To the Editor:

Nonextraction testosterone immunoassays of samples from women are nonspecific because of interference by closely related steroids (1). The reference method, isotope-dilution GC-MS is not suitable for routine use. LC-MS is increasingly being used with women because of its enhanced specificity (2). Not all routine laboratories have access to LC-MS.

Dehydroepiandrosterone sulfate (DHEAS) interferes in several testosterone immunoassays, including the Roche Testosterone immunoassay (3). Roche claims that their Testosterone II immunoassay improves testosterone measurement in women, with reduced DHEAS interference. Some characteristics of this assay have been published (4). The claim that this assay is prone to less DHEAS interference has not been independently verified. We investigated the influence of measured DHEAS in the Roche Testosterone and Testosterone II immunoassays by comparing results obtained with LC-MS.

Serum samples obtained from 184 consecutive women (22–41 years of age) participating in an in vitro fertilization program were stored at −80 °C. None was taking any steroid-containing medication. Body mass index values were >19 kg/m² and <30 kg/m². Serum testosterone was measured with the Roche Testosterone and Testosterone II assays along with serum DHEAS on a Roche E module analyzer according to the manufacturer’s instructions.

The LC-MS method for testosterone was developed in house with deuterated testosterone used as internal standard. Ten microliters of internal standard was added to 200 μL containing sample, calibrator, and QC material in a 2.0-mL microtube containing 1 mL methyl tert-butyl ether and mixed for 10 min. The separated solvent was evaporated under nitrogen, and the sample was reconstituted in 100 μL of a solvent consisting of equal volumes of water and methanol. The sample was then transferred to a 96-well plate, heat-sealed, and loaded onto the autosampler. Chromatography was performed with a Waters 2795 HPLC system. Fifty microliters of the extracted sample was injected at 24 °C onto a SecurityGuard C18 column (4 mm × 2 mm; Phenomenex), which was connected to a Synergi™ 4-μm Hydro-RP column (50 mm × 3 mm; Phenomenex). The initial mobile phase was 700 mL/L methanol for 2 min. That was followed by 950 mL/L methanol mobile phase for 0.5 min before reequilibration with starting conditions for 1 min. The flow rate was 0.6 mL/min. The eluted sample was pumped directly to the electrospray probe of the mass spectrometer (Waters Quattro micro tandem mass spectrometer) without splitting or solvent diversion, in electrospray positive-ionization mode, and with multiple reaction monitoring mode. The total run time was 4.3 min. The process control used Waters MassLynx NT 4.1 software, and data processing used the MassLynx Quantify program. The LC-MS assay was validated according to US Food and Drug Administration guidance (5). The lower limit of quantification was 7.2 ng/dL (0.25 nmol/L) (CV, 15%) with good linearity, recovery (96%–100%), and precision. Intra- and interassay CVs were 5.4%–14% and 6.4%–15%, respectively. There was no ion suppression.

The testosterone immunoassays were compared to each other and to LC-MS. The correlation between the 2 immunoassays was good ($r^2 = 0.79$), with the Testosterone assay giving higher values [mean, 43.2 ng/dL (1.5 nmol/L); range, <2.9–129.6 ng/dL (<0.1–4.5 nmol/L)] than the Testosterone II assay [mean, 34.6 ng/dL (1.2 nmol/L); range, 2.9–83.5 ng/dL (0.1–2.9 nmol/L)].

Both immunoassays showed a lower correlation to the LC-MS method (Testosterone, $r^2 = 0.48$; Testosterone II, $r^2 = 0.43$). There was considerable scatter, with both immunoassays giving higher results than the LC-MS method [mean, 23.0 ng/dL (0.8 nmol/L); range, 7.2–60.5 ng/dL (<0.25–2.1 nmol/L)].

The testosterone concentrations for both immunoassays were positively correlated with the DHEAS concentration (Testosterone, $r^2 = 0.73$; Testosterone II, $r^2 = 0.63$), and the difference between the 2 immunoassays was positively related to the measured DHEAS concentration ($r^2 = 0.38$). The LC-MS assay is less strongly correlated with the DHEAS concentration ($r^2 = 0.23$). The difference between each individual testosterone immunoassay result and the LC-MS result (i.e., immunoassay minus LC-MS) was examined. The results for the Testosterone immunoassay showed a stronger relationship with DHEAS ($r^2 = 0.72$) than those for the Testosterone II immunoassay ($r^2 = 0.45$) (Fig. 1).

Although both immunoassays are prone to interference by DHEAS, this work demonstrates an improved specificity of the Testosterone II immunoassay for...
testosterone with respect to DHEAS.

The DHEAS immunoassay is likely to be susceptible to interference from other endogenous and exogenous steroid compounds that affect the testosterone immunoassay. Further study should include analysis of DHEAS by a specific method, such as LC-MS. It is also possible that differences in the calibration of the 2 assays could contribute to this apparent improvement.

These results confirm that although immunoassay-measured DHEAS interferes less in the Roche Testosterone II assay than in the Testosterone assay, the Testosterone II assay still appears susceptible to interference from DHEAS or by other steroids detected by the DHEAS immunoassay. This work highlights residual specificity problems with the Roche Testosterone II assay and provides further evidence of the problems in developing direct testosterone immunoassays with a specificity adequate for measuring testosterone in women.

References


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