REVIEW ARTICLE

Abnormal Thyroid Test Results in Euthyroid State: An Appraisal of the Role of Drugs

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ABSTRACT

Background: Thyroid function tests (TFTs) are one of the most frequently performed hormonal tests. Abnormal reports are commonly encountered even in the absence of thyroid dysfunction. Concomitantly used medications are often implicated in this effect. **Objectives:** Failure to recognize drug-induced alterations in TFTs can result in misdiagnosis and inappropriate management. This review seeks to increase awareness of the common challenges and pitfalls encountered when interpreting drug-induced changes in TFTs. **Methods:** We did a literature search for eligible publications and articles published before April 2024 on drug-induced alterations in TFTs. **Results:** The common mechanisms by which drugs alter TFTs include assay interference and alterations in levels or affinity of thyroid-binding proteins (TBP). High-dose biotin can result in erroneous TFTs by interfering with immunoassays that utilize streptavidin-biotin immobilizing systems. The TFTs mimic findings seen in thyrotoxicosis after biotin intake. Heparin-induced increase in nonessential fatty acid causes displacement of thyroid hormone from TBP, resulting in an artefactual increase in free hormone levels. Increases or decreases in thyroid-binding globulin (TBG), the major TBP, cause concomitant changes in total thyroxine and triiodothyronine levels. Estrogen, selective estrogen receptor modulators (SERMs), 5-fluorouracil, mitotane, clofibrate, heroin, and methadone increase, while androgens, anabolic steroids, glucocorticoids, nicotinic acid, and L-asparaginase decrease TBG. Some medications like salicylates, frusemide, carbamazepine, and phenytoin displace thyroxine from TBP, producing a decline in the total hormone levels. **Conclusion:** Awareness about these drug-induced TFT fallacies is essential to avoid improper diagnosis and overtreatment.

Keywords: Thyroid function tests, drug interference, biotin, heparin, thyroid-binding globulin

Thyroid function test (TFT) is one of the most commonly performed hormonal evaluations. Abnormalities can be detected in TFT reports in euthyroid individuals due to various reasons. One such important, but overlooked cause is the interference from concomitant medications during testing. Various drugs, from over-the-counter vitamin supplements to specialized anti-cancer medications, can influence the measurement of components of TFTs, including thyroid-stimulating hormone (TSH), total thyroxine (TT4), total triiodothyronine (TT3), free thyroxine (FT4), and free triiodothyronine (FT3).

This review will focus on drugs that produce fallacious TFTs, in the absence of any clinically relevant effect on thyroid physiology. Awareness about drug-induced TFT alterations could avert misdiagnosis and overtreatment of euthyroid subjects.

METHODOLOGY

We searched PubMed, Scopus, and Google Scholar for eligible publications and articles published before April 2024 using the following search strategy. We used one of the following terms: "thyroid function test", "thyroid assay", "thyroid-stimulating hormone", "thyroxine", "tri-iodothyronine", "thyroid-binding globulin", "thyroid-binding proteins" in combination with "drugs", "medications", "assay interference", "biotin", "heparin", "oral contraceptive pills", "selective estrogen receptor modulators", "estrogen", "tamoxifen", "raloxifene", "droloxifene", "fluorouracil", "clofibrate", "heroin", "methadone", "mitotane", "nicotinic acid", "asparaginase", "glucocorticoids", "androgens", "anabolic steroids", "L-asparaginase", "salicylates", "aspirin", "salsalate", "nonsteroidal anti-inflammatory drugs", "frusemide", "phenytoin", and "carbamazepine". References obtained from original papers were also scrutinized and included wherever relevant to the subject of this review.

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DRUGS INTERFERING WITH THE ASSESSMENT OF THYROID FUNCTION TESTS

The principal mechanisms by which drugs can cause inaccurate thyroid test results include assay interference, changes in levels of serum thyroid-binding proteins (TBP), particularly thyroid-binding globulin (TBG), and alterations in the affinity or binding of thyroid hormones to serum TBP. The medications that commonly affect thyroid test results through these mechanisms will be discussed in the following sections.

DRUGS CAUSING ASSAY INTERFERENCE

Medications can interfere with the accurate estimation of the thyroid profile due to interference with components of the assays used. Biotin and heparin are commonly used medications that cause TFT abnormalities in euthyroid individuals.

Biotin

Biotin based immunoassays: Biotin is a water-soluble vitamin belonging to vitamin B family. It is also an essential component of streptavidin-biotin immobilizing systems (SBIS) used for most immunoassays. This system utilizes the interaction between the fungal protein streptavidin and biotin which is one of the strongest noncovalent interactions in nature. It remains undisturbed by multiple washing steps in assays[1].

Furthermore, biotinylation does not alter the biological activity or immunologic specificity when bound to any test molecule. SBIS is widely used in many Food and Drug Administration (FDA) approved immunoassay systems using fully automated platforms, including Access, DxI, and DxC (Beckman Coulter, California, US); the Elecsys, Cobas, and Modular platforms (Roche Diagnostics, Basel, Switzerland); the Isys platform (Immuno Diagnostic System, East Boldon, United Kingdom); the Ortho Vitros platform (Ortho Clinical Diagnostics, New Jersey, US); the Dimension Vista, Exl, Immulite platforms (Siemens Healthineers, Erlangen, Germany), Abbott Architect i2000® (Abbott Diagnostics, Illinois, United States), and Diasorin Liaison XL® (DiaSorin, Saluggia, Italy)[2].

Type of abnormality: The presence of excess biotin in test samples causes abnormalities in accordance with the type of immunoassay. Falsely low readings occur with sandwich immunoassays (glycoprotein hormones like TSH) and falsely high values in competitive immunoassays (e.g., triiodothyronine (T3), thyroxine (T4), steroid hormones, and 25-hydroxyvitamin D)[2].

Mechanism of interference with sandwich immunoassays: When the assay has a "sandwich" design as employed for estimation of TSH, the test serum is incubated with biotinylated monoclonal TSH antibodies and radiolabeled monoclonal antibodies. Immune complex "sandwiches" thus formed are captured by streptavidin-coated magnetic microparticles. Chemiluminescence produced by application of a voltage to these magnetic microparticles is directly proportional to the TSH levels in the test serum. The presence of excess biotin in the serum will saturate the streptavidin-binding sites and reduce binding of these immune sandwiches to the solid phase (magnetic microparticles) resulting in low chemiluminescence and falsely low readings as illustrated in Figure 1[1,2].

Mechanism of interference with competitive immunoassays: When a competitive immunoassay is utilized as in the FT4 assay, the test serum is incubated with biotinylated T4 molecules and radiolabeled anti-T4 monoclonal antibodies. Biotinylated T4 molecules compete with the analyte (or T4) in the test serum for binding with the radiolabeled anti-T4 antibodies. The biotinylated T4 molecules are then immobilized in the streptavidin-coated microparticles. The chemiluminescence signal generated from the application of voltage to these magnetic microparticles is inversely proportional to the levels of FT4. The presence of excess biotin in the test serum will prevent the complexes of biotinylated T4-radiolabeled antibodies from binding to the magnetic microparticles. This would result in lower chemiluminescence, translating to falsely higher levels of T4 in the serum as depicted in Figure 2[1,2].

Biotin dose and assay interference: The recommended daily intake of biotin ranges from 30 to 70 μg daily. While dietary biotin intake does not generate significant enough blood levels to interfere with *in-vitro* diagnostic tests, supraphysiologic levels achieved through the therapeutic use of biotin as supplements for skin and hair growth, multiple sclerosis and rare inherited metabolic disorders like biotinidase deficiency, propionic acidemia, thiamine responsive basal ganglia disease, holocarboxylase synthase deficiency, and mitochondrial disorders, can cause problems with these tests[3]. The minimal dose required and the degree, duration, and magnitude of the interference is variable and might be specific to the analyte being tested and the assay characteristics[4,5].

The level of interference depends on the serum concentration attained rather than the dose of biotin consumed, which in turn is determined by the length of the washout period before retesting[6,7], Grimsey et al

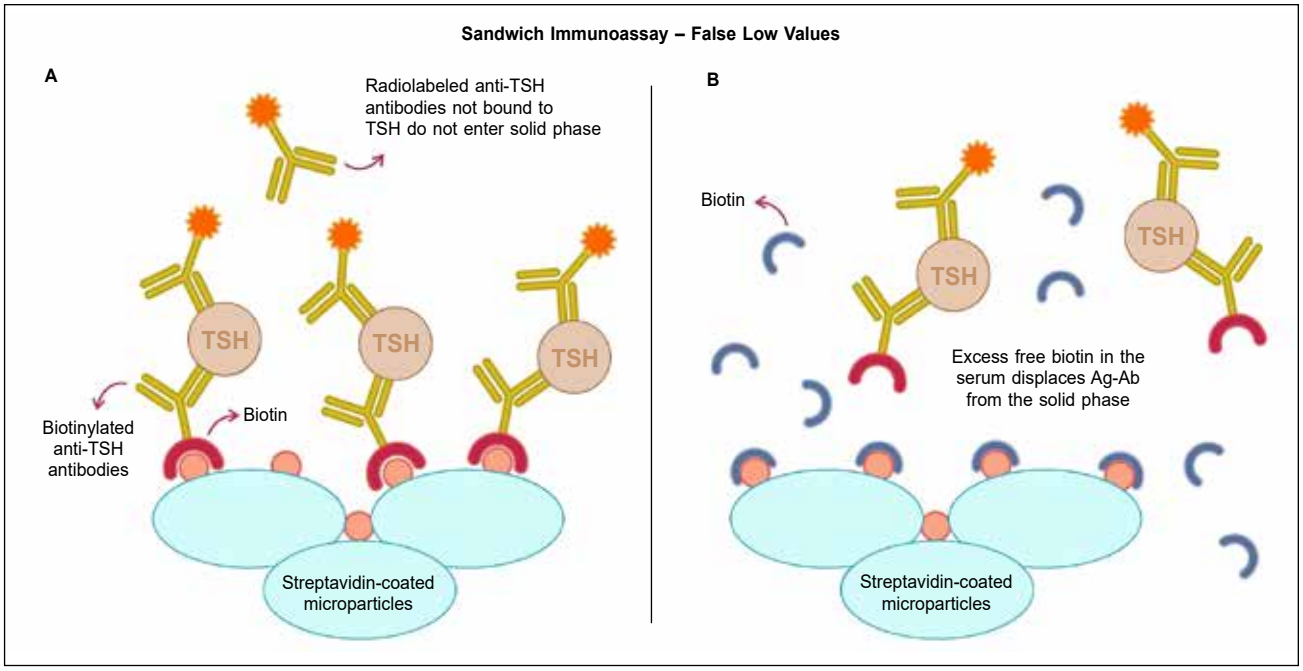

Figure 1. Effect of biotin on sandwich immunoassays utilizing streptavidin-biotin immobilizing systems.

Ag-Ab = Antigen-antibody; TSH = Thyroid-stimulating hormone.

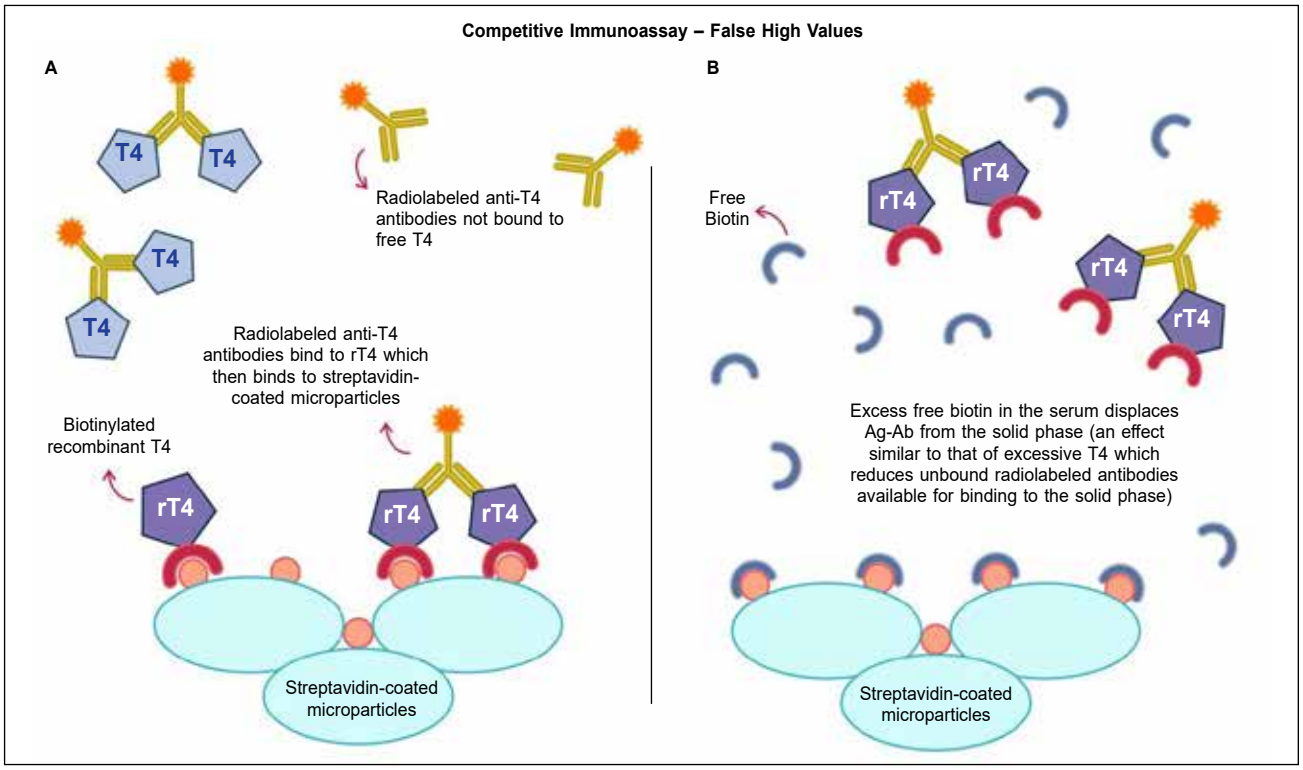

Figure 2. Effect of biotin on competitive immunoassays utilizing streptavidin-biotin immobilizing system.

rT4 = Radiolabeled thyroxine; T4 = Thyroxine.

evaluated washout periods, required for assays with interference thresholds ranging from 10 to 100 ng/mL, at biotin dose regimens ranging from 1 mg once daily to 300 mg 4 times daily[7]. For assays with an *in-vitro* interference threshold of >30 ng/mL, biotin doses of up to 5 mg twice daily or 10 mg once daily, an 8-hour

washout period is sufficient to mitigate the risk. If assays have an *in-vitro* interference threshold of <30 ng/mL, or in rare cases of biotin intake of >10 mg/day, sampling should be delayed for a more extended period (up to 73 h) after the last dose of biotin. Though the effect of biotin on FT3, FT4, and TSH estimation wanes in hours, anti-TSH receptor antibodies (TRAbs), which can also be falsely elevated due to assay interference, may take up to 7 days to normalize[2,8-10].

Clinical correlate: The TFTs could falsely suggest a diagnosis of overt or subclinical thyrotoxicosis or thyroid hormone resistance, and falsely elevated TRAbs can further mislead the diagnosis. History of intake of over-the-counter vitamin supplements containing biotin should be obtained. If an erroneous report is suspected, retesting should be done in serial dilutions (if using the same platform) or on another platform, which does not utilize SBIS (Centaur FT4, Diasorin, Abbott)[11,12]. Another option would be to repeat the test, after stopping biotin supplements for a duration which is determined by the dosage and analyte being assessed. Depletion protocols which involve pretreatment of the test sample with substances (e.g., streptavidin-coated particles) that bind biotin can also be used[13].

Heparin

TFTs performed in individuals receiving heparin, can demonstrate fallaciously elevated FT4 and FT3, with normal TSH levels[14,15].

Mechanism of interference: Lipoprotein lipase (LPL), released from vascular endothelium after heparin exposure, acts on triglycerides, to increase serum levels of nonesterified fatty acids (NEFA). High NEFA levels inhibit binding of thyroid hormone with their TBP, producing an increase in the measured free hormone levels[14]. There is a demonstrable increase in blood levels of LPL as well as free fatty acid (FFA), that persists *in-vitro*, in patients receiving heparin[14,15].

Relation to the type of heparin: The effect has been reported with the use of unfractionated heparin (UFH) as well as low molecular weight heparin (LMWH), irrespective of the route or dose. Standard subcutaneous doses, as well as intravenous doses as small as 0.08 U/kg, can increase the LPL activity of the serum[16]. It takes 10 hours or more after UFH injection for this effect to remit. Greater bioavailability and longer duration of action of LMWH might necessitate an interval of 24 hours after the last dose for this effect to wane[17].

Variations depending on assay and storage: This interference has been observed with different assays, including direct immunoassays, ultracentrifugation, and equilibrium dialysis[18]. Assays that require longer incubation periods (e.g., equilibrium dialysis) are most severely affected. As an extension of the same effect, preanalytical delays due to storage of samples before testing can also worsen the interference as *in-vitro* displacement of FT4 and FT3 continues[14].

Physiologic variations: The TFT abnormalities are not seen in all patients receiving heparin as there are other factors at play, namely serum levels of triglycerides and albumin. The released LPL requires an adequate concentration of the substrate, i.e., triglycerides (>180 mg/dL) in the serum to cause an increase in FFA. Albumin acts as a high-affinity binding protein for FFA; thus, its serum concentration affects the FFA-induced effects on thyroid hormone displacement. FT4 levels remain unaltered, till such time that the molar concentration of FFA is 5 times more than that of albumin, overwhelming its binding capacity[19].

Clinical correlate: Collecting blood samples after an adequate gap (at least 10 hours with UFH), immediate processing and testing and correlation with TSH can mitigate the problem. Estimating TT4 instead of FT4 is more reliable in individuals receiving heparin and also having hypertriglyceridemia[20].

DRUGS AFFECTING THYROXINE-BINDING GLOBULIN

Thyroxine-Binding Globulin Physiology

Thyroxine-binding globulin (TBG) is a high affinity, low concentration TBP that binds 80% of T3 and 75% of T4. Transthyretin (TTR) and serum albumin are the other TBPs in the blood. TBG utilizes both distributive and buffering mechanisms to stabilize the concentration of free thyroid hormones available to target tissues. This ensures a consistent supply of thyroid hormones where they are needed in the body[21,22]. Abnormalities in the TBG can be inherited or acquired, and drugs are a common etiology for acquired alteration.

Increase in Thyroxine-Binding Globulin

Estrogen: An elevated TT4 and TT3 level are observed in patients receiving estrogen, while FT4, FT3, and TSH values remain normal. It causes a dose-dependent increase in TBG, commencing in 2 weeks of initiation and reaching a new steady-state within 4 to 8 weeks. At routine doses of ethinyl estradiol (20-35 μg) or conjugated equine estrogen (CEE) (0.625 mg), there is an approximate increase of 30% to 50% in TBG and 20% to 35% in TT4[23-27]. The progesterone component of

oral contraceptives (OCs) does not alter this estrogen-induced effect; however, the androgenic properties of the progesterone do offset the net effect of the OC on TBG levels. OCs containing antiandrogenic progesterone like dienogest induce a higher rise in TBG (50%-60% vs. 30%) as compared to those containing androgenic levonorgestrel[28]. Transdermal estrogens do not affect TBG levels despite achieving comparable serum estrogen levels to oral formulations suggesting the possibility of a portal threshold for estrogenic stimulation of TBG[24,29].

Estrogen increases TBG levels predominantly by prolonging its half-life, along with a probable slight increase in synthesis. It induces a post-translational modification of TBG, resulting in molecules which are more resistant to hepatic degradation, due to the higher terminal sialic acid content[30]. The stimulatory effect of estrogen on TBG synthesis, has been observed in *in-vitro* studies[31,32].

Selective estrogen receptor modifiers: Selective estrogen receptor modifiers (SERMs) are molecules that can act as agonist or antagonist at the estrogen receptors, depending on the target tissue. Tamoxifen has a weak hepatic estrogen-agonistic effect and can cause a mild increase in serum TBG concentrations. The increases are lower than those observed during pregnancy or with estrogen use[33-36].

Raloxifene at a daily dose of 60 mg is used for postmenopausal osteoporosis. Six months of therapy with raloxifene did not significantly affect TBG or TT4 levels[37]. However, when the same dose of raloxifene was administered for 1 year, it resulted in a small but significant increase in serum TBG[38,39]. In postmenopausal women, droloxifene at the dose of 60 mg daily for 6 weeks, increased TBG by 41% from baseline. The increase in TBG with CEE (used as the comparator) was twice that observed with droloxifene. Although structurally similar to tamoxifen, droloxifene may have a more profound effect on serum TBG. It is unclear whether this is due to greater estrogen agonistic properties on the liver or is dose-related[40].

Heroin and methadone: Serum TBG concentrations are increased in about 25% to 50% of chronic heroin abusers or those under methadone therapy[41-43]. In a series of 285 euthyroid individuals with narcotic addiction, 22% had elevated TT4 and TBG. Normalization of both parameters after successful stabilization with methadone therapy[44]. Inhalational opium use is also associated with an increase in TT3 levels and TBG as assessed by T3 resin uptake (T3RU)[45,46]. T3RU is commonly used to evaluate TBG as there is an inverse association between T3RU and TBG[47].

The mechanisms involved in these changes have not been clearly understood. The rise in TBG could be related to increased synthesis or decreased degradation or both. The widely accepted theory is that of concomitant liver dysfunction, as TBG is synthesized in the liver[41,44,48]. In methadone treated addicts, high T3-binding ratio has been demonstrated to correlate with the degree of hepatic dysfunction directly. The hepatic dysfunction in these patients is multifactorial with contribution from the direct effect of opiates on the liver, dietary deficiencies, chronic and low-grade inflammation due to frequent injections, abuse of other drugs, and OC use[48]. Liver dysfunction, however, is unlikely to be the sole pathogenetic mechanism as these TBG changes are also seen in individuals on narcotics (8%-25%) with normal liver function[44].

Another proposed mechanism is through inhibition of thyroxine-metabolizing microsomal enzymes in the liver by morphine (a heroin metabolite), and contaminants (like quinine) and methadone[41,42]. Alteration in sex steroids by hepatic dysfunction or direct effects of heroin or methadone could also influence TBG levels[41].

5-Fluorouracil: 5-Fluorouracil has been associated with an increase in TT4 and TT3, but FT4 and TSH remain unchanged. It is probably due to an increase in TBG levels. There are not many studies reporting this effect of 5-fluorouracil[49].

Mitotane: Long-term use of mitotane is associated with an increase in the concentration of TBG and also a smaller increment of sex hormone-binding globulin and cortisol-binding globulin. The changes occur as early as 1 month after commencement of therapy and normalize gradually approximately 1 year after cessation of medications. The exact mechanism is not clear, and presumed to be related to reduce degradation due to enhanced sialylation or increased synthesis[50].

Interestingly, unlike the other medications which raise TBG, there is a decrease in TT4 levels by around 38% from the baseline value[50]. This is hypothesized to be due to competition of mitotane for binding sites on TBG or due to alteration in T4-binding characteristics[51].

There are also reports of reduced FT4 levels with prolonged mitotane use, with an inverse correlation between FT4 levels and serum mitotane concentrations. While the exact reason for this is unknown, in patients receiving mitotane, high FT3/FT4 ratios have been demonstrated, which indicate augmented deiodinase activity aiding the conversion of T4 to T3[52,53]. This is considered as a characteristic compensatory thyroid

function change in hypothyroid conditions[54]. Changes in FT3 or TSH levels have not been reported.

Clofibrate: Clofibrate, a hypolipidemic agent, has been discontinued since 2002, given the observed excess mortality despite successful cholesterol-lowering[55]. Increase in TBG and a small decrease in FT4 and FT3 levels were observed with this medication[56]. There was however no relationship between these changes and the lipid response to clofibrates[56,57].

Decrease in Thyroid-Binding Globulin

Androgens and anabolic steroids: Androgens, when used as replacement therapy in hypogonadal men or in supraphysiological doses for anabolic effects in athletes of both sexes, result in decreased TBG, TT4, and TT3 levels without affecting FT4 and TSH levels[58-61]. The rise starts within 1 week, reaching a maximum in 2 to 3 weeks[60].

The effect has also been observed with danazol used in its usual dose of 800 mg daily for treatment of endometriosis[62]. Androgen therapy with the nonaromatizable oral fluoxymesterone for metastatic, hormone-sensitive breast cancer, decreased serum levels of TT4 and TBG in 4 weeks. Normalization occurs within 6 to 12 weeks of therapy cessation. In hypothyroid women, on levothyroxine supplementation, the decrease in TBG caused clinical thyrotoxicosis (with elevated FT4 and suppressed TSH), requiring 25% to 50% dose reduction in thyroid hormone doses. The effect reversed after 8 to 10 weeks of cessation of androgen therapy[63]. In euthyroid female-to-male transsexuals, administration of testosterone esters at a dose of 250 mg intramuscular every 2 weeks, resulted in a 14% decrease in TBG but serum FT4 and TSH levels remained unchanged. The study also reported an increase in T3/T4 ratio indicating a probable upregulation of deiodinase activity by testosterone[29].

Though the exact mechanism is unclear; it is believed that androgens cause desialylation of TBG making it susceptible to degradation[63].

The net effect of a particular androgen on TBG levels depends on its aromatization rate to estrogen[60]. Adequacy of liver function also influences this effect, as observed in a study in males with alcoholic cirrhosis. Oral testosterone treatment resulted in TBG decrease in alcoholic cirrhotic men with mild liver dysfunction (Child-Turcotte Group A) and not in those with more advanced liver disease (Child-Turcotte Group B or C). This could be due to inadequate expression of sex hormone receptors in the liver, increase in serum estrogen concentration and use of antiandrogenic medication like spironolactone[64]. Short-term studies have suggested a possible shift of T4 binding from TBG to transthyretin during therapy with norethandrolone due to lower TBG levels[65]. From a clinical perspective, androgen therapy may unmask or worsen mild hyperthyroidism due to reduction of TBG levels[66].

Chronic glucocorticoid therapy: Endogenous glucocorticoid hypersecretion or exogenous glucocorticoid administration, at a rate of 2 to 20 times the basal adrenal glucocorticoid secretion, results in decreased TBG levels[67-69]. While it can occur with any route of administration, it depends on the dose and duration of the glucocorticoid therapy[69]. It is unclear how glucocorticoids cause this change; it is probably mediated through a decrease in the hepatic synthesis of TBG perhaps at a transcriptional level[68].

L-asparaginase: L-asparaginase decrease TBG levels, with a return to normal after drug withdrawal. The dose-dependent suppression of hepatic synthesis of the glycoprotein as demonstrated in *in-vitro* studies of cultured human hepatoma (HEP G2) cell lines may be responsible[70]. During the induction phase of acute lymphoblastic leukemia (ALL) treatment, L-asparaginase causes a reduction in TBG and TT4[71-73]. It occurs within 2 days to 2 weeks after administration and resolves in 2 to 4 weeks after cessation of medication. Two cases of transient hyperthyroidism occurring after L-asparaginase therapy for ALL, have been reported. There was an increase in FT4 along with TSH suppression accompanied by clinical features of thyrotoxicosis in both the cases[74].

The mechanism involved is presumed to be due to the general ability of L-asparaginase to suppress protein synthesis in the liver along with concomitantly suppressing the production of TBG[70].

Nicotinic acid: Nicotinic acid can result in decreased TBG and TT4 levels without affecting FT4 and TSH values[75-78]. O'Brien et al proposed nicotinic acid associated hepatitis or subclinical liver dysfunction, while Drinka et al attributed a direct drug effect as the cause for the change in TBG[76,77]. Shakir et al ascribed mobilization of fatty acids from the periphery, with subsequent interference to binding of thyroid hormones to TBG as another mechanism[75]. The exact mechanism is not clearly understood.

DRUGS ALTERING THE BINDING OF THYROID HORMONE TO THYROID-BINDING PROTEINS

Displacement of thyroid hormones from TBPs like TBG and TTR can alter thyroid test results.

Salicylates and Nonsteroidal Anti-inflammatory Drugs

Salicylates and salsalate inhibit binding of T4 to all three TBPs with maximum effect on TBG[79,80]. This occurs at doses of >2 g daily of salicylates and 1.5-3 g daily of salsalate. There is an initial and transient increase in FT4 levels at drug initiation, which is then followed by a decrease in TT4 and normalization of FT4 levels as therapeutic concentrations are maintained[81]. Salsalate may produce a more significant decline in T4 levels as compared to salicylate (30%-40% vs. 20%-30%)[80,82].

An analysis of different nonsteroidal anti-inflammatory drugs (NSAIDs) revealed that ibuprofen, naproxen, and indomethacin did not alter hormone levels, while aspirin caused a decrease in TT4, TT3, TSH after 1 week probably due to dilutional artefact. Meclofenamate caused transient fluctuation of hormone levels which normalized in a week. All subjects remained clinically euthyroid during the 1 week study period, and TSH remained within normal range[83].

Frusemide

Frusemide displaces thyroid hormones from TBG, TTR, and albumin, causing a transient increase in FT4 and decrease in TT4[84]. The effect occurs at large intravenous doses of >80 mg and not at usual therapeutic doses[67]. The changes depend on the interval between drug administration and sample collection, occurring 2 to 5 hours after frusemide doses of 80, 120, or 250 mg[85]. Other factors involved are the renal drug clearance rate and serum concentration of albumin, which also binds frusemide[67].

Phenytoin and Carbamazepine

Phenytoin and carbamazepine lower TT4, TT3, FT3, and FT4 levels without affecting serum TSH levels, in euthyroid individuals[86]. These drugs at therapeutic levels displace thyroid hormones from TBP. This is distinct from the class effect of augmented T4 and T3 metabolism, by induction of hepatic microsomal P450 enzymes. Low FT4 levels are presumably artefactual, and is believed to occur due to serum dilution during the estimation process[87]. Direct measurement of FT4 by equilibrium dialysis or indirect assessment by measuring FT4 index can overcome the effect[88].

CONCLUSIONS

Several medications can lead to abnormal TFT results in euthyroid individuals, despite no clinically significant impact on thyroid function itself. This can occur due to assay interference, changes in TBP levels, or alterations in their affinity for thyroid hormones. Awareness of these drug-related effects is crucial because while they may not require medical intervention, they can potentially mislead clinicians.

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