Laboratory assessment of thyroid function

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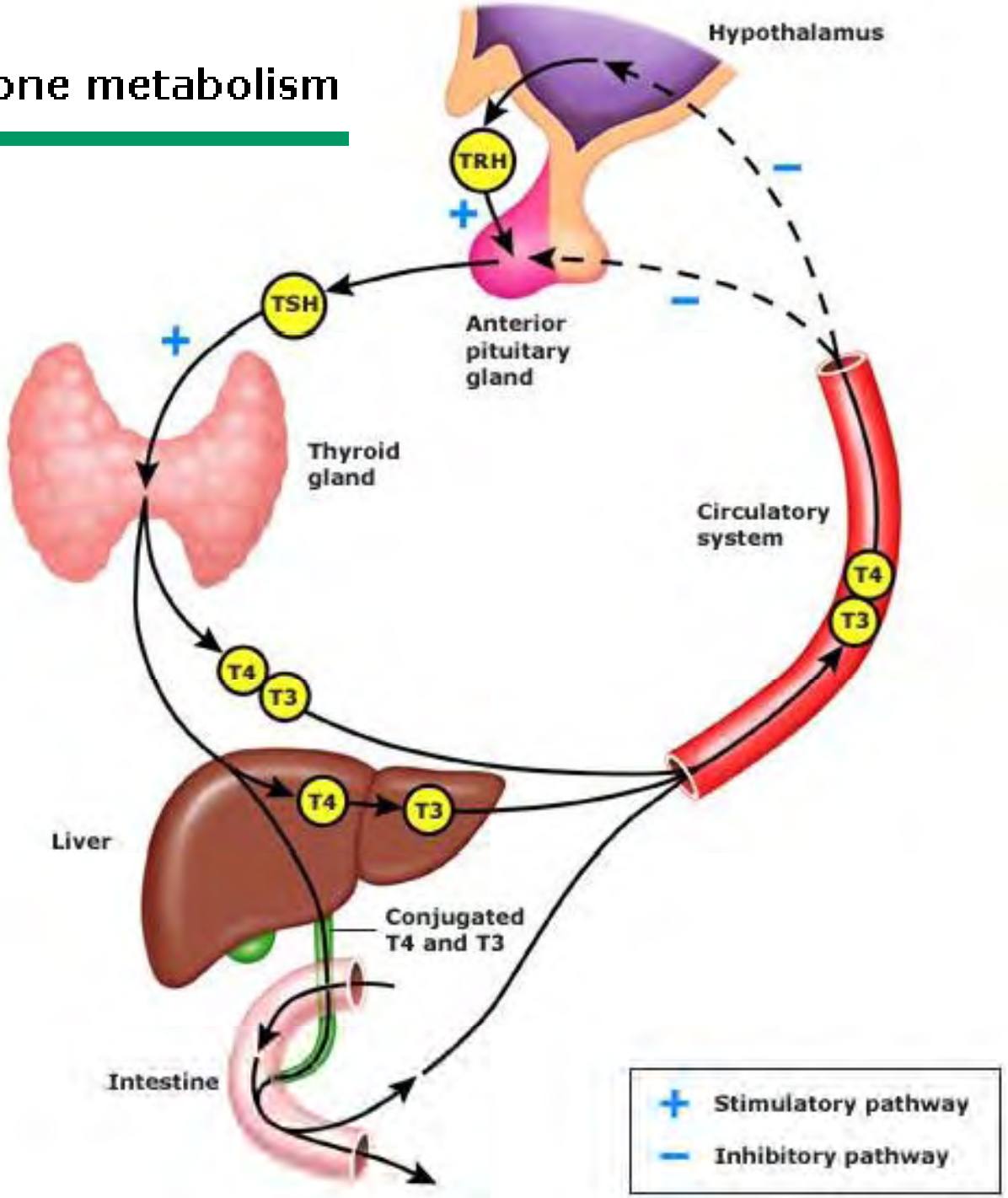
Pathways of thyroid hormone metabolism



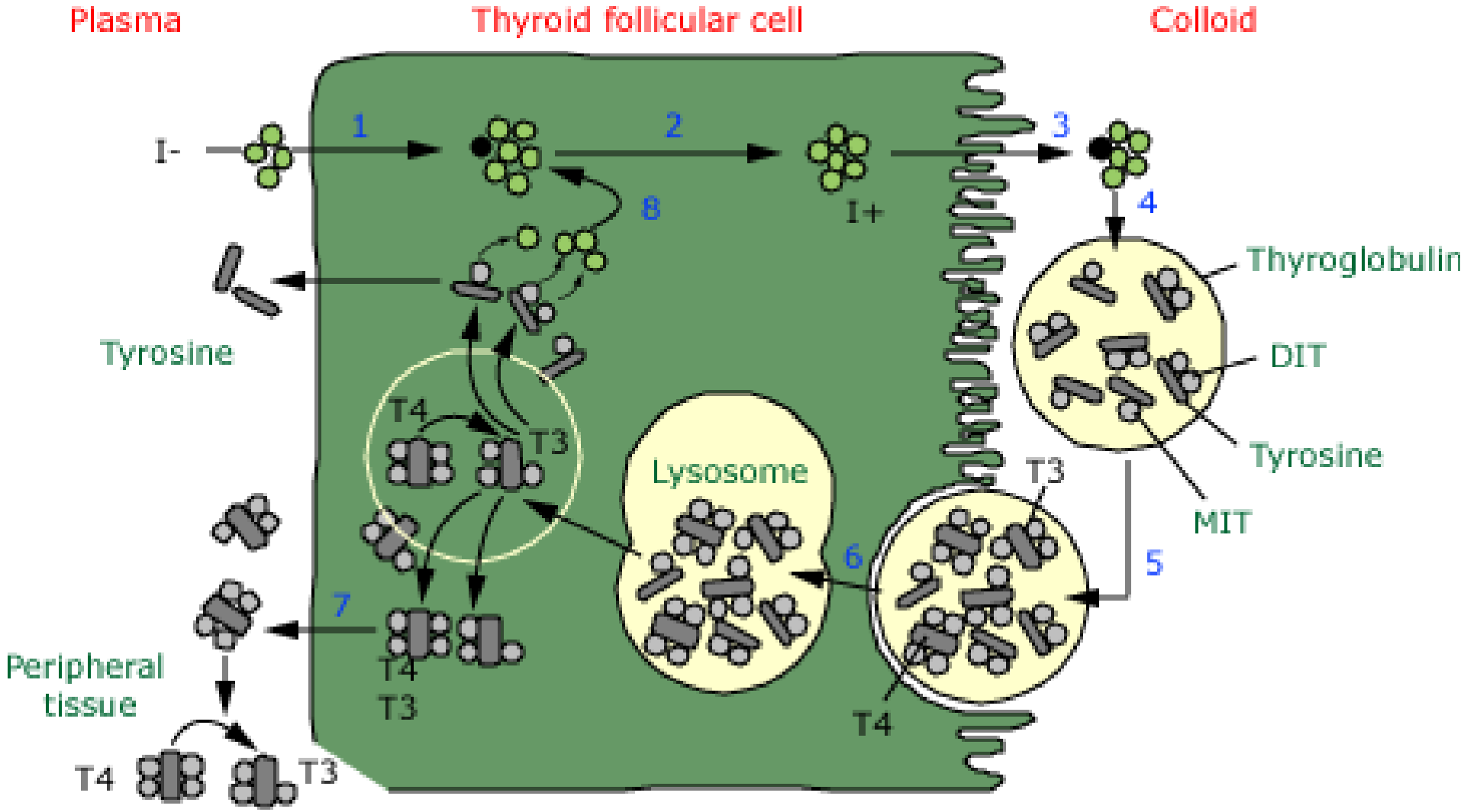
Physiology

- Thyroid gland produces Thyroxine
- Converted to active form T3 in tissue
- Scattered C cells within thyroid
- Thyroid stimulated by TSH from anterior Pituitary
- Anterior Pituitary stimulated by TRH from Hypothalamus

Physiology

- Thyroxine (T4) and Triiodothyronine (T3) secreted by Thyroid Gland
- 80% T4 and 20% T3
- TSH produced from Anterior Pituitary stimulates Thyroid
- T4 Up, TSH Down
- T4 Down, TSH Up

Thyroid hormone biosynthesis



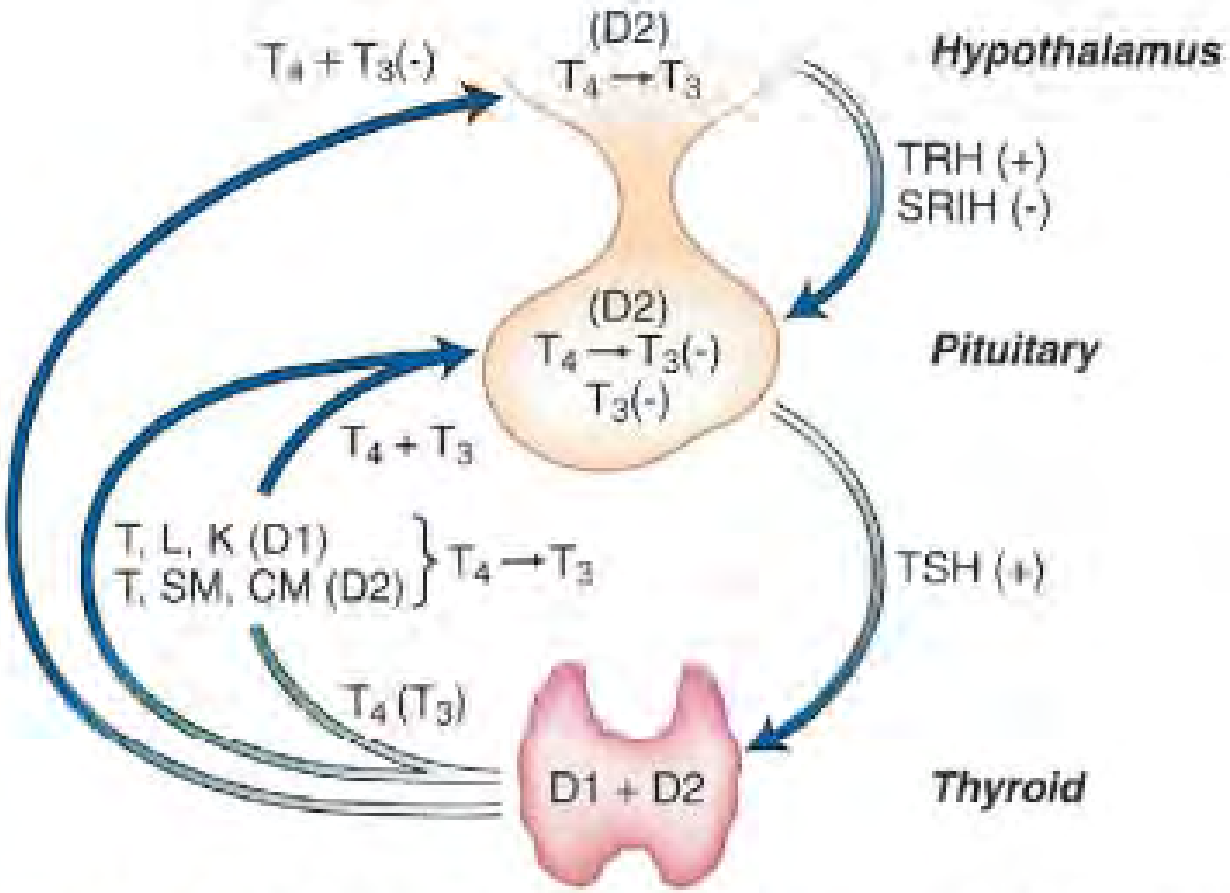


Figure 11-8 Roles of thyroxine (T_4) and triiodothyronine (T_3) in the feedback regulation of secretion of thyrotropin-releasing hormone (TRH) and thyroid-stimulating hormone (TSH). Secreted T_4 must be converted to T_3 to produce its effects. This conversion may take place in tissues such as the liver (L), kidney (K), and thyroid (T) catalyzed by the type I iodothyronine deiodinase, D1. Type 2 (D2) is present in human thyroid (T), skeletal muscle (SM), possibly cardiac muscle (CM), and the pituitary and hypothalamus. SRIH, somatotropin release-inhibiting factor (somatostatin hormone).

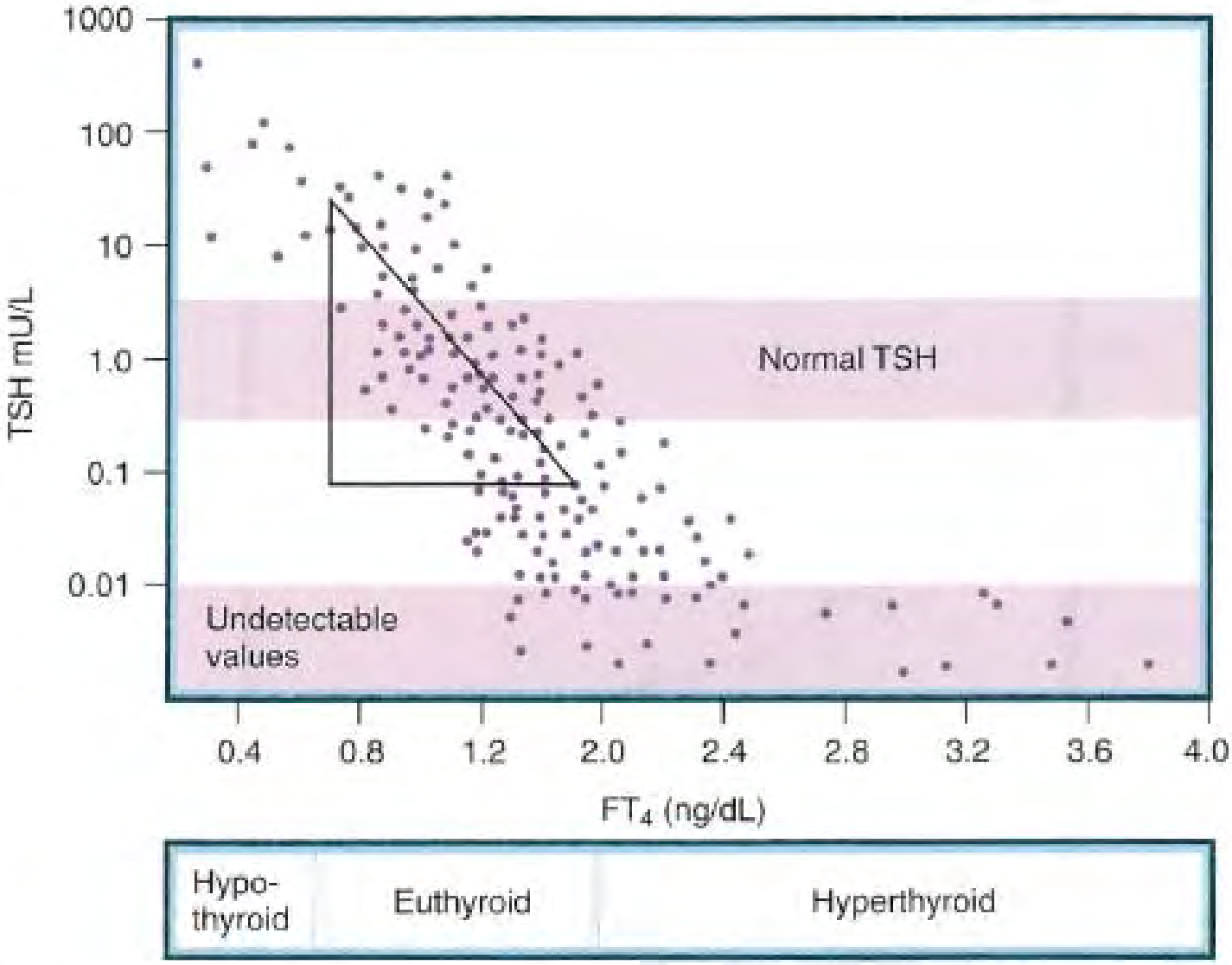


Figure 11-9 The log/linear relationship between thyroid-stimulating hormone (TSH) and the concentration of free T_4 (FT_4) in serum. Typical FT_4 concentrations in hypothyroid, euthyroid, and hyperthyroid patients are shown.

- There is a negative log-linear relationship between serum free T4 & TSH concentrations.
- This means that very small changes in serum free T4 concentrations induce very large reciprocal changes in serum TSH concentrations.
- As a result, thyroid function is best assessed by measuring serum TSH, assuming steady state conditions and the absence of pituitary or hypothalamic disease.
- Direct measurement of serum thyroid hormone levels is still important in many patients, since it may be difficult in some patients to be certain about state of pituitary & hypothalamic function.

Thyroxine metabolism

- Mainly absorbed from Jejunum and Ileum
- Some absorption from Duodenum
- Usually 80% absorbed but large variation
- Large variation in clearance rates

LABORATORY TESTS USED TO ASSESS THYROID FUNCTION —

- **Serum TSH**
- **Serum total T4**
- **Serum total T3**
- **Serum free T4 (or T3)**
- Thyroid Antibodies

Imaging

Thyroid Ultrasound scan

Thyroid Isotope Scan

Case 1

- *56 years old female*
- *Tired & weight gain*
- Thyroxine(T4) = 6 µg/dl (5.5-12.5)
- TSH = 12 mU/l (0.4 – 4)

What is the diagnosis ?

What other tests are required ?

What is the treatment ?

What Precautions are required ?

Answer 1

- Probable primary hypothyroidism
- Thyroid Antibodies
- Thyroxine 50 mcgm 1OD
- Advice re interactions

NO THYROID ULTRA SOUND SCAN

Hypothyroidism

- Common
- Mainly Females
- Usually runs in family
- Other autoimmune conditions (Hyperthyroidism, Addisons Disease, Pernicious Anaemia, Premature Ovarian Failure,Vitiligo)
- Down Syndrome

Hypothyroidism

- Dry skin
- Brittle and lustreless hair
- Weight gain
- Tiredness
- Constipation
- Muscle aches
- Bradycardia
- Cold intolarance
- Depression
- Memory Loss
- Heavy periods

Hypothyroidism

- Conginital absence of gland
- Inherited defeciency of enzymes
- Severe Iodine defeciency
- Goitrogens; cassava to lithium
- Iatrogenic ; surgical or radioiodine therapy
- Secondary to hypopituitarism
- Thyroid hormone resistance
- Thyroiditis
- Auto Immune

Thyroid Antibodies

Thyroid Peroxidase*(thyroid microsomal)*

- *100% in Hashimato thyroiditis*
- *87% with graves disease*

Thyroglobulin Antibody

- *76% of Graves Disease*

Thyroid receptor antibody

- *Normally present in 12 –18 % of female population*

History and Examination

- Symptoms
- Family History
- Other autoimmune disorders
- Cardiac History
- Other drugs
- Viral Infections
- Neck Pain
- Diabetes

Drugs affecting absorbtion

- Iron
- Calcium
- Antacids

Guidelines

- ***The diagnosis of primary hypothyroism requires measurement of both TSH & T4***

Serum TSH

- normal range for serum TSH;0.4 to 5.0 mU/L

First generation TSH radioimmunoassays;

- detection limits ~ 1 mU/L. ;,
- useful for diagnosis of primary hypothyroidism
- not sufficiently sensitive to distinguish between normal TSH & low TSH in most hyperthyroid patients.

Serum TSH

Second generation TSH immunometric assays;

- detection limits ~ 0.1 mU/L.,
- sufficiently sensitive to distinguish hyperthyroidism from euthyroidism and hypothyroidism
- can not distinguish degree of hyperthyroidism,
- poor quality control in many laboratories can lead to erroneous values

Third generation TSH chemiluminometric assays,

- currently in wide use, detection limits ~ 0.01 mU/L.
- Can provide detectable TSH even in mild hyperthyroidism
- even with poor quality control, serum TSH values in patients with overt hyperthyroidism are easily distinguished from those in euthyroid patients. In order to reliably detect values of serum TSH in the hyperthyroid range, one needs a third generation assay with a functional sensitivity of at least ≤0.05 mU/L.

Age-based normal ranges for TSH:

- **are important,**
- as illustrated by an analysis of 16,533 individuals in the National Health and Nutrition Examination Survey III (NHANES III);

  an age-related shift towards higher TSH in older patients, which persisted when those with positive antithyroid antibodies were excluded.

- E.x., 97.5 centile for TSH in **age 20 – 29; 3.56**

  age > age 80 ; 7.49 mU/L,

- 70% of older group with TSH > 4.5 mU/L were within normal range for their age.

Case 2

44 years old female

Tired & weight gain

- Total T4; 7 µg/dl (5.5-12.5)
- TSH ; 8 (range 0.4 – 4)

Strongly positive antibodies

Diagnosis ?

Treatment ?

Subclinical Hypothyroidism

Risk of conversion to HYPOthyroidism over 20 years

- If TSH raised & Antibodies raised ; 50%
- If TSH raised & AB negative ; 33%
- If TSH normal & AB positive ; 25%

Guideline

Patients stabilised on
long term thyroxine treatment
should have TSH checked annually

Drugs causing hypothyroidism

Inhibition of synthesis/release	Thionamides, lithium, perchlorate, aminoglutethimide, thalidomide, iodine, iodine-containing drugs including amiodarone, radiographic agents, expecturants(organidin,combid),kelp tablets, potassium iodine solutions(sski), betadine douches, topical antiseptics
↓ T4 absorption, Possibly medications impair acid secretion	Cholestyramine,colestipol, colsevelam, aluminum hydroxide, calcium carbonate, sucralfate, iron sulfate, omeprazole, lansoprazole, sevelemer, lanthanum carbonate, chromium, malabsorption syndromes
Immune dysregulation	Interferon-alpha, interleukin-2
Suppression of TSH	dopamine
Possible destructive thyroiditis	suntinib
↑T4 clearance &↓of TSH	Bxarotene

Case 3

38 years old female

Palpitation, tremors, etc

- Total T4; 24 µg/dl (5.5-12.5)
- TSH ; < 0.01

Investigations

Treatment

Precautions

Hyperthyroidism
Causes

- Autoimmune
- Thyroiditis
- Iatrogenic
- Solitary Nodule
- Toxic multinodular Goitre

Hyperthyroidism
Clinical Features

- **Palpitations**

Heat intolerance
Nervousness
Insomnia
Breathlessness
Increased bowel movements
Light or absent menstrual periods
Fatigue

Tachycardia
Tremors Weight loss
Muscle weakness
Warm moist skin
Hair loss
Staring gaze

History and examination

- Symptoms
- Family History
- Exclude Thyroiditis
- Asthma ?
- Eye problem
- Thyroid acropachy and PTM

Investigations

- TSH
- T4
- T3
- Thyroid antibodies
- ? Thyroid receptor antibody
- ? Thyroid Isotope Scan

Treatment Options

- Surgery
- Radio-Iodine
- Medications

Case 4

- 38 years old female with vague symptoms

- Total T4 ; 12 µg/dl (5.5-12.5)
- TSH ; <0.01

Causes ?

Investigations ?

Treatment ?

Subclinical hyperthyroidism

T3 Toxicosis

Solitary nodule

Early thyrotoxicosis

Multi nodular goitre

Subclinical Hyperthyroidism

Atrial fibrillation

Osteopenia

What do I do ?

- History
- Thyroid Isotope Scan to exclude Toxic Adenoma (radio iodine treatment) or Thyroiditis
- Bone density
- Document pulse and/or ECG
- Follow up

Consider treatment if;

- severe osteoporosis
- atrial fibrillation

Drugs causing hyperthyroidism

↑ hormone synthesis/release	Iodine, amiodarone
Immune dysregulation	Interferon-alpha, I nterleukin-2, denileukin diftitox

Case 5

- 20 years old man with painful neck

- Thyroxine ; 14 µg/dl (5.5-12.5)
- TSH ; 0.01
- Antibody negative

Case 6

20 years old male

Painful neck and flu like symptoms

- Thyroxine ; 3 µg/dl (5.5-12.5)
- TSH ; 18

Further investigations ?

Dose ?

Drugs causing abnormal thyroid function tests without thyroid dysfunction	
↓ TBG	Androgens, danazol, glucocorticoids, slow-release niacin(nicotinic acid), l-asparaginase
↑TBG	Estrogens, tamoxifen, methadone, 5-fluouracil, clofibrate, heroin, mitotane
↓T4 binding to TBG	Salicylates, salsalate, furosemide, heparin(via free fatty acids), certain NSAIDs
↑T4 clearance	Phenytoin, carbamazepin, rifampin, phenobarbital
↓TSH secrstion	Dobutamine, glucocorticoids, octreotide
↓ T4 to T3 conversion	Amiodarone, glucocorticoids, contrast agents for oral cholecystography (eg, iopanoic acid), propylthiouracil, propranolol, nadol

Thyroid Function in Pregnancy

- Pregnancy affects virtually all aspects of thyroid hormone economy.
- total serum T4 and T3 concentrations rise to levels about 1.5 times those of nonpregnant women

Serum T4—

- total T4 is usually measured by RIA, chemiluminometric assay, or similar immunometric technique.
- Virtually all (99.97 %) of serum T4 is bound to;

1. TBG,
2. transthyretin (also called thyroxine-binding prealbumin),
3. albumin.

- total T4 assays measure both bound & unbound free T4.
- Normal range total T4: 4.6 to 11.2 mcg/dL (60 to 145 nmol/L)

Serum T3 —

- T3 is also measured by RIA, chemiluminometric assay or other immunometric assay.
- T3 is less tightly bound to TBG & TBPA, but more tightly bound to albumin than T4.
- normal range is 75 to 195 ng/dL (1.1 to 3 nmol/L).

Serum free T4 & free T3 —

- estrogen-induced TBG excess in which total T4 are high due to increased TBG-bound hormone, but free T4 are normal.

- It is therefore necessary to estimate free hormone concentrations.

4 different tests to estimate free hormones

1. equilibrium dialysis,
2. "direct" free hormone measurements,
3. calculating the free hormone index by using the thyroid hormone-binding ratio or index (THBR or THBI) via measurement of T3 resin uptake,
4. calculating ratio of total thyroid hormone to TBG

Because none of these methods measure FT4 directly, guidelines suggest that these methods be named **"free T4 estimate tests"**

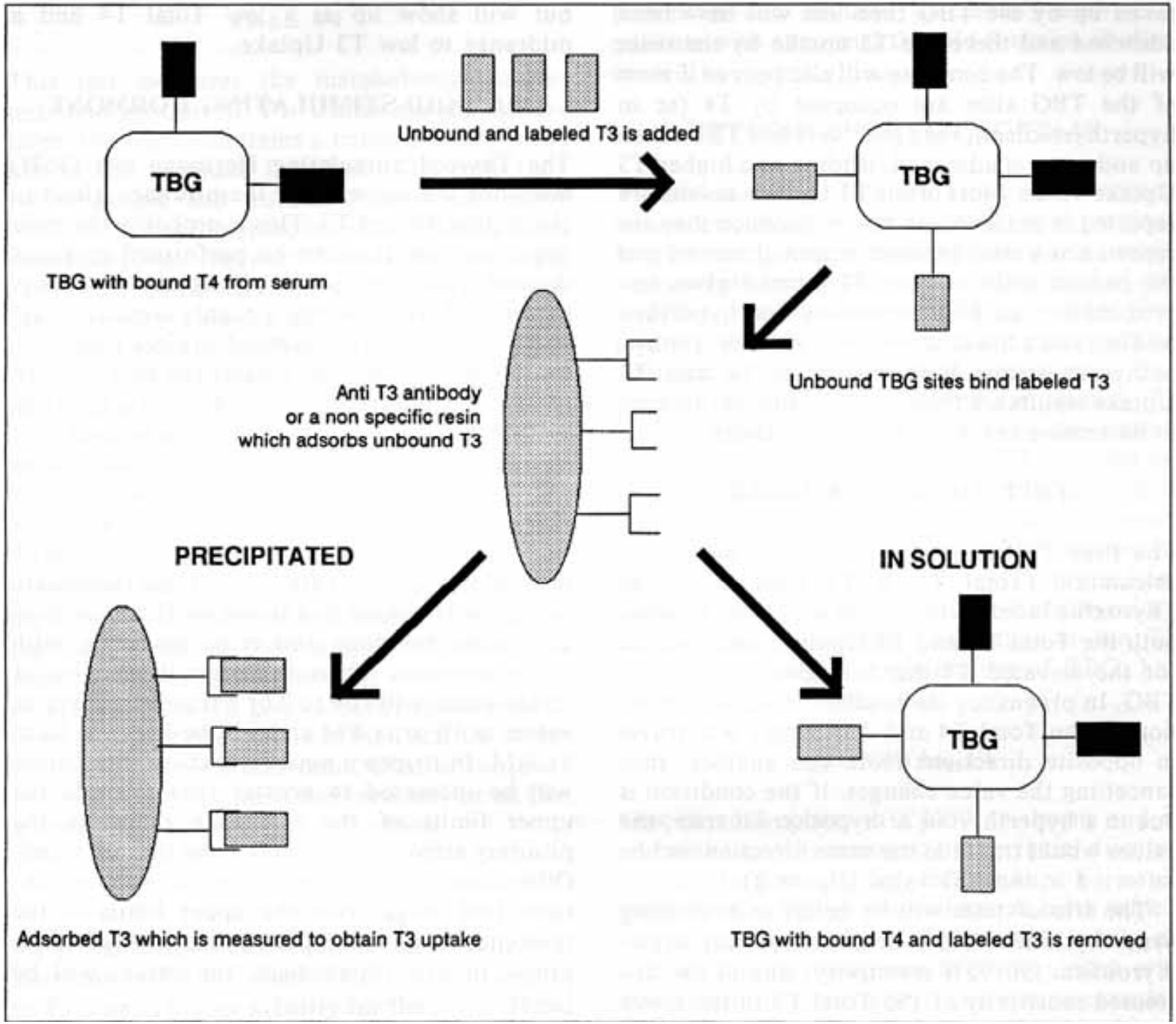
T3-resin uptake —

- This test is performed by incubating patient's serum with radiolabeled T3 tracer, & subsequently adding an insoluble resin that traps remaining unbound radiolabeled T3.

- A typical resin is dextran-coated charcoal.

- value reported is percent tracer bound to resin,

- However, the index may not fully correct at the extremes of binding protein abnormalities.


TBG
Unbound and labeled T3 is added
TBG
TBG with bound T4 from serum
Unbound TBG sites bind labeled T3
Anti T3 antibody
or a non specific resin
which adsorbs unbound T3
PRECIPITATED
IN SOLUTION
TBG
Adsorbed T3 which is measured to obtain T3 uptake
TBG with bound T4 and labeled T3 is removed

T3-resin uptake —

- While many laboratories still report actual measured value for T3 resin, it is preferable to calculate a THBR or THBI, which is simply a normalized T3-resin uptake value .

- THBI = patient's T3 resin ÷ normal pool T3 resin

- mean THBI is therefore by definition 1.00, with a normal range of approximately 0.83 to 1.16.

- Free T4 index = total T4 x THBI

- ***competitive radioimmunoassay;***

  a method in which an antigen (e.g., a hormone) in a specimen competes with radiolabeled reagent antigen for a limited number of binding sites on a reagent antibody.

- **Many commercial kits** & automated immunoassays use nonisotopic signal systems to measure hormone concentrations.

- These assays often use colorimetric, fluorometric, or chemiluminescent signals rather than radioactivity to quantitate the response,

- The advantages of these signals are biosafety, longer reagent shelf life, and ease of automation.

- On the other hand, they are more subject to matrix interferences than radioactive iodine.

TBI

- An excellent pair of linkers is biotin & streptavidin.
- (Sample) + (exogenous T4) + (biotin-T4- polyhapten)
- T4 occupies free sites
- Labeled T4- specific Ab bind to polyheptan
- Add streptavidin that interact to biotin

- Measurement of free T4 by equilibrium dialysis is available only in a few reference laboratories.

- The classic technique measures the distribution of radiolabeled T4 tracer across a dialysis membrane to estimate the unbound fraction.

- The method is too tedious and expensive for routine use.

Screening for thyroid dysfunction —

- There is some debate over cost-effectiveness of screening.
- One decision analysis, shows measurement of serum TSH is as cost-effective as many other preventive medicine.
- Second & third generation serum TSH assays are both more sensitive & specific than serum free T4 measurements for outpatients if a single screening test is utilized.
- some experts recommend both TSH & FT4 be measured in all patients for screening, errors may be made when only TSH is measured in patients with secondary or central hypothyroidism or TSH-mediated hyperthyroidism.
- This approach adds considerable cost to screening, & is likely to pick up few cases of unsuspected pituitary disease.

Screening for thyroid dysfunction —

- TSH & free T4 if pituitary or hypothalamic disease is suspected (eg, a young woman with amenorrhea & fatigue).

- free T4 if patient has convincing symptoms of hyper- or hypothyroidism despite a normal TSH result.

ANTITHYROID ANTIBODIES —

- Several antibodies against thyroid antigens have been described in chronic autoimmune thyroiditis. The antigens include:

- Thyroglobulin (Tg)
- Thyroid peroxidase (TPO, formerly known as the microsomal antigen)
- The TSH receptor

Thank you

Any Questions ?