The role of the laboratory in thyroid disease

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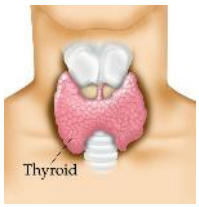
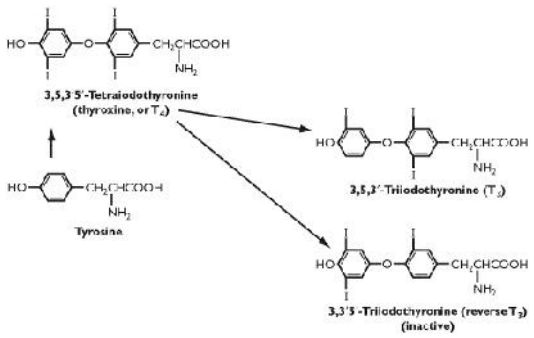
Learning Objectives

- Describe the structure and function of the thyroid gland
- Explain the function of thyroid hormones
- Outline the action of thyroid hormones and control of their secretion from the thyroid gland
- Describe the conditions which lead to abnormal thyroid hormone production
- Discuss the investigation of suspected thyroid dysfuction

Thyroid hormones

- Thyroxine – T4
  - Precursor to T3
- Tri-iodothyronine – T3
  - 10x more active than T4
  - Binds nuclear hormone receptors in target tissues to induce its effects
- Calcitonin originates from thyroid gland



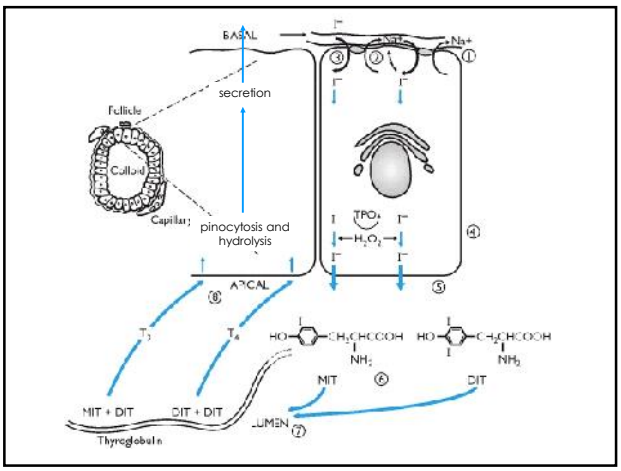


Action of thyroid hormones

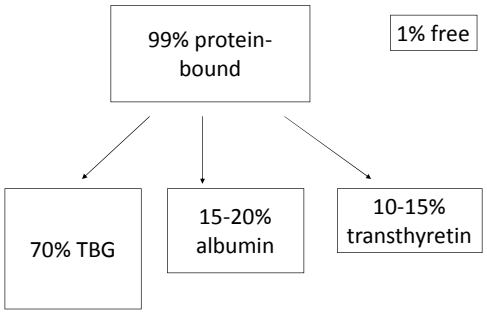
- Increase basal metabolic rate
- Increase activity of Na/K ATPase
- Required for normal bone and brain development
- Increase rate of carbohydrate and fat metabolism
- Increase cardiac output and heart rate
- The effect of thyroid hormones on the mitochondria is under investigation
- Signalling by binding of T3 to nuclear hormone receptors in target tissues to activate transcription of many genes

Thyroid hormone synthesis

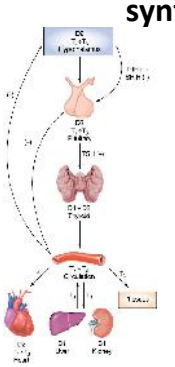
- Iodide uptake by Na/I ATPase then oxidation to iodine catalysed by thyroid peroxidase (thyroid gland v. efficient at trapping and storing iodine)
- Iodine transported to colloid space of thyroid follicle
- Tyr residues on Thyroglobulin (Tg) in colloid are iodinated by TPO to form mono-iodotyronine (MIT) and Di-iodotyronine (DIT)
- Coupling of MIT and DIT within/between thyroglobulin molecules (regulated by TPO) to form T4 & T3
- Tg pinocytosed by follicular cell
- Hydrolysis of Tg molecule to release T4 & T3 following fusion of pinosome with lysosome
- Secretion of T4 (90%, ~100 µg/day) & T3 (10%, ~10 µg/day)



Thyroid hormone transport



Regulation of thyroid hormone synthesis/secretion



D1 & D2: deiodinases, convert T4 to T3

- HPT axis
- Iodide availability
- TPO activity

- TSH stimulates:
  - Iodide uptake
  - TPO synthesis
  - Follicular cell pinocytosis
  - T4/T3 secretion
  - Thyroid gland growth

Thyroid function tests

- TSH 0.35 – 4.94 mU/L
- Free T4 9.0 – 20.0 pmol/L (Abbott)
- Free T3

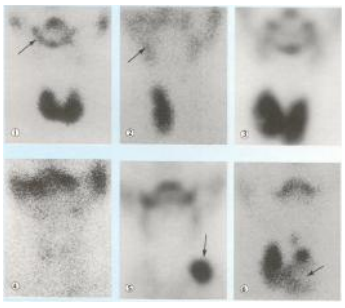
Others:

- Thyroid peroxidase Ab (general marker of thyroid autoimmunity)
- TSH receptor Ab (autonomously stimulates TSH-R in Graves' disease)
- Thyroglobulin (specifically expressed in thyroid tissue
  - used to monitor thyroid Ca after ablation)

Disorders that affect TFTs

- Hyperthyroidism (1°/2°/3°)
- Hypothyroidism (1°/2°/3°)
- Pregnancy
- Drug effects
- Non-thyroidal illness

Thyroid uptake scans

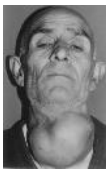


1. Normal thyroid
2. L lobe surgically removed
3. Diffusely increased uptake (e.g. Graves')
4. Decreased uptake (hypothyroidism)
5. Single "hot" nodule with suppression of surrounding tissue
6. "Cold" region in thyroid cancer

Hypothyroidism

Hypothyroidism - causes

- Primary (↑TSH, ↓T4):
  - Hashimoto's thyroiditis: chronic autoimmune lymphocytic destruction of gland (F:M – 4:1)
  - Iodine deficiency (v. common in some parts of the world)
  - These usually present with goitre
  - Iatrogenic: thyroidectomy, radioiodine, anti-thyroid drugs
- Rarer causes of 1° hypothyroidism:
  - Inflammatory thyroiditis (e.g. subacute, post-partum) – usually transient and preceded by hyperthyroidism
  - Genetic disorders of synthesis, gland agenesis
  - TSHR blocking Ab (no goitre)



Multinodular goitre due to iodine deficiency in Kashmir

Hypothyroidism - causes

- Secondary (↓/N TSH, ↓T4):
  - Hypopituitarism (tumours, radiotherapy, surgery)
  - Defective TSH synthesis (genetic)
  - (NB exclude NTI, on T3, hyperthyroid Rx)
- Tertiary (TRH deficiency):
  - Hypothalamic damage (tumours, radiotherapy, surgery)
- Consider assessment of other pituitary hormone axes

Hypothyroidism - signs & symptoms

- Fatigue/lethargy
- Cold intolerance
- Depression
- Constipation
- Aches & pains
- Carpal tunnel syndrome
- Dry skin
- Coarse, brittle, thinning hair
- Bradycardia
- Puffy skin
- Anaemia
- Goitre (depending on aetiology)

Patients at risk of developing hypothyroidism

Check TFTs annually in following pts:

- Type I diabetics (and those with tendency to AI disease)
- Past history of post-partum thyroiditis
- Down syndrome and Turner syndrome
- Patients on lithium and amiodarone (check prior to starting treatment and monitor every 6-12 months)
- Following radioiodine or surgery for thyrotoxicosis
- Following neck irradiation for head & neck cancers

Myxoedema coma

- Rare complication of chronically untreated hypothyroidism
- More common in the elderly
- Signs and symptoms:
  - Hypothermia
  - Stupor/coma
  - Hypotension
  - Respiratory depression
  - Ileus
  - Urinary retention
- Biochemical features:
  - Hyponatraemia
  - Hypoglycaemia
  - Hypoxaemia
  - Acidosis
  - Hypercapnia
- Treat with large doses of T4 (+T3?)
- Correct fluid/electrolyte imbalance

Other abnormalities caused by hypothyroidism

- ↑ CK (can be ~1000)
- Hyponatraemia (↓ free water excretion)
- ↑ LDL-C (↓ LDL-R clearance, ↓ BMR, ↓ bile acid synthesis)
- ↑ Prolactin (lactotrophs of anterior pituitary stimulated by TRH)
- Normocytic normochromic anaemia

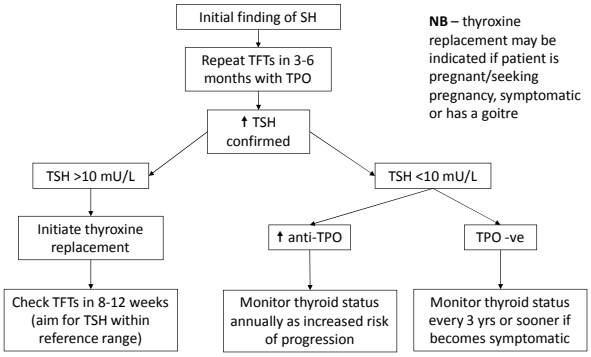
Hypothyroidism - treatment

- Levothyroxine
  - e.g. start with 25 µg/day and titrate upwards with monitoring every 8-12 weeks
  - Monitor 1° hypothyroidism with TSH – aim to get within reference range (lower half of RR?)
  - ↑ risk of AF & osteoporosis if TSH suppressed
  - Use FT4 to monitor 2° & 3° hypothyroidism – aim for upper half of RR (also monitor replacement of other hormones if panhypopit)
- Liothyronine - T3 (or combination of T3/T4)
  - Beware TFTs on T3 – can look like 2° hypothyroidism (normal TSH, ↓ FT4 – but FT3 is normal)
  - Can be used if patients believe that T4 is not working for them

Subclinical (compensated) hypothyroidism

- FT4 within RR with raised TSH
- Failing thyroid gland with increased TSH drive sufficient to maintain FT4 within normal range
- Risk of progression to overt hypothyroidism, especially if TPO +ve
- May or may not be symptomatic
- Results should be confirmed in 3-6 months to exclude NTI
- Also exclude drugs and assay interference

Management of subclinical hypothyroidism



Congenital Hypothyroidism

- UK prevalence of CH 1:4000
- Many causes – abnormal thyroid hormone synthesis, defective or absent gland
- Untreated leads to irreversible IQ deficits, poor development etc
- Bloodspot TSH at ~day 7 of life (TSH surge prior to this)
  - 5-20 mU/L: repeat bloodspot – refer if ↑ on repeat
  - >20 mU/L: request mother and baby serum
  - Baby: TFTs & Tg, Mother: TFTs and TRAbs (to look for blocking Abs and interference)
- If confirmed, commence T4 by day 18
- Will miss cases of 2° and 3° hypothyroidism
  - Hopefully they will soon present clinically due to midline defect
  - Screen by total T4 in USA

TRH test

- Used to differentiate between 2° and 3° hypothyroidism
  - Collect baseline sample
  - Administer thyrotropin releasing hormone (TRH)
  - Collect 30 and 60 min sample
  - Measure TSH
- Interpretation
  - Normal response: TSH rise to >5 mU/L at 30 min
  - Pituitary disease (2°): blunted response
  - Hypothalamic disease (3°): TSH rise but 60 min value exceeds 30 min
- Often performed with other DFTs to assess full pituitary function (ITT, GnRH test)

Hyperthyroidism

Hyperthyroidism - causes

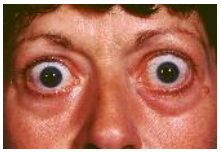
- Primary (↓/suppressed TSH ↑T4)
  - Graves' disease – IgG autoantibodies that bind to and stimulate TSH-R (TRAbs) – unregulated thyrotropic action & goitre
  - Toxic multinodular goitre – aetiology unclear, multiple hyperactive nodules throughout gland
  - NB: T3 toxicosis – ↓ TSH with normal FT4 and ↑FT3
- Rarer causes:
  - Toxic adenoma
  - Thyroiditis (initially hyperthyroid then hypo)
  - Some forms of thyroid hormone resistance

Hyperthyroidism - causes

- Secondary hyperthyroidism - rare
  - TSHoma (can measure alpha subunit)
  - Trophoblastic tumours/severe hyperemesis gravidarum – HCG can stimulate TSH-R
  - Jod-Basedow phenomenon following iodine administration esp. in iodine deficient patient (opposite to Wolff-Chaikoff)
- Factitious – e.g. buying preparations over internet to lose weight

Hyperthyroidism - signs & symptoms

- Palpitations, tachycardia, CCF
- Goitre
- Heat intolerance, sweating
- Opthalmopathy (30% of Graves' patients)
- Anxiety
- Diarrhoea
- Weight loss with no loss of appetite
- Bone demineralisation



Hyperthyroidism - treatment

- Anti-thyroid drugs
- Radioiodine (^{131}I) ablation of thyroid gland
- Thyroidectomy

- Treatment of choice depends on aetiology
- In all cases, can alleviate symptoms with beta-blockers

Anti-thyroid drugs

- Carbimazole
  - Most widely used in UK
  - Inhibits TPO, therefore ↓ T3 & T4 synthesis
- Propylthiouracil
  - Drug of choice in pregnancy as protein-bound so crosses placenta/milk ducts to lesser degree
  - Inhibits TPO and also peripheral conversion of T4→T3

Anti-thyroid drugs

- ATDs used mainly to treat Graves' Disease
- Regimens:
  - Block and replace
  - Titration
- TSH can remain suppressed for several months therefore monitor FT4 initially
- Stop ATDs periodically to see if in remission
- Can remain on ATDs indefinitely

Anti-thyroid drugs

- Side effects:
  - Agranulocytosis (monitor FBC, discontinue immediately if develop sore throat and fever)
  - LFT abnormalities (cholestatic with carbimazole, hepatitic with propylthiouracil)
- Following discontinuation of ATDs, monitor TFTs 6 & 12 weeks post, then 3 monthly for 1st year, then annually
- 60% relapse rate in 1st year
- May develop spontaneous hypothyroidism many years later

Radioiodine ablation

- Used for Graves', Multinodular goitre (MNG) or solitary thyroid nodule (STN) either as first line or post relapse following antithyroid drugs (ATDs)
- Contraindicated in pregnancy
- Pre-treat with ATDs to get FT4/FT3 within RR
- About 10% may require 2nd dose 9-12 months later
- Most will eventually become hypothyroid – monitor and replace
- Can also use following thyroidectomy in Ca thyroid pts to destroy remnants and get undetectable Tg

Thyroidectomy

- Removal of large unsightly goitres/toxic nodules or thyroid cancer
- Partial or complete
- Pre-treat with ATDs to get FT4/FT3 within the reference range
- Monitor subsequently for development of hypothyroidism or relapse
- Check Ca^{2+} and PTH following surgery

Treatment of subclinical hyperthyroidism

- Confirm that low TSH is persistent and exclude non thyroidal illness (NTI) and drugs
- Consider treatment if TSH suppressed, increased age of patient or symptomatic (increased risk of AF and osteoporosis)
- Important to monitor untreated patients every 6-12 months
- Refer to thyroid clinic to determine cause

Thyrotoxic storm

- Rare life-threatening severe thyrotoxicosis
- 50% mortality
- More common in Graves disease
- Precipitated by illness or trauma in patients with undiagnosed or under-treated hyperthyroidism
- Exaggerated features of hyperthyroidism
  - Hyperthermia, tachycardia, psychosis, chronic cardiac failure, jaundice
- Biochemical features:
  - Hypercalcaemia, deranged LFTs, hyperglycaemia leucocytosis

Treatment of thyrotoxic storm

- Reduce thyroid hormone synthesis & secretion:
  - Carbimazole/Propylthiouracil
  - Lithium
  - Inorganic iodide (Wolff-Chaikoff effect: high dose iodine shuts down thyroid hormone synthesis)
- Inhibit conversion of T4 to T3
  - Propylthiouracil
  - Glucocorticoids
- Supportive treatment
  - Beta blockers
  - External cooling
  - Fluid balance
- Treat precipitating cause e.g. infection

Neonatal hyperthyroidism

- Rare medical emergency
- Trans-placental crossing of maternal TRAbs
- May be euthyroid initially due to maternal anti thyroid drugs (ATD'S) in circulation
- Treat with ATDs, monitor FT4
- Transient (weeks-months) as TRAbs are cleared from circulation
- Babies of Graves' mothers may be born with goitre
- Persistent neonatal hyperthyroidism is rare e.g. McCune-Albright syndrome

Thyroid cancer

Thyroid cancer

- Accounts for 1% of all cancers
- Patients with palpable nodules should be referred for assessment, regardless of TFTs
- Increased suspicion of malignancy when nodule present but no TFT abnormalities or signs/symptoms of hyper- or hypothyroidism
- 3 main forms: Papillary, follicular, medullary
- Fine needle aspiration (FNA) will reveal type of tumour, but difficult to distinguish benign from malignant
- Following treatment, administer T4 to obtain TSH <0.1 mU/L – do not want to stimulate growth of any remaining thyroid tissue (papillary and follicular)

Medullary thyroid carcinoma

- 5% of thyroid cancers – aggressive, poorly differentiated, ↑ morbidity and rates of recurrence
- Derived from C-cells of thyroid
- 80% sporadic, 20% familial (Multiple Endocrine Neoplasia (MEN2A, 2B) and familial medullary thyroid cancer (FMTC) – advisable to perform genetic testing for Ret oncogene Autosomal Dominant (AD)
- Raised calcitonin, esp after calcium/pentagastrin stimulation test
- Rx by radical thyroidectomy & removal of lymph nodes (cannot use radioiodine as no uptake by C-cells)
- Monitor for residual tissue or recurrence by calcitonin measurement (also CEA)

Papillary and follicular cancers

- Papillary: 75% Follicular: 16%
- Can treat by ^{131}I or surgery
- Check for remaining tissue by Tg measurement
- Measure Tg annually to monitor recurrence - should be suppressed
- Tg >2 μg/L could indicate recurrence of tumour or persistence of normal thyroid – needs investigating further
- Also monitor by ^{131}I uptake scan

Thyroglobulin monitoring

- More useful to measure thyroglobulin (Tg) in the presence of a TSH drive, as usually patients are on doses of T4 designed to suppress TSH and keep any remaining thyroid tissue dormant
- Done either by 2-week withdrawal of T4 (unpleasant for patient) or administration of recombinant human TSH (recent supply issues)
- Ideally have TSH >30 mU/L to be confident of a suppressed Tg result

Thyroglobulin assay

- ~20% of patients have anti-Tg antibodies that can interfere in Tg assay
- UK thyroid guidelines recommend that labs measuring Tg should have checks in place for interference
- Measure anti-Tg Abs and also perform recovery studies if possible
- Guidelines favour anti-Tg over recovery
- Important to use same Tg assay for monitoring over time, and re-baseline if lab is changing method

Thyroid disease in pregnancy

TFTs in pregnancy

- 1st trimester often see ↓TSH due to thyrotropic effects of human chorionic gonadotrophin (HCG), esp hyperemesis gravidarum (structural similarities between HCG isoforms and TSH – ability to stimulate TSH-R)
- Total T4 increases due to ↑ binding proteins
- FT4 usually lower due to dilutional effect

Hypothyroidism in pregnancy

- Maternal hypothyroidism associated with increased foetal loss, IQ deficit and low birth weight
- In pre-existing hypothyroidism, TFTs should be measured:
  - Pre-conception
  - At diagnosis of pregnancy
  - At antenatal booking appt
  - At least once every trimester
  - 2-4 weeks post-partum
- Thyroxine dose should be increased by 25-50 µg/day due to increased requirements and increased binding proteins
- Maintain TSH <2.5 mU/L in 1st trimester and <3 mU/L in 2nd and 3rd trimester
- Check TFTs 2-4 weeks post-partum to review dose

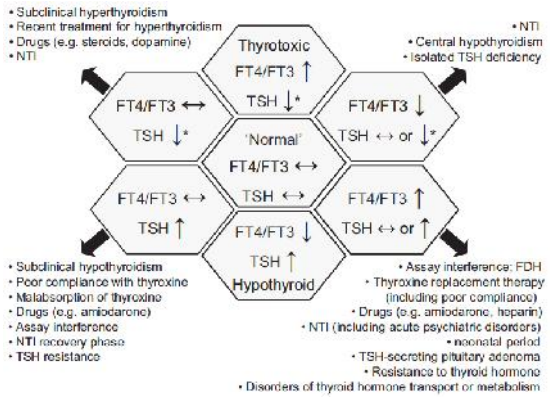
Hyperthyroidism in pregnancy

- Undiagnosed can lead to increased foetal loss, stillbirths, prematurity and intra uterine growth retardation
- Difficult to diagnose as symptoms similar to those normally observed during pregnancy (palpitations tachycardia, heat intolerance, warm moist skin, emotional lability)
- FT4 most useful for diagnosis (TSH can be ↓ due to HCG)
- May also be useful to measure TPO Abs and TRAbs
- Rx with PTU and monitor closely (also switch pre-existing hyperthyroid patients from CBZ to PTU when pregnant and if on block and replace switch to maintenance dose of PTU only) – ideally do this pre-conception

Post-partum thyroiditis

- Likely autoimmune aetiology – similar to Hashimoto's
- Inflammation of thyroid 2-4 months post-partum with painless goitre
- Hyperthyroidism followed by hypothyroidism (rapid release of pre-formed hormones from inflamed gland)
- Not all women experience both phases
- Most will return to normal in 12-18 months, but ~20% will remain hypothyroid
- +ve TPO Ab or other pre-existing autoimmunity indicates increased risk of post-partum thyroiditis
- Rx: symptom control e.g.beta blockers (antithyroid drugs not effective), anti-inflammatories, followed by thyroxine if necessary (eventually taper dose to check for recovery of thyroid function)

Odd TFT results



Non-thyroidal illness

- Altered TFTs secondary to other illness – no thyroid gland dysfunction
- E.g. surgery, infection, Myocardial Infarction (MI), malignancy, starvation
- Mechanisms and pattern of TFTs seen are poorly defined and understood

Non-thyroidal illness

- Typically ↓ TSH initially then ↑ in recovery phase
- FT3 ↓ due to reduced peripheral conversion
- ↓ FT4 due to changes in protein binding (artefact of free hormone assay)
- In reality, any combination of TSH/FT4/FT3 can be seen
- TFTs not indicated in inpatients unless strongly suspect thyroid disease
- Repeat TFTs when patient is well again are usually normal

Amiodarone

- Anti-arrythmic drug
- Contains 37% iodine by weight
- Can induce hypo- or hyperthyroidism, depending on iodine status of patient
- Tends to be hypothyroidism in iodine-replete areas (Wolff-Chaikoff effect). If no escape, replace T4
- Also inhibits hepatic T4 to T3 conversion
- Hyperthyroidism by 2 mechanisms
  - Type I: Jod-Basedow effect – can give anti thyroid drugs (ATDs)
  - Type II: cytotoxic action on thyroid gland, leads to initial thyrotoxicosis, treat with steroids

Lithium

- Treatment for bipolar disorder
- Associated with hypothyroidism and goitre (5-20% of pts)
  - Inhibits thyroidal iodide uptake
  - Inhibits iodotyrosine coupling
  - Alters thyroglobulin structure
  - Inhibits thyroid hormone (TH) secretion
- More likely in anti-TPO +ve pts
- Replace T4 if develop hypothyroidism on lithium
- V. v. rarely hyperthyroidism

Decrease in TSH secretion	**Decreased thyroid hormone secretion**	**Increased thyroid hormone secretion**	**Decreased thyroidal synthesis**	**Displacement of hormone from plasma proteins**	**Impaired T4 to T3 conversion**
Dopamine Dopaminergic agents Glucocorticoids Cytokines Octreotide	Lithium Iodide Amiodarone	Iodide Amiodarone Lithium (rare)	Methimazole Carbimazole Propylthiouracil Lithium	Frusemide Fenclofenac Salicylates Mefenamic acid Carbamazepine Non-steroidal AIDs	Beta antagonists Glucocorticoids Amiodarone Propylthiouracil Iopanoic acid Radiocontrast dyes
Increase TBG, TT3, TT4	**Decrease TBG, TT3, TT4**	**Increased hepatic metabolism of T4**	**Impaired absorption of thyroxine**	**Alter autoimmunity**	**Modify thyroid hormone action**
Oestrogens Tamoxifen Heroin Methadone Clofibrate Raloxifene	Androgens Anabolic steroids Glucocorticoids	Phenytoin Carbamazapine Barbiturates Rifampacin	Cholestyramine Cholestapol Aluminium hydroxide Ferrous sulphate Sucralfate Calcium carbonate Soy protein Proton pump inhibitors	Interleukin 1 Interferon α Interferon β TNF α	Amiodarone

Thyroxine replacement

- Non-compliant patient taking excessive T4 few days prior to clinic visit (not enough time for TSH to normalise) → ↑/N FT4 & ↑ TSH
- Inadequate thyroxine dose: FT4 in normal range & slightly ↑ TSH
- TFTs not improving despite ↑ thyroxine dose and poor compliance has been excluded
  - Malabsorption (GI problems or interference of other drugs e.g. ferrous sulphate)
  - ↑ hepatic clearance (induced by e.g. carbamazepine)

Assay interference

- Familial dysalbuminaemic hyperthyroxinaemia: genetic variants of albumin with altered affinity for thyroid hormones can cause overestimation in FT4 assay although assays can now compensate
- Heterophilic antibodies – anti-animal Abs
  - Block binding sites of capture or detection Ab resulting in negative interference (or +ve interference if competitive)
  - Bridge binding sites of capture and detection Abs resulting in positive interference
- MacroTSH

Free hormone assays

- T4 and T3 are small □ measured by competitive immunoassay
- In free hormone assays, labelled T4/T3 analogue must not disrupt equilibrium between bound and free hormone (i.e. has no affinity for binding proteins)
- Factors in serum which disrupt this equilibrium will affect free hormone measurement
- E.g. heparin causes overestimation of free hormones due to activation of lipoprotein lipase and generation of free fatty acids, which displace T4/T3 from albumin *in vitro*
- Gold standard for free hormones is equilibrium dialysis

Investigating assay interference

- Send to another lab using different kit (different antibody specificities)
- Dilution of Thyroid Stimulating Hormone – non-linear dilution suggests interference
- Heterophile Ab blocking reagents
- PEG precipitation
- Send to specialist thyroid service e.g Addenbrooke's in Cambridge
- If patient is euthyroid, more likely to be interference

Thyroid hormone resistance

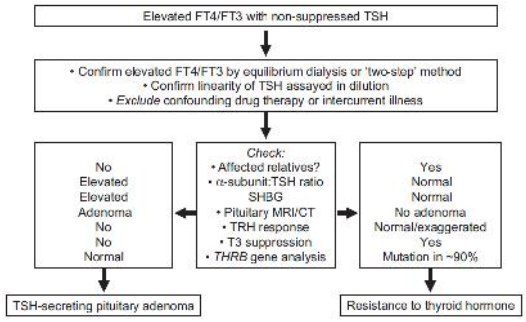
- Dominantly inherited mutation in thyroid hormone receptor β
- ↑ FT3/FT4 with unsuppressed TSH
- Variable thyroid hormone resistance in peripheral tissues
- 2 major forms
  - Generalised thyroid hormone resistance – clinically euthyroid (thyroid hormone rise compensates)
  - Predominant pituitary resistance – thyrotoxic features as –ve feedback loop is impaired
  - Rare form: peripheral thyroid hormone resistance – may have symptoms of hypothyroidism
- Management is complex

Table 1
Clinical features of thyroid hormone resistance

Findings	Frequency (%)
Goitre	86–95
Emotional disturbances	80
Recurrent ear and throat infections	50
Delayed bone age >2 SD below mean	29–47
Attention deficit hyperactivity disorder	40–60
Tachycardia	33–75
Hyperkinetic behaviour	33–68
Low body mass index (in children)	33
Learning disability	30
Short stature (<5th centile)	18–25
Hearing loss (sensorineural)	10–22
Mental retardation (IQ <70)	4–16

From Agrawal *et al* Postgrad Med J 2008 **84,** 473-477

Investigating inappropriate TSH



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