

Biotin Effects on Thyroid Function Tests – Facts, Fancies, and Fallacies.

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ABSTRACT

Many recent reports warn clinicians of possible interferents which may lead to spurious Thyroid Function Test (TFT) results. The effect of exogenous biotin causing false hyperthyroidism has received attention. Clinicians must understand the realities of biotin effects on TFTs. Biotin is used in many automated immunoassay analyzers to label the Thyroid Stimulating Hormone (TSH), free T4 (fT4), free T3 (fT3), total T4 (tT4), total T3 (tT3), Thyroglobulin Antibodies (Tg-Ab), Thyroid Peroxidase Antibodies (TPO-Ab) and TSH receptor antibodies (TRAb) in competitive and non-competitive assay formats. Exogenous biotin competes with the biotinylated antigen/antibody complexes to reduce signals generated by the solid phase resulting in falsely raised fT4/fT3/TRAb and falsely low TSH results that mimics hyperthyroidism in asymptomatic euthyroid patients. Small doses of biotin (30-3000ug) found in over-the-counter multi-vitamins are insufficient to affect TFTs while minor changes are seen with higher doses in life-style biotin-only supplements (2.5-25mg). A clinician must be cautious when biotin is used in therapeutic (20-40mg biotin/d) and supra-therapeutic doses (100-300mg biotin/d) in the treatment of inherited metabolic disorders and multiple sclerosis as they can cause hyperthyroid TFTs. There is marked variability in susceptibility to biotin interference in between individuals, TFT analytes and immunoassay analyzers, as well as varied results with similar doses of biotin taken orally or similar biotin concentrations spiked in serum. However, even in populations with widespread biotin use, the prevalence of significant biotin-related effects is rare. In addition, there are interferents other than biotin (e.g. anti-streptavidin and anti-ruthenium antibodies) that can cause similar hyperthyroid-like effects on TFTs. It is important for doctors to understand that although biotin can affect TFTs, the usual dosage consumed is too small to cause any clinically significant effect on TFTs in most cases. Should biotin interference be suspected, it would be prudent to discuss results with the clinical laboratory.

ABBREVIATIONS

TFT – thyroid function test, TSH - thyroid stimulating hormone, free T4 - fT4, free T3 - fT3, total T4 – tT4, total T3 - tT3, thyroglobulin antibodies - Tg-Ab, thyroid peroxidase antibodies - TPO-Ab, TSH receptor antibodies – TRAb, limit of detection – LoD, Emergency Department – ED, multiple sclerosis (MS), end-stage renal disease – ESRD

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INTRODUCTION

Thyroid Function Testing (TFT) is indispensable in the management of thyroid diseases. They are performed on fully automated immunoassay analyzers. Accurate TFT is essential for the diagnosis and monitoring of all thyroid conditions. Analytical interferences are inherent in immunoassays including those that cause spurious TFT results. Recently there has been a surge in reports of biotin-induced spurious TFT [1-3]. On susceptible analyzers, biotin may be associated with elevated levels of free thyroxine (fT4) and free triiodothyronine (fT3), suppressed Thyroid-Stimulating Hormone (TSH) and elevated levels of TSH receptor antibodies (TRAb) - mimicking thyrotoxicosis [1]. Biotin may also affect results of other hormones and analytes [2,3].

Biotin (also known as vitamin B7) is ubiquitous in the normal daily diet. Thus supplementation is not required. The recommended daily allowance for biotin is 30ug [4,5]. Biotin reaches peak serum levels 1-3h after oral ingestion [6,7]. Normal blood concentrations of biotin range from 0.03-0.09ng/mL [8]. When used in consecutive dosing biotin can accumulate [9]. Its half-life is roughly 15h, reaching a steady state at around 3 days. Biotin, metabolized in part to bisnorbiotin and biotin sulfoxide, is excreted by the kidney [6].

FACTS

Fact - Biotin is used in immunoassays

Biotin is used in many modern automated immunoassay analyzers to label the antigen (e.g. fT4, TSH) or antibody (e.g. TRAb). Biotin's affinity for streptavidin enables the biotinylated antigen/antibody complexes to bind to streptavidin with very high specificity and low non-specific binding [10]. Once bound onto a streptavidin-coated solid phase, the interaction generates a signal that is quantified as the analyte concentration. In sandwich (non-competitive) assays (e.g. TSH) the signal is proportional to the analyte concentration; in competitive assays (e.g. fT4, fT3, TRAb) the signal is inversely proportional to the analyte concentration [10,11]. The major manufacturers employing biotin-based thyroid immunoassays [8] include Roche (TSH, fT4, fT3, total T4, total T3), Siemens (TSH, fT4, fT3), Beckman (fT4, fT3), Ortho (TSH); Abbott does not use biotin in its assays. Between 50-60% of the TFTs in France in 2016 are biotin-based and thus potentially at-risk

from biotin interference [8] - TSH 500/953, fT4 509/810, fT3 442/709.

Fact - Biotin can interfere in TFT immunoassays

Excess biotin in serum prevents the biotinylated antigen/antibody from interacting with the streptavidin-coated solid phase resulting in a low immunoassay detection signal [4,7]. The impaired signal is falsely translated as a low analyte concentration in the sandwich assays (low TSH) and a high analyte value in competitive assays (high fT4, fT3, TRAb) [8,11]. It is this combination of TFT results that resembles a "hyperthyroid" profile. Biotin's propensity to impact TFT results varies with biotin dose, type of immunoassay analyzer, and across different analytes. Manufacturers such as Roche warn of potential assay interferences when biotin consumption exceeds 5-10mg/d; the serum biotin interference threshold is given in the assay package insert - TSH 20ng/mL, fT4 25ng/mL, total T3 10ng/mL, total T4 100ng/mL [9]. This critical biotin interference threshold has been independently verified to be slightly higher - TSH 30ng/mL, fT4 61ng/mL, total T3 19ng/mL, total T4 348ng/mL [9].

Fact - Biotin consumption is common

Exogenous biotin arises from the daily use of multivitamins containing biotin. Most over-the-counter multivitamin supplements contain small quantities of biotin (30-300ug) per tablet [10]. Less frequently used is higher-dose biotin in lifestyle supplementation for hair, nail and skin health; some of these formulations containing up to 10mg of biotin [11]. Mega-dose biotin is prescribed for medical conditions like propionic acidemia or biotinidase deficiency with 10-40mg/d of biotin [7,13]. Ultra-high dose biotin (up to 300mg/d) has been used in some clinical protocols for multiple sclerosis (MS) and demyelinating pathologies [14]. Biotin use is quite common. From an outpatient survey (questionnaire) of 1944 respondents at the Mayo clinic [12], 42% of patients were consuming multivitamins (which contain small doses of biotin) while another 7.7% reported using biotin supplements. In addition, biotin was measurable by tandem mass spectrometry above the Limit of Detection (LoD) of 5ng/mL in 48.9% of serum from 1442 patients from the Emergency Department (ED) at Mayo [12]. Since subjects taking 10mg of biotin/d achieve a serum biotin of 3.6ng/mL [15], some of the 51.1% of the subjects in the

Mayo study with biotin levels below the LoD could well have been consuming biotin. Thus the actual prevalence of exogenous biotin use is likely to be over 50% in the Mayo ED population.

FANCIES

Fancies – Biotin interferes with TFT results

With the volume of reports on biotin and TFTs [1-4,6-8,11-26], it is easy to believe that biotin is a major interferent in thyroid test results and that “biotin-itis” is endemic [27]. However, careful analysis suggests otherwise. The frequency and prevalence of biotin supplementation causing interference in diagnostic tests is difficult to assess [27,28]. Moreover, the levels of biotin at which factitious TFTs occur vary across different immunoassay platforms [11]. Biotin concentrations in serum depends on many other factors including amount of endogenous biotin and metabolites present, type of biotin supplement consumed, dose and duration of biotin intake, and timing of blood draw after biotin ingestion [11]. Consuming 30ug, 300ug or 3000ug (3mg) biotin does not result in significant differences between pre- and post-biotin TFT results [20]. At 10 mg biotin/d for 1 week the serum biotin in 6 healthy volunteers was 3.6ng/mL [15]. In this study baseline TFTs on the Roche platform showed a decline in TSH of 0.72mU/L from 1.92 ($p=0.006$), elevation in fT4 of 0.13ng/dL from 1.24 ($p=0.01$) and increase in fT3 of 0.36pg/mL from 3.21 ($p=0.005$); they were all within the reference intervals. The mean fT3 on the Siemens Vista analyzer also rose by 0.78 pg/mL ($p<0.001$) with 1 value outside the reference range. However, total T3 (Roche) increased from 1.02ng/mL to 1.87 ($p=0.001$) with 3 out of 6 values above the upper reference limit. Mean TSH values on the Ortho Vitros 5600 showed a very significant decrease from 1.77mU/L to 0.1 ($p<0.001$) with all 6 values below the reference range [15]. In another study [23] Biscolla administered 10 mg biotin to 19 volunteers [23]. The mean pre-biotin TSH on the Roche assay decreased from 2.84mU/L to 1.66 ($p<0.005$) after biotin intake; all values were within the reference limits. Mean pre-biotin fT4 measured on the Beckman Access analyzer increased from 0.8ng/dL to 1.2 ($p<0.0001$) post-ingestion; 52% (10/19) of the values were outside the upper reference limit. In the Mayo study [12], 107 of the ED patients (7.4%) had a serum biotin levels exceeding the interference threshold of 10ng/mL; 48

patients had an immunoassay test of which only 1 patient had a biotin concentration that exceeded the interference threshold stated in the manufacturer's package insert for NTproBNP only. The Mayo study [12] also reported that two of the highest plasma biotin (117ng/mL and 280ng/mL) were encountered in patients with End-Stage Renal Disease (ESRD). Another study [16] reported 2 cases of ESRD with unusual levels of TSH and fT4 due to raised biotin levels. It is thus important to actively seek out any history of kidney disease or exogenous biotin intake especially in those with ESRD to help in the interpretation of their TFTs.

In their study, Piketty [17] also noted that the serum biotin concentration achieved in a 2 healthy volunteer who took 15 mg/d of biotin were 31.7 and 43.9 ng/mL respectively while another on 30mg/d of biotin was 56.8ng/mL [17]. At these serum biotin concentrations there was no significant shift in thyroid function tests; fT3, fT4 and TSH remained within the reference range. This underscores the variability in serum levels after biotin ingestion in contrast to the 6 subjects on 10mg/d who attained a similar biotin level of 3.6ng/mL [15]. There is also great variability in how much biotin is required to cause a significant interference for each analyte. Piketty [17] reported that a biotin concentration of over 180ng/mL is needed for a significant effect on TSH, ≥ 233 ng/mL for fT4 and ≥ 363 ng/mL for fT3 respectively. The Piketty study also revealed that only the patients receiving very high doses of biotin (100-300mg/d) had significantly raised serum biotin concentrations. There were 9 MS patients receiving 300mg of biotin daily, and 8 healthy controls who received single biotin doses of 100, 200 or 300mg. The range of biotin concentrations in their blood was 169-1160ng/mL [17]. Other case studies where biotin caused a significant shift in the thyroid function tests [18-20] also involved patients with MS who had been prescribed supra-therapeutic doses of biotin, not patients taking over-the-counter supplements. When serum is spiked with biotin to a level of 15.6ng/mL to simulate a 5mg biotin intake [24] Trambas found minimal effects on fT4 and fT3 ($<5\%$ analytical bias) and TSH (10% negative bias) but larger positive bias in the thyroid antibodies - anti-thyroglobulin (Tg-Ab) 10-20%, anti-thyroid peroxidase (TPO-Ab) 60-70%, and TRAb 80-90%. At a spiked biotin concentration of 31.3ng/mL (simulating a 10mg biotin dose) fT4 and fT3 were unaffected (5-10% bias) but

TSH declined by 20%. The analytical bias in thyroid antibodies was wider – Tg-Ab 50%, TPO-Ab 250%, TRAb 750%. There was marked variation in the susceptibility to biotin interference both in terms of the biotin concentration needed to cause detectable interference and the maximum observed effect. Antithyroid antibodies were exquisitely sensitive to low biotin concentrations (<100ng/mL); fT4/fT3 required 200ng/mL of biotin in serum before large changes in values were noted, but the magnitude in elevation of fT4 and fT3 values differed. At a spiked biotin concentration of 500ng/mL, the percentage increase in fT3 concentrations ranged from 235-427% while that for fT4 ranged from 433-1146%.

In a supplement and recovery study [25] the effect of 11 different biotin concentrations on thyroid function assays using the Cobas e602 (Roche) were studied. Results were altered for TSH, free T4 and free T3, at 80ng/mL, 320ng/mL and 320ng/mL of biotin respectively. However, on the Dimension Vista (Siemens) TSH, fT4, and fT3 were shown to be altered at biotin concentrations of 320, 320, and 160ng/mL respectively. Another study [26] using the Cobas e602 (Roche) system found TSH being significantly affected (-10% change) at 250ng/mL of biotin while fT4/fT3 required biotin concentrations of >500ng/mL. The extent of biotin interference was not dependent on the baseline TSH level. Biotin (250ng/mL) reduced a TSH level of 1.65mU/L by 12.4% while in another case the TSH of 5.86mU/L was reduced by 12.8%.

It is important to note that a spiking study only defines interference from pure biotin as serum also contains biotin metabolites which may in turn also affect TFT results. This could account, in part, for the widely variable and unpredictable effects of biotin on TFTs.

Fancies - Biotin interference is common

One study [12] sought to establish the prevalence of biotin use and biotin concentrations in serum in their patient population. From the previous section the tipping point for biotin effects on TFTs is around 30ng/mL. Out of 1442 samples tested for biotin by mass spectrometry, only 7 samples (0.5%) had a biotin concentration of ≥ 30 ng/mL, despite nearly 42% of their population reported multi-vitamin or supplement use [12]. Thus none of the 1435 samples (99.5%) with biotin concentrations under 30ng/mL will be expected to have impaired TFT results according to the Piketty data [17] alluded to in the previous

section. Thus, despite widespread use of supplements, the proportion of subjects with significant biotin concentrations that can affect laboratory results is actually quite small. As demonstrated in the Katzman study [12] biotin consumption per se will not necessarily lead to interference in test results. Besides, the prevalence of biotin use is likely to be influenced by population demographics, geography and socio-cultural factors. Even in populations with extensive biotin consumption, serum biotin concentrations exceeding 30ng/mL are still rare.

Fancies - Biotin effects in individuals and assays are predictable

There is marked variability in susceptibility to biotin interference, both between individuals and in the assays affected. A review of some studies [8] shows that even for similar daily doses of biotin (300 mg/d), the effects on serum TFT values can vary widely; fT4 ranging from 50 to >100pmol/L have been reported (20-22). Thus, the magnitude of TFT change for similar doses of biotin is also very variable. When using a similar assay and even after trying to standardize the timing of blood sampling [17], serum biotin concentrations can also vary markedly with very uneven effects on TFT levels. Besides, different laboratories may also employ different assay platforms for the different components of TFTs. These studies demonstrate that the amount of biotin required to interfere with TFT immunoassays is highly assay-, platform-, and biotin concentration-dependent. The biotin interference occurred at concentrations significantly higher than those encountered with subjects taking regular over-the-counter vitamin supplements.

FALLACIES

Not all biotin-like aberrant TFTs are due to biotin

Anti-streptavidin antibodies: Anti-streptavidin antibodies can easily cause aberrant readings in a fashion similar to biotin in streptavidin-based assays - reduced signal intensity translating into a raised FT4 with low TSH. One of the first published descriptions of interference from antibodies against streptavidin (used in the streptavidin-biotin assay systems) [29] was in a 61-year-old man with TFTs that appeared hyperthyroid on the Roche assays. However, the result was normal on a non-streptavidin assay platform (Siemens Centaur). Lam [30] reported on a patient with worsening fatigue and symptoms of hypothyroidism while on carbimazole,

despite a TSH of 0.75mU/L and FT4 of 12pmol/L on the Roche assay. Another patient on carbimazole had stopped her antithyroid therapy due to pregnancy and was developing hyperthyroid symptoms; FT4 was elevated at 31.9pmol/L but TSH was not suppressed (0.33mU/L). When tested on an alternative TFT assay platform (Siemens Centaur), results were markedly different. The first patient had TSH of 37mU/L with fT4 of 7pmol/L, and the second patient was shown to be euthyroid. TFTs on the Roche platform normalized after incubation with streptavidin microparticles, yet, there was no history of biotin intake in either patient. It was then discovered that anti-streptavidin antibodies of the IgM isotype were the cause for the discrepancies. Other cases of anti-streptavidin antibodies causing spurious hyperthyroidism or Graves' disease have also been reported [31].

Anti-ruthenium antibodies: Another possible interferent is antibodies directed against ruthenium, the label used to generate signals in the Roche streptavidin-biotin assays. A series of cases in one study [32] showed inappropriately low TSH levels with raised levels of FT3/FT4 despite being symptomatically euthyroid. Only after Roche improved their assay reagents to counter this antibody activity did the FT3 show any correction. In another study [33] two patients had inappropriately high FT4 despite being symptomatically euthyroid. When tested with two different assays (Roche vs. Architect), the Roche assay showed a spuriously high FT4. This was subsequently proved to be due to anti-ruthenium antibodies.

REALITY

It is prudent to appreciate that the consumption of the common over-the-counter supplements containing biotin rarely results in biotin levels significant enough to interfere with TFTs. However, the managing clinician must be wary of patients with a history of kidney disease, MS, or the rare in-born errors of metabolism since their biotin levels can be high enough to cause aberrant TFT results. When such risk factors are present, it is advisable to follow the latest 2016 American Thyroid Association guidelines on hyperthyroidism [34]. They recommend that patients stop taking biotin for 2 days before TFTs are ordered. When biotin effects are suspected it would be appropriate to discuss the results with the laboratory to clarify the assay used. Biotin has different propensity to affect different assays and analytical

platforms. The Roche package insert warns of biotin interference in patients treated with >5mg/d of biotin. It is also important to remember that the effects of biotin are extremely variable.

MINIMIZING RISKS

What can institutions do to minimize the risk of biotin interference? When biotin interference is suspected, the affected samples should be retested on another non-biotin platform in the laboratory (if available) or sent to a referral laboratory. Alternatively, the laboratory may neutralize the biotin in the sample and re-test the sample thereafter. One such method has been described by Piketty et al [17] who utilized magnetic microparticles coated with streptavidin to absorb excess biotin. All MS patients in their study who had been treated with up to 300mg/d of biotin had biotin concentrations below the limit of quantitation after these samples were so treated. Post-treatment the fT4 of the patients with MS in the study normalized to between 13.0-18.6pmol/L (1.0-1.4ng/dL); pre-treatment fT3 of 5.21-15.3pmol/L (0.34-1.00ng/dL) normalized to 4.5-5.8pmol/L (0.29-0.38ng/dL) while pre-treatment TSH (<0.01-0.66mU/L) increased to 0.83-3.9mU/L. Trambas et al [35] also used a similar method to good effect.

CONCLUSION

Clinicians need to be cognizant of the effects of biotin on thyroid testing. Laboratorians also need to communicate to their clinicians the potential effects of biotin on the analytical platforms that they employ. Both the clinic and the laboratory need to be aware of when and how likely biotin will cause a genuine discrepancy in TFT results. When a patient has TFTs that appear hyperthyroid but do not correlate with clinical findings, combined with a history of no biotin intake, it would be prudent to discuss the case with the laboratory to elucidate the cause.

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