trations in second-trimester maternal serum. This finding suggests that the fetal DNA in maternal serum is strongly influenced by the pathologic and biologic status of placent al trophoblasts. Although the origin of fetal DNA in maternal serum has yet to be elucidated, our findings suggest that it is from placent al trophoblasts rather than from nucleated fetal cells circulating in maternal blood. Furthermore, increased fetal DNA and hCG concentrations in maternal serum might be associated with certain pregnancy-related complications.

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References


pretreated the serum with [3H]testosterone before AS precipitation. BAT was then calculated from the percentage of the tracer in the supernatant multiplied by the total testosterone concentration, which was usually measured by RIA (with or without an extraction step) (3, 9). A novel approach used extraction and chromatography of the precipitate before RIA (11). The development of a standard BAT assay is required because current reports suggest that BAT can range from 20% to 70% of total testosterone for men (3) and 10% to 34% for women (3, 12).

We investigated the measurement of BAT using the Bayer ImmunoTM 1 immunoassay system (an enzyme immunoassay; Bayer Corporation, Diagnostics Division) after precipitation with AS. Serum samples collected from 48 men during routine clinical investigation or monitoring of hypogonadism at the Kingston General Hospital Urology Clinic were stored at −20 °C until analysis. Total testosterone was measured on the Immuno 1 System with a 1:1 dilution of serum with diluent supplied by RIA (with or without an extraction step) (3)

We found that the presence of either saline or AS in the final sample increased testosterone results by the Immuno 1 system. We speculate that AS in particular interferes with one of the reagents (possibly one of the antibodies) used in the immunoassay. Interference of AS in the final measurement of testosterone has not been reported previously in BAT assays because the previous reports used isotope detection in contrast to the enzyme immunoassay used in our method.

To eliminate this interference by AS, we decided to determine BAT indirectly by measuring testosterone in the precipitate after AS pretreatment. The precipitate was reconstituted in Bayer Diluent-B Reconstitution Buffer and then assayed for testosterone. A control sample for total testosterone was measured by 1:1 dilution of serum with diluent to control for any minimal interference in measurement of testosterone by the diluent. With the saturated AS solution, testosterone in the precipitate was >90% of the total testosterone, indicating that ALB-T was also precipitating or that testosterone was being displaced from ALB and then binding to SHBG. Indeed, ALB was detected in the precipitate by the Synchron CX7 Clinical System (Beckman Coulter, Inc.).

Confirmation that the concentration of AS being used was also precipitating ALB led to a study to optimize the AS concentration. Various concentrations of AS were added to equal volumes of serum, and the precipitate was dissolved in the diluent. Both testosterone and ALB were then measured. The recovery of testosterone in the precipitate increased as a function of AS concentration (Table 1). In particular, large increments in the recovery of testosterone were evident at AS saturations of 65% and 75%. However, experiments at 73% and 75% AS saturation, revealed that ~6 g/L ALB was typically precipitated, whereas ALB was undetectable (<10 μg/L) at 68% and 65% AS saturation. It appears that 70% AS saturation was a threshold because of the greater degree of variation in results for samples treated with this concentration (within-assay CVs, 13–21%; Table 1). Decreasing the AS concentration by only 2%, from 70% to 68% saturation, produced results with moderate within-assay CVs (5–7%) and no precipitation of ALB. A similar approach of reconstituting the AS precipitate was used by Ratajczak et al. (12) in 1981, followed by dichloromethane extraction and measurement of testosterone by RIA. They reported

| Table 1. Testosterone in reconstituted pellet after AS precipitation. |
|---------------------|---------------------|--------|
| Saturation of AS solution, % | Mean recovery ± SD, % | CV, % |
| 60 | 20.5 ± 1.3 | 6.3 |
| 65 | 36.0 ± 1.5 | 4.2 |
| 68 | 47.0 ± 2.7 | 5.7 |
| 70 | 61.6 ± 13.5 | 21 |
| 70 | 72.0 ± 9.3 | 13 |
| 75 | 83.4 ± 4.7 | 5.6 |
| 80 | 89.8 ± 1.2 | 1.3 |
that at a final AS saturation of 41%, the supernatant was completely free of SHBG, whereas no ALB was found in the precipitate. A clear conclusion of the work reported here is the sensitivity of the BAT assay to AS concentration and ALB precipitation. Each laboratory should establish the critical concentration of AS that does not precipitate ALB.

In 48 samples from men undergoing routine clinical investigation or monitoring of hypogonadism, the mean percentage of testosterone recovered as BAT (68% AS saturation) was 62.1% ± 8.6%, with the percentage of BAT being independent of the total testosterone concentration. In 20 assay precipitates, no ALB was detected. Previous reports have indicated BAT percentages of 20–70% (3), 30–80% (13), and 45% (9) in healthy men, whereas older men have slightly lower percentages of 30%, and impotent men have percentages of ~20% (9). Although we did not perform a method comparison of our BAT method with another non-SHBG-bound-T method in this current study, we did include this in a subsequent study of 119 men and 21 women who were being treated or investigated for hypogonadism (unpublished data). Our BAT method demonstrated good correlation not only with total testosterone and FT, but also with FT as calculated by Vermeulen et al. (14), which was based on the measurement of testosterone, ALB, and SHBG.

The high correlation between BAT and testosterone and the good correlation between FT and testosterone (Fig. 1) are consistent with data reported by Winters et al. (13) in 28 thin or obese, but otherwise healthy, men in which the correlation between FT and testosterone was slightly better than the correlation between BAT and testosterone. Several studies have noted good correlations between BAT, FT, calculated FT, and total testosterone (7, 12, 13). If BAT continues to demonstrate good correlation with total testosterone for other patient groups and in studies with substantially larger study groups, the question must be raised whether BAT adds any useful information to the total testosterone concentration or if it is a redundant test. The same issue has recently been raised for FT (13).

In summary, with the Immuno 1, BAT cannot be measured accurately on the supernatant, which contains AS, but can be measured indirectly in the precipitate after reconstitution with the manufacturer’s diluent. The optimal concentration of AS for the selective precipitation of SHBG was 34% AS saturation. AS solution was added slowly during vortex-mixing to minimize the microprecipitation of ALB, and samples were kept at 4 °C for 1 h after AS precipitation before the final centrifugation was performed. The analytical reliability of selective SHBG-T precipitation with AS needs to be carefully investigated when establishing BAT assays. The very good correlation between BAT and total testosterone (and between FT and total testosterone) raises the question of whether these newer tests add any useful clinical information to total testosterone results. To define the usefulness of these new tests, large studies on a variety of well-characterized patient populations are needed.

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References

Fig. 1. Correlations between FT and total testosterone (top panel) and BAT and total testosterone (bottom panel) in men being investigated or treated for hypogonadism (n = 48).