Accuracy of calculated free testosterone differs between equations and depends on gender and SHBG concentration

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ABSTRACT

Serum free testosterone (fT) concentrations are often calculated, however different equations often yield discrepant results. This study explores the sources of this variability. We compared three established and two new equations that differed only by their testosterone association constants with isotope dilution equilibrium dialysis in two patient groups with different gender distributions. Equation components were examined to determine how they impacted correlation with isotope dilution equilibrium dialysis. Association constants derived for each patient group correlated best with isotope dilution equilibrium dialysis for that group and not the other set. Samples with the poorest correlation between isotope dilution equilibrium dialysis and calculated fT results had significantly higher SHBG concentrations. Regardless of equation, ≥25% of samples showed unacceptable deviation from isotope dilution equilibrium dialysis. Association constants and gender makeup and SHBG concentration of the patient groups used to establish an equation all significantly impact correlation with isotope dilution equilibrium dialysis. Application of many fT equations to wider populations will therefore frequently yield results that differ substantially from isotope dilution equilibrium dialysis.

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1. Introduction

Measurement of serum testosterone concentrations is a central component of the assessment of androgen status. Increased concentrations can be seen in women with congenital adrenal hyperplasia or polycystic ovarian syndrome (PCOS), and in both genders due to exogenous testosterone administration, adrenal-, testicular-, or ovarian tumors, and in children with precocious puberty [1]. Conversely, decreased serum testosterone concentrations are the hallmark of both primary and secondary hypogonadism in men, and, to a lesser degree, women, regardless of the underlying etiology. Androgen deficiency in males is believed to be a particular common problem, affecting an estimated 4.7 million men between the ages of 30 and 79 in the United States in 2000, and predicted to rise to 6.5 million by 2025 [2,3]. Most of these cases represent mild to moderate deficiency, often referred to as “andropause” or “late-onset hypogonadism”, a state that is blamed for anergia, sexual dysfunction, declining mental ability, diminished muscle strength and reduced bone density [4,5]. Decreased testosterone concentrations in women present with fewer and less specific symptoms [6,7].

Measurement of total testosterone, often combined with gonadotropin measurements, is generally sufficient to diagnose significant androgen excess or deficiency. However, for suspected mild to moderate deficiency, measurement of the free, bioactive, fraction of testosterone (fT) is believed to be of superior diagnostic value, especially under circumstances of altered sex hormone binding globulin (SHBG) serum concentrations or binding affinity, as may be found in hyper- or hypothyroidism, liver cirrhosis, obesity, or exogenous sex hormone use, especially estrogen treatments [8–11]. Serum concentrations of SHBG also increase with age, often affecting fT levels disproportionately to total testosterone concentrations [12].

Because the majority of testosterone is bound to SHBG or albumin, fT accounts for only 1–4% of the total testosterone levels. Furthermore, the protein-bound distribution of testosterone is gender dependent because of differences in sex steroid distribution, with 44% of testosterone bound to SHBG in men and 66% bound to SHBG in women [13]. These factors conspire to make measurements of fT challenging. The gold standard method of measurement is isotope dilution equilibrium dialysis [14–16]. While this method is considered the most accurate, its technical and laborious nature can result in high assay variability [17]. Analog immunoassays that
detect fT have been proposed as an alternative. However, they have been widely criticized for their lack of accuracy and variability of results with fluctuating SHBG concentrations [18–20], suggesting that they do not truly measure fT.

Another alternative is to calculate fT using equations based on the law of mass action. These equations typically incorporate the results of serum measurements of total testosterone, albumin, and SHBG. The equation originally described by Sodergard et al. [21] and derived by Vermeulen et al. [22] also includes the testosterone association constants for albumin and SHBG. However, calculated results for fT have often been found to vary significantly from isotope dilution equilibrium dialysis. Potential sources for this variation include biological factors such as inter-individual variability in the concentrations of competing hormones and binding proteins, and in the affinity of testosterone for SHBG or albumin, and laboratory factors including different SHBG and testosterone methodologies, equations, and association constants used [23–26]. Several different constants have been published and some studies have used empirically derived constants and equations to provide the best fit for their individual combinations of patient population, and total testosterone, SHBG and albumin assays [15,27–31].

Overall, there appears to be little consensus on the best method for calculating fT, likely because of the wide variability of performance of these equations between studies, patient groups, and laboratories and a lack of understanding of how these factors impact calculated fT measurements. The purpose of this study was to improve our understanding of how these parameters affect calculated fT results, and to determine whether satisfactory agreement with isotope dilution equilibrium dialysis can be achieved. To this end we assessed the performance of three established equations and two new equations, generated using different patient groups. These equations differed only in their testosterone association constants for SHBG and albumin. Results calculated with each of these five equations in these two patient groups were compared with those obtained by isotope dilution equilibrium dialysis performed on the same specimens. Demographic characteristics of the two patient populations and equation variables including albumin, SHBG, and choice of association constants were examined to determine how they affected the correlation with isotope dilution equilibrium dialysis results. Samples with poor correlation between calculated and measured fT results were examined further to find common characteristics that could explain the deviations.

2. Experimental

2.1. Subjects

The Mayo Clinic Institutional Review Board approved this study. Non-fasting serum samples were obtained from two groups of patients that were seen for fT testing at Mayo Clinic Rochester. Group A consisted of 209 patients (171 males/38 females) seen between June and July, 2003. Group B consisted of 191 patients (93 males/98 females) seen between March and August, 2007. Group A consisted of all consecutive patients, while patients for Group B were intentionally selected to yield an equal number of males and females. Characteristics of patients from each group are shown in Table 1.

2.2. Methods

There was no change in relevant laboratory methodologies during the study. Serum was isolated by centrifugation and assayed immediately.

Total testosterone was measured by liquid chromatography–tandem mass spectrometry (LC–MS/MS, API 5000, Applied Biosystems-MDS Sciei, Foster City, CA; inter-assay CV 2.2–11.6%, reportable range 7–2000 ng/dL) as previously described [32]. The results were used for both the calculation of fT concentration from the isotope dilution equilibrium dialysis–determined fT percentage and for calculating fT with the various equations (see below).

SHBG was measured using a solid phase, two-site chemilumino-nometric assay on the Immulite 2000 automated immunoassay analyzer (Siemens, Deerfield, IL). Inter-assay CV of daily controls averaged over a month was 6.5 and 5.5% for 5 and 75 nmol/L SHBG, respectively, and the reportable range of the assay was 2–180 nmol/L. Lot-to-lot variability over the past three years has averaged 3% CV. Albumin was assumed to be 43 g/L for all patients except for an initial experiment comparing measured and assumed albumin concentrations and their effect on the fT calculations in patient group B. The albumin measurements for this subgroup were performed using a bromocresol green dye photometric assay (Roche Diagnostic Corp., Indianapolis, IN) on the Roche Chemistry Modular System. Inter-assay CV was <3% for 2.5 and 5 g/L albumin and the reportable range of the assay was 1–7 g/dL.

As gold standard reference for fT concentrations, the percentage of fT was measured by isotope dilution equilibrium dialysis as previously described [14,33]. Briefly, undiluted serum samples were prepared in a pH 7.4 phosphate buffer using HPLC-purified 30 ng/dL [1H]-testosterone per sample (Amersham, San Francisco, CA, as the tracer. Radioactivity was measured in tubing and dialysate following a 17 h dialysis at 37 °C on a Taurus liquid scintillation counter (Micro-Medic, Huntsville, AL). The resulting fT percentage was then multiplied by the total testosterone concentration measured by LC–MS/MS (see above) to obtain the fT concentration. Total error for the measurement of fT concentration is therefore the sum of the error in fT percentage and total testosterone concentration measurements. Inter-assay CV for the measurement of percent fT was determined in 100 additional patient samples (50 M/50 F) and ranged from 1 to 5%. Combining this error with the inter-assay CV for the measurement of total testosterone described above resulted in a total inter-assay error of 19 and 10% CV for concentrations of 1 and 5 μg/dL, respectively.

\[
fT (\text{mol/L}) = \frac{-b + \sqrt{b^2 + 4a[TT]}}{2a}
\]

For the calculated fT, total testosterone, SHBG and albumin (assumed or measured) values were entered into the mass action equation originally described by Sodergard et al. [21] and into the

### Table 1

Clinical characteristics of patient groups A and B.

<table>
<thead>
<tr>
<th></th>
<th>Group A</th>
<th>Group B</th>
</tr>
</thead>
<tbody>
<tr>
<td>n</td>
<td>209</td>
<td>191</td>
</tr>
<tr>
<td>Male</td>
<td>171</td>
<td>93</td>
</tr>
<tr>
<td>Female</td>
<td>38</td>
<td>98</td>
</tr>
<tr>
<td>Age</td>
<td>55, 57 (13–88)</td>
<td>49, 51 (14–90)</td>
</tr>
<tr>
<td>Male</td>
<td>58, 59 (13–88)</td>
<td>55, 57 (14–90)</td>
</tr>
<tr>
<td>Female</td>
<td>42, 39 (16–78)</td>
<td>42, 43 (15–75)</td>
</tr>
<tr>
<td>TT (ng/dL)</td>
<td>322, 335 (11–944)</td>
<td>238, 655 (7–1350)</td>
</tr>
<tr>
<td>Male</td>
<td>378, 377 (13–944)</td>
<td>459, 420 (7–1350)</td>
</tr>
<tr>
<td>Female</td>
<td>417, 33 (11–97)</td>
<td>26, 20, 5 (7–115)</td>
</tr>
<tr>
<td>Dialysis fT (ng/dL)</td>
<td>8.8, 9.0 (0.21–23.9)</td>
<td>5.73, 1.51 (0.07–41.8)</td>
</tr>
<tr>
<td>Male</td>
<td>10.1, 9.79 (0.21–23.9)</td>
<td>11.2, 10.0 (0.19–41.8)</td>
</tr>
<tr>
<td>Female</td>
<td>0.82, 0.63 (0.21–2.84)</td>
<td>0.48, 0.33 (0.07–2.82)</td>
</tr>
<tr>
<td>Albumin (g/L)</td>
<td>Not performed</td>
<td>43.8, 44 (24–55)</td>
</tr>
<tr>
<td>Male</td>
<td>43.8, 44 (24–55)</td>
<td>43.8, 44 (24–55)</td>
</tr>
<tr>
<td>Female</td>
<td>43.8, 44 (30–51)</td>
<td>43.8, 44 (30–51)</td>
</tr>
<tr>
<td>SHBG (nmol/L)</td>
<td>37.7, 35.5 (2.6–112)</td>
<td>46.3, 37.5 (7.7–200)</td>
</tr>
<tr>
<td>Male</td>
<td>38.3, 34.0 (2.6–98)</td>
<td>35, 33 (7.7–98)</td>
</tr>
<tr>
<td>Female</td>
<td>56.3, 55.3 (11–112)</td>
<td>57.2, 45 (11–200)</td>
</tr>
</tbody>
</table>

Results are given as mean, median, and full range in parentheses. ng/ml testosterone and free testosterone can be converted to nmol/L by multiplying by 0.0347.
The agreement between calculated and isotope dilution equilibrium dialysis measured FT concentrations varied depending on patient groups and association constants used in various equations (Fig. 1). As expected, the derived constants yielded the best fit with isotope dilution equilibrium dialysis results for the respective groups they had been derived from, surpassing the published constants (cA, group A: y = 1.05x – 0.41, cB, group B: y = 1.06x – 0.53). However, the derived constants yielded a worse fit when used on the other group (cA, group B: 0.83x – 0.25, cB, group A: y = 1.35x – 0.69), indicating that custom-derived constants are less effective outside of their designated patient set. Bland-Altman analysis yielded similar results (Supplementary data Fig. 1).

Since the major difference between the two groups was gender composition, differences between the sexes appeared to be responsible for this variation. To examine this further, males and females from both groups A and B were examined separately. Agreement between calculated and isotope dilution equilibrium dialysis measured FT results was expressed as percent difference. Percent differences above 20% were deemed unacceptable. Histogram analysis was performed to determine the distribution of patients with acceptable and unacceptable agreement (Fig. 2). This distribution varied both between genders and between equations. A significant percentage of patients displayed unacceptable agreement of calculated FT with isotope dilution equilibrium dialysis FT for at least one of the equations used; depending on the equation, this percentage of patients ranged from 32–72% in males to 29–57% in females. Some patients showed unacceptable agreement of calculated FT with isotope dilution equilibrium dialysis FT for multiple calculation equations, with 14.9% of males and 11.1% of females showing poor fit by all five calculations (data not shown). A further 13.1% of males and 28.5% of females showed poor agreement by four equations, 28.9% of males and 6.4% of females by three equations, 22.4% of males and 15.8% of females by two equations, and 20.5% of males and 38% of females by a single equation. This high percentage cannot be explained solely by variability of the isotope dilution equilibrium dialysis assay. Repeating 100 patient samples (50 M/50 F) by isotope dilution equilibrium dialysis yielded a linear regression slope of y = 0.92 + 0.10, r² = 0.95 (Supplementary data Fig. 2). Only 4% of these repeats had percent differences greater than 20% (data not shown).

In order to determine other sources of the variation between calculated and measured FT and between the different calculations, each of the components of the equations was examined. Age and concentration of albumin, total testosterone, and FT did not significantly correlate with percent difference (data not shown), indicating that those factors do not contribute to the observed variation. However, the SHBG concentration was related to the goodness of fit between calculated and isotope dilution equilibrium dialysis FT. Linear regression of a plot of SHBG concentration versus percent difference yielded a positive slope for most equations, indicating that increasing SHBG correlated with increasing percent differences and poorer fit (Fig. 3). The exception was the Sodergard equation, which had a neutral slope indicating a minimal impact of SHBG on the fit. Certain equations, such as the Emadi-Konjin and cA equations, were more affected by SHBG than other equations, such as the cB equation, as indicated by a larger slope. Fits for males and females were similarly impacted by SHBG concentrations when the Sodergard, Emadi-Konjin, and cB equations were used as indicated by their similar slopes. By contrast, the fit for males was affected more by SHBG compared to the fit for females when the Vermeulen and cA equations were used. These results indicate that both SHBG and gender can significantly affect the goodness of fit between calculated and isotope dilution equilibrium dialysis FT results.

### Table 2

<table>
<thead>
<tr>
<th>Testosterone association constants.</th>
<th>kA (L/mol)</th>
<th>kB (L/mol)</th>
<th>Ref.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vermeulen</td>
<td>3.6 x 10⁴</td>
<td>1 x 10⁹</td>
<td>[22]</td>
</tr>
<tr>
<td>Sodergard</td>
<td>4.06 x 10⁴</td>
<td>5.97 x 10⁹</td>
<td>[22]</td>
</tr>
<tr>
<td>Emadi-Konjin</td>
<td>1.3 x 10⁴</td>
<td>1.4 x 10⁹</td>
<td>[34]</td>
</tr>
<tr>
<td>cA</td>
<td>2.4 x 10⁴</td>
<td>1.2 x 10⁴</td>
<td></td>
</tr>
<tr>
<td>cB</td>
<td>1.8 x 10⁴</td>
<td>1 x 10⁹</td>
<td></td>
</tr>
</tbody>
</table>

Where kA is the association constant of testosterone binding to albumin and kB is the association constant of testosterone binding to SHBG.

- Constants cA and cB were derived from patient groups A and B in this study, respectively. These constants were the only variables used in the mass action equation described in Section 2 to generate the similarly named five equations.

### Variation of this equation derived by Vermeulen et al. [22] and Emadi-Konjin et al. [34]:

\[
a = k_A + k_A + (k_A \times k_B) ([\text{SHBG}] + [\text{alb}] - [\text{TT}])
\]

\[
b = 1 + k_A [\text{SHBG}] + k_B [\text{alb}] - (k_A + k_B) [\text{TT}]
\]

where kA is the association constant of testosterone binding to albumin (L/mol), kB is the association constant of testosterone binding to SHBG (L/mol), [SHBG] is the concentration of SHBG (nmol/L), [alb] is the concentration of albumin (converted from g/L to mol/L using the molecular weight of 69,000 g/mol), and [TT] is the concentration of total testosterone (mol/L). Different association constants were entered into the above mass action equation to generate five equations. The three sets of association constants of testosterone with SHBG and albumin that have been previously published by Vermeulen et al. [22], Sodergard et al. [21], and Emadi-Konjin et al. [34] were used for the calculations. In addition, new constants (cA and cB) were empirically derived for both patient groups to yield the best agreement with the isotope dilution equilibrium dialysis data, defined as a regression slope as close to 1 as possible. These constants used in the study are the only variable in the above mass action equation and are summarized in Table 2.

### 2.3. Data analysis

Results in Table 1 are shown as mean, median, and full range. Deming regression and Bland-Altman analysis was performed using Analyse-It version 3.0 (Analyse-It Software Ltd., United Kingdom) to compare FT results measured by isotope dilution equilibrium dialysis assay. Repeating 100 patient samples (50 M/50 F) by isotope dilution equilibrium dialysis yielded a linear regression slope of y = 0.92 + 0.10, r² = 0.95 (Supplementary data Fig. 2). Only 4% of these repeats had percent differences greater than 20% (data not shown).

In order to determine other sources of the variation between calculated and measured FT and between the different calculations, each of the components of the equations was examined. Age and concentration of albumin, total testosterone, and FT did not significantly correlate with percent difference (data not shown), indicating that those factors do not contribute to the observed variation. However, the SHBG concentration was related to the goodness of fit between calculated and isotope dilution equilibrium dialysis FT. Linear regression of a plot of SHBG concentration versus percent difference yielded a positive slope for most equations, indicating that increasing SHBG correlated with increasing percent differences and poorer fit (Fig. 3). The exception was the Sodergard equation, which had a neutral slope indicating a minimal impact of SHBG on the fit. Certain equations, such as the Emadi-Konjin and cA equations, were more affected by SHBG than other equations, such as the cB equation, as indicated by a larger slope. Fits for males and females were similarly impacted by SHBG concentrations when the Sodergard, Emadi-Konjin, and cB equations were used as indicated by their similar slopes. By contrast, the fit for males was affected more by SHBG compared to the fit for females when the Vermeulen and cA equations were used. These results indicate that both SHBG and gender can significantly affect the goodness of fit between calculated and isotope dilution equilibrium dialysis FT results.
The relationship between SHBG concentration, gender, and fit was further examined by dividing males and females into quartiles (Q1–Q4) based on percent difference (Fig. 4). Group Q1 consisting of patients with the best agreement between isotope dilution equilibrium dialysis and calculated fT, with percent differences in group Q1 ranging from 0 to 18% in males and from 0 to 11% in females depending on the equation used. Group Q4 consisted of patients with the worst agreement, with percent differences increasing to 23–103% for males and 22–105% for females. Similar to the regression analysis of percent difference and SHBG described above, patients in group Q4 had significantly higher concentrations of SHBG, with average concentrations in group Q4 of 26.4–41.8 nmol/L in males and 30.6–58.9 nmol/L in females (data not shown), while the corresponding values in group Q4 were 33.4–47.4 nmol/L and 60.5–97.7 nmol/L, respectively (p < 0.05 or <0.001, depending on equation). The percentage of male and female patient samples tested at our institution over the past year with SHBG concentrations greater than 47.4 nmol/L in males and 97.7 nmol/L in females is 13% (n = 586) and 11% (n = 881), respectively (data not shown). This relationship between SHBG and fit was again observed for all equations except for the Sodergard equation.

4. Discussion

During the past decade, many laboratories have shifted away from isotope dilution equilibrium dialysis because of the cost and labor required and the amount of variability inherent in the assay. Measuring fT by ultrafiltration, while showing promising results in several studies [35–37], was found to have an unacceptable rate of device failure at our institution. The problems with these methods make calculated fT an attractive alternative. However, several publications have pointed out the variability of calculated fT results between studies, patient groups, and laboratories [23–26]. There is currently no consensus on which equation and constants are considered ideal. Instead the recommendation has been to tailor equations to individual laboratories and patient populations to obtain the best results with the gold standard. This approach would make it difficult to compare results between laboratories and potentially complicate any future attempts to standardize calculated fT assays.

In this study, we compared calculated fT results, derived by using several variation of the mass action equation first published by Sodergard et al., to two patient groups with matched results obtained by the gold standard methodology of isotope dilution equilibrium dialysis. Sodergard-derived equations incorporate serum concentrations of albumin, SHBG, and total testosterone and association constants for testosterone binding to albumin and SHBG. As previously reported, albumin concentration had little effect on the calculated result [22]. Total and fT concentrations were also found to minimally impact equation performance. However, regardless of the equation used, there were a significant proportion of samples that had highly discrepant results between isotope dilution equilibrium dialysis and calculated testosterone. The high number of discrepant samples cannot be explained solely by inherent variabilities in the isotope dilution equilibrium dialysis assay.

This study shows a significant number of patients with discrepant calculated and isotope dilution equilibrium dialysis fT results. Even though the Sodergard equation was minimally affected by SHBG, it still displayed poor performance overall, with 29% of females and 34% of males in this study showing unacceptable agreement between that equation and isotope dilution equilibrium dialysis. When examining all male and female patient samples tested at our institution over the past year, 11% of male and 13% of female samples had SHBG concentrations above the average con-
Fig. 2. Histogram distribution of percent difference between calculated and isotope dilution equilibrium dialysis measured fT. Histogram plots were created for females and males from both groups A and B (F = 136, M = 264) based on percent difference between free testosterone results measured by isotope dilution equilibrium dialysis and calculated using Vermeulen (A), Sodergard (B), Emadi-Konjin (C), cA (D), and cB (E) equations. Percentages of total males or females with a percent difference greater than the acceptability limit of 20% are indicated in insets for each equation.
Fig. 3. Linear regression of fit between calculated and isotope dilution equilibrium dialysis fT results and SHBG concentration. Female (●) and male (○) patients from both groups A and B (F = 136, M = 264) were plotted for SHBG concentration versus percent difference of free testosterone measured by isotope dilution equilibrium dialysis compared with calculated using Vermeulen (A), Sodergard (B), Emadi-Konjin (C), cA (D), and cB (E) equations. Linear regression lines were determined for both female (dashed line) and male (dotted line) samples. Slope, intercept, and correlation coefficient are indicated in insets.

Consistent methodologies for isotope dilution equilibrium dialysis, SHBG, and total testosterone analysis were used throughout this study. The number of discrepant samples would undoubtedly change with the introduction of other methods and platforms to measure SHBG and total testosterone. For example, recent CAP PT survey results for SHBG indicate that the Immulite 2000 used in this study had 5.8–6.8% CV between laboratories, while the Immulite 1000 had up to 13.4% CV (Y Ligand Special, 2009). This higher variability will result in higher variability in the fT calculation and larger numbers of samples with discrepant calculated and isotope dilution equilibrium dialysis fT results. The use of different testosterone and SHBG assays over serial fT calculations of a patient would introduce additional variability and potentially complicate interpretation of the patient’s status. Several studies have demonstrated that the use of different assays to measure SHBG yield different calculated fT results and different correlations between measured and calculated fT [17,38]. The wide range of SHBG values in this study, attributable to inter-individual factors including gender, age, alterations in hormonal and metabolic factors, and polymorphisms in the SHBG gene, may also contribute to the variation between equations [39].
Testosterone association constants and SHBG, the remaining components of the \( fT \) equation, were both found to impact equation performance. Systematic and random errors varied widely depending on the association constants used. Choice of association constants significantly affected calculated results, as constants derived specifically for this study were found to correlate better with isotope dilution equilibrium dialysis results than previously published constants. However, these derived constants only yielded a superior fit with isotope dilution equilibrium dialysis results when used on the patient groups from which they had been derived. These findings indicate that there is significant variability in the performance of the constants between groups of as small as 200 patients. It is not fully understood why certain sets of constants correlated better with isotope dilution equilibrium dialysis than others, though the performance of certain constants may be affected by other circulating hormones including estradiol and DHT.

Males and females with the poorest fit between calculated and measured \( fT \) results had significantly higher serum concentrations of SHBG. This relationship between fit and SHBG was dependent on which equation was used. Results calculated using the Sodergard equation were not affected by SHBG, while results calculated using the other equations were affected to various degrees. This characteristic of the Sodergard equation may be because the assumed affinity of testosterone for SHBG used in the equation was the lowest of the five equations examined, therefore samples with high SHBG would display less discrepancy between calculated and measured \( fT \) results. In addition, the large range of SHBG concentrations in this study, a consequence of examining both males and females in this study as well as other inter-individual factors, may have contributed to the variation between measured and calculated \( fT \) results.

Gender was also a factor in variability between calculated and assayed results. Deming regression demonstrated that the equations performed differently in patient groups A and B, which had different compositions of males and females. Males and females also had a different distribution of percent differences between calculated and assayed \( fT \) results. In addition, a higher SHBG concentration was found to affect fit in males more than in females when certain equations were used. While the exact cause of this gender variability is not known, it is possible that differences between genders including the distribution of sex steroids play a role. These and similar equations may depend too much on SHBG concentration and neglect the contribution of other binding proteins and steroids, such as estradiol and DHT, which vary between the genders and may affect testosterone association constants. More detailed equations may show better agreement with isotope dilution equilibrium dialysis.

In conclusion, variability in performance of the family of testosterone mass action equations examined in this study is affected by several factors, chiefly gender, SHBG concentration and choice of association constants. Because of this variability, a large percentage of patients show a substantial and unacceptable deviation of calculated \( fT \) results from those obtained by the reference methodology, isotope dilution equilibrium dialysis. Designing different equations specifically for patient subgroups, e.g. male or female patients or patients who have elevated SHBG concentrations, may improve performance. However, these equations that use only total testosterone and SHBG measurements may be too simplistic to encompass the entire range of patients referred for \( fT \) testing, resulting in a percentage of patients that will be discrepant from isotope dilution equilibrium dialysis regardless of alterations to the equation. While additional analysis of other hormones may yield more accurate results, this will increase overall cost, labor, and variability, reducing or eliminating the advantages of using the equations in the first place. Currently, isotope dilution equilibrium dialysis appears the only valid approach for \( fT \) measurements.
otherwise accuracy of individual measurements can be severely compromised in many patients.

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.steroids.2010.08.008.

References


