serum thyroid-stimulating hormone with normal serum free thyroxine and free tri-iodothyronine concentrations (Surks et al, 2004). It can be categorized into those with low but detectable serum thyroid-stimulating hormone (0.1–0.45 mU/litre) and those with thyroid-stimulating hormone <0.1 mU/litre (Surks et al, 2004).

A low thyroid-stimulating hormone can be a result of mild thyroid hormone excess, hypothalamic or pituitary disease, non-thyroidal illness syndrome, normal pregnancy or ingestion of drugs inhibiting thyroid-stimulating hormone secretion (Surks et al, 2004; Wilson and Curry, 2005). In non-thyroidal illness syndrome, however, free thyroxine is usually low–normal rather than the high–normal seen in subclinical hyperthyroidism and approximately 1–2% of cases of subclinical hyperthyroidism where thyroid-stimulating hormone is <0.1 mU/litre will progress to overt hyperthyroidism (Surks et al, 2004).

If the thyroid-stimulating hormone level is 0.1–0.45 mU/litre, and the subject is not on thyroxine, non-thyroidal illness syndrome and drug effects should be excluded and thyroid-stimulating hormone measurement repeated with free

Table 5. Possible causes of primary hyperthyroidism

Graves' disease
Toxic multinodular goitre
Recurrence following partial thyroidectomy
Exogenous iodine (contrast media, amiodarone, over-the-counter preparations)
Functioning thyroid adenoma
Thyroiditis (viral, lymphocytic, post-irradiation, or post-partum in the early phase)
Tri-iodothyronine toxicosis
Human chorionic gonadotropin-mediated (hyperemesis gravidarum, trophoblastic disease)
Neonatal thyrotoxicosis
Follicular carcinoma (functioning)

thyroxine and free tri-iodothyronine to exclude overt hyperthyroidism. In most instances, serum thyroid-stimulating hormone will have returned to within the reference interval. If thyroid-stimulating hormone is still 0.1–0.45 mU/litre, with normal free thyroxine and/or free tri-iodothyronine, then a thyroid function test should be repeated every 6–12 months (*Figure 3*).

Central hyperthyroidism

In some cases an elevated free thyroxine and/or free tri-iodothyronine level occurs in the presence of an 'inappropriately' detectable or elevated thyroid-stimulating hormone (Beck-Peccoz et al, 2009). It most commonly results from poor compliance or assay interference in one or more assays; however, there are rare pathological causes of central hyperthyroidism, namely thyroid-stimulating hormoneoma and resistance to thyroid hormone, where determination of a high circulating alpha-subunit and sex-hormone-binding globulin is helpful in identifying thyroid-stimulating hormone-secreting adenoma (Beck-Peccoz et al, 2009).

Drugs affecting the thyroid

There is an extensive list of drugs that can affect thyroid function tests (Haugen, 2009; Stockigt and Lim, 2009; Smellie et al, 2010). Some of the drugs in common use are outlined in *Tables 4* and *5*; those of particular note are amiodarone and lithium (Haugen, 2009; Stockigt and Lim, 2009). Patients taking amiodarone or lithium require thyroid function tests before commencement of treatment, and every 6 months thereafter, and in the case of amiodarone up to 12 months after cessation of therapy (Association of Clinical Biochemistry et al, 2006).

Conclusions

Thyroid function tests are a common laboratory request in primary and secondary care. Appropriate interpretation requires an awareness of non-thyroidal disorders and medications that can affect test results. Thyroid function tests should not be used for population screening, however, it is important to monitor thyroid function in particular patient populations. Non-specialist doctors ordering thyroid function tests in clinical practice should be informed by the algorithms provided herein and by national guidelines. **BJHM**

KEY POINTS

- Serum thyroid-stimulating hormone is the most sensitive and specific marker for thyroid dysfunction.
- Serum free tri-iodothyronine measurement is usually used as a second line investigation in patients with equivocal thyroid-stimulating hormone and free thyroxine results.
- Unless clinically indicated caution is required in the investigation of thyroid function in acute psychiatric disturbances and clinical depression since non-thyroidal illness and medication that affect thyroid function are both common and may prompt inappropriate intervention.
- The measurement of serum thyroglobulin has no role in the diagnosis of thyroid cancer.
- Investigation algorithms exists as national guidelines and should be referred to appropriately

TOP TIPS

- Do not request routine testing of thyroid function in patients admitted acutely to hospital as a screening tool unless specific clinical indications exist.
- Liaise with endocrinology and hospital biochemist early in suspected thyroid dysfunction to best guide investigation and treatment.
- Provide accurate and relevant clinical details and relevant drug history to the biochemical department to facilitate correct tests and result interpretation.
- Perform baseline thyroid function tests before starting antithyroid medications, amiodarone and lithium to allow for relevant monitoring interpretation.

Conflict of interest: none.

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