THYROID HORMONES & THYROID FUNCTION TESTS

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What are the Thyroid Hormones?

- **Thyroid Hormones are**:
  - **Thyroxine** : {3,5,3',5' – Tetra-Iodothyronine} (**T4**)
  - **Tri-Iodothyronine:** {3,5,3' – Tri-Iodothyronine} (**T3**)
- **T4** contains Four Iodine atoms,
- **T3** contains Three Iodine atoms,
- T3: Biological active form of Thyroid hormones, because it binds to receptors and trigger end-organ effects;
- Thyroid hormones are unique because they contain the trace element **Iodine** for Biological activity;
- **Reverse T3:** {3,3',5'-Tri-Iodothyronine} (**rT3**):
  - Is the **Biological Inactive** form of Thyroid hormones;

How are Thyroid Hormones biosynthesized?

- Biosynthesis of Thyroid hormones occurs in the Thyroid gland (**Fig. 1**);
- Process involves:
  - Trapping of Iodide ($\mathbf{I^-}$),
  - Iodination (Organification) of Tyrosine residues to form MIT & DIT on **Thyroglobulin** (**TG**),
  - Coupling of DIT and MIT on TG to form Thyroid hormones;
- **The process can be separated into Two Major Stages;**

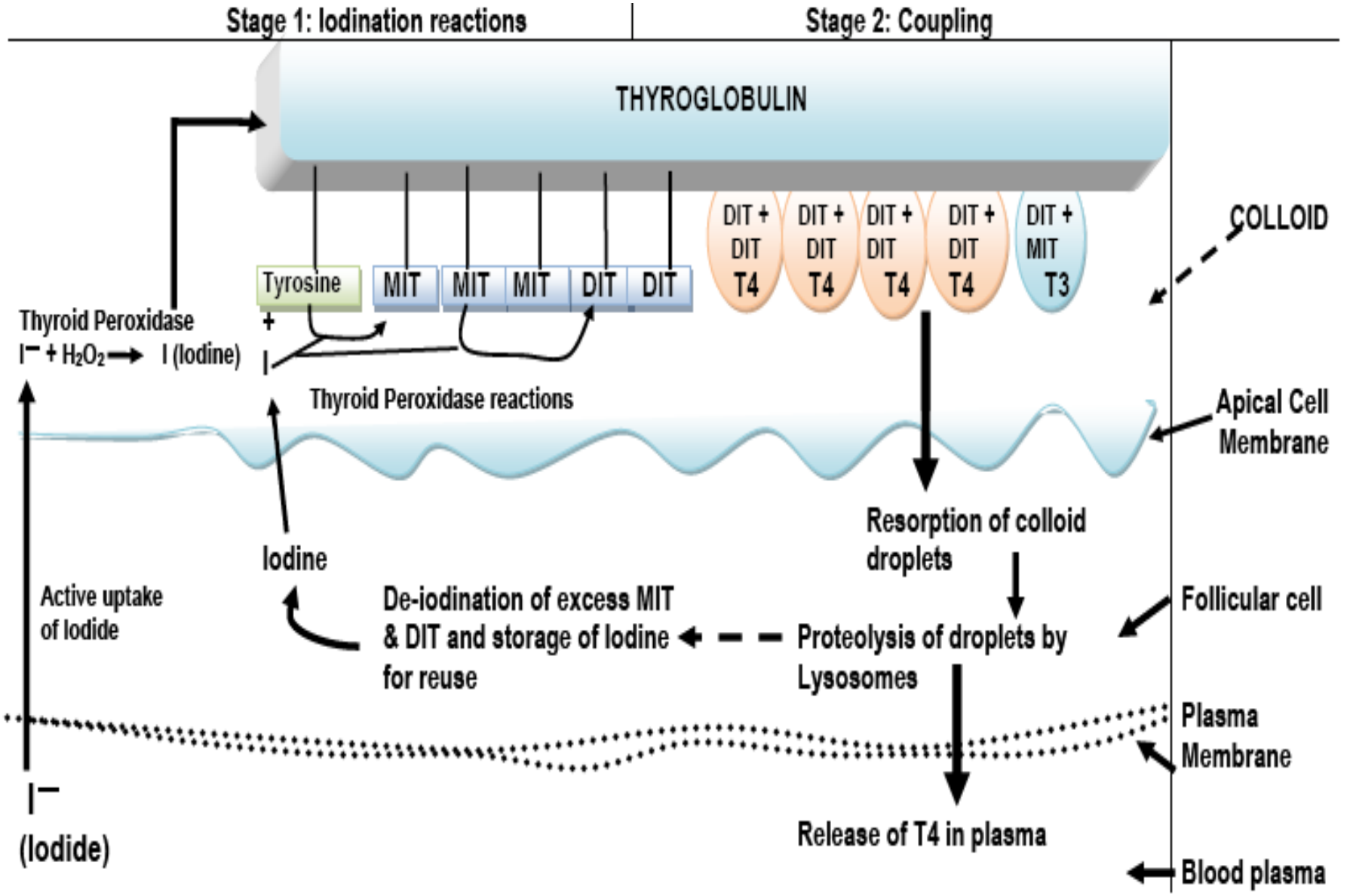
Stage One: Iodination Reactions (or Organification):

- Trapping of **Iodide** from plasma by Thyroid gland,
- Oxidation of Iodide ($\mathbf{I^-}$) to Iodine (**I**) by **Thyroid Peroxidase** using Hydrogen Peroxide ($H_2 O_2$),
- Thyroid Peroxidase then uses Iodine to Iodinate Tyrosine residues attached to Thyroglobulin (TG), forming **3-Monoiodotyrosine** (**MIT**) residues,
- Thyroid Peroxidase iodinate MIT residues **Second time** to form **3,5-Diiodotyrosine (DIT)**;
- Both **MIT** and **DIT** still remain attached to TG;

Stage Two: Coupling Reactions:

- Thyroid Peroxidase cleaves off **MIT** or **DIT** and **Couples** it to **Acceptor DIT residues on TG,**
- Three combinations can occur:
  - **DIT + DIT** coupling gives **T4**,
  - **MIT + DIT** coupling gives **T3,**
  - **DIT + MIT** gives **r T3** (inactive hormone),
- **Major coupling reaction is formation of T4**,
- Finally, T4 and T3 are released into plasma,
- Thyroid gland secretes mostly T4 into plasma;
- **Fig. 1: Diagram of biosynthesis of Thyroid Hormones**

Fig. 1: Schematic diagram: Biosynthesis of Thyroid Hormones



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

- **T4:** Pro-hormone produced by Thyroid gland,
- **Biologically active Thyroid hormone is T3**,
- Liver and Kidneys have **De-Iodinase** that De-iodinate **T4** to produce about two-thirds of **T3** in plasma,
- **De-Iodinase** that catalyses conversion of **T4 to T3** requires trace element **Selenium**, because it contains a specific Amino Acid called "**Seleno-Cysteine"**,
- **5'-De-Iodinase** that **does not** require **Selenium**, catalyses the conversion of **T4 to Reverse T3,**

- Deficiency of **Selenium** causes **decrease** in conversion of **T4 to T3**, resulting at the same time in increased conversion of **T4 to reverse T3** (rT3) by **5'-Deiodinase** that **does not** contain **Seleno-Cysteine**,
- Other body cells containing Deiodinase can convert T4 to T3,
- Alternatively, T4 can be metabolised to Reverse T3 (rT3), which is biologically inactive,
- By modulating relative production of T3 and rT3, tissues can "**Fine Tune**" their local Thyroid Status,

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

- Several factors affect conversion of T4 to T3 in cells,
- Some factors decrease activity of De-Iodinase, thus increasing rT3/ T3 ratio, less T4 to T3 conversion,
- Other factors that affect T4 to T3 conversion include:
  - Pregnancy or oral contraceptive pills,
  - Fasting,
  - Stress,
  - High plasma Cortisol,
  - Catabolic diseases,
  - Hepatic and Renal diseases,
  - Thiouracil drugs (inhibits Thyroid Peroxidase activity)

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

- T4 & T3 are bound to specific plasma proteins:
  - Thyroxin-Binding Globulin (TBG),
  - Transthyretin (Thyroxin-binding pre-albumin or TBPA),
  - Plasma Albumin,
- TBG: important binding protein for Thyroid hormones,
- TBG is synthesized in the Liver;
- TBG binds about 70% of T4 and about 80% of T3,
- About 0.05% of T4 and 0.2% of T3 are Free in plasma (i.e., unbound to protein in plasma),
- Estrogens (pregnancy and birth control pills) increase the biosynthesis of TBG,

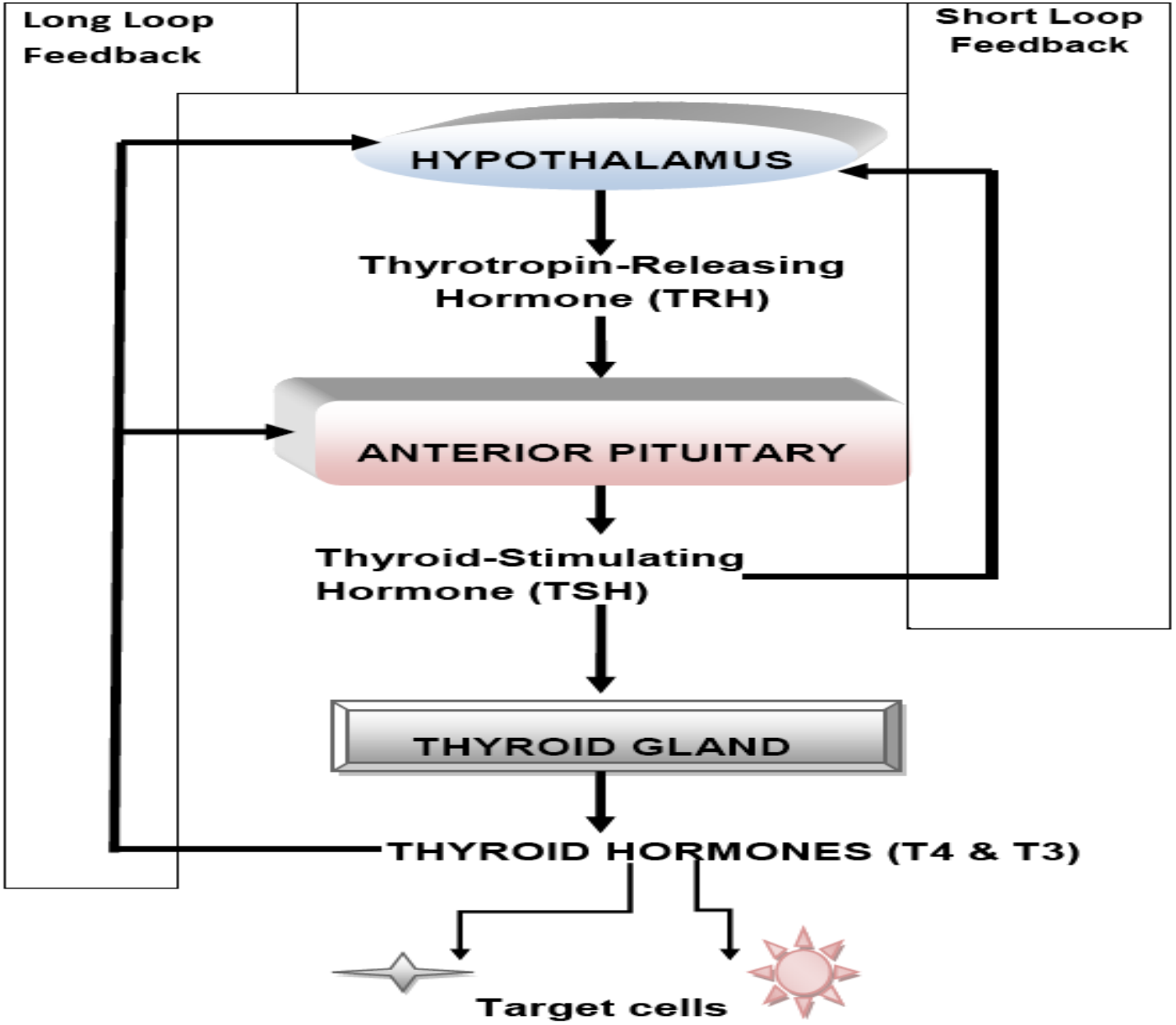
IMPORTANT TO NOTE

- Plasma contains both Bound and unbound (Free) Thyroid hormones,
- Amount of **unbound or "Free" T4 and T3** (**FT4 and FT3**) are important for biological effects of Thyroid hormones, including feedback control to the Anterior Pituitary and Hypothalamus, (**Why?**)
  - Because only the Free Fractions can cross the cell membrane and affect intracellular metabolism;

How is the secretion of Thyroid hormones regulated?

- Feedback regulation of Thyroid hormones occurs via the **Hypothalamic-Pituitary-Thyroid axis** (**HPT axis**), {**Fig. 2**}
- Hypothalamus secretes Thyrotropin-Releasing Hormone (TRH),
- TRH stimulates Anterior Pituitary to synthesize and release Thyroid-Stimulating Hormone (TSH),
- TSH stimulates Thyroid glands to produce T4 and T3,
- Excess FT4 and FT3 act via long loop feedback to block production of TSH and TRH,
- TSH blocks TRH production via short loop feedback,
- Knowledge of feedback regulation of HPT axis is essential for interpretation of results in investigation of thyroid status,

Fig. 2: Negative Feedback regulation of HPT-axis



IMPORTANT TO NOTE

- If Thyroid gland of a patient is producing too much Thyroid hormones, then the circulating TSH will be suppressed (**Why?);**

- If 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;
- **Non-Thyroidal illness** (**NTI**): a number of hormones and other agents inhibit the release of TSH;
- These include the following:
  - Dopamine, Somatostatin, Glucocorticoids, Interleukins

What are some cellular actions of Thyroid hormones?

- FT3 binds to high affinity receptors on membranes of target cells, and are actively transported into cells by ATP-dependent mechanism;
- In cells, FT3 enters Nucleus, binds to Hormone Response Elements (HRE) in DNA, which then cause activation of T3-responsive Genes;
- These genes 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,
- General Development, which are essential for the normal maturation and metabolism of all tissues,
- High plasma Thyroid hormone levels may cause increased Metabolic State by:
  - Increasing Mobilization of Endogenous Protein, Fat and Carbohydrate for production of substrates needed for Energy Production,

- Effects of Thyroid hormones on tissue maturation are 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,
- Example of the effect of Thyroid hormones on lipid metabolism is High Serum Cholesterol in some Hypothyroid Patients,
  - Due to reduction in cholesterol metabolism, caused by down regulation of LDL receptors on Liver cells with subsequent failure of Sterol excretion via GIT,

Summary of the actions of Thyroid Hormones on whole body metabolism

- 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 as 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 CNS in Foetus,

THYROID FUNCTION TESTS

- **How can Thyroid function be investigated?**
- Tests for investigation of Thyroid dysfunction can be separated into Two categories:
- Tests to established Thyroid status:
  - Measurement of **[TSH]** in Plasma or Serum,
  - Measurements of **[Thyroid Hormones]** {T4 and T3} in Plasma or Serum;

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

- **IMPORTANT TO NOTE**:
- Thyroid status MUST be determined before using tests to elucidate cause of dysfunction;

What tests are 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 dysfunction,
  - Can be used to diagnose Primary Hypothyroidism,
  - Can be used to differentiate Primary from Secondary Hypothyroidism,

- **Thyroid-Releasing Hormone (TRH)**:
  - Test to evaluate patients with Hyperthyroidism and Hypothyroidism;
  - Helpful in differential diagnosis of Hypothyroidism;

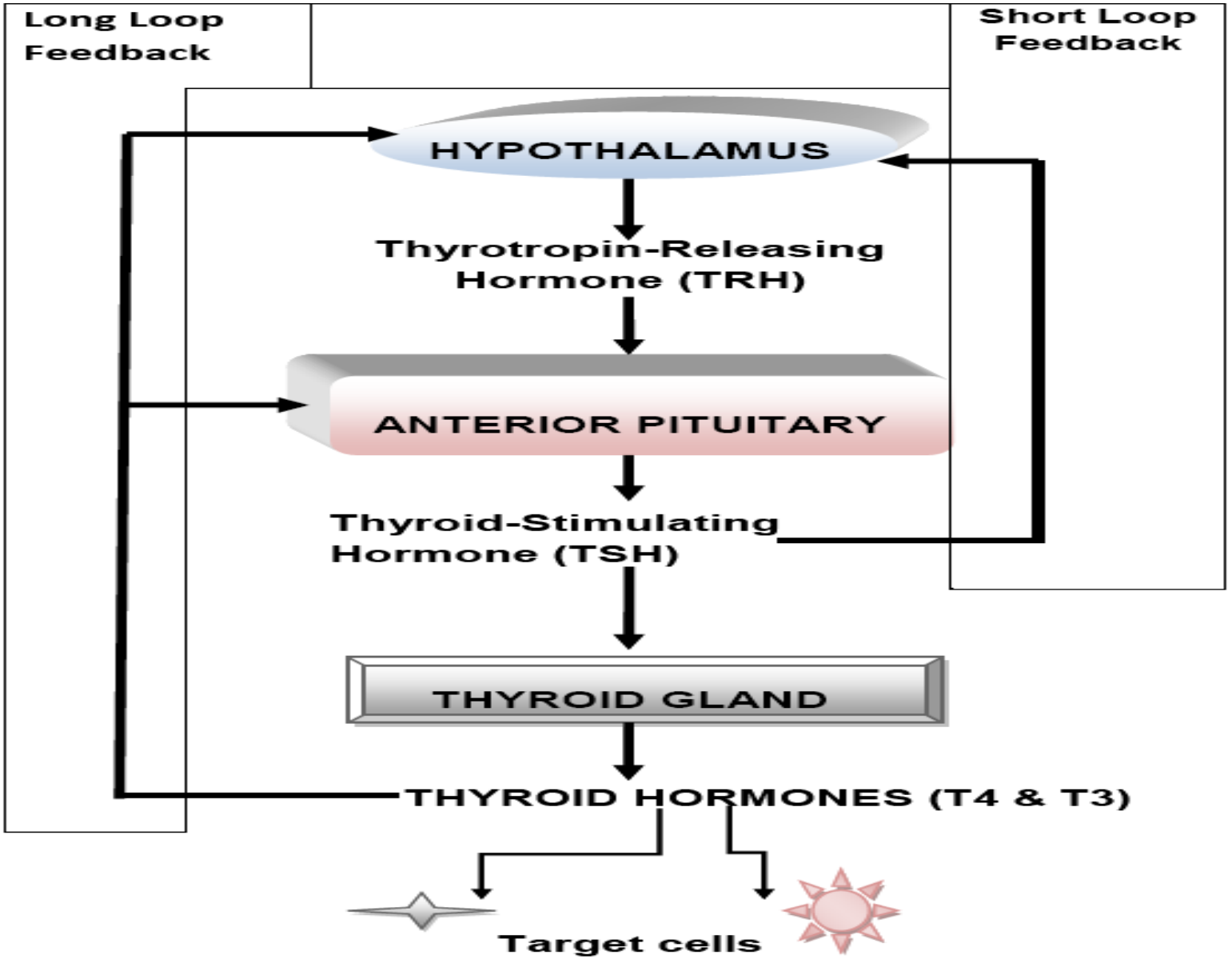
- **Free Thyroxine (FT4)**:
  - Used to evaluate Thyroid Function,
  - Used to diagnose Hyperthyroidism or Hypothyroidism,
- **Free Triiodothyronine (F T3)**:
  - Used to diagnose Thyroid Function,
  - Used to monitor replacement and suppressive thyroid therapy;

- **Thyroid-Binding Globulin (TBG):**
  - Plasma [TBG], major carrier protein of thyroid hormones,
  - Used to evaluate patients with abnormal Total [T4] or [T3],
  - Must be done with Total [T4] and [T3], for interpretation;
- **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 Replacement and Suppressive therapy,

How significant is plasma TSH test (TSH, Thyrotropin)?

- Reference range of [TSH]: 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 (**Fig. 1**)

Fig. 1: HPT-axis



- Measurement of [TSH] in basal blood sample provides one of the single most sensitive, specific and reliable test of Thyroid status in both Overt & Subclinical Thyroid dysfunction;
  - 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?**),

- In Thyrotoxicosis plasma [TSH] is low; **Why?**
  - Thyroid produces too much T4 and T3, which then suppresses release of TSH via Negative Feedback control of HPT-axis;

- **TAKE NOTE**:

1. When lab result shows raised plasma [TSH], then plasma FT4 should be measured;
2. When lab result shows low plasma [TSH], then both plasma FT4 and FT3 should be measured;

Why should FT4 & FT3 be measured in the second case?

- Because the Thyroid gland over secretes only T3, in patients with **T3 Toxicosis,** thus both FT4 & FT3 should be measured to diagnose this form of Thyrotoxicosis;
  - Such condition occurs 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] may be seen in some Euthyroid patients;

How are results of plasma or serum [TSH] tests interpreted?

- Use High Sensitivity TSH Assay to determine [TSH];
  - Normal Range: Plasma [TSH] is 0.4 to 4.5mIU/L,
- **TSH is under**:
  - Negative Feedback Control of plasma FT4 & FT3,
  - Positive Control of TRH from Hypothalamus;
- Deficiency of FT4 or FT3: Plasma [TSH] increases;
- **Plasma [TSH] greater than 20mIU/L** is good indicator of **Primary Thyroid Failure**;
- Plasma [TSH] between 4.5 and 15mIU/L is borderline thyroid dysfunction, it requires careful evaluation;

- In **Secondary Hypothyroid** status, plasma [TSH] may be low, normal or borderline range;
- Plasma [TSH] above 15mIU/L is good evidence for Primary Hypothyroidism;
- Plasma [TSH] below 5 is very good evidence against Primary Hypothyroidism;
- Presence of Low [FT4] with [TSH] less than 10mUI/L strongly suggests Secondary Hypothyroidism;
- High plasma [FT4] and [FT3] suppresses plasma [TSH] level, in almost all case of Hyperthyroidism, thus, [TSH] is falls below 0.3mUI/L or less than 0.1mIU/L,

Interpreting the use of plasma [TSH] for monitoring

- Plasma [TSH] can be used to follow patients being treated with Thyroid Hormones;
  - High plasma [TSH] indicates under-treatment,
  - Low plasma [TSH] usually indicates over-treatment,
- Abnormal [TSH] should be interpreted with [FT4] or [FT3] before modifying therapy, because plasma [Thyroid Hormones] changes faster than [TSH],
- Patients recently started using Thyroid Hormone, or who are non-compliant until shortly before a visit to the doctor may have normal [FT4] and [FT3], though their [TSH] may still be elevated;

- Plasma [TSH] may be affected by acute illness and several medications, including Dopamine and Glucocorticoids (Non-Thyroidal Illness, NTI);
- **TAKE NOTE**:
- Plasma [TSH] & [FT4] are used to differentiate Secondary and Primary Thyroid dysfunctions;
  - Decrease [FT4] and Normal or Elevated [TSH] may indicate Primary Thyroid disorder; **Why?**
  - Decrease [FT4] with decreased [TSH] indicates Secondary Thyroid disorder; **Why?**
    - **Always refer to HPT-axis for answers!!**

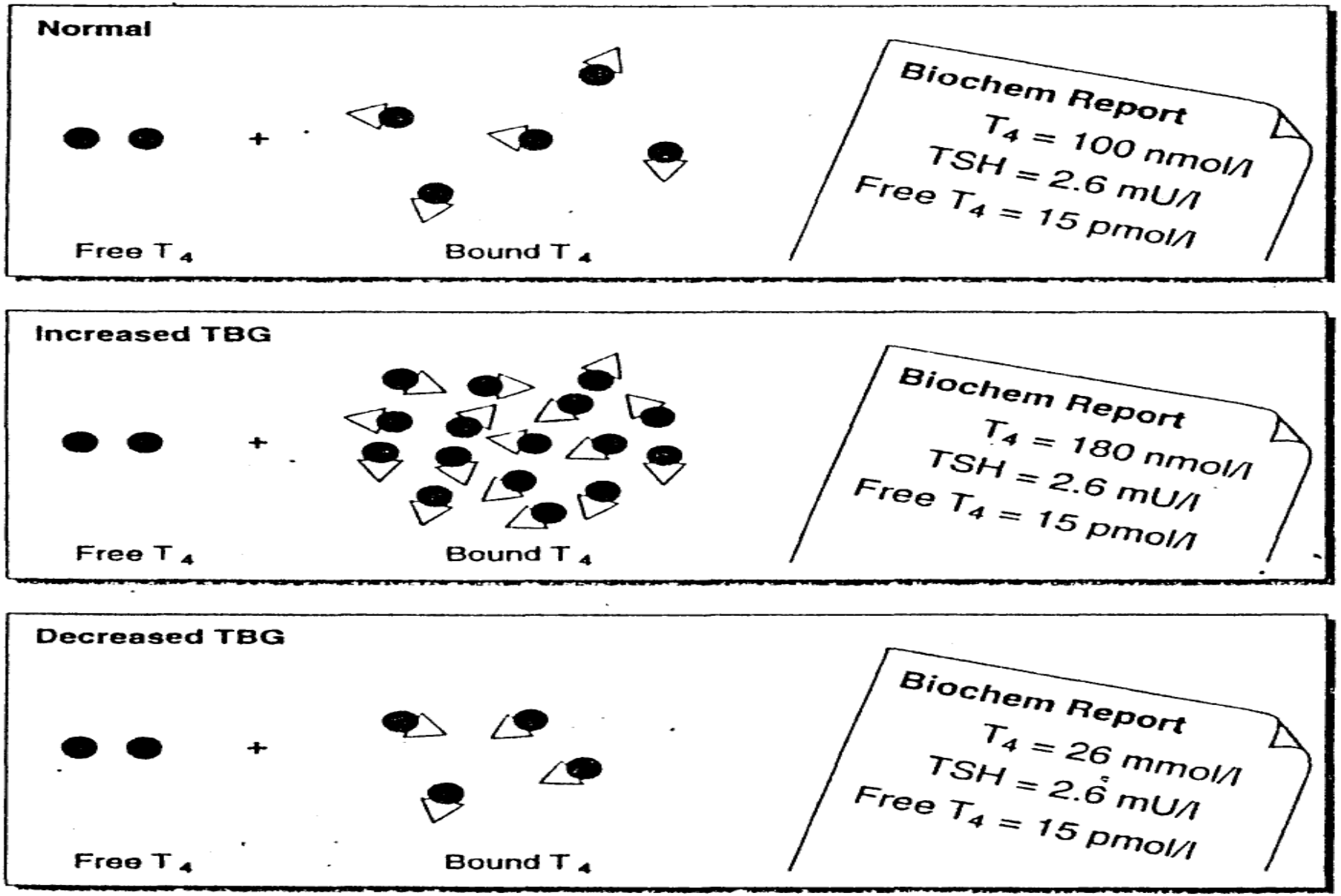
Significance of FT4 & FT3 tests for Thyroid Function

- Plasma [FT3] (Reference range: 3 to 9pmol/L);
- Plasma [FT4] (Reference range: 10 to 27pmol/L);
- Plasma [FT3] and [FT4] can be determined by:
  - Radioimmunoassay (RIA),
  - Enzyme-Linked Immunosorbent Assay (ELISA),
  - Enzyme Immunoassay (EIA),
  - Microplate Enzyme Immunoassay (MEIA);
- Plasma FT4 is reliable test in combination with [TSH],
- Plasma FT3 in combination with [TSH] are the recommended tests in most cases;
- Final choice of test should be made by the Physician;

What is the Thyroxine Binding Globulin (TBG) test?

- TBG Test include the following:
  - Determination of Plasma [TBG],
  - Determination of Plasma [Total T4],
  - Determination of Plasma [FT4],
- INTERPRETATION OF Results of TBG Test:
- See **Fig. 2**

Fig. 2: TBG Test and Interpretation of results (Gaw et al 1999)



The interpretation of thyroid hormone results when TBG concentration changes.

IMPORTANT TO NOTE

- Conditions that causes increase in Plasma [TBG]:
  - Pregnancy,
  - Hormone Replacement Therapy,
  - Oral Contraceptives,
  - Infections,
  - Hepatitis,
- Conditions that causes decrease in Plasma [TBG]:
  - Hypoproteinemia,
  - Nephrotic syndrome,
  - Malnutrition
- **Plasma [FT4] & [FT3] are not affected by changes in plasma [TBG],**

How significant is plasma Total Thyroxine (T4) test?

- Plasma [Total T4] (Reference range: 70 to 150 nmol/L);
- Plasma [Total T4] can be determined by:
  - Radioimmunoassay (RIA),
  - Enzyme-Linked Immunosorbent Assay (ELISA),
  - Enzyme Immunoassay (EIA),
  - Microplate Enzyme Immunoassay (MEIA);
- All labs should **STOP** measuring Plasma [Total T4], because it is affected by many factors;

What factors affect Interpretation of [Total T4] results?

- 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 plasma [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 Reference range, but plasma [FT4] may be normal;
- Plasma [FT4] is recommended in conditions where [TBG] may be altered, e.g., Pregnancy, users of Oral Contraceptive Pill, patients with Nephrotic Syndrome

How significant is plasma Total Tri-Iodothyronine (T3) test?

- Plasma [Total T3] (Reference range: 1.2 to 2.8nmol/L);
- Plasma [Total T3] can be determined by:
  - Radioimmunoassay (RIA),
  - Enzyme-Linked Immunosorbent Assay (ELISA),
  - Enzyme Immunoassay (EIA),
  - Microplate Enzyme Immunoassay (MEIA);
- Gradually laboratories are moving over to FT3 measurements as more FT3 assays become available;

IMPORTANT TO 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;

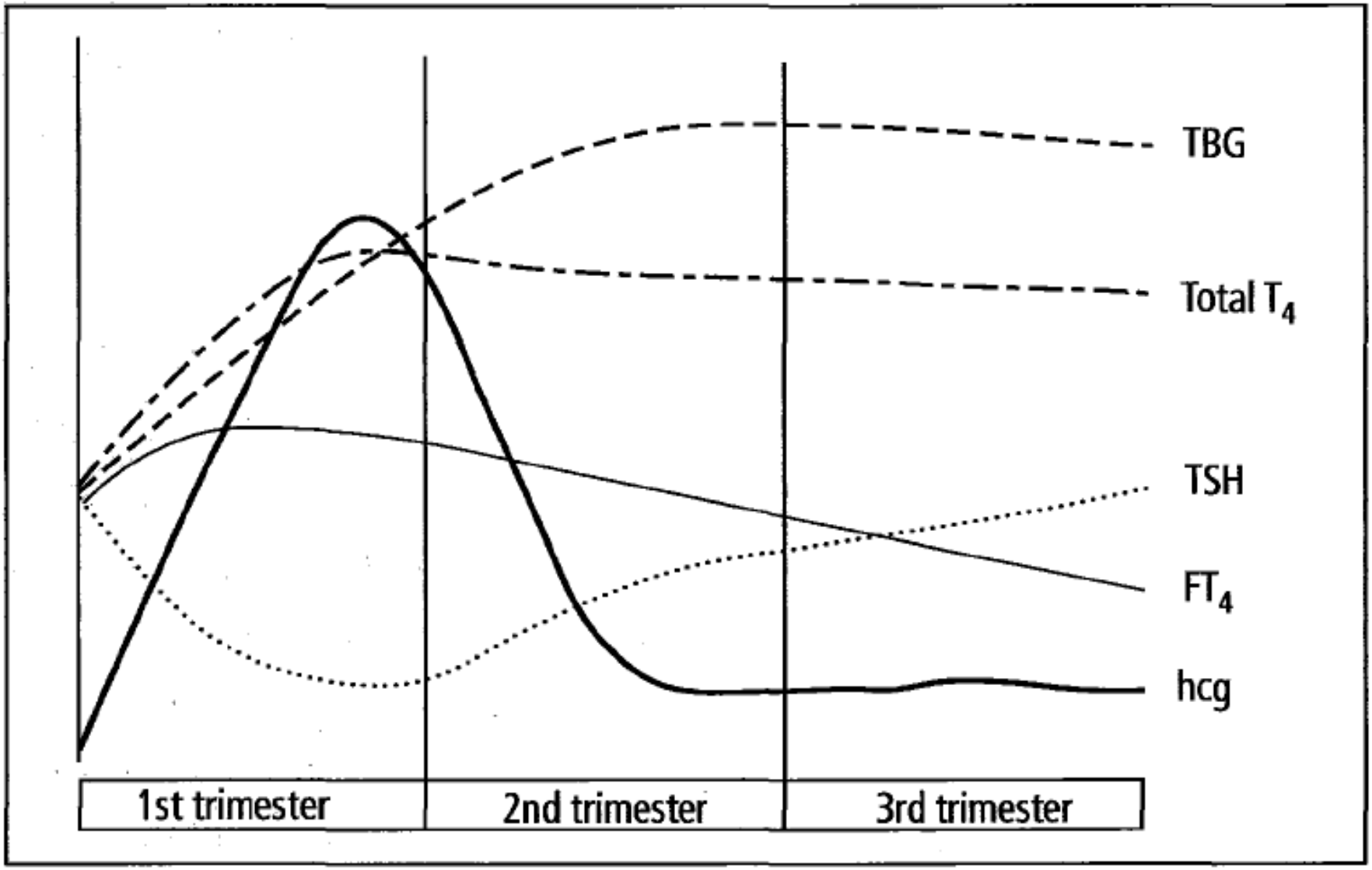
- Plasma [Total T3] is useful test for Hyperthyroidism, because values are often raised proportionately more than Plasma [Total T4];
- Plasma [Total T3] assay is of no value in investigating patients with suspected Hypothyroidism, because plasma [Total T4] is usually low;

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

- Plasma [TSH] is reliable indicator of Thyroid status during the **2nd and 3rd** Trimesters of pregnancy;
- Plasma [TSH] is not a reliable indicator during the 1st Trimester (**Why?**)
  - Because Plasma [TSH] is usually low,
  - May be due to weak Thyrotrophic effect of Placental hCG (Human Chorionic Gonadotrophin), which is high during 1st Trimester;

- Plasma [FT4] increases during 1st Trimester, then decline later; (**Fig. 3**)
- Plasma [TBG] increases during pregnancy, causing elevation in Plasma [Total T4] and [Total T3];

Fig. 3: Changes in plasma [TSH], [FT4], [TBG] & [h CG] during pregnancy (Beckett et al 2008)



SUMMARY

- Plasma [TSH] assay is the single best test for assessing Thyroid Status;
- Plasma [TSH] is elevated in Primary Hypothyroidism;
- Plasma [TSH] is low 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;

SOME STUDY QUESTIONS

- Give a brief outline of the biosynthesis of thyroid hormones
- List some factors that can affect the conversion of T4 to T3
- How are Thyroid hormones transported in plasma?
- How is the secretion of Thyroid hormones regulated?
- Briefly state the cellular functions of Thyroid hormones
- Briefly describe the HPT-Axis for regulation of Thyroid hormones secretion
- What do you understand by the following:
  - Primary Hypothyroidism,
  - Primary Hyperthyroidism,
  - Secondary Hypothyroidism;

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