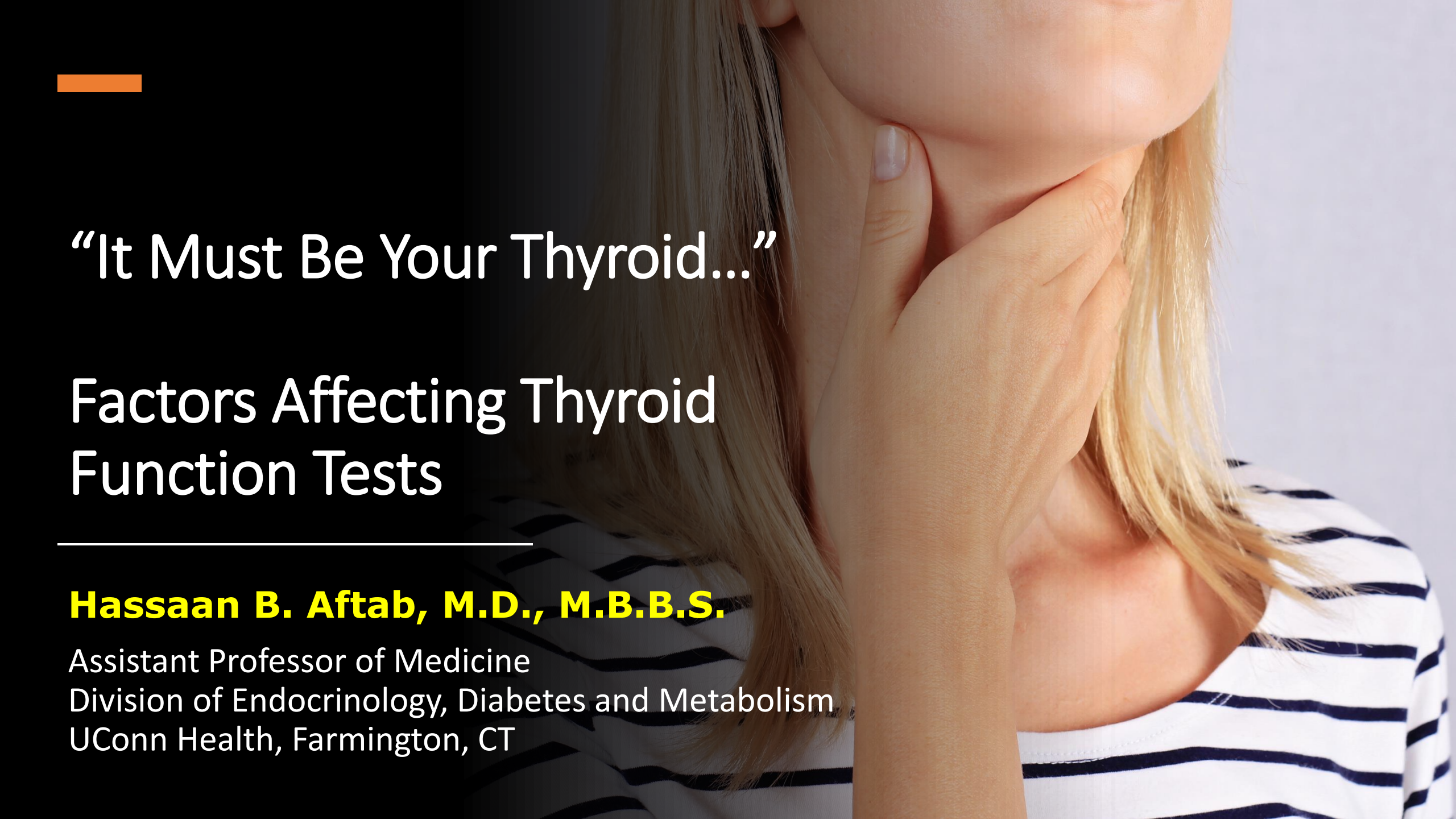
“It Must Be Your Thyroid…”

Factors Affecting Thyroid Function Tests



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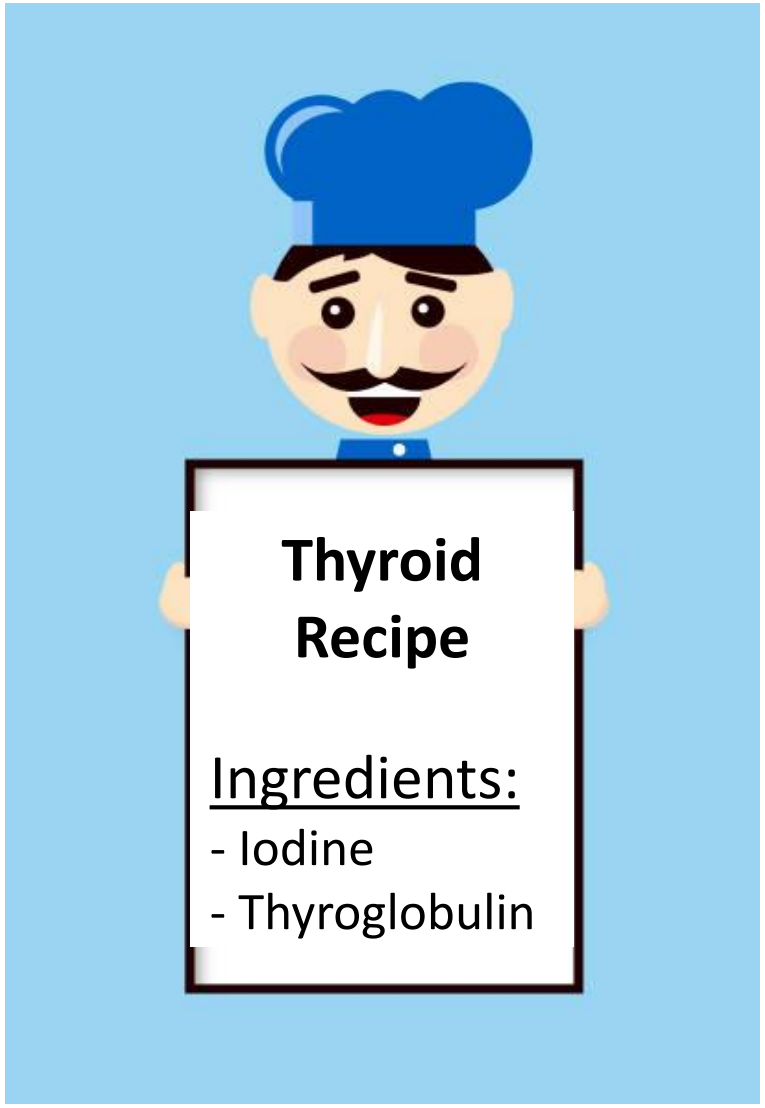
No conflicts of interest

Learning objectives

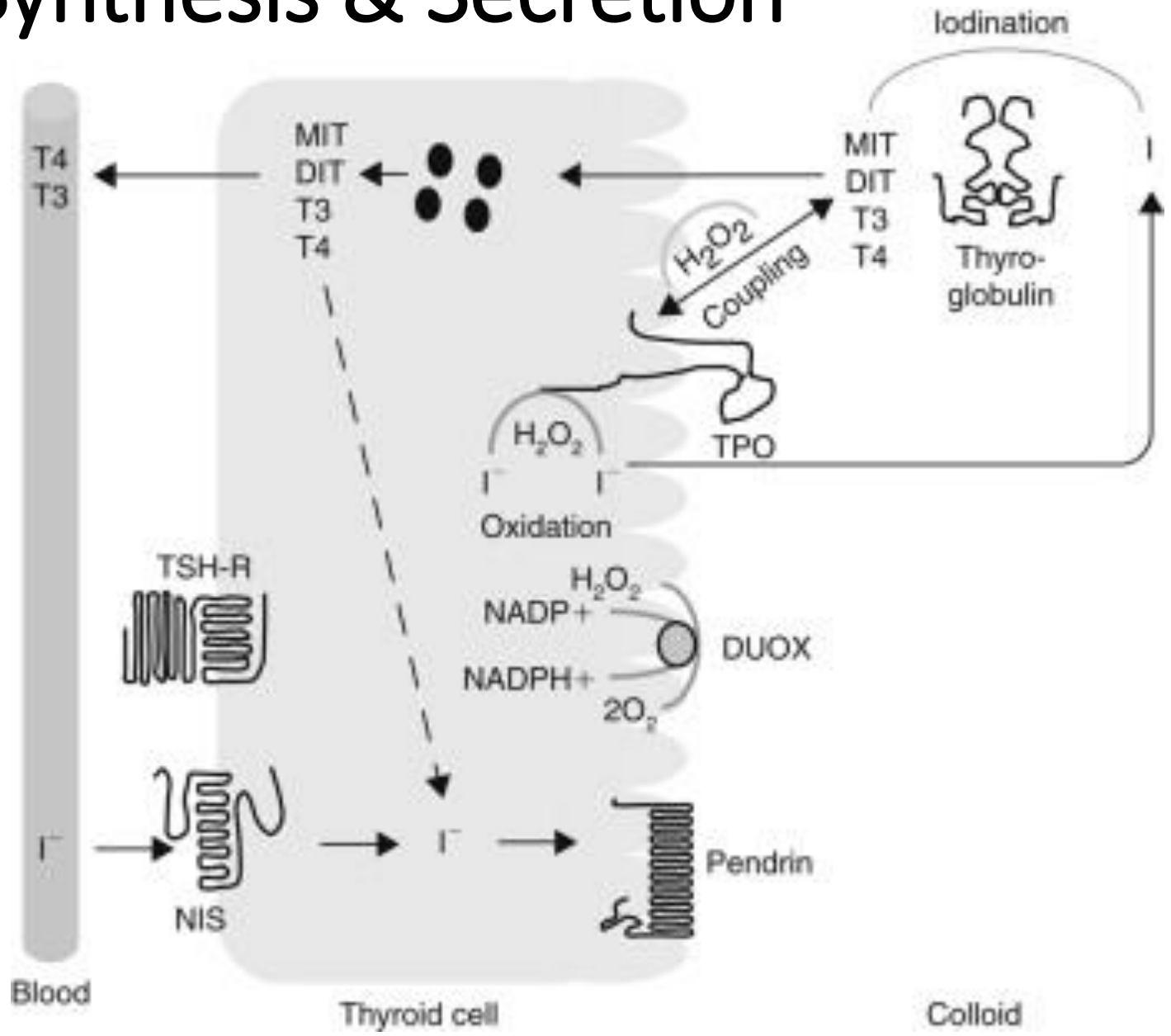
- Review **physiology** of thyroid hormone synthesis and secretion
- Identify the **effects of various factors** on thyroid function tests, including:
  - Illness
  - Glucocorticoids
  - Age
  - Iodine and iodinated contrast
- Review **different assays** used to evaluate thyroid function and identify the effect of **biotin** on these assays.

Physiology of thyroid hormones



- Synthesis
- Secretion
- Plasma transport
- Metabolism
- Regulation (HPT-Axis)

Synthesis & Secretion



- Iodide uptake (active transport)
- Thyroid peroxidase (TPO) actions
  - Oxidation of iodide to iodine
  - Iodination of thyroglobulin (Tg) to form MIT and DIT
  - Coupling of MIT+DIT to form T3 and T4
- Translocation and proteolysis of Tg to release T3 and T4 in circulation

Preedy, V. R., Burrow, G. N., & Watson, R. R. (Eds.). (2009). *Comprehensive handbook of iodine: nutritional, biochemical, pathological and therapeutic aspects*. Academic Press.

Plasma transport - Major thyroid binding proteins

- T3/T4 are poorly soluble in water and thus bind reversibly to plasma proteins.
- Relative abundance in serum: Albumin = 100 x TTR and 2,000 x TBG.
- Relative affinity for T4: TBG = 50 x TTR and 7,000 x albumin.

	Thyroxine-Binding Globulin (TBG)	**Transthyretin (TTR)**	**Albumin**
Distribution of iodothyronines (% protein)			
T_4	**68**	11	20
T_3	**80**	9	11

T_3, Triiodothyronine; *T_4*, thyroxine.

Melmed, Shlomo, et al. *Williams textbook of endocrinology (14th edition)*.

Examples of effects on TBG

- DECREASE IN TBG:
  - Hepatic failure or nephrotic syndrome leads to decreases in all three
  - L-Asparaginase blocks the synthesis of TBG
- INCREASE IN TBG:
  - Estrogen (pregnancy, OCPs) and acute hepatitis increase TBG
- INHIBITION OF TBG Binding of T4 and T3: e.g. phenytoin, salicylate, furosemide, and mitotane.

Free thyroid hormones

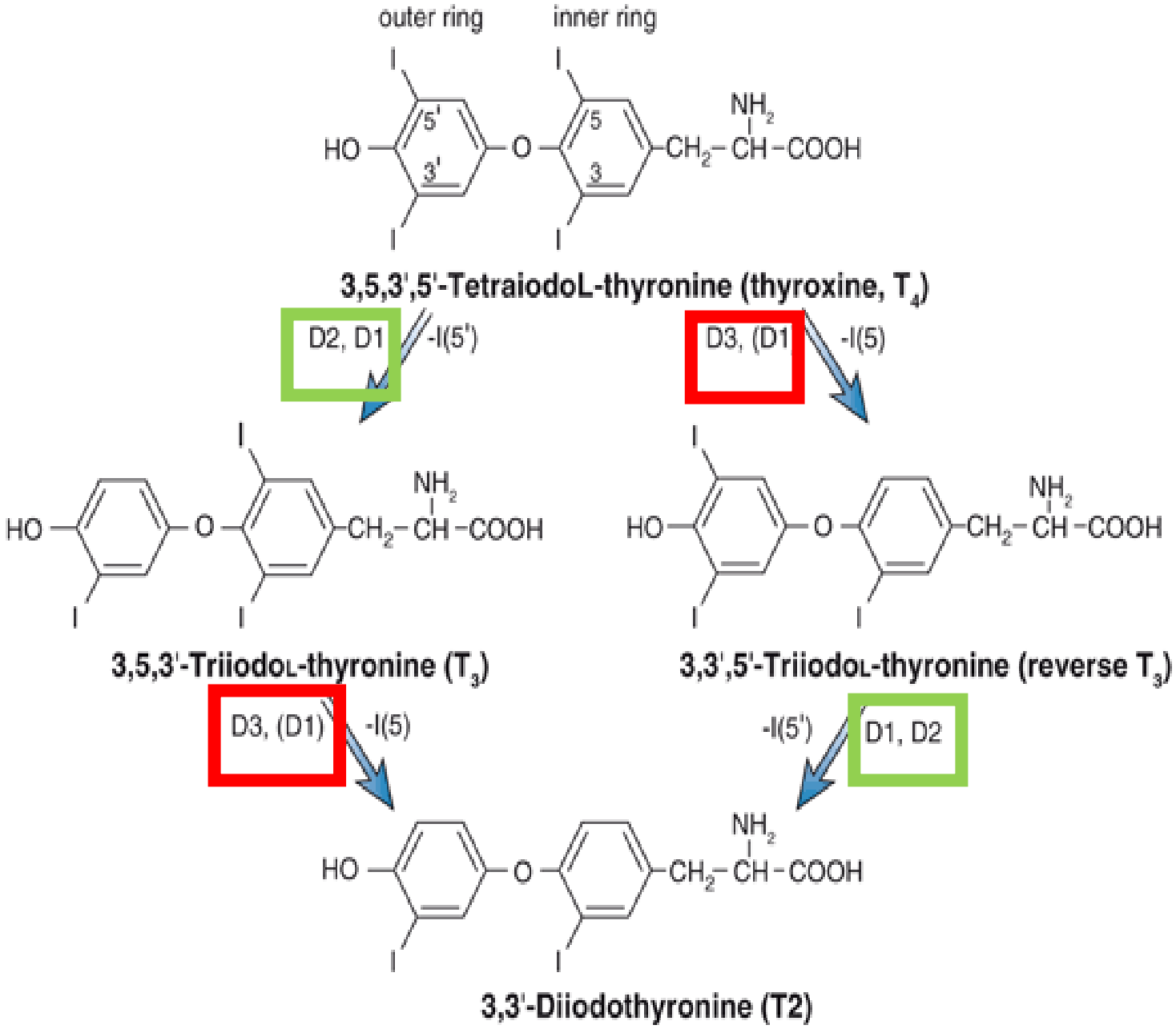
- Approx. 0.03 % of the total serum T4, and 0.3 % of the total serum T3 are present in free or unbound form.
- Concentration of the free hormones is the determinant of the metabolic state, and it is this concentration that is defended by homeostatic mechanisms.

Parameter	T_3	T_4
Production rate (nmol/day)	50	110
Fraction from thyroid	20%	100%
Relative metabolic potency	1.0	0.3
Serum concentration		
Total	117 ng/dL	7.77 mcg/dL
Free	3.2 pg/mL	1.55 ng/dL
Fraction of total hormone in free form ($\times 10^{-2}$)	0.3	0.02
Distribution volume (L)	40	10
Fraction intracellular	0.64	0.15
Half-life (days)	**0.75**	**6.7**

Melmed, Shlomo, et al. *Williams textbook of endocrinology (14th edition)*.

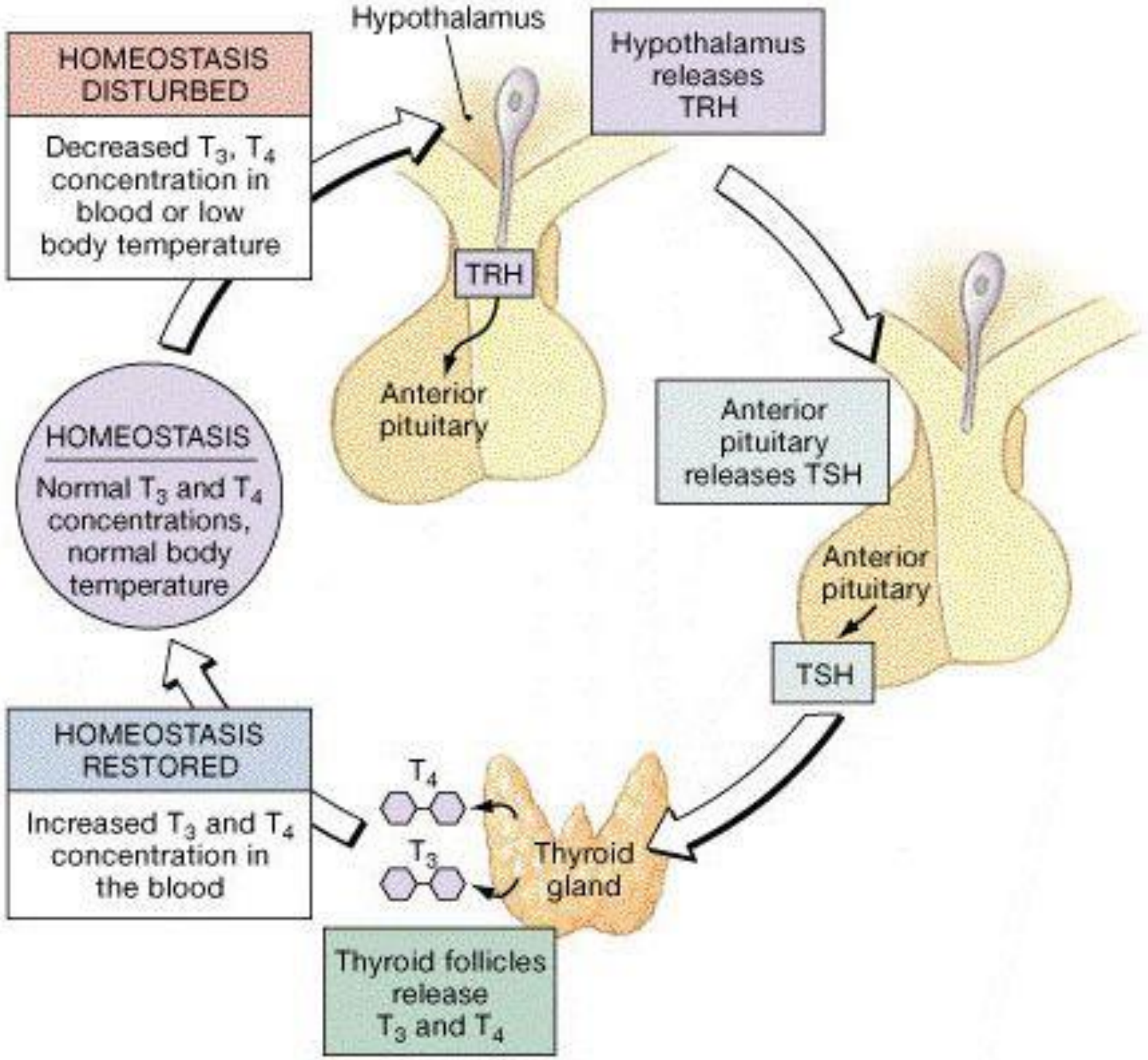
Metabolism

- Glucuronidation, sulfation, and deiodination - latter being most clinically relevant.
- 3 major enzymes catalyzing deiodination:
  - type 1 (D1), type 2 (D2) and type 3 (D3) iodothyronine deiodinases.
- **D1 and D2** have outer ring deiodinase activity, converting the prohormone T4 to its bioactive form T3 and degrading rT3 to 3,3’-T2.
- **D3** has inner ring deiodinase activity and degrades T4 to rT3 and T3 to 3,3’-T2



Regulation – Hypothalamic-Pituitary-Thyroid Axis

- TRH – source - paraventricular nuclei (PVN) of the hypothalamus .
- TSH (aka Thyrotropin) – source – thyrotroph cells in anterior pituitary.
- Classic negative feedback loop relationship with T3/T4



Lab measurements

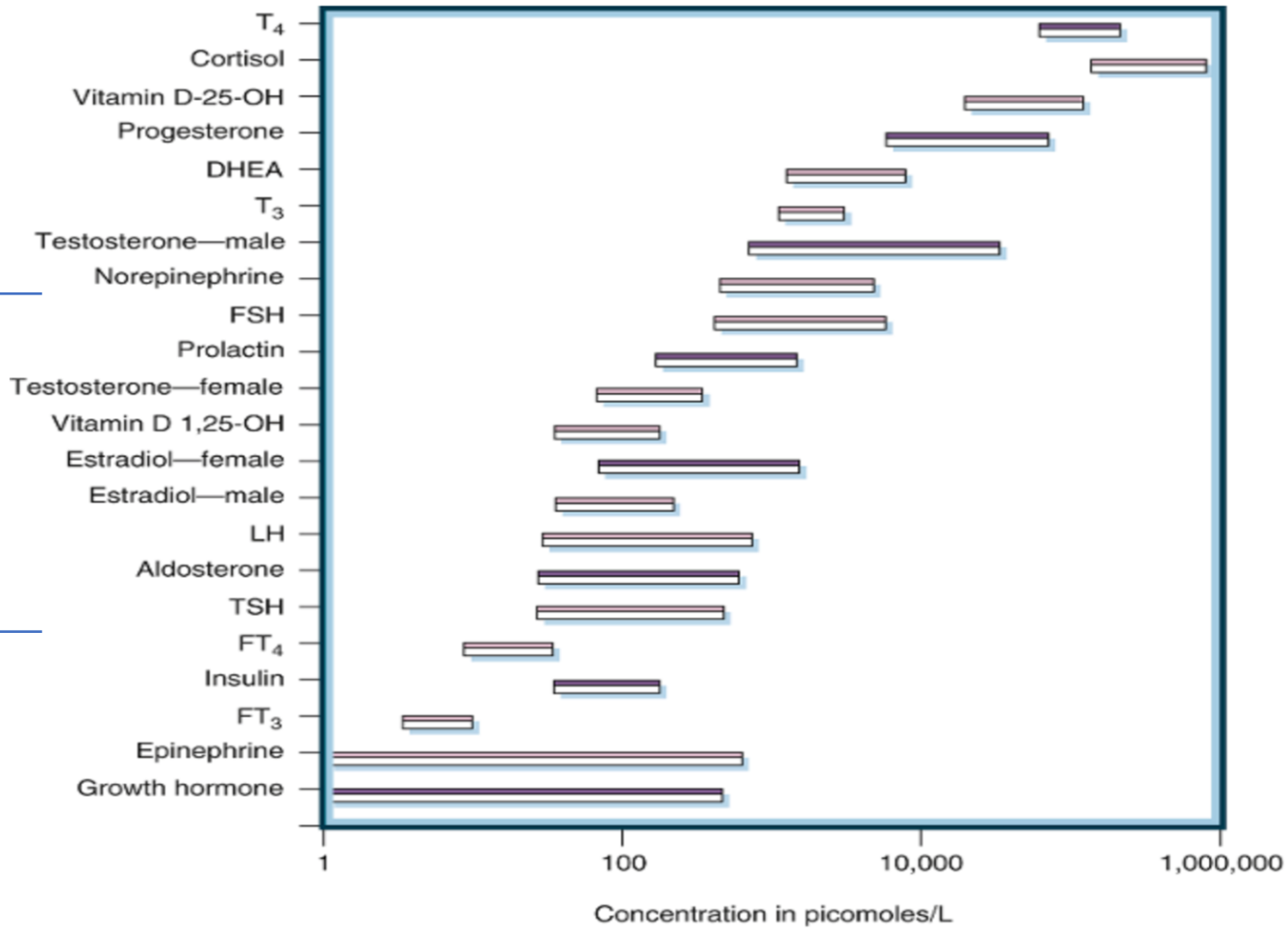
- Small changes in hormone levels are often early specific indicators of disease.
- Rate of TSH secretion is exquisitely sensitive to the plasma concentrations of free thyroid hormones.

Challenges in hormone assays

Concentrations of most hormones are much lower than those of general chemistry analytes.

Analytic methods must have exquisite sensitivity.

Range of concentrations is very broad (often several orders of magnitude), necessitating methods with a very wide dynamic range of measurement.



Melmed, Shlomo, et al. *Williams textbook of endocrinology (14th edition)*.

Immunoassay

- Current method of choice in clinical laboratories for TFTs.
- Full automation, short turnaround time, and high specificity and sensitivity.
- Vulnerable to different types of interference.

Burlacu and Gruson . Endocrine Reviews.2018

Biotin

www.healthoxide.com

Biotin

- Treatment of various hair, nail, and skin disorders.
- Limited clinical evidence on its efficiency, except in biotin deficiency.
- Recommended intake of 30 µg/day of biotin is easily obtained from a regular balanced diet.
- Most patients fail to mention taking this supplement to their physician

Biotin effects on lab assays

- Widely available OTC oral biotin supplements (1–30 mg/day).
- 5 to 10 mg of a day can cause spurious results in these assays.

- Since Feb 2019, the European Medicines Agency imposed including a warning message concerning the risk of erroneous laboratory results, which must accompany any biotin supplements containing biotin dose higher than 150 µg.
- FDA has issued a similar warning message.

Biotin streptavidin interaction

To help separate the reagent antibodies from the reaction mixture, the streptavidin–biotin interaction is a rapid, efficient, and convenient method.

The biotinylated Ab or Ag is separated on a streptavidin-linked solid phase, and this method has been widely adopted by many manufacturers.

Any interferent that prevents the separation of immune complexes, will affect this interaction and potentially disturb multiple immunoassays.

Ghazal et al. Ann Lab Med 2022

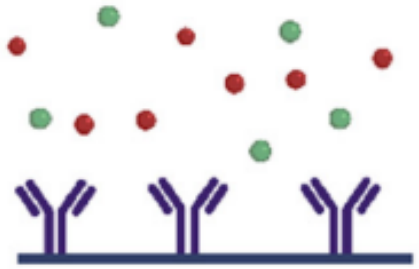
In TSH sandwich assays;

Excess biotin displaced biotinylated antibody-antigen complexes from streptavidin-coated microparticles.

Resulting in falsely low TSH levels.

In contrast, in competitive assays of FT4 and FT3, excess biotin caused overestimation of both hormones.

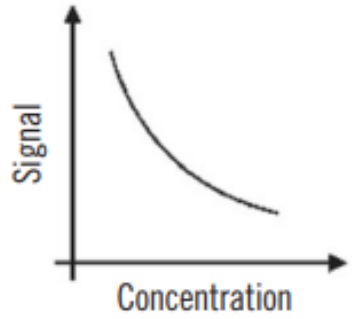
A Competitive immunoassay



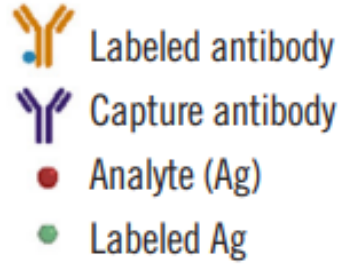
① Sample and labeled Ag added to capture Ab fixed on solid phase

② Sample and labeled Ag compete for capture Ab

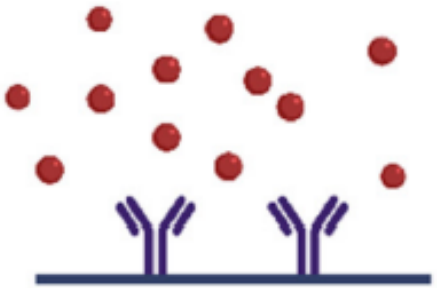
Generated signal intensity: negative correlation with analyte concentration



Competitive immunoassay calibration curve



B Sandwich immunoassay

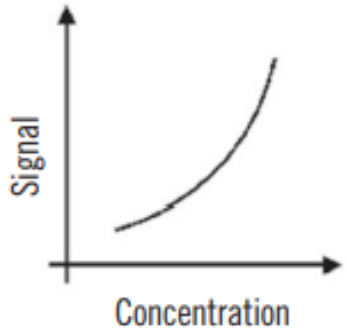


① Sample added to capture Ab fixed on solid phase

② Antigen binds to capture Ab

③ Labeled Ab binding to another site on Ag and immunocomplex formation

Generated signal intensity: positive correlation with analyte concentration



Sandwich immunoassay calibration curve

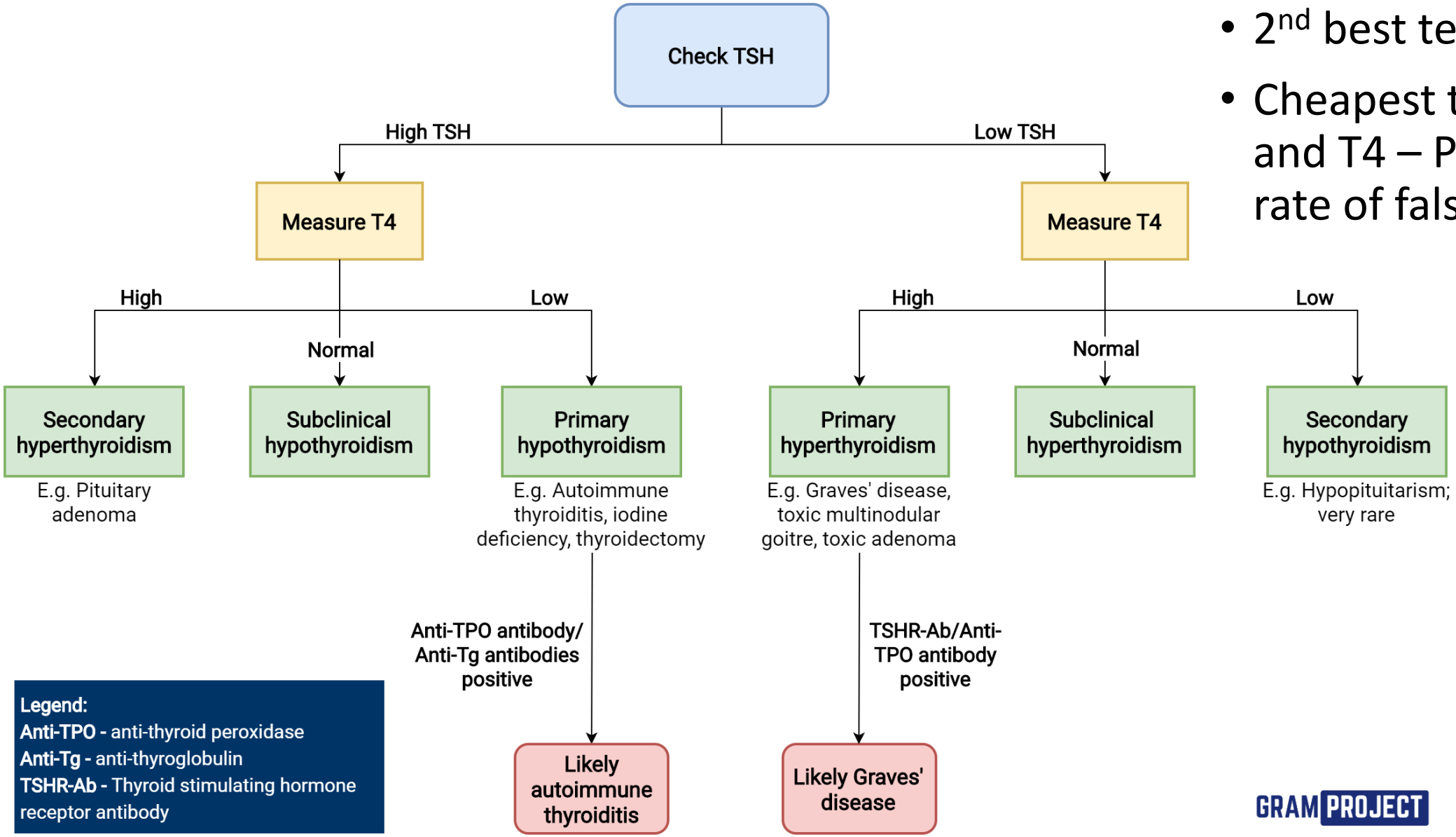
If biotin interference is suspected-

- A dilution test with the manufacturer’s diluent.
- Another method not using the biotin-streptavidin interaction can be used.

- (MOST CONVENIENT) A washout period may be advisable to be free of this interference.

Burlacu and Gruson . Endocrine Reviews.2018

Thyroid function tests (TFTs)



Legend:
Anti-TPO - anti-thyroid peroxidase
Anti-Tg - anti-thyroglobulin
TSHR-Ab - Thyroid stimulating hormone receptor antibody

- **KEY POINTS**
- Most sensitive: TSH
- 2nd best test – Free T4
- Cheapest tests - total T3 and T4 – Problem: High rate of false high/low

GRAM PROJECT

Fun Fact(s)

- Brown-throated 3-toed sloth has lowest rate of daily energy use of any mammal.
- Moss can grow in sloth hair grooves.
- A species of moth has evolved to exclusively inhabit the fur of sloths.

Thyroid function during illness

- HPT axis maintains T3 levels within the normal range with notable exceptions of when it is not meant to be in that range (i.e., during fasting or illness).

- Evolutionary hypothesis - beneficial energy-sparing and nitrogen-sparing adaptation during nutritional deprivation or illness.

- All compensatory mechanisms are reduced and serum T3 may drop to almost undetectable levels.

- Low T3 syndrome,
  Sick Euthyroid Syndrome (ESS)
  Non-thyroidal illness (NTI)

Causes of low T3 (NTI)

- Sepsis
- Burns
- Trauma
- Surgery
- Myocardial infarction
- Malignancy
- **Starvation – (?Caloric restriction)**

Effect of Long-Term Calorie Restriction with Adequate Protein and Micronutrients on Thyroid Hormones FREE

Luigi Fontana ✉, Samuel Klein, John O. Holloszy, Bhartur N. Premachandra

The Journal of Clinical Endocrinology & Metabolism, Volume 91, Issue 8, 1 August 2006, Pages 3232–3235, https://doi.org/10.1210/jc.2006-0328

Published: 01 August 2006 **Article history** ▾

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Abstract

Context: Caloric restriction (CR) retards aging in mammals. It has been hypothesized that a reduction in T_3 hormone may increase life span by conserving energy and reducing free-radical production.

Objective: The objective of the study was to assess the relationship between long-term CR with adequate protein and micronutrient intake on thyroid function in healthy lean weight-stable adult men and women.

Pathophysiology

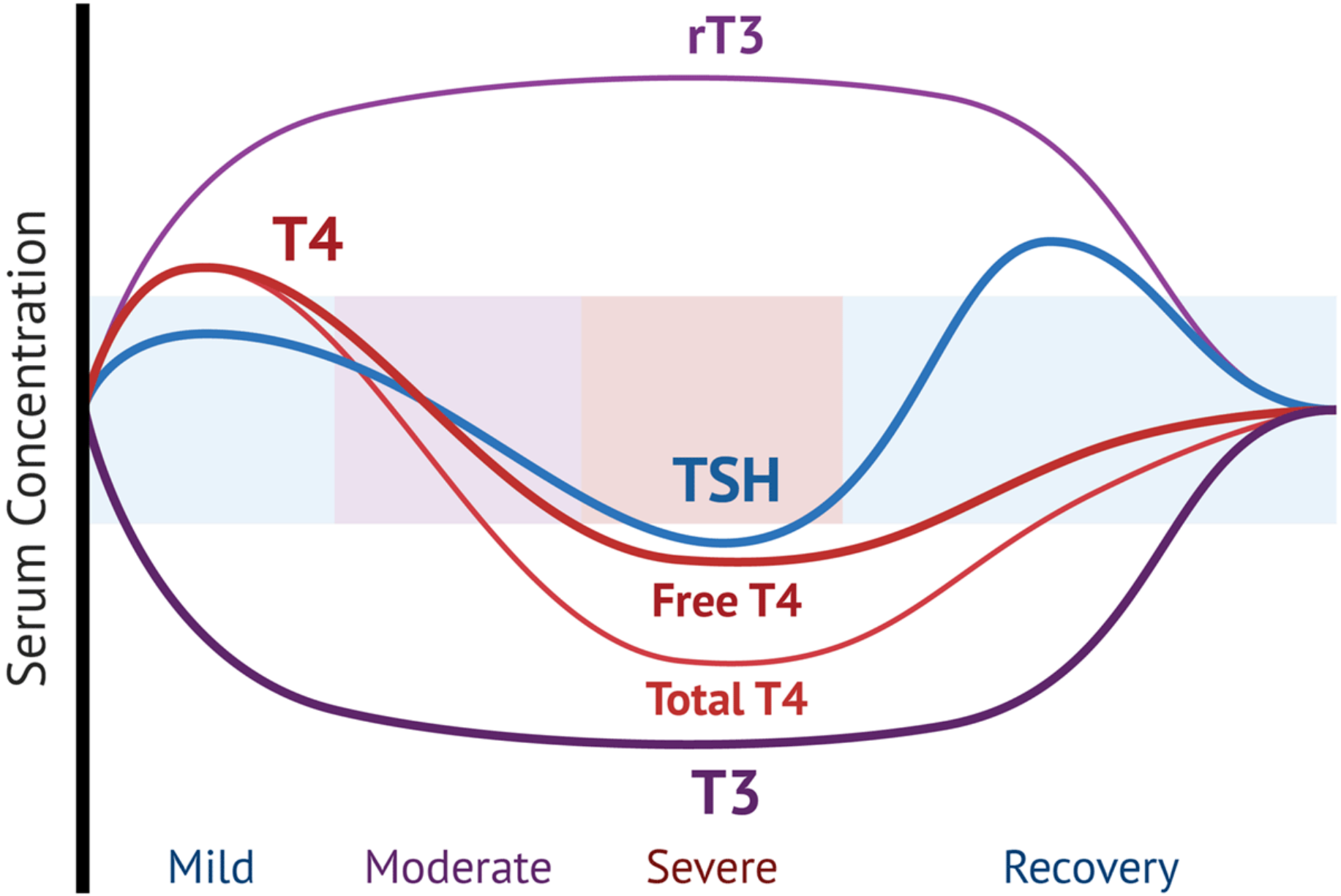
- Decreased D1/D2 expression or cofactor levels
- Diminished hepatic uptake of T4 and rT3
- As illness progresses
  - Reduction in TBG
  - Reduced hypothalamic secretion of TSH
- Severity of reduction in T4 in NTI is associated with risk of mortality.

Thyroid levels in NTI

Severity of Illness	Free T_3	Free T_4	Reverse T_3	TSH	Probable Cause
Mild	↓	N	↑	N	↓ D2, D1
Moderate	↓↓	N, ↑↓	↑↑	N, ↓	↓↓ D2, D1, ? ↑ D3
Severe	↓↓↓	↓	↑	↓↓	↓↓ D2, D1, ↑ D3
Recovery	↓	↓	↑	↑	?

D1 through *D3,* Iodothyronine deiodinases; *N,* no change; *T_3,* triiodothyronine; *T_4,* thyroxine; *TSH,* thyroid-stimulating hormone (thyrotropin).

Recovery phase of NTI



- High TSH , Low T3/T4 = ?Primary hypothyroidism
- rT3 assay does not differentiate
- **Clinical context is the only differentiator!!**
- Clue
  in NTI, TSH < 20
- Follow-up labs 4-8 weeks.

Burmeister LA. Reverse T3 does not reliably differentiate hypothyroid sick syndrome from euthyroid sick syndrome. Thyroid. (1995) 5:435–41. doi: 10.1089/thy.1995.5.435

Courtesy: MedSchool.com

To treat or not to treat?

- Biochemical improvement – no change in mortality.
- Suggests that biochemical dysfunction is marker of the severity of the illness rather than its cause.

- Brent GA, Hershman JM: Thyroxine therapy in patients with severe nonthyroidal illnesses and lower serum thyroxine concentration, J Clin Endocrinol Metab 63:1–8, 1986.
- Becker RA, Vaughan GM, Ziegler MG, et al: Hyper-metabolic low triiodothyronine syndrome of burn injury, Critical Care Med 10:870–875, 1982.

- ?possible beneficial effect of T3 therapy in patients after CABG
- Cautious use of thyroid hormonal therapy. none of the studies found evidence of damage caused by treatment.

- Novitzky D1, Cooper DK2. Thyroid hormone and the stunned myocardium. J Endocrinol. 2014 Oct;223(1):R1-8. doi: 10.1530/JOE-14-0389.
- Bennett-Guerro E, et al: Duke T3 Study Group, Cardiovascular effects of intravenous triiodothyronine in patients undergoing coronary artery bypass graft surgery. A randomized, double-blind, placebo-controlled trial, J Am Med Assoc 275:687–692, 1996

ATA guidelines

- **In hospitalized adult patients exhibiting the "nonthyroidal illness syndrome," should thyroid hormone replacement be instituted with levothyroxine?**

*recommend **against** the use of levothyroxine*
Strong recommendation. Moderate quality evidence

- **with liothyronine?**

*recommend **against** the use of liothyronine*
Weak recommendation. Moderate quality evidence

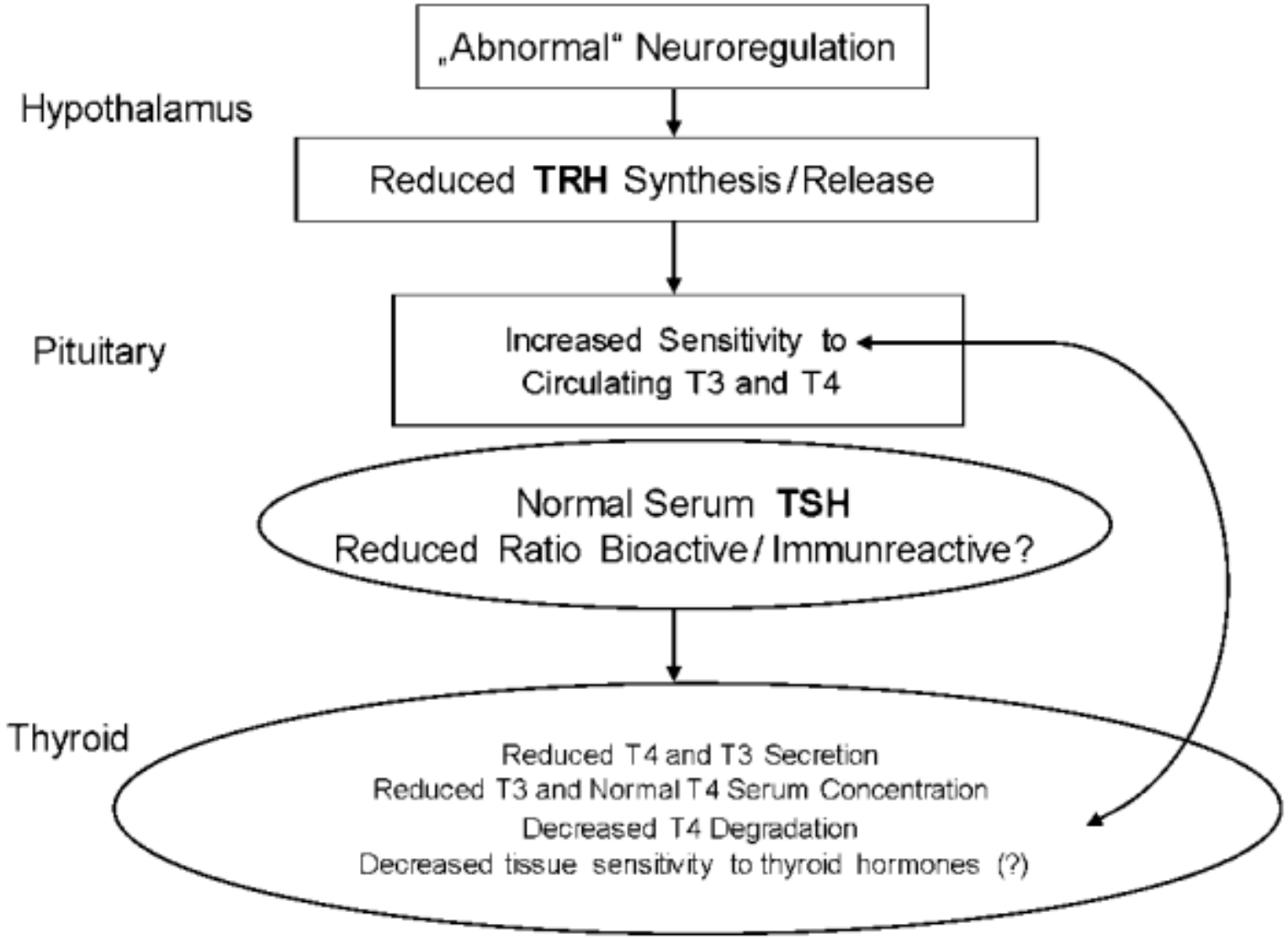
Jonklaas, Jacqueline, et al. "**Guidelines for the treatment of hypothyroidism: prepared by the american thyroid association task force on thyroid hormone replacement.**" thyroid 24.12 (2014): 1670-1751.

Glucocorticoid effect on TFTs

- Acute administration of steroids (effects within 24hrs):
  - **Decrease TSH**, TBG, TTR (high dose)
  - **Decrease serum T3/T4 and increase rT3/T4 ratios**
  - Increase rT3 production (? ↑ D3)
  - Low thyroidal uptake of iodide
  - Increase renal clearance of iodide

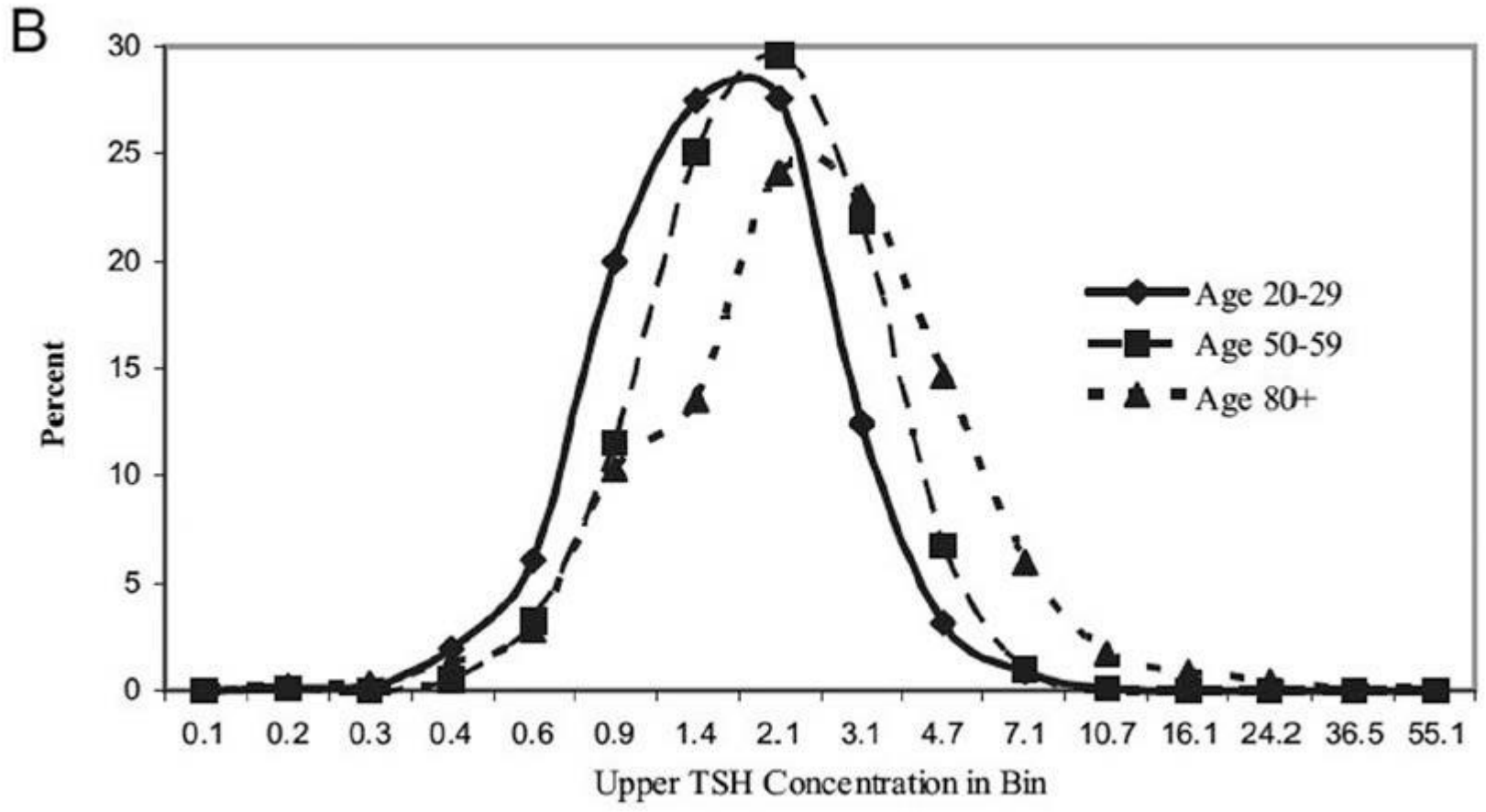
- These effects resolve during long-term therapy
- Conversely – primary adrenal insufficiency ~ elevated TSH

Thyroid and aging



Weissel, Michael. "Disturbances of thyroid function in the elderly." Wiener Klinische Wochenschrift 118.1 (2006): 16-20.

- A study of the effect of age on the TSH reference range showed that the 97.5th percentile of TSH values for individuals aged
- 50-59 y, 60-69 y, 70-79 y, and 80+ was
- 4.04, 4.33, 5.90, and 7.45 mIU/L, respectively.



Surks, Martin I., and Joseph G. Hollowell. "Age-specific distribution of serum thyrotropin and antithyroid antibodies in the US population: implications for the prevalence of subclinical hypothyroidism." The Journal of Clinical Endocrinology & Metabolism 92.12 (2007): 4575-4582.

Treatment of subclinical thyroid dysfunction in elderly

- No definitive conclusions with regard to subclinical thyroid disease.
- **A resetting of the TSH normal range according to age is strongly recommended** before any diagnosis is made and treatment is implemented.
- Pronounced subclinical thyroid disease (TSH <0.1 mU/L or TSH >10 mU/L) treatment should be considered, (dependant on assessment of symptoms and risk factors).
- Mild subclinical thyroid disease (TSH 0.1-0.45 mU/L and TSH 4.5-10 mU/L)
  - the treatment of subclinical **hyper**thyroidism should be considered in the elderly
  - whereas **subclinical hypothyroidism in the very old may be protective** and should therefore not be treated.

Peeters, Robin P. "Thyroid hormones and aging." Hormones 7.1 (2008): 28-35.

Iodine

In susceptible individuals, exposure to supraphysiological amounts of iodine can result in thyroid dysfunction.

Sources of iodine excess include radiographic iodinated contrast media, medications, diet, skin cleansers, and nutritional supplements.

TABLE 11.1 Recommended and Typical Values for Dietary Iodine Intake

Recommended Daily Intake	
Adults	150 µg
During pregnancy[a]	220 µg
Children	90–120 µg
Median Urinary Iodine Concentrations[b]	
United States (2010)	213 µg
China (2017)	239 µg
Belgium (2011)	113 µg
Switzerland (2015)	137 µg
Russia (2004)	78 µg

The World Health Organization (WHO), United Nations Children's Fund (UNICEF), and the International Council for the Control of Iodine Deficiency Disorders (ICCIDD) recommend a slightly higher iodine intake for pregnant women of 250 µg/day.

TABLE 11.7 Iodine Content of Various Iodinated Pharmaceuticals [a]

Agent	Iodine Content
Saturated solution of potassium iodide	38 mg/drop
Lugol solution	6 mg/drop
Iodized salt (1 part KI/10,000 NaCl)	760 μg/10 g
Amiodarone (200-mg tablet)	75 mg organic iodine, 8–17% is released as iodide
Iopanoate, ipodate	350 mg/tablet
Angiographic and CT dyes	400–4000 mg/dose
Povidone-iodine	10 mg/mL
Kelp tablets	150 μg/tablet
Prenatal vitamins	150 μg/tablet
Iodinated glycerol	25 mg/mL
Quantity of iodine required to suppress radioactive iodine uptake to <2%	>30 mg/day

CT, Computed tomography; *KI,* potassium iodide; *NaCl,* sodium chloride.

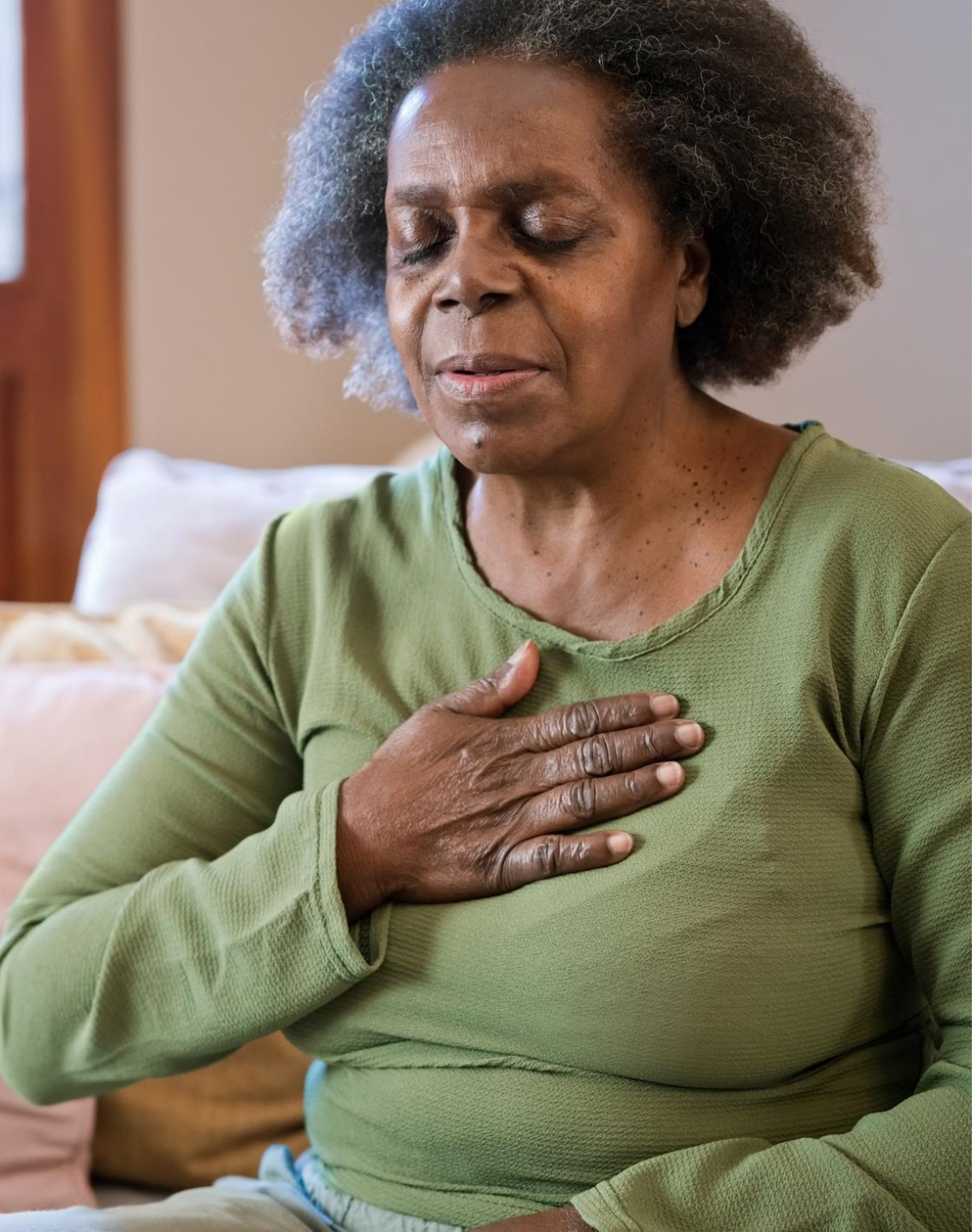
Pearce et al. J Clin Endocrinol Metab. 2015

Iodinated Contrast Media (ICM)

ICM is an increasingly common source of excess iodine.

The iodine content in ICM (320 to 370 mg/mL) is much higher than the US recommended daily allowance of 150 µg for adults and 220–290 µg for pregnant + lactating women.

The tolerable upper limit (the approximate threshold below which significant adverse effects are unlikely to occur in a healthy population) for iodine is 1100 µg/d in adults.



High risk groups

- Most cases of contrast-induced thyroid dysfunction are **transient**.
- Risk for atrial fibrillation with hyperthyroidism and myxedema coma with hypothyroidism, especially in **elderly** patients.
- The **fetus and neonate are susceptible to developing iodine-induced hypothyroidism** after maternal or neonatal ICM exposure.
- Patients with **impaired renal function**, **underlying thyroid disease**, or a **history of thyroid dysfunction**.

Pearce et al. J Clin Endocrinol Metab. 2015

Hyperthyroidism after ICM

- May occur up to several weeks after ICM exposure.
- Thyroid nuclear imaging can help determine the etiology of hyperthyroidism.
- Low radioiodine uptake is seen in iodine-induced hyperthyroidism because the radioiodine uptake is inversely related to plasma iodine concentrations.
- A spot urinary iodine concentration may be helpful to support iodine excess as the cause of hyperthyroidism.

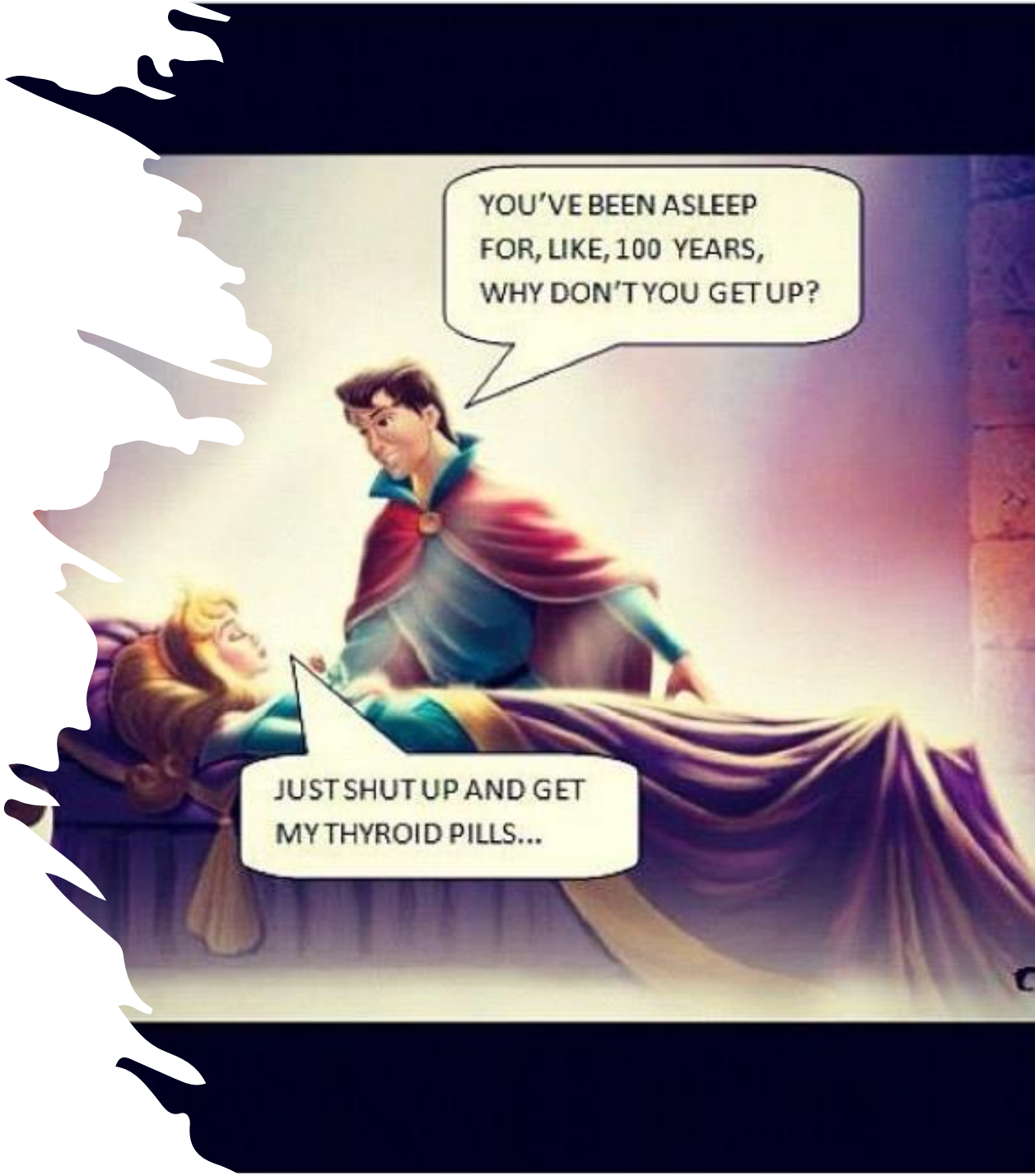
Pearce et al. J Clin Endocrinol Metab. 2015

TREATMENT

- Generally transient and self-resolves after withdrawal of iodine
- Symptomatic patients may be treated with a β-blocker and/or methimazole.
- In selected patients at high risk of developing contrast-induced hyperthyroidism or in patients with underlying cardiac disease such as atrial fibrillation, may consider prophylactic antithyroid drug therapy with methimazole or perchlorate.

Pearce et al. J Clin Endocrinol Metab. 2015

Thank you!
Questions ?



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