EDITORIAL

Total or free, that is the question

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In this issue of the Netherlands Journal of Medicine, Vastbinder et al.[1] describe the effect of oestrogen-containing oral contraceptives on the results of the dexamethasone screening test (DST) in normal women. The DST is one of the tests used to exclude the diagnosis of Cushing's syndrome. The test erroneously showed cortisol levels above the cut-off value of 50 nmol/l in 8 out of 13 women, presumably due to increased levels of the liver-produced glycoprotein cortisol-binding globulin (CBG), which are stimulated by the oestrogenic component of the contraceptive pills. Results of the DST normalised one week after oestrogen withdrawal in all but one of the women, and in all women after another five weeks.

The authors did not estimate actual levels of CBG, presumably because the mechanism leading to the increased CBG levels and therefore to the disturbed DST result after oestrogen administration is well known.[2] However, a number of other factors, as mentioned below, may also affect the results of measurements of protein-bound hormones and the interpretation of related function tests. Many of these depend on changes in the level of binding proteins. This has clearly been recognised to be the case for thyroid hormones; measurement of free thyroxin has superseded the estimation of total thyroxin for a long time already. The situation for steroid hormones is different, probably because estimation of free steroid concentrations by dialysis is time consuming, direct assays for serum free steroids are notoriously unreliable, and the number of available CBG assays is limited. Nevertheless, there are possibilities to overcome these problems, which will also be discussed below.

FACTORS AFFECTING CONCENTRATIONS AND EFFECTS OF CBG

Circulating cortisol is partly bound to CBG (ca 70%), to albumin (ca 10-15%) and is partly free in the circulation. Biological effects are presumably exerted by the sum of albumin-bound and free cortisol (i.e. non-CBG-bound cortisol), since the binding to albumin has a low affinity and is readily disrupted while the CBG-cortisol complex will not be separated during the time necessary to pass the vascular bed of a target organ. In dialysis experiments, only free cortisol is measured.

Apart from the above-mentioned stimulating effect of oestrogens on serum CBG levels, CBG concentrations can be suppressed by increased levels of immune modulators such as interleukin-6, by insulin, thyroxin, by growth hormone treatment through increased levels of IGF-1 and by liver disease, e.g. cirrhosis.[3] All of these conditions may lead to erroneously low levels of total cortisol suggesting hypocortisolaemia or falsely suppressed cortisol in DSTs. Furthermore, mitotane treatment for adrenal cortical carcinoma will stimulate CBG levels, possibly leading to misinterpretation of total cortisol levels in patients with this disease.

A different reason for misinterpretation of total serum cortisol concentrations may be the presence of mutations in the gene coding for this protein[3] leading to suppressed total, but normal non-CBG-bound or free cortisol levels in serum. Interestingly, one of these mutations is prevalent in Han Chinese, where it leads to a so far unexplained preference for female offspring.[4] Finally, increased levels of total and free cortisol in the absence of signs and symptoms of hypercortisolaemia can be found in patients with mutations in the gene coding for the glucocorticoid receptor.[5]

ALTERNATIVES FOR DIRECT ESTIMATION OF FREE CORTISOL

Apart from the measurement of serum levels of free cortisol by dialysis, an approximation can be made by calculation, using the levels of total cortisol, CBG and albumin in the formula described by Dorin et al.[6] Alternatively, salivary levels of cortisol are strongly correlated with free cortisol levels in serum samples taken at the same time,[7] whereas the cortisol level measured in hair reflects the mean serum free cortisol concentration as present during a longer period.[8] Assuming a hair

growth rate of 1 cm/month, it is possible to study changes in mean cortisol levels during illness or periods of stress by measuring cortisol in subsequent centimetres of hair.

TOTAL TESTOSTERONE CONCENTRATIONS ARE STRONGLY AFFECTED BY SHBG LEVELS

Like CBG, sex hormone-binding globulin (SHBG) is a glycoprotein, produced and secreted by the liver, which binds testosterone, 5α-dihydrotestosterone and oestradiol. Approximately 50% of testosterone is SHBG-bound in men; in women the SHBG-binding amounts to 80%. Total testosterone levels in men are strongly related with single nucleotide polymorphisms (SNPs) in the SHBG gene[9] and with the serum level of SHBG,[10] whereas the concentration of serum non-SHBG-bound testosterone is independent of the SHBG level. In its turn, the SHBG concentration is partly dependent on SNPs in a larger number of other genes, which encompass multiple biological pathways, including hepatic function, lipid metabolism, carbohydrate metabolism, type 2 diabetes, and androgen and oestrogen receptor function.[11] These observations are in line with earlier findings on direct effects of oestrogens, androgens and insulin on SHBG levels, and follow a similar pattern compared with the factors affecting CBG concentrations. Finally, one case of an inactivating mutation in the SHBG gene has been described in a man with an inadequately low level of total testosterone but normal gonadal development and spermatogenesis.[12]

ALTERNATIVES FOR DIRECT ESTIMATION OF FREE TESTOSTERONE

Similar to the situation for cortisol, methodologies for the calculation of free or non-SHBG bound testosterone have been developed. However, the concordance between the various methods was only limited,[13] indicating that valid conclusions can only be drawn from comparisons with reference values obtained using the same method. A much simpler approximation of the concentration of non-SHBG-bound testosterone is the calculation of the free androgen index, defined as total testosterone x100/SHBG, where concentrations of both testosterone and SHBG are expressed as nmol/l. This method might yield meaningful results in women, where total testosterone levels are much lower than SHBG concentrations.[14] However, in men, where testosterone levels exceed SHBG concentrations by a factor between 1.5 and 2, this will not lead to meaningful results. Relatively new developments are estimations of testosterone in saliva[15] and hair,[16] which might also reflect the serum concentration of non-SHBG-bound testosterone.

CONCLUSIONS

Changes in the concentrations of specific steroid-binding proteins or in the affinity of their binding to steroids will become visible in the total concentration of the steroid, while the non-protein bound concentration will only be affected slightly. For this reason, if unexpected results of determinations of steroid hormones are encountered, it may be possible to resolve these discrepancies by investigation of the quantity and quality of the specific steroid-binding proteins.

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