SCHOOL OF MEDICINE AND HEALTH SCIENCES
DIVISION OF BASIC MEDICAL SCIENCES
DISCIPLINE OF BIOCHEMISTRY AND MOLECULAR BIOLOGY
PBL SEMINAR MBBS III; BMLS & BDS Year 3

THYROID HORMONES: An Overview

What are the Thyroid Hormones?

- Thyroid Hormones are:
  - Thyroxine (T4, also called 3,5,3',5' – Tetra-Iodothyronine) and
  - Tri-Iodothyronine (T3 also called 3,5,3' – Tri-Iodothyronine)
  - T4 contains Four Iodine atoms while
  - T3 contains Three Iodine atoms.
  - T3 is the biological active form of the Thyroid hormone, because it is the hormone that binds to receptors and trigger the end-organ effects
  - Thyroid hormones are unique in that they require the trace element Iodine for Biological activity.
  - Biological inactive form of the thyroid hormone is called **Reverse T3** (rT3, 3,3',5'-Tri-Iodothyronine)

Outline the biosynthesis of the Thyroid Hormones (Fig. 2):

- Thyroid hormones are synthesized in the Thyroid gland
- The biosynthesis of Thyroid involves the Iodination and Coupling of Tyrosine molecules attached to a Complex Protein called **Thyroglobulin (TG)**.

Biosynthesis process can be separated into two stages as follows:

Stage one: Iodination reactions (or Organification):

- The process begins by Trapping of Iodide from plasma by the Thyroid gland
- The Iodination reactions (Organification) of the Tyrosine residues in Thyroglobulin are carried out by the enzyme Thyroid peroxidase, which catalyses the oxidation of Iodide (I^-) to Iodine using locally generated Hydrogen Peroxide ($H_2 O_2$).
- The Thyroid Peroxidase then uses the Iodine to Iodinate Tyrosine residues in Thyroglobulin forming 3-Monoiodotyrosine (MIT) residues.
- The 3-Monoiodotyrosine can then be Iodinated a Second time to form 3,5-Diiodotyrosine (DIT) in a reaction catalysed by Thyroid Peroxidase.
- Both MIT and DIT still remain attached to Thyroglobulin.

Stage two: Coupling reactions:

- At this stage of the process Thyroid Peroxidase cleaves off a molecule of MIT or DIT and Couples it to an Acceptor DIT molecule.
- Three combinations are possible:
  - DIT + DIT coupling gives T4;
  - MIT + DIT coupling gives T3
  - DIT + MIT coupling gives reverse T3 (which is not active)

- The major coupling process that occurs is the formation of T4.
- Finally, T4 and T3 are released into the plasma, (note that the thyroid gland secretes mostly T4 into the plasma).

How is T4 utilized in peripheral tissues?
(Production of T3 in peripheral tissues)

- T4 is a Pro-hormone and it is produced exclusively by the Thyroid gland.
- **The biologically active Thyroid hormone is T3**.
- The liver and kidneys have a lower affinity, higher capacity enzyme system (De-Iodinase) that catalyzes the de-iodination of T4.
- The Liver and Kidney, De-iodinate T4 to produce approximately two-thirds of the T3 present in plasma.
- The De-Iodinase enzyme that catalyzes the conversion of T4 to T3 requires the trace element Selenium, because it contains a specific Amino Acid called **Seleno-Cysteine**.
- There is also an enzyme called 5'-De-Iodinase that does not require Selenium. This enzyme catalyzes the conversion of T4 to Reverse T3
- Deficiency of the trace element Selenium can result in a decrease in the conversion of T4 to T3, thus causing at the same time an increase in the conversion of T4 to reverse T3 (rT3), by the enzyme 5'-Deiodinase that does not contain the amino acid Seleno-Cysteine.
- Other body cells are capable of taking up T4 and De-iodinating it to form the more biologically active T3.
- Alternatively, T4 is metabolised to Reverse T3 (rT3) that biologically inactive.
- By modulating the relative production of T3 and rT3, tissues can "Fine Tune" their local Thyroid Status.

What are some of the factors that can affect the conversion of T4 to T3?

- A number of factors can affect the conversion of T4 to T3 in body cells.
- Some of these factors act by decreasing the activity of the enzyme 5'- De-Iodinase, resulting in increased rT3/ T3 ratio, less T4 to T3 conversion.

Some of these factors include the following:

- Pregnancy or oral contraceptive pills,
- Fasting, Stress, High plasma Cortisol, Catabolic diseases, Hepatic and Renal diseases
- Thiouracil drugs (blocks thyroid peroxidase activity**)**

How are the Thyroid hormones transported in plasma?
(Thyroid Hormone Binding in Plasma):

Both T4 and T3 circulate in plasma bound to specific binding proteins:

- Thyroxine-Binding Globulin (TBG),
- Transthyretin (also called Thyroxine-binding pre-albumin or TBPA)
- Some of the Thyroid hormones are also bound to plasma Albumin.
- In the plasma TBG is quantitatively the most important binding protein for the Thyroid hormones.

- TBG binds about 70% of plasma T4 and about 80% plasma T3.
- Approximately 0.05% of plasma T4 and 0.2% of plasma T3 are Free (i.e., unbound to protein in plasma).
- TBG is synthesized in the Liver;
- Estrogens (pregnancy and birth control pills) increase the synthesis of TBG.

TAKE NOTE:

- Plasma contains both Bound and unbound (Free) Thyroid hormones
- It is the amount of **unbound or "Free" T4 and T3** (i.e., **FT4 and FT3**) that are important for the biological effects of the Thyroid hormones, including the feedback to the Pituitary and Hypothalamus.
- This is because only the Free Fractions can cross the cell membrane and affect intracellular metabolism.

How is the secretion of Thyroid hormones regulated?
(See Figs. 3 & 4):

Feedback regulation of Thyroid hormones occurs via the Hypothalamic-Pituitary-Thyroid axis (**HPT axis**).

- Hypothalamus secretes a tripeptide called Thyrotropin-Releasing Hormone (TRH, also called Thyroliberin).
- TRH then stimulates the Anterior Pituitary to synthesize and release a large Glycoprotein Hormone, called Thyroid-Stimulating Hormone (TSH, also called Thyrotropin).
- TSH then stimulates the Thyroid glands to produce the Thyroid hormones.
- Excess circulating unbound Thyroid hormones (FT4 and FT3) act via the long loop feedback mechanism to block the production of TSH and TRH.
- TSH can also act via the short loop feedback mechanism to block the production of TRH by the Hypothalamus.
- Knowledge of the regulation of Thyroid hormones via the feedback loops is essential for interpretation of results in the investigation of thyroid status.

TAKE NOTE:

It is import to note the following:

- If the Thyroid gland of a patient is producing too much Thyroid hormone, then the circulating TSH will be suppressed. (**Why?)**
- If the Thyroid gland of a patient is not secreting enough Thyroid hormone, the TSH level will be very high in an attempt to stimulate the Thyroid gland to secrete more Thyroid hormone.
- In **Non-Thyroidal illness** (NTI) a number of hormones and other agents have been shown to be responsible for inhibiting the release of TSH.

These include the following:

- Dopamine, Somatostatin, Glucocorticoids, Interleukins

What are some of the cellular actions of the Thyroid hormones?

- Plasma FT3 is the biologically active form of Thyroid hormone.
- After binding to high affinity binding sites on the membranes of target cells, the Thyroid hormones are actively transported into the cell by an ATP-dependent mechanism.
- In the cell, T3 acts mainly at the Nucleus, where it binds to specific receptors (Hormone Response Elements, HRE) that in turn activate T3-responsive Genes.
- These genes appear to exert a number of effects on cell metabolism, which include:
  - Stimulation of Basal Metabolic Rate
  - Metabolism of Lipids, Carbohydrates and Proteins
- Regulation of Gene Expression,
- Regulation of Tissue Differentiation and General Development, which are essential for the normal maturation and metabolism of all the tissues in the body.

- High Thyroid hormone concentration causes increased Metabolic State by:
  - Increasing the Mobilization of Endogenous Protein, Fat and Carbohydrate for the production of substrates needed for Energy Production
- The effects of Thyroid hormones on tissue maturation are most dramatically seen in Congenital Hypothyroidism, a condition, which unless treated within a short time after birth, may results in permanent brain damage.
- Hypothyroid children have delayed skeletal maturation, short stature and delayed puberty.
- An example of the effect of Thyroid hormones on lipid metabolism is the observation of High Serum Cholesterol in some Hypothyroid Patients.
  - This is a consequence of the reduction in cholesterol metabolism, due to down regulation of Low-Density Lipoprotein (LDL) receptors on Liver cell membranes, with a subsequent failure of Sterol excretion via the gut.

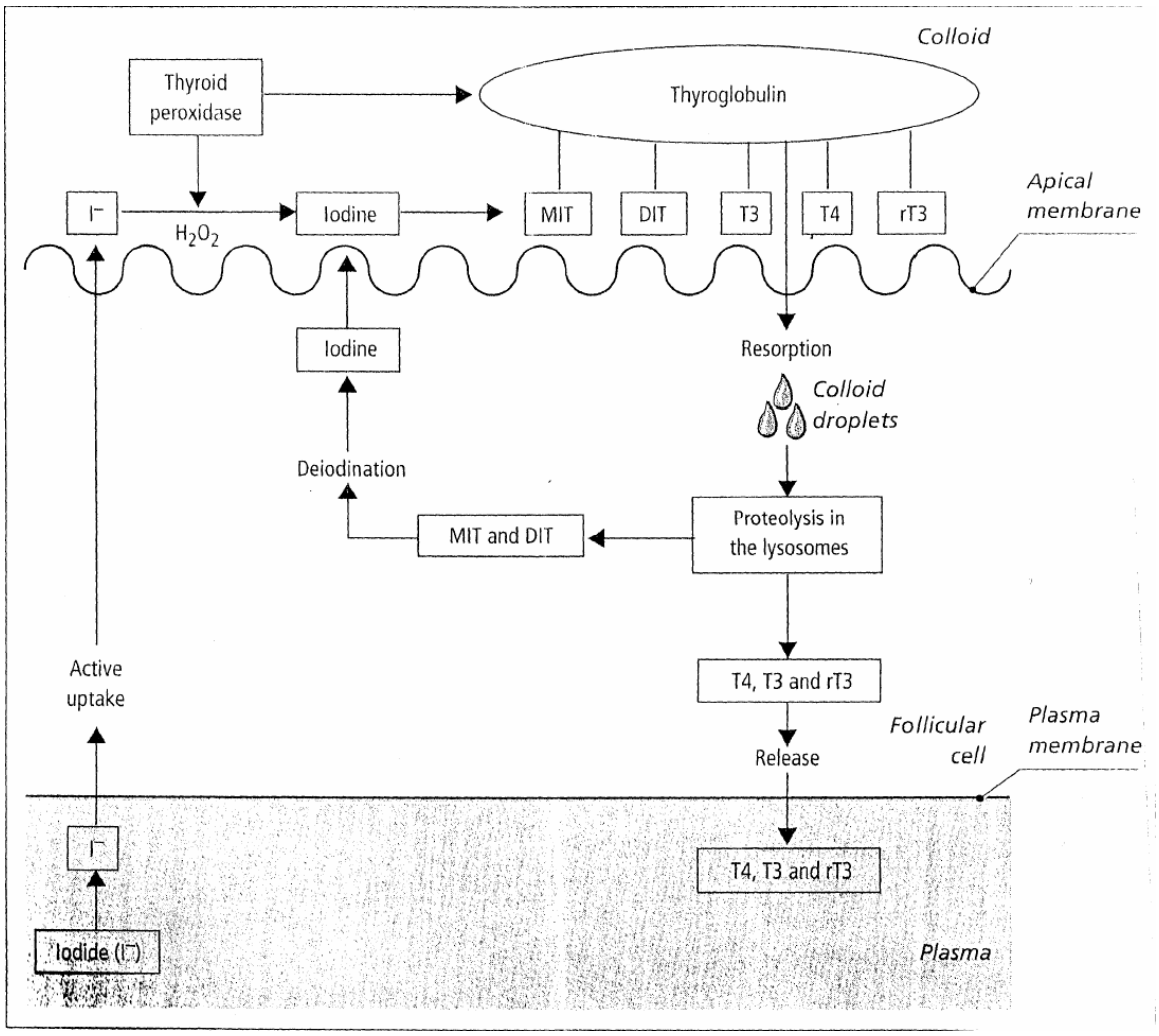
Briefly state the actions of Thyroid hormones

- Thyroid hormones:
  - Increase Basal Metabolic Rate (BMR)
  - Increase Oxygen consumption
  - Increase Thermogenesis (heat production in the body)
  - Activate Na^+-K^+-ATPase in cells
  - Increase number of Mitochondria in cells
  - Increase mobilization of endogenous Carbohydrate, Fat and Protein to produce substrates for energy metabolism
    - Increase Glycolysis, Glycogenolysis, Gluconeogenesis,
    - Increase Lipolysis and Protein degradation

  - Decrease Muscle mass
  - Decrease Adipose Tissue

  - Increase Beta-Adrenergic receptors, which leads to increase Cardiac Output
  - Increase Systolic blood pressure only
  - Increase Ventilation Rate
  - Required for maturation of Ovary and Testis

- Required for Actions of Growth Hormone (GH) to promote linear growth / bone formation
- Required for development of the CNS in the Foetus



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THYROID FUNCTION TEST – An Overview

How can Thyroid function be investigated?

- Test for investigation of Thyroid dysfunction can be separated into Two categories:
  - Groups of Tests to established Thyroid status:
    - Measurement of [TSH] in Plasma or Serum
    - Measurements of [Thyroid Hormones] {T4 and T3} in Plasma or Serum

- Groups of Test to elucidate cause of Thyroid dysfunction:
  - Thyroid Auto-antibody, Serum [Thyroglobulin], Thyroid Peroxidase, Biopsy of the Thyroid, Ultrasound and Isotopic Thyroid Scanning

TAKE NOTE:
- Thyroid status MUST be determined before tests to elucidate cause of dysfunction

What are the tests used to determine Thyroid status?

- **Thyroid-Stimulating Hormone (TSH)**:
  - Single most sensitive, specific and reliable test of Thyroid status in both overt and subclinical thyroid disease,
  - Can be used to diagnose Primary Hypothyroidism and to differentiate it from Secondary Hypothyroidism

- **Thyroid-Releasing Hormone (TRH)**:
  - Test assists in evaluation of patients with Hyperthyroidism and Hypothyroidism
  - Especially helpful in differential diagnosis of Hypothyroidism

- **Thyroid-Binding Globulin (TBG)**:
  - Measurement of TBG, the major thyroid hormone protein carrier
  - Used in evaluation of patients who have abnormal Total T4 and T3 levels
  - Can be done concurrently with Total T4 and Total T3 test, for proper interpretation of Total T4 and Total T3

- **Total Thyroxine (Total T4)**:
  - Used in assessing Thyroid Function
  - Used to monitor Replacement and Suppressive Therapy

- **Total Triiodothyronine (Total T3)**:
  - Used to evaluate Thyroid Function
  - Mainly used to diagnose Hyperthyroidism
  - Used to monitor Thyroid Replacement and Suppressive therapy

- **Free Thyroxine (FT4)**:
    - Used to evaluate Thyroid Function
    - Used to diagnose Hyperthyroidism and Hypothyroidism

- **Free Triiodothyronine (F T3)**:
    - Used to diagnose Thyroid Function and to monitor replacement and suppressive therapy

How significant is TSH test (TSH, Thyrotropin)?

{**Reference range: 0.4 to 4.5mU/L**}

- TSH release is very sensitive to alterations in plasma [Thyroid Hormones]
- **Decrease** in Plasma [Thyroid Hormones] causes **Increase** secretion of TSH
- **Increase** in Plasma [Thyroid hormones] suppresses secretion of TSH
    - **Feedback control mechanism in HPT axis**
- Measurement of Plasma [TSH] in basal blood sample by Immuno-metric Assay provides one of the single most sensitive, specific and reliable test of Thyroid status in both Overt and Subclinical Thyroid Disease
- In Primary Hypothyroidism, Plasma [TSH] is increased above Normal reference range {**Why?}**
- In Primary Hyperthyroidism (e.g., Thyrotoxicosis) Plasma [TSH] is reduced below Normal reference range {**Why?}**

Take note of following questions and answers

- **Thyrotoxicosis {Low [TSH], Why?}**:
    - Thyroid automatically manufactures too much T4 and T3, which suppresses production of TSH via Feedback Mechanism
- When laboratory result shows raised TSH level, then FT4 should be measured
- When laboratory result shows a suppressed TSH level then FT4 and FT3 should be measured

    - **Why should both FT4 and FT3 be measured in the second case**?
        - In some patients Thyroid over secrete only T3, a condition called **T3 Toxicosis** and both hormones need to be measured to detect this form of Thyrotoxicosis
        - Condition is usually seen in patients who previously had Thyroidectomy or had been treated with Radioactive Iodine for Thyrotoxicosis in the past
    - **Exceptions**: both raised and undetected plasma TSH concentration may be seen in some Euthyroid patients

How are the results of TSH tests interpreted?

- High sensitivity TSH assay measures the [TSH] in Serum
- "Healthy" individuals: Serum [TSH] is usually between 0.4 and 4.5m U/L
- TSH is under:
    - Negative Feedback Control of FT4 and FT3 in circulation
    - Positive Control of TRH in Hypothalamic

- Thyroid hormone deficiency should cause elevated Serum [TSH]
- Serum [TSH] greater than 20m U/L is a good indicator of Primary Failure of Thyroid Gland

- Serum [TSH] between 4.5 and 15m U/L is borderline thyroid dysfunction, which requires more careful evaluation
- In **Secondary Hypothyroid** status, [TSH] may be low, normal or occasionally borderline range
- Serum [TSH] above 15m U/L is very good evidence for Primary Hypothyroidism
- Serum [TSH] below 5 is very good evidence against Primary Hypothyroidism
- Presence of Low [FT4] with [TSH] less than 10m U/L strongly suggests a Secondary Hypothyroidism
- High [FT4] and [FT3] will suppress TSH levels, in almost all case of Hyperthyroidism, thus, [TSH] is usually below 0.3m U/L and may be less than 0.1m U/L

Interpreting the use of Serum [TSH] for monitoring

- Serum [TSH] can be effectively used to follow patients being treated with Thyroid Hormone
- High [TSH] indicates under-treatment,
- Low [TSH] usually indicates over-treatment
    - Abnormal [TSH] should be interpreted with [FT4] or [FT3] before modifying therapy because Serum Thyroid Hormone levels change more quickly than TSH levels
    - Patients who recently started using Thyroid Hormone, or who have been non-compliant until shortly before a visit to the doctor may have normal [FT4] and [FT3], though their TSH levels are still elevated
- Serum [TSH] may be affected by acute illness and several medications, including Dopamine and Glucocorticoids (Non-Thyroidal Illness, NTI)

TAKE NOTE:

- Serum [TSH] and [FT4] are frequently measured to differentiate between Secondary and Primary Thyroid dysfunction
    - Decrease [FT4] and Normal or Elevated [TSH] indicates Primary Thyroid disorder {**Why?**}
    - Decrease [FT4] with a decreased [TSH] indicates Secondary Thyroid disorder {**Why?**}

What is the Thyroxine Binding Globulin (TBG) test?

- Determination of Plasma [TBG]
- Determination of Plasma [Total T4]
- Determination of Plasma [FT4]
- Used in evaluation of patients with abnormal Plasma [Total T4]
- Conditions that causes increase in Plasma [TBG] include:
    - Pregnancy, Hormone Replacement Therapy, Oral Contraceptives, Infections, and Hepatitis,
- Conditions that causes decrease in Plasma [TBG]:
    - Hypoproteinemia, Nephrotic syndrome, and Malnutrition

Fig. 1: Strategy for investigation of Low TSH

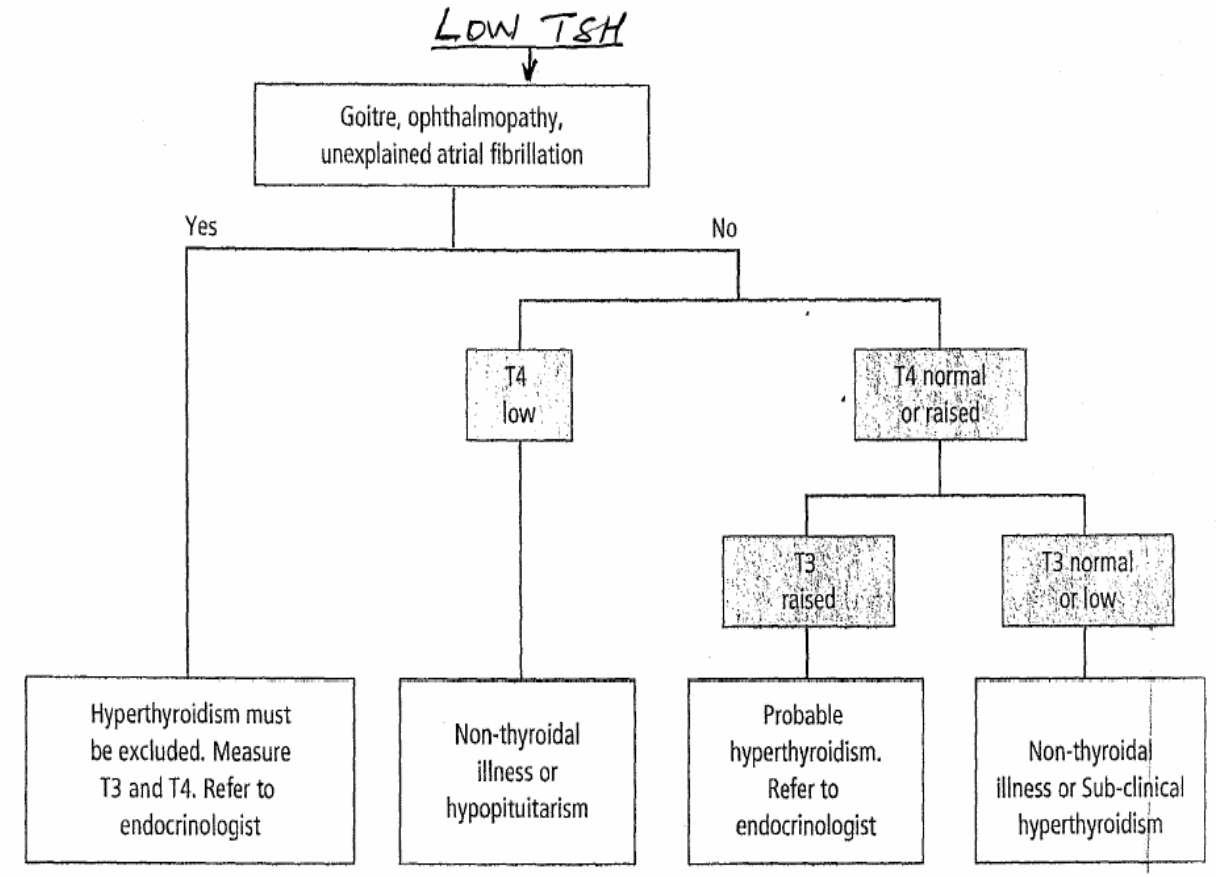
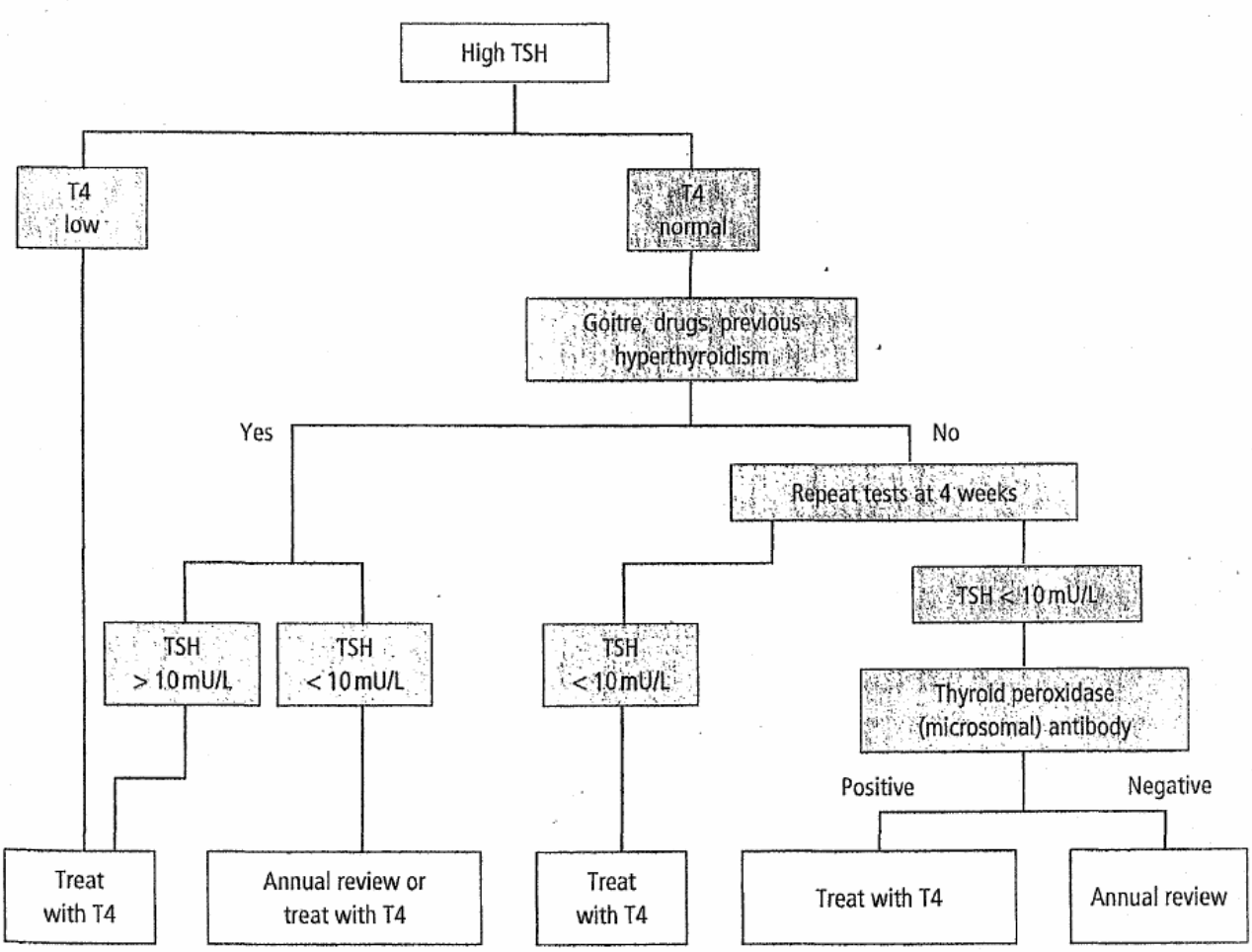
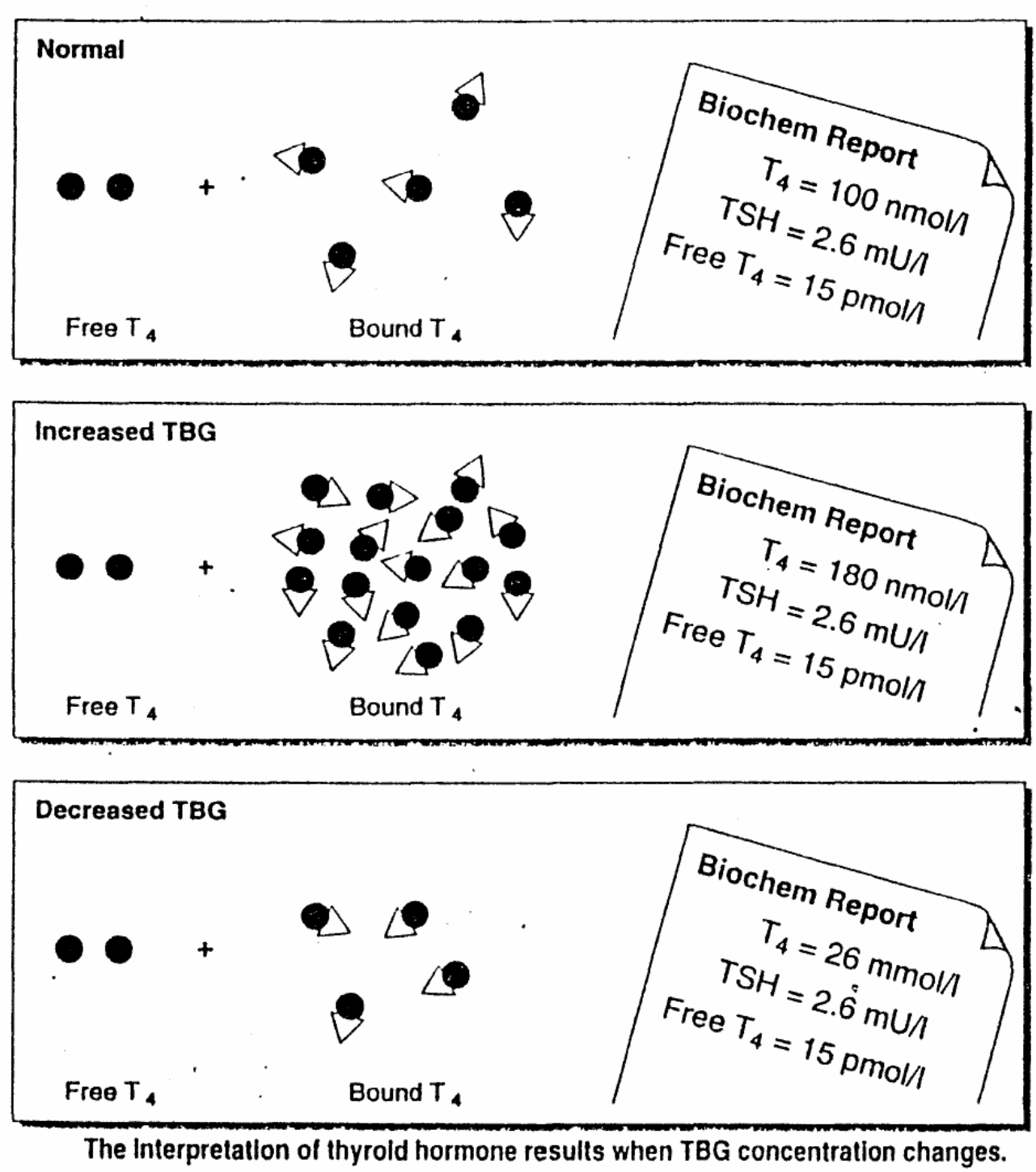


Fig. 2: Strategy for investigating High TSH



- Plasma [FT4] and [FT3] are independent of changes in Plasma [TBG]
- Provide a more reliable means of diagnosing Thyroid Dysfunction than measurement of Plasma [Total T4] alone **(Fig. 3)**

Fig. 3: TBG Test and Interpretation of results



The Interpretation of thyroid hormone results when TBG concentration changes.

How significant is the Thyroxine (T4) screen?

- Plasma [FT4] (**Reference range: 10 to 27 pmol/L**)
- Plasma [Total T4] (Reference range: 70 to 150 nmol/L)
- Plasma [Total T4] or [**FT4**] can be determined by several methods:

    - Radioimmunoassay (RIA),
    - Enzyme-Linked Immunosorbent Assay (ELISA),
    - Enzyme Immunoassay (EIA),
    - Microplate Enzyme Immunoassay (MEIA)

- **All laboratories should be encouraged to measure Plasma or Serum [FT4]**

What factors can affect Interpretation of Plasma [Total T4] results?

- Some laboratories still measure Plasma [Total T4],
- **Results of Plasma [Total T4] depends on Plasma [TBG], thus results should be interpreted with care**
- Plasma [TBG] may be Low in some patients with Inherited but harmless deficiency
    - Plasma [Total T4] is Low in these patients, but [FT4] may be Normal

- Plasma [TBG] may be elevated in Pregnant women and in Women using Oestrogen-containing Oral Contraceptive Pill,
    - Plasma [Total T4] may be elevated well above the Reference range, but [FT4] may be normal
- Determination of Plasma [FT4] is recommended in conditions where [TBG] may be altered, e.g., Pregnancy, users of Oral Contraceptive Pill and patients with Nephrotic Syndrome

How significant is the Tri-Iodothyronine (T3) test?

- Plasma [**FT3**] (Reference range: 3 to 9 pmol/L)
- Plasma [Total T3] (Reference range: 1.2 to 2.8 nmol/L)
- Test is primarily used to diagnose Hyperthyroidism and to monitor thyroid replacement and suppressive therapy
- Plasma [Total T3] or [**FT43**] can be determined by several methods:

    - Radioimmunoassay (RIA),
    - Enzyme-Linked Immunosorbent Assay (ELISA),
    - Enzyme Immunoassay (EIA),
    - Microplate Enzyme Immunoassay (MEIA)

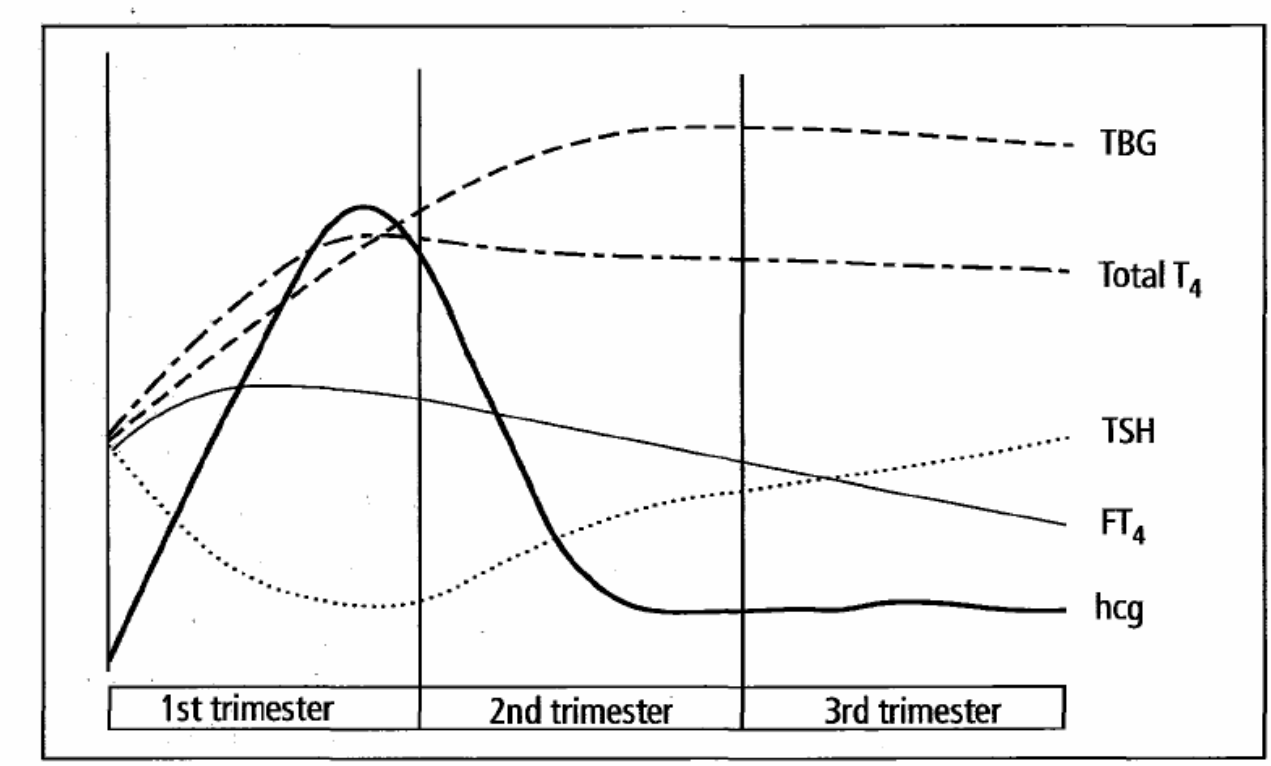
- FT3 comprises about 0.3% of Total circulating T3 in blood
- Gradually laboratories are moving over to FT3 measurements as more reliable FT3 assays become available

TAKE NOTE:

- Conversion of T4 to T3 depends on a number of situations such as Chronic illness or Surgical stress, which cause a fall in T4 to T3 conversion (called low T3 syndrome)
- Starvation can alter T4 to T3 conversion with a fall in T3 as the body tries to reduce its metabolism to conserve energy
- Plasma [Total T3] provides a useful test for Hyperthyroidism, as values are often raised proportionately more than Plasma [Total T4]
- Determination of Plasma [Total T3] is of no value in investigating patients with suspected Hypothyroidism, as normal results are often found

How reliable is Thyroid function test for assessing Thyroid status during Pregnancy?

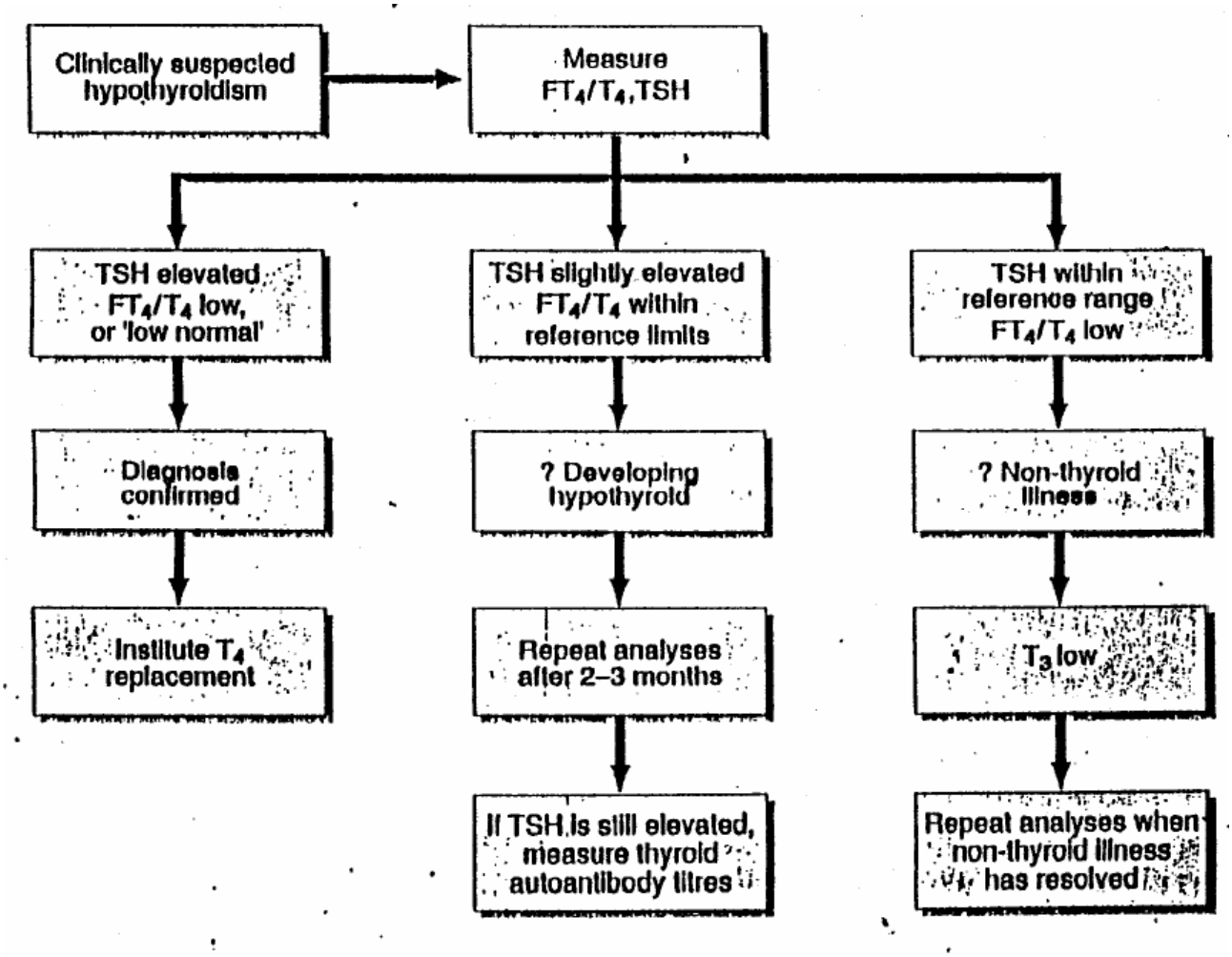
- Plasma [TSH] is reliable indicator of Thyroid status in 2nd and 3rd Trimester of pregnancy
- Plasma [TSH] is not a reliable indicator during 1st Trimester of pregnancy (**Why?**)
- Plasma [TSH] is usually low, may be due to weak Thyrotrophic effect of Placental hCG (Human Chorionic Gonadotropin), which is high during 1st Trimester
- Plasma [Free Thyroid hormone] increases during 1st Trimester, then decline later
- Plasma [TBG] increase during pregnancy, causing elevated in Plasma [Total T4] and [Total T3]



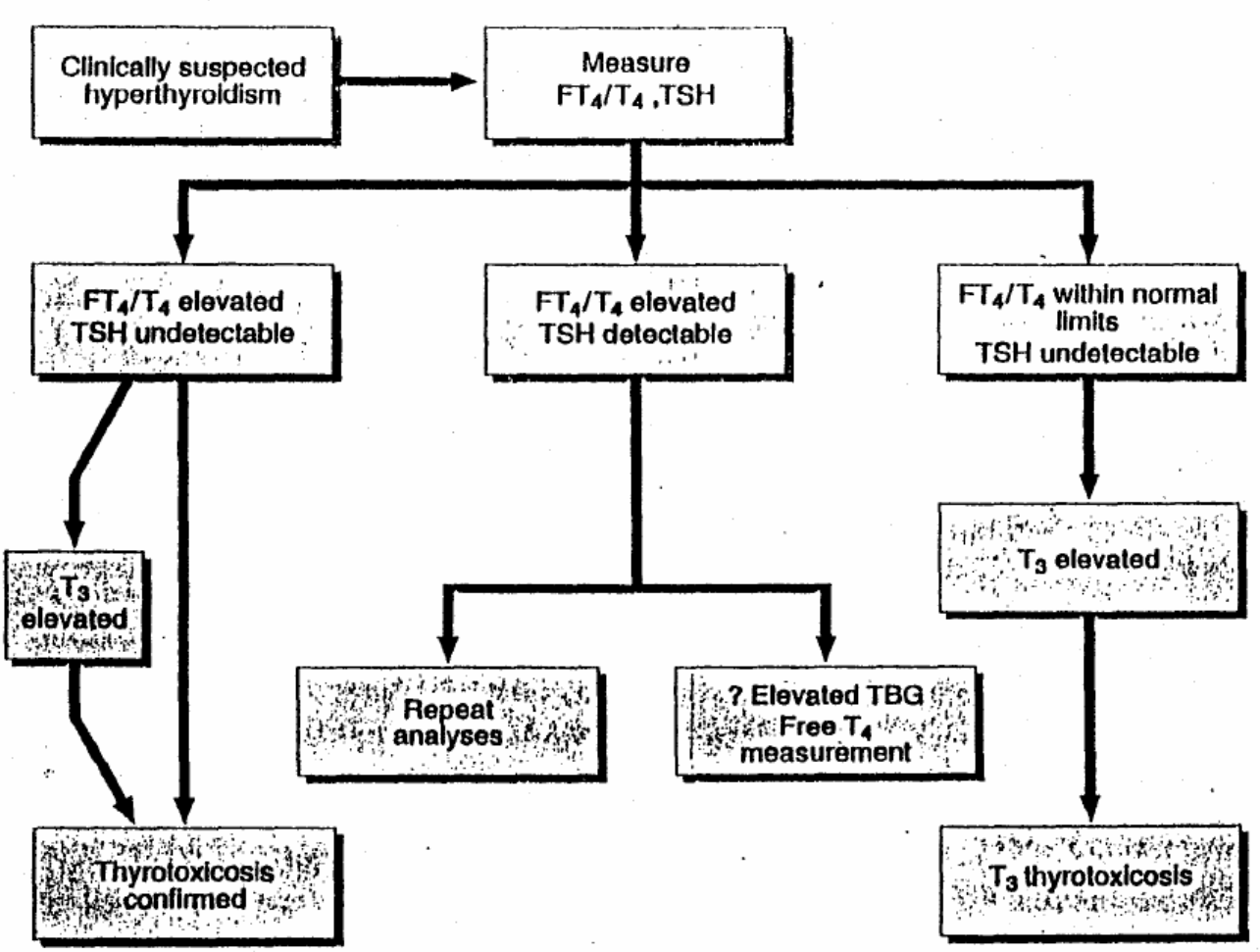
In Summary:

- Plasma [TSH] is the single best test for assessing Thyroid Status
  - Plasma [TSH] is elevated in Primary Hypothyroidism
  - Plasma [TSH] is suppressed in Primary Hyperthyroidism
  - Normal Plasma [TSH] usually excludes Primary Thyroid Disorder

- Plasma [FT4] and [TSH] can be used to assess severity of Thyroid disease and distinguish Subclinical from Overt disease
- Plasma [FT3] and [TSH] can be used to determine severity of Hyperthyroidism and to identify patients with T3 Hyperthyroidism
- Plasma [Free Thyroid Hormones] correlates more closely with Thyroid Status than Plasma [Total Thyroid hormones], which are heavily influenced by changes in Plasma [TBG]
- Thyroid Function Tests (TFT) are often abnormal in patients with Non-Thyroidal Illness (NTI), and should not be requested in hospitalised patients unless the presenting complaint is due to Thyroid Disease



Strategy for the biochemical investigation of suspected hypothyroidism.



Strategy for the biochemical investigation of suspected hyperthyroidism:

Major References for further reading:

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3. V. L. Davidson and D. B. Sittman, Biochemistry Clinical Cases and Correlations 3rd Edition; Harwal Publishing