

## CLINICAL QUESTION

# What should be done when thyroid function tests do not make sense?

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## Summary

Interpretation of thyroid function tests (TFTs) is generally straightforward. However, in a minority of contexts the results of thyroid hormone and thyrotropin measurements either conflict with the clinical picture or form an unusual pattern. In many such cases, reassessment of the clinical context provides an explanation for the discrepant TFTs; in other instances, interference in one or other laboratory assays can be shown to account for divergent results; uncommonly, genetic defects in the hypothalamic–pituitary–thyroid axis are associated with anomalous TFTs. Failure to recognize these potential ‘pitfalls’ can lead to misdiagnosis and inappropriate management. Here, focusing particularly on the combination of hyperthyroxinaemia with nonsuppressed thyrotropin, we show how a structured approach to investigation can help make sense of atypical TFTs.

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## Introduction

Thyroid function tests (TFTs) are amongst the most commonly requested laboratory investigations.<sup>1</sup> Fortunately, in most patients the interpretation of TFTs is straightforward with the combination of thyroid hormone (TH) and thyrotropin (TSH) measurements confirming euthyroidism, thyrotoxicosis or hypothyroidism consistent with their clinical status. However, in a small but significant

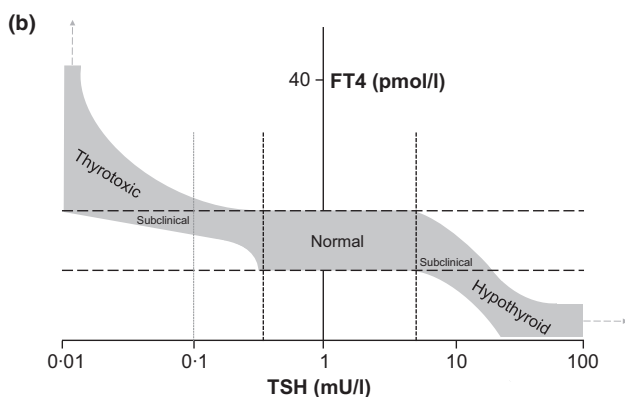
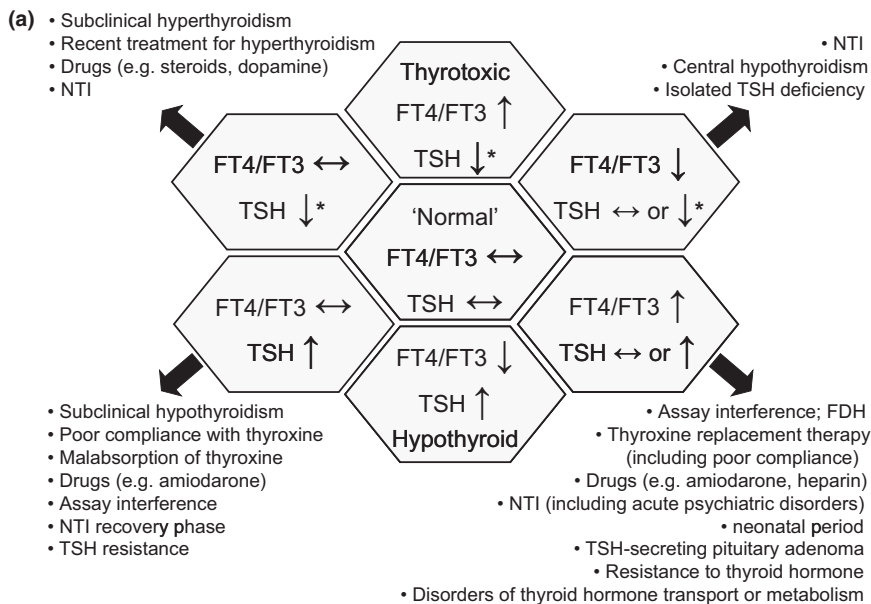
group of contexts the laboratory results either do not ‘fit’ with the clinical picture and/or form an unusual, nonphysiological pattern – so-called ‘funny TFTs’. Establishing the correct diagnosis in these cases is critically dependent on careful clinical assessment combined with focused laboratory, radiological and genetic testing – failure to adopt a structured approach may result in an incorrect diagnosis and inappropriate management.

Figure 1a shows the pattern of TFTs typically seen in classical thyrotoxicosis and hypothyroidism, together with various ‘deviations’ from these patterns and possible causes. A detailed review of all of these anomalous profiles is beyond the scope of this article, and the reader is directed to other resources [e.g. Association for Clinical Biochemistry/British Thyroid Association/British Thyroid Foundation joint guidelines; National Academy of Clinical Biochemistry (NACB) guidelines; Thyroid Disease Manager]<sup>2–4</sup> for a more general discussion of how to investigate/manage discrepant TFTs. Here, we focus on the differential diagnosis of conditions associated with elevated TH [thyroxine (T4) and/or triiodothyronine (T3)] together with nonsuppressed (inappropriate) thyrotropin levels and ask the question ‘what should be done when TFTs do not make sense?’ Our approach is based on two decades of experience investigating such cases and proposes an algorithm that combines clinical, laboratory, radiological and genetic analyses. Formulation of this algorithm has been guided by knowledge of the following: (i) physiology of the hypothalamic–pituitary–thyroid (HPT) axis and the factors that govern TH action at a tissue/cellular level; (ii) the principles underpinning laboratory measurement of T4, T3 and TSH, and potential mechanisms of assay interference; and (iii) causes of hyperthyroxinaemia with nonsuppressed TSH. Importantly, many of the guiding principles outlined here (e.g. exclusion of assay interference or confounding drug therapy) are also applicable to other clinical contexts where discordant TFTs are encountered.

## HPT axis physiology and TH action

The production of T4 and T3 is stimulated by pituitary TSH whose synthesis is regulated by hypothalamic thyrotropin-releasing hormone (TRH). In turn, T4 and T3 inhibit TRH and TSH production by negative feedback, thus establishing an equilibrium: this ‘set point’ of the HPT axis is tightly regulated within an individual,<sup>5</sup> but varies between subjects, likely reflecting genetic and other factors. The actions of TH are mediated by two genes (*THRA*, *THRB*),

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**Fig. 1** Relationship between free thyroid hormone and thyrotropin levels in physiological and pathological states. (a) Schematic representation of different patterns of thyroid function tests and their causes. FDH, familial dysalbuminaemic hyperthyroxinaemia; FT4, free thyroxine; FT3, free triiodothyronine; NTI, nonthyroidal illness; TSH, thyrotropin [\*signifies that TSH may be either fully suppressed (for example as seen in classical primary hyperthyroidism) or partially suppressed (i.e. measurable but below the lower limit of normal); historically, the lower limit of detection of TSH assays was 0.1 mU/l, but modern assays can detect levels an order of magnitude lower than this]. (b) Schematic representation of the log-linear relationship between thyrotropin and free thyroxine, illustrating how relatively small changes in FT4 (even within the normal range) lead to marked excursions in TSH. Horizontal and vertical black dotted lines denote upper and lower limits of the respective reference ranges for FT4 and TSH, respectively. Grey dotted line indicates TSH 0.1 mU/l.

which encode three nuclear receptor subtypes with differing tissue expression (TR $\alpha$ 1: central nervous system, cardiac and skeletal muscle; TR $\beta$ 1: liver and kidney; TR $\beta$ 2: pituitary and hypothalamus).<sup>6</sup> At a pre-receptor level, cellular deiodinases (DIO) mediate hormone metabolism: in the hypothalamus and pituitary, type 2 deiodinase (DIO2) converts T4 to T3; hepatic type 1 deiodinase (DIO1) mediates peripheral hormone conversion, contributing significantly to circulating T3 levels; in contrast, type 3 deiodinase (DIO3) converts T4 to inactive metabolites [reverse T3 (rT3) and T2], thus limiting TH action.<sup>6</sup> Recently, membrane proteins [e.g. monocarboxylate transporter 8 (MCT8)] have been shown to mediate cellular influx/efflux of TH.<sup>6</sup>

### Laboratory measurement of T4, T3 and TSH, and mechanisms of assay interference

The choice of first-line TFTs varies between laboratories; measurement of TSH alone (using a highly sensitive assay with a limit of detection <0.1 mU/l) is the most efficient test for primary thyroid disease because of the log-linear relation between T4 and TSH (Fig. 1b). Thus, in an individual with an intact HPT axis, small alterations in thyroid status are typically associated with a signifi-

cant fall (hyperthyroidism) or rise (hypothyroidism) in TSH levels, whereas the corresponding changed T4/T3 level may still remain within its reference range [as is the case in subclinical hyper- and hypothyroidism respectively (Fig. 1b)]. However, some laboratories routinely provide a combination of TSH and T4 ( $\pm$ T3) measurements as a more comprehensive evaluation of the axis, although others reason that this is only required if central thyroid dysfunction is suspected, as TSH is often 'inappropriately normal' in the latter setting.<sup>1</sup> Knowledge of local laboratory practice is central to understanding how misleading TFT results may arise.

### Altered serum binding proteins

*Quantitative.* Thyroid hormones circulate bound to carrier proteins [thyroxine binding globulin (TBG), albumin, transthyretin (prealbumin)] and increased concentrations of these can result in elevated total T4 or T3 measurements.<sup>7</sup> Pregnancy, oestrogens (oral contraceptive, hormone replacement therapy, tamoxifen) and hepatic disorders, or rarely hereditary TBG excess, can raise TBG levels.<sup>7</sup> However, the advent of assays which measure nonprotein bound or free TH (FT4, FT3) has largely eliminated this problem,

although there are still situations in which misleading results can arise (discussed in more detail later).

**Qualitative.** Dominantly inherited genetic variants of albumin, e.g. familial dysalbuminaemic hyperthyroxinaemia (FDH) [or transthyretin: transthyretin-associated hyperthyroxinaemia (TTR-AH)], which alter its affinity for iodothyronines, can cause FT4 (and less frequently FT3) to be overestimated, particularly in 'one-step' analogue hormone assays; even some 'two-step' assays are susceptible to interference.<sup>7,8</sup> Hormone measurement by equilibrium dialysis can circumvent this (see later). Genetic diagnosis of FDH is facilitated by its common association with a restricted repertoire (e.g. R218H/P, L66P) of albumin gene mutations.<sup>8</sup>

### Assay interference

**TSH measurement.** The majority of commercially available TSH assays use a noncompetitive or 'sandwich' format with two antibodies – capture and (labelled) detection – directed against different epitopes on TSH, with the TSH moiety essentially acting as a bridge between the two. The capture antibody is typically immobilized to a solid phase to ensure good separation between bound and unbound label, thus increasing sensitivity; several different detection antibodies may also be employed to further improve assay sensitivity.

The presence of human anti-animal antibodies (HAAs) in a patient's serum can interfere with TSH measurement if directed against the same species as the assay antibodies: thus, a HAA that blocks TSH binding to either capture or detection antibodies will result in 'negative interference', causing a falsely low TSH readout; conversely, a HAA that is capable of cross-linking the capture and detection antibodies may cause 'positive interference', leading to a falsely high TSH.<sup>9,10</sup> Many manufacturers include panels of antigens or pre-immune serum from source animals to 'mop up' HAAs. However, heterophile antibodies (which are weak polyspecific antibodies that are similarly capable of causing negative or positive interference)<sup>11,12</sup> can prove more difficult to remove. Such interference in the TSH assay can be seen in cases of Graves' Disease and in patients with positive rheumatoid factor (RhF).<sup>13</sup>

Interfering antibodies may also bind the analyte (TSH) rather than the assay antibodies. An extreme example of this type of interference is the 'macro hormone' complex, in which a specific anti-TSH immunoglobulin binds TSH and neutralizes its biological activity, but leaves epitopes exposed for interaction with the assay antibodies.<sup>14,15</sup> The consequence is analogous to the high prolactin levels seen in patients with macroprolactinaemia.

If interference is suspected, it is best to seek the advice of the laboratory as there are several ways to confirm this such as showing the following:

- discordant TSH results in an assay that utilizes different antibody pairs;
- altered TSH result following immunosubtraction [using polyethylene glycol (PEG) or protein G/A]; and
- nonlinear TSH measurement following sample dilution: if either TSH or the assay reagents are weakly bound by interfering anti-

bodies, this interaction may be disrupted by dilution and a non-linear dilution series will result.<sup>9</sup>

**Free T4/T3 measurement.** Determination of FT4 (and FT3) is particularly challenging as the assay must detect very low levels of 'free' hormone relative to a vast excess of protein-bound analyte (>99.5%). The relatively small size of T4 (and T3) precludes use of a 'sandwich' assay format, so 'competition assays' are commonly used; here, labelled T4 (the tracer) competes with serum T4 for a fixed number of anti-T4 antibody binding sites. Free hormone assays are designed such that the equilibrium between T4 and its binding proteins is conserved during measurement, so that the amount of tracer displaced reflects the 'free' rather than 'total' hormone concentration. Clearly, the presence of factors in serum which affect this equilibrium will confound hormone measurement. Examples include the following:

- both fractionated and unfractionated heparin can cause an artefactual elevation in measured concentrations of FT4/FT3 by displacement of T4 and T3 from their carrier proteins.<sup>16</sup> The mechanism is poorly understood, but is likely to involve generation of free fatty acids (FFAs) via heparin-mediated activation of endothelial lipoprotein lipase (LPL), with FFAs displacing T4 from albumin. The extent to which FFAs rise is variable and, as displacement continues *in vitro*, pre-analytical delay can compound the situation.<sup>16</sup> Thyroid function testing prior to administration of heparin or by measurement of total rather than free T4 (as the artefact is secondary to hormone displacement) can circumvent the problem;
- anti-iodothyronine antibodies which can bind the tracer;<sup>17,18</sup>
- HAAs or heterophile antibodies that block the assay antibody;<sup>9</sup> and
- variant thyroid hormone binding proteins (e.g. albumin in FDH) with altered affinity for T4.<sup>7,8</sup>

The use of a 'two-step' ('back titration') assay method, with a wash step prior to tracer addition, may reduce but not completely eliminate such interference.<sup>9</sup> If erroneous results are suspected with a 'one-step' assay, then re-measurement using a 'two-step' assay would be a logical step. If the problem persists, hormone measurement following equilibrium dialysis (ED) remains the gold-standard for eliminating FT4 assay interference.<sup>19,20</sup> However, ED is still not widely available and measurement of total hormone levels with estimation of T4/T3 binding capacity is advocated by some clinicians/laboratories as an alternative.<sup>21</sup>

### Other causes of hyperthyroxinaemia with nonsuppressed TSH

#### Poor compliance or thyroxine therapy

Poor compliance with thyroxine replacement commonly causes anomalous TFTs: owing to their differing half-lives, intermittent hormone ingestion may result in normal or even elevated TH levels, but fails to normalize TSH.<sup>22</sup> Even more confusingly in this context, a seemingly appropriate increase in thyroxine dosage to perhaps nonphysiological levels can normalize TSH, then raising the possibility of the patient being hormone 'resistant'.

However, distinct from the aforementioned context, it is also recognized that thyroxine replacement in physiological dosage to optimize TSH can be associated with mildly elevated FT4 but normal FT3 levels, with this pattern being ascribed to DIO2 in mediating TH feedback.

### Drug treatment

**Amiodarone.** The pleiotropic effects of amiodarone on thyroid function include significant inhibition of DIO1 and hepatic T4 to T3 conversion. Patients on amiodarone alone, or in combination with exogenous thyroxine, can exhibit elevated FT4 with normal TSH, but FT3 levels are usually normal.<sup>23</sup>

**Other agents.** Propylthiouracil, glucocorticoids, propranolol and some iodinated contrast media or iodine-containing supplements/nonprescription medications can also diminish T4 to T3 conversion via a similar mechanism to amiodarone.

### Nonthyroidal illness

Raised TH levels with nonsuppressed TSH are a recognized pattern during nonthyroidal illness (NTI) including acute psychiatric states, but the abnormalities (which reflect a secondary adaptive response rather than primary HPT dysfunction) usually revert with recovery.<sup>24</sup> Where available, measurement of TH levels on a sample taken just prior to/at the onset of NTI may confirm previously normal thyroid status, strongly suggesting this diagnosis.

### Resistance to thyroid hormone (RTH) vs TSH-secreting pituitary tumour (TSHoma)

As outlined earlier, the commonest causes of raised TH and nonsuppressed TSH levels include analytical interference, or in association with readily recognized clinical or drug therapy contexts. Following their exclusion, the differential diagnosis is between RTH and a TSHoma or, uncommonly, other disorders of TH transport or metabolism.

**Clinical features.** RTH (estimated incidence one in 50 000 live births)<sup>6</sup> and TSHoma (estimated incidence 1 per million)<sup>25,26</sup> occur in patients of a similar age range and either gender. A subset of patients with predominant central/pituitary resistance (PRTH) also exhibit thyrotoxic symptoms and signs, such that these features are not discriminatory.<sup>6</sup>

**Pituitary imaging.** An obvious lesion (macroadenoma) on magnetic resonance imaging (MRI) or computed tomography (CT) can be diagnostic; however, potential 'pitfalls' include the rising incidence of TSH-secreting microadenomas (perhaps because of earlier diagnosis of this disorder), and dynamic MRI and/or octreotide scintigraphy may be required to visualize these.<sup>25,26</sup> Conversely, as in other contexts, patients with RTH do harbour 'incidental' abnormalities on imaging, causing diagnostic confusion; finally, persistently elevated TSH levels, either in the context of chronic noncompliance in hypothyroidism or following thyroid

ablation in RTH, results in thyrotroph hyperplasia and pituitary enlargement which is reversible.<sup>27</sup>

**Serum  $\alpha$ -subunit.** Excess serum levels of pituitary glycoprotein  $\alpha$ -subunit ( $\alpha$ -SU) are associated with TSHomas,<sup>25,26</sup> but also found in nonfunctioning and GH-secreting pituitary tumours. Furthermore, normal  $\alpha$ -SU levels, but an elevated  $\alpha$ -SU/TSH molar ratio ( $>1.0$ ), are a recognized finding in TSH-secreting microadenomas;<sup>25,26</sup> however, the latter needs careful interpretation with clinical context – elevated molar ratios are seen in some normal subjects, especially postmenopausal women.

**Tissue markers of thyroid hormone action.** Amongst various peripheral markers of TH action, basal levels of serum sex hormone binding globulin (SHBG) – analysed using age and gender-specific reference ranges) are the most discriminatory, being elevated in TSHoma and normal in RTH.<sup>26</sup> Falsely low SHBG levels can occur in mixed GH/TSHoma because of inhibition of its synthesis by growth hormone; conversely, synthetic oestrogen therapy in RTH can falsely elevate SHBG. Basal levels of other biochemical markers (e.g. serum cholesterol, creatine kinase) are less useful, but comparison of changes in these parameters following T3 administration (see later) with reference data from normal subjects can discriminate refractoriness or resistance to hormone action.

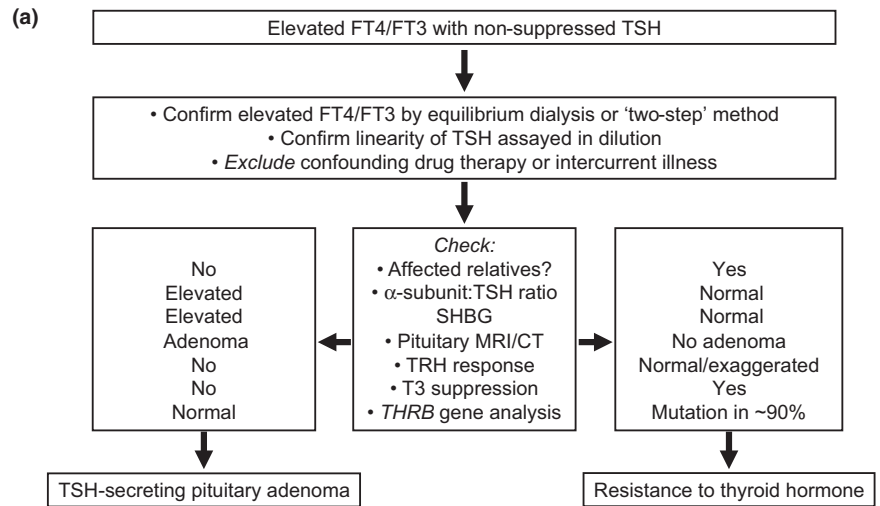
**Dynamic testing.** 80–90% of patients with TSHoma show a blunted or absent TSH response (TSH increment  $<150\%$  of baseline) following TRH stimulation;<sup>26</sup> conversely, in RTH the TSH response is either preserved or even exaggerated.<sup>6</sup> Protocols of T3 administration to suppress TSH secretion involve giving either a fixed (100  $\mu\text{g/day}$ ) or graded doses (50 then 100 then 200  $\mu\text{g/day}$  each for 3 days) of liothyronine (L-T3) over a ten day period, with TRH testing and measurement of other biochemical markers of thyroid hormone action.<sup>6,26,28</sup> TSH secretion in pituitary tumour patients remains autonomous, while in RTH there is qualitatively normal inhibition of TSH secretion, but the degree of suppression is not complete. An acute octreotide test (100  $\mu\text{g s.c.}$ ) reduces TSH, FT4 and FT3 levels in both RTH and TSHoma patients, but chronic administration of long-acting somatostatin analogue maintains reduced TH levels in TSHoma, whereas subjects with RTH are refractory.<sup>29</sup>

**TFTs in relatives.** In total, 75% of RTH cases are dominantly inherited, with the remainder being caused by sporadic, 'de novo' gene defects.<sup>6</sup> Thus, a similar pattern of abnormal TFTs in first-degree relatives strongly suggests RTH.

**Genetic testing.** RTH is associated with *THRB* gene defects and their identification by gene sequencing can confirm the diagnosis; however,  $\sim 15\%$  of RTH cases are not associated with *THRB* mutations, such that absence of an abnormality in this gene does not exclude the diagnosis.<sup>6</sup>

### Other genetic disorders of TH transport or metabolism

**MCT8 mutations.** An X-linked disorder of childhood-onset, with psychomotor retardation including speech and developmental



**Fig. 2** Investigation into elevated thyroid hormones with nonsuppressed TSH. (a) An algorithm to exclude assay artefact or other confounding causes and differentiate RTH from TSHoma. (b) Typical TFT patterns and clinical features in genetic disorders associated with raised thyroid hormones. \*FT3 is raised with a rare albumin gene variant (L66P) causing FDH; CT, computed tomography; FT4, free thyroxine; FT3, free triiodothyronine; MRI, magnetic resonance imaging; rT3, reverse T3; SHBG, sex hormone binding globulin; *THRB*, thyroid hormone receptor  $\beta$  gene; TRH, thyrotropin-releasing hormone; TSH, thyrotropin.

(b)

Disorder	Familial dysalbuminaemic hyperthyroxinaemia (FDH)	Resistance to thyroid hormone (RTH)	Allan Herndon Dudley syndrome	Selenoprotein Deficiency disorder
GENE	<i>ALB</i>	<i>THRB</i>	<i>SLC16A2 (MCT8)</i>	<i>SECISBP2</i>
FT4	Raised	Raised	Normal or low	Raised
FT3	Normal*	Raised	Raised	Normal or low
TSH	Normal	Normal or mildly raised	Normal or mildly raised	Normal
rT3	Raised	Raised	Low	Raised
SHBG	Normal	Normal	Raised	Normal
Clinical features	Euthyroid	Goitre; tissue-selective hyperthyroidism	Mental and psychomotor retardation	Growth retardation, male infertility, skeletal myopathy, hearing loss

delay and spastic quadriplegia, is caused by defects in the *MCT8* (*SLC16A2*) gene, encoding a membrane transporter. In addition to neurological abnormalities, male patients exhibit a characteristic pattern of abnormal TFTs with elevated FT3, low FT4 and normal TSH levels.<sup>6,30,31</sup>

**Functional deiodinase deficiency.** The deiodinase enzymes are part of a larger family of 25 human proteins containing selenocysteine. Recently, a multisystem selenoprotein deficiency disorder, manifesting with growth retardation in childhood or other features (male infertility, skeletal myopathy, photosensitivity, hearing loss) in adults, has been associated with a thyroid signature – raised FT4, normal/low FT3 and normal TSH levels, because of functional DIO deficiencies.<sup>6,32–34</sup>

An algorithm summarizing the approach to investigation and differential diagnosis of patients with hyperthyroxinaemia and nonsuppressed TSH levels is shown in Fig. 2a, together with a summary of the key laboratory and clinical features of genetic disorders that may be associated with elevated TH levels and detectable TSH (Fig. 2b).

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The authors have nothing to disclose.

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