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High Prevalence of Low Serum Biologically Active Testosterone in Older Male Veterans

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Abstract

Objectives: Assess the prevalence of hypogonadism in older male Veterans by comparing direct measurements of total testosterone (T) and bioavailable testosterone (BioT) versus indirect BioT values derived from existing and newly developed regression analyses.

Design: Cohort study.

Setting: Malcom Randall VA Medical Center, Gainesville, FL.

Participants: Community-dwelling male Veterans aged 60 and older (n = 203).

Measurements: Total T, BioT, albumin, sex hormone–binding globulin (SHBG), and body mass index were evaluated. Blood values were assessed via liquid chromatography–tandem mass spectrometry (LC-MS/MS) and clinical or commercially available immunoassays to compare accuracy among assessment techniques. Existing and newly developed multiple regression analyses were evaluated to assess accuracy in predicting BioT.

Results: Total T was 13.80 ± 6.25 nmol/L (398 ± 180 ng/dL) and was low (≤10.4 nmol/L or ≤300 ng/dL) in 34% of participants. SHBG was 58 ± 35 nmol/L and elevated (≥62 nmol/L) in 36% of participants. BioT was 1.94 ± 0.97 nmol/L (56 ± 28 ng/dL), with 72% of participants below the

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clinical cutoff (≤2.43 nmol/L or ≤70 ng/dL). Albumin was within the normal clinical range. Total T and BioT measured via immunoassay and LC-MS/MS were moderately to highly correlated, with no differences between assessment methods. Several existing predictive equations overestimated BioT by 74% to 166% within our cohort ($P < .001$). A newly developed regression model that included total T, SHBG, albumin, and age more accurately predicted BioT, with values correlated ($r = 0.508$, $P < .001$) and comparable to LC-MS/MS.

Conclusion: In our cohort, the prevalence of low total T was higher and low BioT was markedly higher than reported in the general age-matched population, indicating a greater incidence of hypogonadism in older male Veterans. In addition, existing empiric formulae, derived from other populations produced BioT values that were considerably greater than those directly measured, whereas our newly developed regression analysis provides improved predictive capabilities for older male Veterans.

Keywords

Testosterone; bioavailable testosterone; SHBG; hypogonadism; multiple regression

The prevalence of hypogonadism [ie, low serum testosterone (T)] in men increases with age[1,2] and is associated with a number of deleterious effects, including osteopenia, sarcopenia,[3] and higher all-cause mortality rates in older Veterans.[4] Sex hormone–binding globulin (SHBG) increases with age, further contributing to hypogonadism by lowering the fraction of T that is biologically active.[1,2] Typically, hypogonadism is assessed by evaluating total serum T which is composed of that bound to SHBG or albumin, and that which circulates in the unbound state (ie, free T).[3] Bioavailable testosterone (BioT) is the fraction of total T that circulates either free or loosely bound to albumin, representing the proportion that is able to bind with androgen receptors, whereas T that is tightly bound to SHBG is biologically inactive.[3]

Total T is most readily assessed in clinical laboratories by immunoassay or liquid chromatography–tandem mass spectrometry (LC-MS/MS). However, measurement of BioT is rarely available in the clinical setting as the method typically requires separation of SHBG-bound testosterone via ammonia sulfate precipitation or other methods and radioactive isotope tagging prior to assessment.[5–7] Thus, BioT is often estimated using one of several prediction equations, such as the Vermeulen[8] or Morris[9] equations. The Vermeulen BioT prediction equation was derived from the direct measurements of serum albumin, SHBG, and total T in a cohort of apparently healthy ambulatory men.[8] However, the Morris equation[9] was validated using a population of men of any age undergoing coronary angiography and relies on measured concentrations of total T and SHBG to estimate BioT. Of interest, neither of these commonly used equations included age as a factor in their models, were validated using an entirely elderly male population, nor evaluated a Veteran population where comorbidities are often higher than the general population.[10] Age and existing comorbidities may alter total T, SHBG, and/or subfractions of total T,[11] potentially biasing the estimation of BioT in the older male Veteran population. Because chronic disease risk is higher in Veterans than in the general age/sex-matched US population,[12] we hypothesized that a greater incidence of hypogonadism may be present in older male Veterans, and moreover, that the existing BioT prediction equations may not

accurately reflect true BioT concentrations. Our primary purpose was to assess the prevalence of low total T and low BioT in a cohort of community-dwelling older male Veterans. We also determined if existing BioT prediction equations accurately estimate measured BioT in our population and we assessed the accuracy of newly developed BioT regression models that are specific to our older male Veteran population.

Methods

Study Design

This study was approved by the Institutional Review Board of the University of Florida. All participants provided written informed consent. Participants were community-dwelling male Veterans ≥60 years of age who had been seen as patients at North Florida/South Georgia Veterans Health System (NF/SG VHS) and who volunteered to undergo screening for a randomized clinical trial of intramuscular TRT plus finasteride treatment.[13] A pre-screening health history questionnaire and medical records check was completed by 1117 male volunteers to determine eligibility, of which 203 qualified/enrolled. To ensure our findings remained eligible to the general older male Veteran population, participants were not screened for signs/symptoms associated with low testosterone prior to enrollment. We excluded individuals who had standard contraindications for intramuscular TRT, including a history of prostate or breast cancer, severe benign prostate hyperplasia, American Urological Association International Prostate Symptom Score (AUA/IPSS) ≥25, class 3 or 4 congestive heart failure, diagnosed sleep apnea, body mass index (BMI) > 35, who were taking coumadin or failed the MiniCog test. In addition, those who had received TRT within the previous 4 weeks, or finasteride/dutasteride within the previous 6 months, were excluded because these agents may alter endogenous sex-steroid concentrations. Participants who qualified/enrolled underwent a structured medical history and physical examination, as previously reported.[13] Blood samples were acquired twice between 08:00AM and 10:00AM, separated by at least 30 minutes, according to the Endocrine Society Guidelines for assessing androgen deficiency.[14]

Serum Analysis

Serum was separated by centrifugation and assayed immediately or stored at −80°C until analysis. Total T was analyzed in the Clinical Laboratory at the North Florida/South Georgia Veterans Health System (NF/SG VHS) using an automated Cobas electrochemiluminescence immunoassay, which is the clinical standard within the VA Healthcare System. Reliability of the Cobas immunoassay was determined by analyzing 22 serum samples (across the normal physiologic total T range) in duplicate, with the CV being 5.3%. The NF/SG VHS Clinical Laboratory also measured hemoglobin and hematocrit (Sysmex XE2100 automated CBC system) and albumin (Roche-Cobas 501 system for automated chemistry). Total T measures were then validated by LC-MS/MS, which is recommended by the Endocrine Society guidelines[14] and clinical chemistry experts[15] because it is the gold standard assessment method. The bioavailable fraction of total T (BioT) was then separated by ammonium sulfate precipitation of samples spiked with [^{3}H]testosterone (PerkinElmer Life Sci, Boston, MA) according to the method of Trembley,[5] and analyzed both by LC-MS/MS and by commercially available enzyme-linked immunosorbent assay (ELISA) (ALPCO, Salem,

NH). Reliability of the ammonium sulfate precipitation method was determined by extracting 6 serum samples (across the normal physiologic range) 3 times each and analyzing BioT a total of 4 times per extract, resulting in intra- and inter-extraction CVs of 2.4% and 5.9%, respectively. SHBG was analyzed by ELISA (ALPCO, Salem, NH).

In addition, BioT was estimated using 2 standard equations.

Morris equation (uses total T and SHBG)[9]:

$$BioT\,nmol/L = EXP(-0.266 + (0.955 * LN(total\,Tnmol/L)) - (0.228 * LN(SHBG\,ng/L)))$$

Vermeulen equation (uses total T, SHBG, and albumin):[8]

$$fT = (T - [N * fT])/(K_S(SHBG - T + [N * T]))$$

from which BioT is determined using

$$BT = fT + AT$$

where T = molar concentration of total T, fT = molar concentration of free T, BT = molar concentration of BT, SHBG = molar concentration of SHBG, K_s = affinity constant of SHBG for T (1.0×10^9 L/mol), N = K_a * Ca + 1 (where Ca = albumin concentration), and AT = molar concentration of albumin-bound T (~K_a * Ca * fT), as reported by Dechaud et al.[6] The Vermeulen calculator is available at http://www.issam.ch/freetesto.htm.

LC-MS/MS Methods

Serum samples were analyzed on a Bruker EVOQ elite triple-quadrupole mass spectrometer in positive-ion mode (heated electrospray ionization) with selected reaction monitoring (SRM) to assess total T and BioT. Separation was achieved with an ACE Super C18 UHPLC column (100 × 2.1 mm, 2 μm) using gradient elution on an Advance UHPLC. Mobile phase A was 0.1% formic acid in water, and mobile phase B was 0.1% formic acid in methanol. The flow rate was 300 μL/min. To increase sensitivity, serum samples were derivatized using an Ampliflex Keto reagent Kit (AB Sciex LLC, Redwood City, CA). Total T and BioT were quantified with an external calibration curve with the addition of deuterated internal standards.

Definition of Low Total and Bioavailable Testosterone

Low T was defined as a serum total T concentration ≤10.40 nmol/L (≤300 ng/dL) or serum BioT concentration ≤2.43 nmol/L (≤70 ng/dL), per the Endocrine Society Guidelines.[14] These same criteria were used for enrollment in the clinical trial associated with this analysis.[13,16,17]

Statistical Analysis

All statistical analyses were conducted using SAS 9.3 (SAS Institute, Cary, NC). Descriptive statistics are reported as means ± standard deviation in SI units (and conventional units),

with the threshold for significance defined as $P < .05$ (2-sided). Normal distribution of BioT and each independent hormone variable was checked using a Shapiro-Wilks test in combination with graphical methods. Data were transformed using the natural logarithm (ln) for all statistical analyses, because of the inherently skewed distributions in all hormone concentrations. Pearson correlation coefficients were used to determine associations among assessment methods for total T and for BioT. Paired-samples *t* tests were used to determine differences among assessment methods for total T and for BioT. One-sample *t* tests were used to analyze differences in SHBG between our cohort and several other large cohorts of elderly men.[18–23]

We used a multiple linear regression modeling approach to develop and validate a formula for estimating BioT from measured hormone values. For each model, the ln of each independent hormone including total T, SHBG, and albumin were entered as candidate predictors. In the final equation, inclusion of age and BMI were determined through a stepwise selection procedure. The SAS default for removal in stepwise model selection was $P = .15$. The regression model with the highest R^2 was retained as the final model. BioT concentrations measured via LC-MS/MS and by our predictive regression model were then individually compared to determine the agreement in the proportion of individuals that exhibit low BioT. Bland-Altman plots[24] were used to compare hormone concentration agreement in samples measured via LC-MS/MS to those measured with other techniques or predicted using our regression model and other published BioT prediction equations.[9,25]

Results

Total Testosterone Comparisons for LC-MS/MS and Cobas Assay

Descriptive statistics for the entire cohort and for the population subsets that exhibited low total T or low BioT are reported in Table 1. Within the entire cohort, 34% of elderly male Veterans exhibited low total T (≤10.40 nmol/L or ≤300 ng/dL) when assessed by either LC-MS/MS or the Cobas assay. LC-MS/MS and Cobas methods exhibited an 88% agreement in identifying individuals with low total T and values were highly correlated within the entire cohort ($r = 0.883$, $P < .001$) and for all population subsets (Table 2), with no differences in total T concentrations present among assessment methods. Bland-Altman analysis of the agreement between LC-MS/MS and Cobas log-transformed values indicated a high level of agreement between these assessment methods, with a mean total T difference of 0.027 ± 0.22 ln(nmol/L) (Supplementary Figure 1).

Bioavailable Testosterone Comparisons for LC-MS/MS and ELISA

Within the entire cohort, 72% and 81% of elderly male Veterans exhibited low BioT when assessed by LC-MS/MS or by ELISA, respectively, with 81% agreement among methods in identifying individuals with low BioT. BioT concentrations assessed via LC-MS/MS and ELISA were moderately correlated within the entire cohort ($r = 0.622$, $P < .001$) and for all population subsets analyzed (Table 3). No differences were present among BioT values assessed via LC-MS/MS or ELISA within the entire cohort or the cohort with low total T. However, the ELISA resulted in an 11% higher BioT concentration (in SI units) than LC-MS/MS in the population subset exhibiting low BioT (Table 3, $P < .001$). Bland-Altman

analysis of the agreement between LC-MS/MS and ELISA log-transformed values indicated a high level of agreement between these assessment methods, with a mean BioT difference of −0.002 ± 0.449 ln(nmol/L) (Supplementary Figure 2).

Comparison of Predicted Bioavailable Testosterone With LC-MS/MS

BioT measured via LC-MS/MS was positively correlated with predicted BioT from the Morris ($r = 0.537$, $P < .001$) and Vermeulen equations ($r = 0.604$, $P < .001$) for the entire cohort and for all population subsets (Table 3). However, the predicted BioT concentrations from the Morris and Vermeulen equations were 96% to 153% higher than that measured by LC-MS/MS (in SI units) for the entire population ($P < .001$) and were 50% to 200% higher for the population subsets ($P < .001$), with the largest variances occurring for the population subset with low BioT. In all populations subsets analyzed, predicted BioT concentrations from the Morris and Vermeulen equations were highly and positively associated with one another (Supplementary Table 1), with the Vermeulen equation producing values that were 25% to 36% higher than the Morris equation ($P < .001$). Bland-Altman analysis of the agreement between LC-MS/MS and Morris and Vermeulen log-transformed BioT values indicated a positive bias for both prediction equations, with mean BioT differences of 0.729 ± 0.462 ln(nmol/L) for the Morris equation and 0.946 ± 0.454 ln(nmol/L) for the Vermeulen equation (Supplementary Figure 3A, B).

SHBG and Albumin Concentrations

In our cohort of elderly male Veterans, SHBG concentrations were 17% higher than that reported in a separate study that assessed SHBG (using a similar immunoassay method) in a cohort of 1657 elderly men[19] ($P = .001$) and 34% higher than the weighted mean of 6 recent studies (n = 3664) that assessed SHBG in elderly men using a variety of analytical methods.[18–23] Albumin concentrations were within the normal clinical reference range for the total cohort and for the population subsets (Table 1).

Multiple Regression Analysis

We used multiple linear regression to develop an equation that estimates BioT concentrations from the entire cohort of elderly male Veterans who had directly measured values for total T, BioT, SHBG, albumin, age, and BMI (n = 194). The inclusion of ln total T, ln SHBG, ln albumin, and age each significantly improved the regression model (adjusted $R^2 = 0.41$; $P < .001$); however, BMI was not retained in the final model (Table 4). Regression (β) coefficients were calculated from the regression model and applied to the corresponding values, as follows:

$$\ln BioT = -2.113 - 0.009(age) + 0.753(\ln Total\,T) - 0.445(\ln SHBG) + 0.821(\ln Albumin)$$

(ln = natural log, units are nmol/L for T and SHBG, g/L for albumin, and years as closest whole number for age).

Using this regression model, we observed that a similar proportion of individuals exhibited low total T (34%) and low BioT (74%) when compared to that determined by LC-MS/MS. BioT calculated from our model was positively correlated with BioT measured via LC-

MS/MS ($r = 0.649$, $P < .001$) and was not different from the values measured via LC-MS/MS for the entire population or for the subset with low total T (Table 5). Ultimately, the regression equation indicated that 84% of the population exhibited low BioT, with 66% agreement in identifying individuals with low BioT when compared with LC-MS/MS. In contrast, the BioT predicted by our regression equation was 14% higher than that measured by LC-MS/MS in the subset of participants with low BioT ($P < .001$, Table 5). The BioT values predicted from our regression equation were also correlated with BioT predicted from the Morris ($r = 0.817–0.849$, $P < .001$) and Vermeulen equations ($r = 0.952–0.961$, $P < .001$). However, the predicted BioT concentrations (in *SI Units*) derived from the Morris and Vermeulen equations were 105% and 166% higher than that derived from our regression equation for the entire population ($P < .001$), respectively, and 74% to 164% higher than that derived from our regression analysis for the population subsets ($P < .001$). Bland-Altman analysis of the agreement between LC-MS/MS and our regression model indicated a moderate level of agreement between methods, with a mean BioT difference of 0.022 ± 0.411 ln(nmol/L) (Supplementary Figure 3C).

Discussion

The prevalence of low serum T increases with age[1,2] and is associated with loss of muscle[26] and bone,[3,27] along with increased all-cause mortality rates in older male Veterans.[4] Although serum total T is the most readily available measure of hypogonadism, serum BioT may be more meaningful, as it represents the fraction of total T that exerts biological action.[3] However, in most clinical settings, direct BioT measurements are not available, because of the complexity of measurement,[15] so predictive equations are commonly used. In this regard, we followed the recommendations of the Endocrine Society[14] and of clinical chemistry experts[15] to ensure accurate biochemical analysis in this study. These steps included (1) performing 2 assessments of morning total T (before 11:00AM), (2) assessing SHBG and albumin, (3) calculating BioT, and (4) utilizing a gold standard approach (ie, LC-MS/MS) to ensure accuracy and reliability of all testosterone assessments. In addition, we directly measured BioT (via ammonium sulfate precipitation followed by LC-MS/MS)[8] to assess the validity of existing BioT regression models in our population and to determine our own population-specific regression model because Veterans exhibit higher chronic disease incidence than the general population,[28] which may affect androgen status. Using these methods, our total and BioT measurements were highly reproducible across the physiologic testosterone range for adult males. We report that our cohort of older male Veterans displayed a higher prevalence of low circulating total T and a much higher incidence of low BioT when assessed directly via LC-MS/MS, in comparison to men of similar ages within the general population.[11] In addition, 2 commonly used predictive equations[8,9] greatly overestimated BioT concentrations and underestimated the proportion of older Veterans with low BioT. To address this, we developed and validated a BioT regression model specific to the older male Veteran population that produces improved predictive value in comparison with previous equations that used data derived from either the general population of healthy men[8] or from men undergoing coronary angiography.[9]

Testosterone circulates in 3 subfractions, of which 1%–2% is unbound (free T), 40%–50% is loosely bound to albumin, and 50%–60% is tightly bound to SHBG.[11] Of these, BioT

includes the fractions of free T and albumin-bound T, ranging from 15% to 50% of total T.[11] In order to determine prevalence of hypogonadism, we evaluated circulating total T and BioT using several analytical methods, including the automated Cobas electrochemiluminescence immunoassay (common in many VA Medical Center Clinical Laboratories) or commercially available ELISA and subsequently validated our findings using gold standard LC-MS/MS methods. Cross-comparison of immunoassays used for sex-steroid hormone analysis is an essential step to determine assay validity and has been recommended by Collier et al,[15] because significant cross-reactivity may exist between androgens evaluated with this methodology.[25] In our older male Veteran cohort, the prevalence of hypogonadism, as assessed by total T, was moderately higher than what is typically observed in the general age-matched population,[11] and strong correlations were present for total T concentrations with no discernible differences between analytical methods. Few clinical laboratories regularly evaluate BioT because the assessment method is labor intensive,[15] typically requiring ammonium sulfate precipitation to remove SHBG-bound proteins from serum samples spiked with radiolabeled internal standards, prior to analysis of the supernatant.[5,6] Nevertheless, other methods to separate non-SHBG-bound T also exist.[19] We used ELISA to evaluate BioT and observed a much higher prevalence of low BioT in comparison to what is typically observed in other age/sex-matched populations, [11] results that we validated via LC-MS/MS. BioT may be significantly lowered by conditions, such as aging, that can result in elevated SHBG or by other comorbidities,[13] that may lower total T or circulating albumin. In our cohort, the high prevalence of low BioT appears to be a product of increased SHBG, compared with published findings from several other non-Veteran specific age/sex-matched cohorts,[18–23] and a moderately higher prevalence of low total T, whereas albumin remained within the normal reference range.

BioT is often determined using predictive equations because of the lack of clinical laboratories that perform direct BioT assessments. As a result, empirical formulas have been developed to predict BioT from measurements of total T and SHBG[9] or from total T, SHBG, and albumin.[8] In our older male Veteran cohort, differences existed between directly measured BioTconcentrations and BioT predicted by either the Morris or Vermeulen equations, with both equations significantly overestimating BioT concentrations and underestimating the number of individuals with low BioT (≤2.43 nmol/L or 70 ng/dL). Importantly, these equations are population specific and were derived from the general population of men (n = 28) across the age span[8] or from men (n = 1072) undergoing coronary angiography, more than half of whom were under the age of 60,[9] which does not represent the characteristics of our older male Veteran cohort. Interestingly, others have reported age-associated discrepancies between measured and calculated BioT using these predictive equations,[6] suggesting that inclusion of age may be an important characteristic to include in BioT regression models.[15]

We used multiple regression analysis to develop equations from our Veteran cohort that more accurately predicted BioT from measured concentrations of total T, SHBG, albumin, and age. Overall predicted mean BioT values from our equation were moderately correlated and not different from BioT concentrations measured via LC-MS/MS for the entire Veteran cohort and for the subpopulation that exhibited low total T. However, our regression analysis slightly overestimated BioT for the subpopulation of Veterans that exhibited low BioT

assessed via LC-MS/MS. In contrast, the Morris and Vermeulen equations produced BioT values that were 74%–166% higher than our regression analysis for the entire cohort and for all subpopulations, with Bland-Altman plots displaying positive bias for both equations. Despite the improved predictive capabilities of our regression analysis, the Bland-Altman plot indicates that variation also exists in individual BioT concentrations assessed via our regression analysis and those derived from LC-MS/MS. This suggests that our regression equation is well suited for studies assessing overall mean BioT concentrations in older male Veteran populations, but may be somewhat less suited to estimate BioT concentrations from individual participants in a clinical setting. Regardless, improving accuracy of BioT assessments may allow better determination of the appropriateness of TRT in older men who may or may not appear hypogonadal based on assessment of total T alone.

Testosterone replacement therapy for older hypogonadal men is controversial, and the risk/benefit ratio is a topic of intense debate.[29,30] Currently, the Endocrine Society recommends TRT for men who are frankly hypogonadal (serum total T <250 ng/dL or <10.40 nmol/L) and who exhibit signs/symptoms associated with hypogonadism.[14] The rationale for these dual criteria are that serum total T and BioT inherently reflect circulating androgen concentrations but are not necessarily indicative of tissue androgen action, which is influenced by (1) the concentrations of bioavailable androgens in circulation, (2) androgen receptor expression within tissue, and (3) sex steroid hormone metabolism within tissue.[26,27] Interestingly, meta-analyses report that TRT produces musculoskeletal benefits in men with low T who do not meet the Endocrine Society standard for frank hypogonadism.[31,32] In our cohort of older male Veterans, low total T was present in 34% of men, while low BioT was present in 72% of men. These results suggest that assessment of BioT may identify a larger group of individuals who exhibit hypogonadism and require TRT. However, the difficulty in directly determining serum BioT and in developing highly accurate predictive models represent challenges in identifying older men who may require TRT. Regardless, the decision to treat with TRT should follow clinical guidelines and include measurement of circulating androgen concentrations, along with assessing signs/symptoms of hypogonadism.[14,15]

In summary, we report that older male Veterans exhibit hypogonadism in a slightly higher prevalence when assessed via total T and a much higher prevalence when assessed via BioT, in comparison to the age-matched general population. Several existing BioT prediction equations greatly overestimated BioT in our Veteran cohort. As such, we developed an empirical equation from a cohort of older male Veterans and found that it more accurately predicted the mean BioT from our entire cohort than previous prediction models. However, our regression model was not sufficiently accurate for determination of BioT for all individuals within our cohort. This suggests further research is necessary to develop improved techniques to directly measure circulating BioT and/or to predict BioT via regression analysis. In conclusion, our findings indicate that BioT assessments may be beneficial in identifying individuals that are hypogonadal and suggest a greater need for TRT in the older male Veteran population.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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Table 1

Participant Characteristics From the Total Cohort and From Subsets With Low Total T (≤10.40 nmol/L) or Low BioT (≤2.43 nmol/L)*

	Total Cohort (n = 203)	Low Total T (n = 70)	Low BioT (n = 147)
Age, y	70.0 ± 7.1	67.8 ± 7.8	67.9 ± 7.6
Weight, kg	88.2 ± 15.4	95.1 ± 14.9	88.2 ± 15.0
BMI	28.9 ± 4.5	30.6 ± 3.6	29.0 ± 4.5
Total T, nmol/L	13.79 ± 6.51	8.00 ± 1.77	13.33 ± 6.49
BioT, nmol/L	1.93 ± 0.99	1.63 ± 1.10	1.48 ± 0.53
SHBG, nmol/L	58.4 ± 34.5	44.0 ± 26.2	61.7 ± 33.9
Albumin, g/L	43.67 ± 2.40	43.66 ± 2.43	43.45 ± 2.48
Low total T, %	34	100	38
Low BioT, %	72	80	100

*Values are means ± standard deviation in SI units, unless indicated otherwise. Total T and BioT were assessed by LC-MS/MS. To convert T or BioT to nanograms per deciliter, multiply value by 28.84, to convert albumin to grams per deciliter, divide value by 10.

Table 2

Comparison of Total T Assessed via LC-MS/MS or Cobas Assay for the Total Cohort and From Subsets With Low Total T (≤10.40 nmol/L) or Low BioT (≤2.43 nmol/L) *

	Total T via LC-MS/MS, nmol/L	Total T via Cobas, nmol/L	Difference, nmol/L	Correlation
Total cohort	13.79 ± 6.51	13.41 ± 5.96	−0.37 ± 3.20, $P = .098$	$r = 0.883$, $P < .001$
Low total T	8.00 ± 1.77	8.37 ± 2.90	−0.36 ± 2.25, $P = .182$	$r = 0.789$, $P < .001$
Low BioT	13.33 ± 6.49	12.99 ± 5.86	0.35 ± 3.37, $P = .216$	$r = 0.872$, $P < .001$

* Values are reported as means ± standard deviation in SI units for ease of interpretation, but statistical outcomes were calculated using the natural logarithm of these values. To convert T to nanograms per deciliter, multiply value by 28.84.

J Am Med Dir Assoc. Author manuscript; available in PMC 2021 August 08.

Table 3

Comparison of BioT Measured via LC-MS/MS or ELISA or Predicted by the Morris or Vermeulen Equations for the Total Cohort and From Subsets With Low Total T (≤10.40 nmol/L) or Low BioT (≤2.43 nmol/L)*

	Direct Measurements		Prediction Equations	
	LC-MS/MS	**ELISA**	**Morris**	**Vermeulen**
Total cohort				
BioT, nmol/L	1.93 ± 0.99	1.88 ± 0.79	3.78 ± 1.49	4.89 ± 2.56
Difference vs LC-MS/MS	N/A	−0.05 ± 0.83, $P = .369$	1.85 ± 1.44, $P < .001$	2.96 ± 2.31, $P < .001$
Correlation vs LC-MS/MS	N/A	$r = 0.622$, $P < .001$	$r = 0.537$, $P < .001$	$r = 0.604$, $P < .001$
Low total T				
BioT, nmol/L	1.63 ± 1.10	1.52 ± 0.66	2.43 ± 0.55	3.30 ± 1.23
Difference vs LC-MS/MS	N/A	−0.10 ± 1.03, $P = .416$	0.81 ± 1.03, $P < .001$	1.68 ± 1.28, $P < .001$
Correlation vs LC-MS/MS	N/A	$r = 0.666$, $P < .001$	$r = 0.597$, $P < .001$	$r = 0.663$, $P < .001$
Low BioT				
BioT, nmol/L	1.48 ± 0.53	1.65 ± 0.60	3.58 ± 1.46	4.46 ± 2.36
Difference vs LC-MS/MS	N/A	0.17 ± 0.49, $P < .001$	2.09 ± 1.27, $P < .001$	2.98 ± 2.15, $P < .001$
Correlation vs LC-MS/MS	N/A	$r = 0.724$, $P < .001$	$r = 0.606$, $P < .001$	$r = 0.651$, $P < .001$

*Values are means ± standard deviation in SI units for ease of interpretation, but statistical outcomes were calculated using the natural logarithm of these values. To convert T or BioT to nanograms per deciliter, multiply value by 28.84.

J Am Med Dir Assoc. Author manuscript; available in PMC 2021 August 08.

Table 4

Summary of Regression Analyses Indicating the Relationship of Different Variables to BioT Concentration [ln(nmol/L)]

Variable	*R*	R^2	*P*
Total T	0.417	0.1742	<.001
Age	0.316	0.0998	<.001
Albumin	0.222	0.0491	.002
SHBG	0.199	0.0396	.005
BMI		0.0164	.074

Table 5

Comparison of BioT Assessed via LC-MS/MS or via Our Prediction Equation for the Total Cohort and From Subsets With Low Total T (≤10.40 nmol/L) or Low BioT (≤2.43 nmol/L)*

	N	BioT via Prediction Equation, nmol/L	Difference vs LC-MS/MS, nmol/L	Correlation
Total cohort	194	1.84 ± 0.65	−0.10 ± 0.87, $P = .126$	$r = 0.508$, $P < .001$
Low total T	66	1.40 ± 0.42	−0.21 ± 1.01, $P = .089$	$r = 0.663$, $P < .001$
Low BioT	143	1.69 ± 0.59	0.21 ± 0.52, $P < .001$	$r = 0.673$, $P < .001$

*Values are reported as means standard deviation in SI units for ease of interpretation, but statistical outcomes were calculated using the natural logarithm of these values. To convert BioT to nanograms per deciliter, multiply value by 28.84.

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