CCTC Clinical Chemistry Trainee Council | AACC Better health through laboratory medicine.

PEARLS OF LABORATORY MEDICINE

www.traineecouncil.org

TITLE: Thyroid Hormone Synthesis and Transport

PRESENTER: Rob Nerenz

Slide 1: Hello, my name is Rob Nerenz and I am an assistant professor of pathology and laboratory medicine at Dartmouth-Hitchcock Medical Center. Welcome to this pearl of laboratory medicine on thyroid hormone synthesis and transport.

Slide 2: Thyroid hormones play important roles in a number of different biological functions. Their primary role is to regulate the basal metabolic rate, which they do primarily by stimulating mitochondrial respiration. Thyroid hormones also enhance synthesis and breakdown of carbohydrates, proteins and lipids, stimulate CNS development and physical growth in the developing fetus and throughout childhood and adolescence, increase heart rate, support reproductive health and promote drug clearance, as well as many other activities too numerous to list here. It's important to note that both thyroid hormone excess and insufficiency can lead to abnormalities in these biological processes.

Slide 3: Thyroid hormones are secreted by the thyroid gland, which is a butterfly shaped, bilobal structure typically located anterior to the trachea. Pictured here is follicle, the functional unit of the thyroid gland. The thyroid gland is composed of millions of these repeating units. Follicles are defined by an outer membrane of follicular cells, which produce and secrete thyroxine (T4) and a smaller amount of triiodothyronine (T3). At the interior of the follicle is colloid, an amorphous substance composed almost entirely of thyroglobulin, which serves as the protein backbone for thyroid hormone synthesis.

The thyroid gland also contains parafollicular cells or C-cells, which are interspersed between follicles. Parafollicular cells aren't involved in thyroid hormone synthesis but they do secrete calcitonin, which is a protein involved in calcium homeostasis that is most frequently measured clinically as a tumor marker for patients with medullary thyroid cancer.

Slide 4: Thyroid hormones are derived from the amino acid tyrosine and are synthesized through sequential iodination of the tyrosine phenol rings. First, iodine is added to the phenol ring meta positions, resulting in monoiodotyrosine if a single site is iodinated or diiodotyrosine if two sites are iodinated. This is followed by the coupling of one iodinated phenol group to another, resulting in a diphenyl ether. The coupling of two diiodotyrosines formsT4 (which contains two iodine residues on

both the inner and outer rings) while the coupling of a monoiodotyrosine and a diiodotyrosine forms T3 (which contains two iodine residues on the inner ring but only one iodine residue on the outer ring). Reverse T3, a product of peripheral deiodination of T4, contains two iodine residues on the outer ring and a single iodine on the inner ring.

Of the compounds shown on this slide, T4 and T3 are the biologically active hormones while the others are nonfunctional.

Slide 5: Thyroid hormone synthesis is controlled by the hypothalamic-pituitary-thyroid axis, with initial stimulation coming from the hypothalamus in the form of thyrotropin releasing hormone, or TRH. TRH signals the anterior pituitary to release thyroid stimulating hormone, or TSH. TSH in turn stimulates the thyroid to produce primarily T4, with smaller amounts of T3.

To prevent uncontrolled production of T4 and T3, free T4 (the small fraction of T4 not bound by carrier proteins, to be discussed later in the presentation) feeds back primarily on the pituitary and suppresses TSH production. Lower plasma TSH concentrations result in decreased production of T4 and T3 by the thyroid, thereby decreasing circulating free T4 concentrations. Conversely, in response to high metabolic demand, peripheral tissues increase their rate of T4 uptake and conversion of T4 to T3. This decreases the circulating concentrations of free T4, stimulating TSH production by the pituitary, which increases T4 and T3 production by the thyroid gland. This continuous communication between peripheral tissues, the thyroid gland and the pituitary keeps free T4 concentrations within the physiologically appropriate range.

Slide 6: Given the involvement of thyroid hormones in many biological functions, the concentration of free T4 (which is the portion of circulating thyroid hormone the body “sees”, meaning it’s available for use by the tissues) is very tightly regulated and minor changes in the free T4 concentration elicit substantial changes in the TSH concentration. As demonstrated here, the relationship between TSH and free T4 is described as log-linear, meaning that a 2-fold increase in free T4 results in a 100-fold decrease in TSH. Similarly, a 2-fold decrease in free T4 produces a 100-fold increase in TSH.

Slide 7: The first, and rate limiting, step in thyroid hormone synthesis is the active transport of negatively charged iodide from circulating plasma into thyroid follicular cells. Stimulated by TSH binding to TSH receptors on the follicular cell surface, transport is performed by the sodium-iodide symporter against a concentration gradient in which iodide concentrations are typically 30-40 times higher in thyroid follicular cells relative to circulating plasma. Notably, iodide uptake is inhibited by lithium (which competes with sodium) but is not inhibited by antithyroid medications, such as methimazole and propylthiouracil that block subsequent steps in thyroid hormone synthesis.

Slide 8: In the second step, also stimulated by TSH, iodide is transported through the follicular cell and into the colloid by pendrin, a transporter protein located at the follicular cell surface. Once in the colloid, iodide is oxidized (or “organified”) by thyroperoxidase to form an iodine radical, with hydrogen peroxide serving as the electron acceptor.

Slide 9: In the third step, organified iodine is incorporated into thyroglobulin tyrosine residues by thyroid peroxidase to form diiodotyrosine and lesser amounts of monoiodotyrosine. These iodinated

tyrosine residues are then coupled, also by thyroid peroxidase, to form predominantly T4 with smaller amounts of T3. T4 is formed by coupling two diiodinated tyrosines while T3 is formed by coupling a monoiodotyrosine and a diiodotyrosine.

To illustrate the importance of thyroglobulin in thyroid hormone synthesis, thyroglobulin is the dominant protein product of thyroid follicular cells and is estimated to account for approximately 75% of the total protein content of the thyroid gland.

Slide 10: In the fourth step, also stimulated by TSH, colloid is taken up by thyroid follicular cells by pinocytosis, the colloid vesicle is fused with a primary lysosome and thyroglobulin is digested, releasing T4, T3, diiodotyrosine, monoiodotyrosine and non-iodinated amino acids. T4 and T3 are released into circulation while the remaining thyroglobulin degradation products are recycled.

Slide 11: The last step in thyroid hormone synthesis occurs outside the thyroid gland and consists of peripheral deiodination of T4 to T3 and reverse T3. T4 is often referred to as a "prohormone" because it has 10-fold lower biological activity than T3.

Approximately 40% of T4 is converted to the more biologically active T3 by removal of an iodine from the outer ring and 45% of T4 is inactivated by conversion to reverse T3 by removal of an iodine from the inner ring. The vast majority of T3 (around 80%) is formed from peripheral deiodination and only 20% is produced and secreted directly by the thyroid gland.

The conversion of T4 to T3 or reverse T3 is largely determined by the body's desired metabolic rate. For a high metabolic rate, T4 is preferentially converted to T3 rather than reverse T3. By contrast, for a low metabolic rate, T4 is preferentially converted to reverse T3 rather than T3.

Slide 12: Once in circulation, T4 and T3 are either protein-bound or free and the overwhelming majority of T4 and T3 is protein-bound. 99.97% of T4 and 99.7% of T3 is bound to carrier protein, leaving only 0.03% of T4 and 0.3% of T3 free. This is important because free hormone is what the body "sees" as the pool of hormone available for use. For this reason, the concentration of free T4 typically correlates more closely with patient symptoms than does total T4.

Thyroid hormones exert their biological activity by binding to specific intranuclear receptors (TRα and TRβ) that recognize DNA sequences in the regulatory regions of target genes. Following binding to these regulatory regions, TRs recruit cellular machinery that increases or decreases the rate of transcription of thyroid hormone-responsive genes.

Slide 13: There are three proteins responsible for binding T4 and T3 in circulation: thyroxine-binding globulin, or TBG, transthyretin, also known as prealbumin due to its migratory properties during serum protein electrophoresis and albumin. TBG is present at the lowest concentration but binds T4 with the highest affinity, while at the other end of the spectrum, albumin is present at the highest concentration but binds T4 with the lowest affinity. Transthyretin represents the middle ground, as it is present at intermediate concentrations and binds T4 with modest affinity.

Factors that affect the half-lives of the thyroid binding proteins can have an important impact on thyroid hormone concentrations. For instance, estradiol prolongs the half-life of TBG, resulting in increased total

thyroid hormone concentrations while testosterone shortens TBG's half-life, resulting in decreased total thyroid hormone concentrations.

Importantly, TBG has a 10-fold higher binding affinity for T4 relative to T3, which means that most T4 is tightly bound while a relatively larger proportion of T3 exists in a loosely bound state. As a result, T4 has a relatively long half-life of 5-7 days while that of T3 is only 1 day.

Slide 14: As discussed in a previous slide, free T4 typically correlates more closely with patient symptoms than total T4. This is due largely to the fact that the concentrations and binding capacities of thyroid hormone binding proteins can change in response to other drugs. For instance, estrogen increases the concentration of TBG. This increased number of thyroid hormone binding sites briefly reduces the free T4 concentration and stimulates the thyroid to produce larger amounts of T4 to maintain free T4 concentrations within the physiologically appropriate range. During pregnancy, when TBG concentrations remain elevated due to high concentrations of estradiol, total T4 concentrations increase while free T4 remains within the non-pregnant reference interval.

Free T4 concentrations within the reference interval can also be observed in the context of a decreased total T4 in patients whose TBG concentrations are suppressed by excess androgens or glucocorticoids, or in patients treated with compounds that compete with T4 for carrier protein binding sites, including salicylates and phenytoin.

Slide 15: In summary, thyroid hormones are essential for many biological functions and follicular cells are responsible for producing the biologically active thyroid hormones, T3 and T4, but peripheral deiodination is also required to convert T4 to the more biologically active T3. In circulation, T4 and T3 are almost entirely protein-bound and only the free fraction is available for use by thyroid-responsive tissues. It is important to remember that although the total T4 concentration may fluctuate due to changes in the concentrations of thyroid hormone binding proteins, the positive stimulus and negative feedback of the hypothalamic-pituitary-thyroid axis maintains the concentration of free T4 within a very narrow range. For this reason, patient symptoms are typically more consistent with the concentration of free T4 than total T4.

Slide 16: References used in this pearl.

Slide 17: Disclosures and Potential Conflicts of Interest – None Declared

Slide 18: Related Pearls of Laboratory Medicine

Slide 19: Thank you for joining me for this pearl of laboratory medicine on thyroid hormone synthesis and transport.