



FREE TESTOSTERONE

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$$cFT = \frac{-[k_1 + k_2 \times (C_{SHBG} - C_{TT})] + \sqrt{[k_1 + k_2 \times (C_{SHBG} - C_{TT})]^2 - 4k_1k_2(-C_{TT})}}{2 \times k_1 \times k_2}$$

insight

- The Vermuelen equation is now the preferred method of estimating free testosterone.
- Under Medicare rules, doctors should request both testosterone and SHBG in order to obtain a calculated free testosterone level.
- Reference intervals should be appropriate to assays used – see overleaf.

The free hormone hypothesis

Steroid hormones are hydrophobic molecules and therefore highly bound to proteins in serum. It is generally accepted that only the small fraction of free steroid hormone is 'active' and taken up by the cell to exert its effect.

Sex Hormone Binding Globulin

Most testosterone in men and women is specifically bound to a protein called Sex Hormone Binding Globulin (SHBG). In women the concentration of SHBG usually greatly exceeds the total testosterone level and very little free testosterone is present. The amount of free testosterone in women can be roughly estimated as a simple ratio of total testosterone level to SHBG level, also known as the Free Androgen Index (FAI).

However, if total testosterone levels are similar or greater than SHBG levels, as in men, clearly the amount of free testosterone will be higher, even after accounting for some albumin binding of steroid hormones. For these reasons, the FAI becomes unreliable when total testosterone and SHBG levels are similar. This applies when either total testosterone levels are high (eg normal men) or when SHBG levels are low (eg in insulin resistance including most women with PCOS).

Vermuelen calculated free testosterone

To overcome the inaccuracies of the FAI ratio, Vermuelen et al created a binding formula that accounts for the concentrations of both SHBG and albumin. The formula is called 'the Vermuelen equation' or 'calculated free testosterone' (cFT). (This is not the same as an empirical formula called 'bioavailable testosterone' that tries to predict the concentration of testosterone following laboratory precipitation of binding proteins.)

While the Vermuelen method is preferred for estimating free testosterone, it does have some weaknesses. Firstly, the result includes any imprecision in the measurement of total testosterone and SHBG (albumin imprecision is usually more than adequate). Secondly, both total testosterone assays and SHBG assays often measure differently from laboratory to laboratory and this leads to significant biases in the cFT results. Until improvements occur in the way manufacturers standardise their assays, it is very important to ensure that reference intervals and clinical decision limits are appropriate to the particular assays used.

Other measures of free testosterone

Other approaches to measuring free testosterone levels are salivary steroid hormone assays and direct analysis of competition between free hormone and a free hormone analogue. The latter technique is similar to the way laboratories routinely measure free thyroid hormones. Generally both these methods are imprecise because of the difficulties in measuring the very low concentration of free testosterone (10^{-12} moles / litre).

Requesting calculated free testosterone levels

Doctors need to request testosterone and SHBG in order to obtain a calculated free testosterone level as Medicare rules do not currently recognise 'calculated free testosterone' as a request. Although albumin can also be ordered to improve the calculation, it has little impact unless albumin levels are markedly lowered.

Because there is a diurnal variation of testosterone with higher levels in the morning (particularly in men), samples should be taken between 8am and 10am, as that is the time used in reference interval studies.

Finally, low testosterone levels in men usually require Luteinising Hormone (LH) measurement to determine if testicular pathology is likely.

Reference intervals for cFT in men

Andrology Australia recently published the reference intervals for total testosterone and cFT in young fertile men (aged 20 to 35) and included the variations that can be seen with different method combinations. The table below shows the Andrology Australia reference intervals for young men using the Roche E170 total testosterone and SHBG assays. For older men, it is generally acknowledged that testosterone levels fall with age. While less data is available to guide us on how to set reference intervals or clinical decision limits in older men, we will be using the manufacturer's reference intervals as they include older men and suit the distribution of our patient results.

Reference intervals for cFT in women

Reference intervals for women are available from the assay manufacturer. We have assessed these as appropriate for our result distribution and note that testosterone levels in women also show a gradual age-related decline. The clinical importance of this age-related decline is under investigation. In any case, the most common clinical group of women with abnormalities of testosterone and SHBG are young women with possible PCOS where it is vital to understand two important features of the syndrome - insulin resistance (which lowers SHBG) and androgenisation (which is associated with elevated cFT).

References

- Vermuelen A et al, J Clin Endocrinol Metab 1999; 84:3666-72
 Sikaris KA et al, J Clin Endocrinol Metab 2005; 90:5928-36
 Sikaris KA et al, Clin Biochem Rev 2006; 27:S21.



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Dr Sikaris is Director of Chemical Pathology at Melbourne Pathology.

He has been involved in several research projects regarding the application and reliability of testosterone measurements in

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REFERENCE INTERVALS FOR MELBOURNE PATHOLOGY USING ROCHE E170 ANALYSER

	Younger Men	Women	Older Men
	<i>Andrology Australia</i>	<i>Roche E170</i>	<i>Roche E170</i>
Total Testosterone	12.0 – 31.9 nmol/L	0.4 - 2.9 nmol/L	9.9 – 27.8 nmol/L
SHBG	12 – 57 nmol/L	26 – 110 nmol/L	15 – 48 nmol/L
cFT (Vermuelen)	260–740 pmol/L	3 – 37 pmol/L	91-579 pmol/L

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