

**Supplementary Fig. 1.** Bland-Altman plot showing the level of agreement between serum total testosterone [In(nmol/L)] measured via liquid chromatography–tandem mass spectrometry and the automated Cobas immunoassay in the total cohort (n = 203). Absolute agreement would have a variation of zero.



**Supplementary Fig. 2.** Bland-Altman plot showing the level of agreement between serum BioT [ln(nmol/L)] measured via liquid chromatography–tandem mass spectrometry and commercially available enzyme-linked immunosorbent assay in the total cohort (n = 203). Absolute agreement would have a variation of zero. LC-MS/MS, liquid chromatography–tandem mass spectrometry.

## Supplementary Table 1

Comparison of BioT Assessed via Morris and Vermeulen Equations for the Total Cohort and From Subsets With Low Total T ( $\leq\!10.40$  nmol/L) or low BioT ( $\leq\!2.43$  nmol/L)\*

	BioT Difference Morris vs Vermeulen, nmol/L	Correlation
Total cohort Low total T Low BioT	$\begin{array}{l} 1.10 \pm 1.57, \mathit{P} < .001 \\ 0.88 \pm 0.79, \mathit{P} < .001 \\ 0.88 \pm 1.40, \mathit{P} < .001 \end{array}$	$\begin{array}{l} r=0.831, P<.001\\ r=0.865, P<.001\\ r=0.852, P<.001 \end{array}$

BioT, bioavailable testosterone; T, total testosterone.

\*Values are means  $\pm$  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.



**Supplementary Fig. 3.** Bland-Altman plot showing the level of agreement between serum BioT [ln(nmol/L)] measured via liquid chromatography–tandem mass spectrometry and predicted with the Morris (A) and Vermeulen (B) equations or with our regression model (C) for the total cohort of participants with directly measured values of total T, SHBG, and albumin (n = 194). Absolute agreement would have a variation of zero. T, total testosterone; SHBG, sex hormone–binding globulin.