Factitious Graves’ Disease Due to Biotin Immunoassay Interference—A Case and Review of the Literature

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Context: Biotin (vitamin B7) is an essential co-factor for four carboxylases involved in fatty acid metabolism, leucine degradation, and gluconeogenesis. The recommended daily intake (RDI) of biotin is approximately 30 μg per day. Low-moderate dose biotin is a common component of multivitamin preparations, and high-dose biotin (10 000 times RDI) has been reported to improve clinical outcomes and quality of life in patients with progressive multiple sclerosis. Biotin is also a component of immunoassays, and supplementation may cause interference in both thyroid and non-thyroid immunoassays.

Objective: To assess whether biotin ingestion caused abnormal thyroid function tests (TFTs) in a patient through assay interference.

Design: We report a patient with biotin-associated abnormal TFTs and a systematic review of the literature.

Setting: A tertiary endocrine service in Hamilton, New Zealand.

Results: The patient had markedly abnormal TFTs that did not match the clinical context. After biotin cessation, TFTs normalized far more rapidly than possible given the half-life of T4, consistent with assay interference by biotin. Multiple other analytes also tested abnormal in the presence of biotin.

Conclusion: Biotin ingested in moderate to high doses can cause immunoassay interference. Depending on the assay format, biotin interference can result in either falsely high or low values. Interference is not limited to thyroid tests and has the potential to affect a wide range of analytes. It is important for clinicians to be aware of this interaction to prevent misdiagnosis and inappropriate treatment. (J Clin Endocrinol Metab 101: 3251–3255, 2016)

Biotin (vitamin B7) is an essential cofactor for carboxylases involved in fatty acid metabolism, leucine degradation, and gluconeogenesis. The recommended daily intake (RDI) of biotin is 30 μg per day (1). Biotin doses up to 100 times the RDI are frequently encountered in nutritional supplements promoted for conditions such as type 2 diabetes mellitus (2) and are reported to be beneficial for skin and hair. High-dose biotin (10 000 times RDI) has been documented to improve clinical outcomes and quality of life in patients with secondary progressive multiple sclerosis (MS) (3, 4).

Many immunoassays use the biotin-streptavidin interaction as an immobilizing system. Streptavidin binds biotin with high affinity (5). Biotin, being a small molecule, can readily be incorporated into a hormone or an antibody to that hormone. Depending on whether the assay is a competitive immunoassay or an immunometric (sandwich) assay, high circulating levels of biotin may result in falsely high or low levels of the ligand being measured. As such, ingestion of biotin interferes with both thyroid and non-thyroid immunoassays.

Abbreviations: FT₄, free T₄; MS, multiple sclerosis; TFT, thyroid function test; TRAb, TSH receptor antibody.
We report here on our experience of assay interference by biotin mimicking Graves’ disease and the systematic review of the literature we conducted.

### Subject and Methods

A systematic review of articles published before April 6, 2016, was conducted independently by two authors (J.V.C. and M.S.E.). OVID MEDLINE and PubMed search engines were used to identify relevant articles using the keywords “biotin,” “thyroid,” and “interference.” References of relevant articles were also reviewed to identify additional papers not detected by the search engines.

### Case

A 63-year-old New Zealand European woman was referred for assessment of new-onset thyrotoxicosis by her primary practitioner. She had a background of secondary progressive MS diagnosed 15 years earlier. She had never received β-interferon.
or other immunotherapy. Her neurologist had recently started her on biotin 100 mg three times daily (Pharmaceutical Compounding NZ Limited). Apart from fatigue, she had no symptoms of thyrotoxicosis. There was no past or family history of thyroid disease. On examination, she was clinically euthyroid with no goiter or thyroid eye disease. Given the discordance between the clinical and biochemical results, assay interference was suspected.

Thyroid function tests (TFTs) measured 6 months earlier were within the reference interval. TFTs performed using two different immunoassay platforms (Cobas 6000) and Beckman-Coulter DxI are shown in Table 1. At the time of presentation TSH receptor antibodies (TRAbs) were markedly elevated at 40 IU/L (reference interval, 1.3 IU/L). Biotin was stopped, and repeated TFTs were normal 3 days later. The patient had noted symptomatic benefit of her MS after starting biotin, so biotin was reintroduced. TFTs taken after an overnight fast and 16 hours after her last dose of biotin again showed evidence of biotin interference (Table 1).

Systematic review

A total of 585 articles were identified. After review of titles and abstracts, 17 papers were reviewed in more detail. Of these, six papers were selected for inclusion (6–11). The process whereby articles were selected for the systematic review is shown in Supplemental Figure 1. The six papers described eight cases. These are summarized in Table 2. Refs. 6–9 were published in biochemistry journals; however, the Endocrine Society’s Endocrine News also recently featured biotin interference of immunoassays (12).

**Results and Discussion**

This patient had markedly abnormal TFTs in the absence of clinical features of thyrotoxicosis. For thyroid immunoassays, biotin interference may result in a biochemical picture of severe hyperthyroidism indistinguishable biochemically from true hyperthyroidism or an atypical pattern with elevated free thyroid hormone levels and an inappropriately normal TSH level. Fortunately in this case, the likely diagnosis was recognized at first assessment, thus avoiding unnecessary treatment. In addition, the cessation of biotin normalized TFTs faster than the 7-day half-life of clearance of T4 consistent with assay interference by exogenous biotin.

Biotin-induced interference can affect other assays, as shown by the results in Table 1, potentially causing a very confusing biochemical picture. Biotin binds to streptavidin with high affinity and high specificity, making it useful as a general bridge system (5). Biotin-induced interference in immunoassays depends on the assay format. Free T4

### Table 2. Summary of Cases Identified in Systematic Review

<table>
<thead>
<tr>
<th>First Author (Ref.) Year</th>
<th>Case</th>
<th>Biotin Dose</th>
<th>Analyte/s Affected</th>
<th>Direction of Interference</th>
<th>Clinical Consequences</th>
</tr>
</thead>
<tbody>
<tr>
<td>Henry (6) 1996</td>
<td>Newborn baby</td>
<td>10 mg OD</td>
<td>FT4, TSH</td>
<td>↓</td>
<td>Delay in treating hypothyroidism</td>
</tr>
<tr>
<td>Meany (7) 2009</td>
<td>64-year-old female, ESRF</td>
<td>10 mg OD</td>
<td>PTH</td>
<td>↓</td>
<td>Delay in treating severe secondary hyperparathyroidism</td>
</tr>
<tr>
<td>Kwok (8) 2012</td>
<td>3-year-old girl</td>
<td>10 mg QID</td>
<td>FT4, FT3, TSH</td>
<td>↔ ↔ ↑</td>
<td>Nil</td>
</tr>
<tr>
<td>Wijeratne (9) 2012</td>
<td>1-wk-old baby</td>
<td>10 mg TDS</td>
<td>FT4, FT3</td>
<td>↑ ↔ ↔</td>
<td>Nil</td>
</tr>
<tr>
<td>Wijeratne (9) 2012</td>
<td>Male</td>
<td>30 mg single dose</td>
<td>FT4</td>
<td>↑</td>
<td>Peak interference at 2 h, duration and magnitude varied according to analyte</td>
</tr>
<tr>
<td>Waghray (10) 2013</td>
<td>60-year-old female, PHPT</td>
<td>1.5 mg OD</td>
<td>PTH</td>
<td>↓</td>
<td>Nil</td>
</tr>
<tr>
<td>Waghray (10) 2013</td>
<td>62-year-old female, PHPT</td>
<td>5 mg OD</td>
<td>PTH</td>
<td>↓</td>
<td>Nil</td>
</tr>
<tr>
<td>Barbesino (11) 2016</td>
<td>55-year-old man, MS</td>
<td>100 mg TDS</td>
<td>FT4</td>
<td>↑</td>
<td>Nil</td>
</tr>
</tbody>
</table>

Abbreviations: OD, once daily; ESRF, end stage renal failure; QID, four times daily; TDS, three times daily; PHPT, primary hyperparathyroidism; TG, thyroglobulin; DHEAS, dehydroepiandrosterone sulfate; E2, estradiol.

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biotinylated T4 antibody complex. The microparticles coated microparticles are also added, capturing the biotinylated T4 antibody complex. The microparticles are captured magnetically, and unbound substances are washed away. Application of voltage generates a signal via the Ru-complex (13). In competitive assays, the signal is inversely proportional to analyte (FT4) concentration. Biotin in the sample competes with biotinylated T4 for binding with streptavidin (8), causing a low signal and falsely high result.

TSH is a sandwich assay. The assay principle on the Cobas is as follows: during the first incubation, biotinylated TSH-specific antibody and Ru-complex-labeled antibodies bind TSH, forming a sandwich complex. During the second incubation, streptavidin-coated microparticles are added, capturing the biotinylated sandwich complex. The microparticles are magnetically captured, unbound substances are washed away, and voltage is applied, generating a signal via the Ru-complex (14). In sandwich assays, the signal is proportional to analyte (TSH) concentration. Biotin in the sample competes with the biotinylated sandwich complex for binding with streptavidin (8), causing a low signal and falsely low result.

Few previous studies have evaluated biotin-immunoassay interference. Previous assay studies were based on the ingestion of a single 30-mg dose of biotin by a male author (9). Biotin levels of 50 and 125 µg/L have been demonstrated to result in interference of TSH (sandwich assay) and FT4 on a competitive assay, respectively (6), whereas biotin levels of ≥ 20 µg/L have been reported to falsely lower TSH levels in the Cobas e601 sandwich assay (8). Based on the levels of observed assay interference, the authors calculated that the 10-mg dose received by the newborn baby was likely to result in biotin levels > 400 µg/L (6). The duration of interference appears to vary according to analyte (9). The Roche Cobas package insert recommends that samples should not be taken from patients receiving doses of biotin for at least 8 hours after the last biotin administration (13). Here we show that there is still marked biotin interference of TFTs even 16 hours after the last dose. It is also important to be aware that not only TFTs are affected by biotin interference. Common immunoassays that may be affected by biotin ingestion are listed in Supplemental Table 1. Interference by antibodies to streptavidin have also been reported and so may result in a similar pattern without exogenous biotin intake (15).

Antibodies against biotin have also been reported (16) and could potentially interfere with the binding of biotin to streptavidin, thus also resulting in assay interference.

Conclusions
It is likely, with recent data demonstrating a potential benefit of high-dose biotin in MS and with the promotion of biotin use in complementary medicine, that increasing numbers of cases of assay interference with biotin will be seen. It is important that clinicians be aware of the potential for immunoassay interference by biotin in order to avoid misdiagnosis and patient harm due to inappropriate treatment.

Acknowledgments
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Disclosure Summary: The authors have nothing to disclose.

References
9. Wijeratne NG, Doery JC, Lu ZX. Positive and negative interference


