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Medications that distort *in vitro* tests of thyroid function, with particular reference to estimates of serum free thyroxine

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The combination of serum thyroid-stimulating hormone (TSH) with measurement of circulating thyroid hormones greatly improves sensitivity and specificity of thyroid diagnosis, but these assays are not impeccable. Estimation of serum free T4 conveniently accommodates variations in the concentration of thyroxine-binding globulin (TBG), but no current technique reliably reflects the *in vivo* free T4 concentration in numerous other situations. The effect of circulating competitors that increase T4 and T3 *in vivo*, in particular, many medications, is under-estimated by current free hormone estimates that involve sample dilution. Non-esterified fatty acids generated during sample storage and incubation can spuriously increase the measured free T4 estimate, especially after *in vivo* treatment with heparin. These artefacts are unlikely to be overcome by current assay strategies. Total serum T4, corrected for alterations in TBG concentration, gives a more robust estimate of thyroxine concentration than current methods of free hormone estimation and should now be reintroduced as the 'gold standard'.

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Introduction

The aim of *in vitro* testing of thyroid function is to accurately reflect the *in vivo* activity of key analytes such as thyroid-stimulating hormone (TSH), thyroxine and triiodothyronine. For these key laboratory parameters, it is highly desirable for results to be interpretable in relation to consensus reference intervals that do not vary depending on the choice of method. In this article, we consider whether current assays satisfy these objectives and outline situations in which the results may be potentially misleading, with particular attention to the *in vitro* effects of medications. Drugs that alter the secretion of TSH or thyroid hormones *in vivo* are excluded, as are the syndromes of thyroid hormone resistance[1] and situations in which immunoreactivity of TSH may not accurately reflect the biologic activity of this glycoprotein.[2,3]

The major part of this review will consider how free T4 and T3 estimates are influenced by serum constituents that cause discrepancies between the *in vivo* hormone level and the apparent concentration in the assay tube. These effects reflect the difficulty of estimating free hormone concentrations in the presence of proteins that bind numerous other ligands in addition to T4 and T3. Free hormone estimates may be *spuriously high* if components that displace T4 and T3 from protein binding, such as non-esterified fatty acids (NEFA), are generated during sample storage or incubation, a particular problem in heparin-treated patients. Conversely, the measured free hormone concentration may be *falsely low* in diluted samples that contain medications that compete for protein binding of T4 or T3.

Assays for TSH can be subject to interference by heterophilic antibodies, which may also distort the measurement of other analytes. Assays for T4 and T3, either total or free, are vulnerable to interference from circulating auto-antibodies, which produce divergent artefacts in the assay, depending on the technique that is used to separate bound from free hormone (see below).

It is important to re-emphasise that all current assays for thyroid hormones and TSH involve the comparison of known and unknown samples, based on the assumption that the assay signal is solely a function of the concentration of analyte in the sample at the moment of collection. If any of the following three conditions is breached, the assay is likely to give a spurious result.

a. Competition for antibody binding between the assay tracer and free hormone is identical in samples and standards.
b. Any constituent, generated during sample storage and incubation, that influences the concentration of analyte or the assay signal, is similar in samples and standards.
c. Dilution-dependent dissociation of bound hormone is similar in samples and standards.

The impact of misleading individual assay results can be minimised by considering serum T4 and TSH together, taking account of their characteristic feedback relationship and the assumptions and limitations that underpin this diagnostic relationship.[4] While there is no single artefact that concurrently distorts the results for serum free T4 and TSH to simulate a false diagnosis, the combination of a low free T4 estimate with suppressed serum TSH is a challenging and ambiguous finding that may be attributable to severe illness, or to the *in vivo* and *in vitro* effects of medications. While true central hypothyroidism can occur as a neuroendocrine effect of critical illness[5], a similar combination of results can be induced by medications, for example, when high dose furosemide is given together with dopamine or glucocorticoids. The latter two each suppress serum TSH[4], while the former displaces T4 from its binding proteins[6] and also leads to spuriously low estimates of free T4 due to a dilution-dependent *in vitro* artefact (see below). Whenever the cause of an apparent thyroid abnormality is unclear, it is crucial to take account of medications.[6]

Effects of sample storage

It is widely accepted that thyroid hormones and TSH are stable when serum is stored at 4 °C for up to 1 week.[7] Stability on T4 and TSH of filter paper spots used for neonatal screening when stored at 4 °C has been confirmed for at least 1 year.[8]