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Kinetics of Removal of Intravenous Testosterone Pulses in Normal Men

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Abstract

Background—Testosterone (T) is secreted into the bloodstream episodically, putatively distributing into total, bioavailable (bio) [nonSHBG-bound] and free T moieties. The kinetics of total, bio and free T pulses are unknown.

Design—Adrenal and gonadal steroidogenesis was blocked pharmacologically, glucocorticoid was replaced, and T was infused in pulses in 4 distinct doses in 14 healthy men under 2 different paradigms (total of 220 T pulses).

Methods—T kinetics were assessed by deconvolution analysis of total, free, bioavailable, SHBG-bound and albumin-bound T concentration-time profiles.

Results—Independently of T dose or paradigm, rapid-phase half-lives (min) of total, free, bioavailable, SHBG-bound and albumin-bound T were comparable at 1.4 ± 0.22 min [grand mean ± SEM of geometric means]. Slow-phase T half-lives were highest for SHBG-bound T (32 min) and total T (27 min) with the former exceeding that of free T (18 min), bioavailable T (14 min) and albumin-bound T (18 min)[$P < 0.001$]. Collective outcomes indicate that (a) the rapid phase of T disappearance from point sampling in the circulation is not explained by T dose; (b) SHBG-bound T and total T kinetics are prolonged; and (c) the half-lives of bioavailable, albumin-bound and free T are short.

Conclusion—A frequent-sampling strategy comprising an experimental hormone clamp, estimation of hormone concentrations as bound and free moieties, mimicry of physiological pulses, and deconvolution analysis may have utility in estimating the *in vivo* kinetics of other hormones, substrates and metabolites.

Keywords

androgen; human; pulsatile; male; elimination; distribution

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Introduction

The availability of systemic testosterone (T) and estradiol (E_2) to target organs depends upon glandular secretion into the bloodstream, transportation in plasma, entry into tissue fluids, retention at target cells, and metabolic transformation. Secretion of gonadal sex steroids proceeds via an admixture of continuous low basal release and superimposed bursts that reflect pulsatile gonadotropin drive (1–3). Pulsatile T secretion has been corroborated by direct testicular-vein sampling in the human (4). In principle, a pulse of T secreted into systemic blood diffuses within the aqueous compartment, distributes among plasma proteins, exits the vascular tree, and/or is degraded and transformed (5). Thus, gonadal secretion of a pulse of T would be expected to yield time-varying concentrations of total, SHBG-bound, albumin-bound, bioavailable (nonsex steroid-binding globulin (SHBG)-bound) and free (nonSHBG and nonalbumin-bound) T. However, in species like the human high-affinity transport proteins exist in plasma, which greatly damp pulsatile T profiles making kinetic estimates more difficult (6–8). We postulated that if endogenous T were depleted, infusion of (exogenous) T pulses would allow one to directly calculate T moiety-specific disappearance rates, thereby estimating physiological kinetics.

The present studies introduce a model for quantifying the dynamics of T pulses *in vivo*. In particular, the paradigms comprise pharmacological inhibition of adrenal and testicular steroidogenesis at the level of CYP11A (cholesterol side-chain cleavage) with glucocorticoid addback (because of concomitant cortisol depletion), and i.v. infusion of dose-varying T pulses either superimposed or not superimposed upon basal T infusion. Total, bioavailable (bio), SHBG-bound, albumin-bound, and free T concentrations were first estimated in successive 10-min serum samples, using subject-specific measurements of SHBG and albumin. Deconvolution analysis was then applied to each train of moiety-defined T pulses to quantify elimination kinetics. Thereby, we could assess the relative contributions of SHBG and albumin-bound T moieties to total and free T kinetics. The outcomes were similar in both paradigms, and therefore have relevance to understanding the physiology of regulated T egress from the circulation.

Methods

Subjects

Fourteen healthy men ages 21 – 50 yr (range) with body mass indices (BMI) of 19 – 31 kg/m^2 participated in the study. Volunteers were healthy, community-dwelling unmedicated men who provided written informed consent approved by the Mayo Institutional Review Board. The protocol was reviewed by the U.S. Food and Drug Administration. Outpatient history and physical examination excluded any recent medical illness, systemic disease, liver, renal or hematological abnormalities or concurrent drug use. Screening laboratory tests were normal, including baseline glucose, creatinine, hepatic transaminases, complete blood count, morning cortisol, LH, FSH, prolactin, TSH, total T, IGF-I, estradiol, SHBG, albumin, minerals and electrolytes.

Protocol

In the first protocol, volunteers were admitted to the Mayo Clinic Translational Science Unit (CRU) on 3 separate randomly ordered evenings scheduled at least 2 wk apart. At 2000 hr an indwelling i.v. catheter was inserted in each forearm, and kept patent by saline infusion (10 mL/hr). At 2400 hr a constant i.v. infusion of T 1.7 μmol/hr was begun and continued for 18.5 hr (N = 9 subjects). The other i.v. catheter was used for 10-min blood sampling starting at 0500 hr the next morning and continuing for 13.5 hr. T pulses were injected starting at 0800 hr, one dose level (0.46, 1.4 or 4.2 μmol/bolus) per admission. A pulse was delivered i.v. over 30 min

every 90 min for a total of 7 pulses. This schedule emulates the inferred pattern of endogenous T secretion (9). In the second protocol, another cohort of 5 subjects received overnight saline infusion followed by 9 consecutive 1-min i.v. bolus injections of 1.7 μmol/bolus crystalline T beginning at 0800 hr. The rapid-bolus format mimics most earlier studies in pharmacology. Blood was sampled concurrently every 10 min for 13.5 hr. This protocol addition allowed us to assess whether half-life estimates are similar after 30-min and 1-min bolus injections and in the presence and absence of basal T infusion. Thus, 14 subjects were studied altogether. T infusions were prepared as described earlier. Three oral doses of ketoconazole, a steroidogenic inhibitor (10), were administered as follows: 1000 mg at 2200 hr, 400 mg at 0600 hr and 400 mg at 1200 hr. Dexamethasone (0.75 mg) was given orally at 2200 hr with the first ketoconazole dose and again (0.5 mg) at the end of sampling, since ketoconazole blocks cholesterol side-chain cleavage and depletes cortisol (10). Dinner, lunch and breakfast were provided. Lights were extinguished at 2230 hr. Ambulation was permitted within the sampling room. Alcohol use was disallowed.

Sex-steroid measurements

Total T was measured by immunochemiluminescence technology (ACS 180, Bayer, Tarrytown, NY; interassay coefficient of variation [CV] 6 – 11%, lower limit of detection 0.17 nmol/L). Cross-reactivity of this assay was 5% with 5 alpha-dihydrotestosterone and < 1% for all other T metabolites. Total E_2 was measured using a double-antibody radioimmunoassay (Diagnostic Products Corporation, Los Angeles, CA; interassay CV 4.5 – 8%, lower limit of detection 18 pmol/L). Cross-reactivity was 12% with estrone and 6% or less with other estrogen metabolites. Liquid chromatography-tandem mass spectrometry (LC-MS/MS, API 5000, Applied Biosystems-MDS Sciex, Foster City, CA) was used for corroborative measurements in a set of 30 samples. T was prepared by acetonitrile precipitation and high-throughput liquid chromatography (HTLC) extraction. Analysis was performed by MS/MS equipped with a heated nebulizer ion source. Deuterated d_3-testosterone served as internal standards. Values as low as 0.035 nmol/L were detectable by this method. For T values of 0.28, 0.14, 0.07 and 0.035 nmol/L, respective CVs were 7.5, 2.2, 6.3 and 28.8%. The coefficient of determination for total T in the two assay systems was $R^2 = 0.95$ with a slope of 0.96 and y intercept of −0.347 nmol/L.

Albumin was measured in serum collected hourly during the T infusions by the Roche/Hitachi 912 System [Basel, Switzerland]. SHBG was quantified in hourly samples using a chemiluminescence immunoassay (Diagnostic Products Corporation, Los Angeles, CA; interassay CV 4.8 – 8%). The nonSHBG-bound (bioavailable) fraction of total T and E_2 was measured using a modification of the technique of O'Connor *et al.* (11) and Tremblay *et al.* Percentage bioavailable T or E_2 was multiplied by total T or E_2 determined by immunoassay or mass spectroscopy to obtain respective bioavailable T or E_2 concentrations. Free T was estimated by equilibrium dialysis of undiluted plasma at 37C (12).

Other screening hormones were assayed as described (13).

Calculation of bio and free T concentrations

Free and bio T as well as SHBG- and albumin-bound T concentrations were calculated in each 10-min serum sample using measured total T concentrations, albumin and SHBG (13). The equation system was adapted from Sodergard *et al.* (14). The association constants were estimated empirically for T-SHBG and T-albumin as respectively $1.78 \times 10^9\ M^{-1}$ and $1.80 \times 10^4\ M^{-1}$ based upon optimizing the correlation between calculated and directly measured bio and free T concentrations [see supplemental data in (13) on Endocrine Society's Journals Online web site at http://jcem.endojournals.org].

Deconvolution analysis

T concentration time series were analyzed using a recently developed automated deconvolution method. The algorithm was verified mathematically by direct statistical proof and validated empirically using hypothalamo-pituitary sampling and simulated pulsatile time series (15). The Matlab-based program first detrends the data and normalizes concentrations to the unit interval [0, 1]. Second, a smoothing process (a nonlinear adaptation of the heat-diffusion equation) creates multiple successively decremental potential pulse-time sets, each containing one fewer burst. Third, a maximum-likelihood expectation (MLE) estimation method computes all secretion and elimination parameters simultaneously conditional on each of the candidate pulse-time sets. Deconvolution parameters comprise basal secretion (β_0), two half-lives (α_1, α_2), secretory-burst mass (η_0, η_1), random effects on burst mass (σ_A), procedural and measurement error (σ_ε), and a 3-parameter flexible Gamma secretory-burst waveform (β_1, β_2, β_3). The slow half-life of T was represented as 63% of the decay amplitude (16). The fast and slow T half-lives were estimated from the data simultaneously with the other parameters. Statistical model selection was performed to distinguish among the deconvolution fits of the candidate pulse-time sets using the Akaike information criterion (17). The deconvolution parameters (and units) reported here comprise fast and slow half-lives (min) and mass of T infused per burst (concentration units). The apparent distribution volume is the dose infused divided by the calculated mass delivered per pulse.

Statistical analysis

Estimates of the deconvolution parameters were transformed to the natural logarithmic scale to produce symmetric distributions and equalize measurement variability. Logarithmic measurements were analyzed via mixed-effects 2-way ANOVA for repeated measures. For each deconvolution variable, the ANOVA model specification included 2 classification factors to estimate the main effect of T dose (4 factors) and T moiety (5 factors). Model parameters were evaluated via residual maximum likelihood, and the variance-covariance matrix was modeled in the compound symmetry form (18). *A priori* comparisons were formulated by way of linear contrasts of the least-squares means. Tukey's honestly significantly different (HSD) criterion was utilized to maintain an overall two-sided multiple-comparisons type I error of 0.05. Half-life values are reported as the geometric mean (95% confidence interval), and other data (including grand means) as the arithmetic mean ± SEM.

Linear regression was used to test for an effect of the 3 doses on T half-life in the subjects so studied. Standardized slopes were tested against the null hypothesis of a zero-mean unit standard-deviation distribution of z scores by the Kolmogorov-Smirnov statistic.

Results

Baseline hormone data in the 14 subjects included normal concentrations of LH (mean 4.2 ± 0.5 IU/L), FSH (3.8 ± 0.6 IU/L), prolactin (9.6 ± 0.7 μg/L), SHBG (25 ± 3.0 nmol/L), E_2 (107 ± 11 pmol/L), total T (18 ± 1.3), bio T (5.2 ± 0.87) and free T (0.52 ± 0.073) nmol/L. Two subjects dropped out for scheduling reasons before completing the 0.46 μmol T dose. Nine subjects completed both the 1.4 and 4.2 μmol T doses. Five other individuals received 1.7 μmol T as 1-min bolus injections, so as to compare kinetic estimates with those following the 30-min infusions.

Mean and peak T concentrations, which were measured in serum collected every 10 min after 0.46, 1.4 and 4.2 μmol T injections given over 30 min during basal infusion, increased with T dose ($P < 0.001$ by 1-way ANOVA). This was true for each of total, bio, free, SHBG-bound and albumin-bound T concentrations: Supplemental Table 1. Injection of 1.7 μmol T by 1-min bolus with no basal T infusion yielded mean and peak total T concentrations lower than those

observed after 1.4 as well as 4.2 μmol T given as 30-min pulses, indicating that the manner (not just the dose) of T infusion influences T concentrations. Peak T concentrations exceeded the normal range after the highest T dose. Time profiles of total, bio and free T are illustrated for all 3 T pulse sizes in Figure 1A, and for SHBG-bound and albumin-bound T in Figure 1B in one subject.

Two-way ANOVA in a 4 × 5 factor design was used to test the influence(s) of T dose (4 independent variables) and T moiety (5 types) on the rapid-phase T half-life (dependent variable): Supplemental Table 2. The T-dose effect was significant ($P = 0.007$), but only due to a slight prolongation of the rapid T half-life at a T dose of 1.4 μmol compared with 0.46 or 1.7 μmol ($P \leq 0.028$). There was no effect of T moiety and no dose × moiety interaction. Rapid-phase T half-lives (min) averaged across all 4 T doses are shown for each T moiety in: Figure 2 (*Panel A*). The grand arithmetic mean ± SEM of (geometric mean) rapid-phase T half-lives was 1.4 ± 0.22 min for all 5 T moieties and all 4 T doses (N = 14 subjects).

Two-way ANOVA of slow-phase T half-lives in a 4 × 5-factor design (N = 14 subjects) revealed significant main effects of T dose ($P = 0.004$) and T moiety ($P < 0.001$). There was no significant interaction ($P = 0.83$): Supplemental Table 3. The only dose-related contrast was a shorter half-life of bio T after injection of the lowest compared with the highest dose ($P = 0.002$). Total T and SHBG-bound T slow half-lives were statistically similar T. Estimated slow-phase half-lives of albumin-bound, bio and free T averaged across the 4 T doses did not differ ($P = 0.19$). Respective grand means were 18 (albumin-bound), 14 (bio T), and 18 (free T) min. The slow half-life of SHBG-bound T of 32 min exceeded that for each of free, albumin-bound and bio T ($P \leq 0.01$): Figure 2 (*Panel B*). The slow half-life of total T (27 min) exceeded that of bio and albumin-bound (but not free) T in the 14 subjects studied. By linear regression, T dose (3 doses given to each of 7 subjects) did not correlate with the half-life of any T moiety (all $P > 0.10$).

Deconvolution estimates of the mass of T infused per pulse per unit distribution volume are summarized in Supplemental Table 4. Two-way ANOVA in a 4 × 5-factor design (N = 14 subjects) of deconvolution-calculated T pulse-mass values disclosed main effects of T dose ($P < 0.001$) and T moiety ($P < 0.001$), as well as a major interaction between the two factors ($P < 0.001$). With respect to T dose, values for each of the 4 doses differed from each of the 3 others by *post hoc* Tukey's test ($P \leq 0.004$). With respect to T moiety, estimated (infused) free-T mass was less than that of all others ($P < 0.001$). Deconvolution-calculated mass values for albumin- and SHBG-bound T were similar as were values for albumin-bound and bio T at all T doses.

The distribution volume (Vd) was calculated as the quotient of the known mass of T injected per pulse (μmol) and the deconvolution-estimated mass of T infused per pulse (μmol/L). Data were expressed as L/m^2 body-surface. Calculated T moiety-specific Vd values are given by dose in Figure 3 for 0.46, 1.4 and 4.2 μmol T injections in the 9 subjects given 30-min T boluses. According to two-way ANOVA, dose of T and T moiety influenced apparent Vd (both $P < 0.001$ main effects) with a weak interaction ($P = 0.04$). With respect to dose effects, calculated Vd was about twofold greater for the 4.2 than 1.4 μmol T dose ($P < 0.001$). Estimated Vd values for the 1.4 and 0.46 μmol T doses were comparable for all moieties except for total and SHBG-bound T. In relation to T moieties, mean Vd for total T was less than that of all other moieties (each $P < 0.001$). Vd for free T was greater than that of all other moieties ($P < 0.001$). *Post hoc* testing by Tukey's procedure indicated that Vd estimates were similar for the following 3 pairs independently of T dose: (i) albumin- and SHBG-bound T; (ii) bio and albumin-bound T; and (iii) bio and SHBG-bound T. In contrast, estimates of Vd made after 1.7 μmol T given by 1-min bolus injection without basal T infusion (N = 5) were elevated by 2.5-6-fold over those after 4.2 μmol T given by 30-min infusion. An exception was SHBG-

bound T. In particular, 1-min bolus T injections yielded Vd estimates for total, bio, free, SHBG-bound and albumin-bound T of respectively 102 ± 17, 391 ± 111, 4451 ± 1265, 145 ± 17 and 428 ± 122 L/m^2.

Discussion

Deconvolution analyses of 220 discrete pulses of T delivered i.v. across 4 T doses in 14 healthy men during pharmacological inhibition of adrenal and testicular steroidogenesis disclosed that rapid-phase T half-lives are essentially independent of duration of T-infusion pulse (1 min *vs* 30 min), T dose (9-fold range), plasma T moiety (5 moieties assessed) and a > 100-fold range in peak-T concentrations. In contradistinction, estimated slow-phase half-lives were dependent upon T moiety (total, free, bio, SHBG-bound, and albumin-bound), and T dose pulse size (0.46, 1.4, 1.7 and 4.2 μmol). Two-way ANOVA disclosed that total and SHBG-bound T half-lives are statistically comparable (grand mean 30 ± 2.5 min for N = 14 men), whereas half-lives of free, albumin-bound and bio T are similar (grand mean 16 ± 2.8 min). The collective data indicate that estimated rapid and slow kinetics of total, bio and free T pulses have distinct dependencies upon SHBG and albumin.

A grand mean rapid-phase T half-life of 1.4 ± 0.22 min was estimated for total, SHBG-bound, free, bio and albumin-bound T. The consistency of individual estimates across T moieties (Supplemental Table 2) suggests to us a common limiting step in rapid-phase T disappearance, such as intravascular mixing by diffusion and advection (19). One other analytically based estimate of the rapid-phase half-life of total T in 15 men was 2.8 min, which was obtained indirectly without inhibiting endogenous T production or injecting exogenous T (3). Estimation of i.v. radiolabeled T kinetics in 5 normal young men yielded a fast-phase half-life of total T of 7.0 min (16). Our recalculation from the mean data in that study predicted a value of 4.95 min. In a third study, 1 μmol unlabeled T was infused i.v. over 20 min in 11 men ages 72 ± 5 yr in the presence of endogenous T, yielding a rapid-phase total T half-life of 7.5 min (20). The unlabeled-T infusion study did not take the precaution of depleting endogenous T to avoid confounding by endogenous T pulses, which predictively would artificially prolong the rapid-phase half-life estimate. No previous study to our knowledge has estimated the kinetics of SHBG-bound, albumin-bound, free and bioavailable T. Moreover, the many studies that infused T continuously are restricted in physiological interpretation, since T is normally secreted in pulses.

The grand mean slow-phase half-life of total T was estimated here as 27 ± 2.1 min, from geometric mean values of 21, 27, 27 and 31 min for respective T doses of 0.46, 1.4, 1.7 and 4.2 μmol/bolus. Linear-regression analysis showed that T dose did not correlate with slow-phase T half-lives, but the study was not powered to test this idea. Earlier analytical model-based predictions of the slow-phase half-life of endogenously secreted total T averaged about 45 min in healthy men (3). Our estimate at higher T doses is similar to that inferred by Horton *et al*. after injecting a single dose of tritiated T in 5 men, *viz*., 34 min (16), and less than that calculated by White *et al*. after injecting a single dose of unlabeled T in older eugonadal men, *viz*. 56 min (20). In addition to clear methodological differences, disparate estimates of the physiological range of total-T kinetics could reflect (i) unequal hepatic extraction of T, (ii) genetic variations in SHBG concentrations, and (iii) variable activity of sex steroid-metabolizing cytochrome P450 enzymes (21–26). The first notion reflects the major role of the liver in the transformation and excretion of T. The second hypothesis is based upon the capability of SHBG, but not albumin, infusion to prolong total T half-life in animal models (26). Estrogen administration elevates SHBG concentrations and also prolongs the half-life of total T in humans (25). The third postulate arises from known genetic polymorphisms in T metabolism (23).

Our data support the possibility that SHBG-bound rather than albumin-bound T contributes primarily to the slow-phase half-life of total T (Figure 2B). Indeed, the SHBG-bound T half-life was greater than that of total T in 13 of the 14 volunteers. Half-lives of total T were nearly twofold greater than those of albumin-bound, bio and free T. No other published estimates exist for comparison. For the aggregate data, the absolute rank order of decreasing T half-lives was slow-phase SHBG-bound T = slow-phase total T (any T dose) > slow-phase free, bio or albumin-bound T > rapid phase (any T dose and any moiety). One could conjecture that the consistent dose- and moiety-independent rapid-phase half-life of T disappearance during pulsatile T infusions principally reflects initial intravascular distribution of T. In contrast, the nearly 10-fold longer slow-phase half-lives of albumin-bound, bio and free T may mainly reflect rate-limiting loss of T from the bloodstream into interstitial fluids. This postulate remains to be proven directly. It does not require that either SHBG or albumin leave the circulation rapidly, given that nominal dissociation half-times for T-SHBG and T-albumin complexes at 37C are ≤ 12 sec and ≤ 0.35 sec, respectively, at least *in vitro* (14;24;27;28). Since T bound to SHBG and T bound to albumin may be extracted by certain tissues, the extent to which T must be free or selectively protein-bound to be removed from the circulation may be organ-specific.

The present estimate of the median Vd of total T (25 L/m^2) in 9 men receiving the two lower doses of T pulses superimposed upon basal T infusion under steroidogenic inhibition compares with a mean value of about 16 L/m^2 reported in a study of 5 young men given a single injection of radiolabeled T (16) and about 40 L/m^2 in 11 older men given a single 20-min injection of 1 μmol T (20). Another analysis of single-bolus tritiated-T decay curves in 6 men yielded an overall Vd of about 33 L/m^2 (29). In marked contrast to total T, estimates of bio T, free T, albumin-bound T, and SHBG-bound T Vd in the present paradigm were 46, 501, 47 and 79 L/m^2, respectively. To our knowledge, no other published estimates exist for comparison. The apparent Vd of free T is 2-fold was larger than the total body-water space, thus suggesting significant extravascular sequesteration. Whereas T exists in plasma as about 2% free, the remainder is bound to SHBG (approximately 55%), albumin (approximately 50%), and CBG (approximately 3%). Extravascular tissues putatively sequester T via T's binding to interstitial SHBG, albumin, CBG and other extra- and intercellular proteins, T-transforming enzymes and androgen receptors (1;30–32).

By way of caveats, *in vivo* T-SHBG association and dissociation constants are not known. In addition, estimates of *in vitro* binding constants vary by up to 10-fold (13;14;24;27;28). Nonetheless, the facts that *in vitro* equilibrium dissociation half-times are 12 sec or less and that dexamethasone (used here as glucocorticoid replacement) does not interfere with T-SHBG binding should allow reasonable computational estimates of SHBG-bound, albumin bound, bio and free T concentrations in each 10-min sample using measured total T, SHBG and albumin concentrations. For such calculations, SHBG and albumin concentrations were measured every hr during the pulsatile-T clamp, and association constants ($1.78 \times 10^9\ M^{-1}$ for T-SHBG and $1.80 \times 10^4\ M^{-1}$ for T-albumin) were obtained by iterative regression of computed on measured free and bio T concentrations in a cohort of healthy men (*Methods*). Results of the chemiluminescence T assay were confirmed by sequential liquid chromatography and tandem mass spectrometry.

Limitations of the present analyses include the need to ultimately measure T concentrations more frequently; extend the range of ages and BMI's evaluated; ascertain *in vivo* association and dissociation rates of T to and from SHBG and albumin; measure interstitial T concentrations; and compare kinetics of various T-infusion waveforms. One strategy would be to inject fewer T pulses but sample at 2.5- or 5-min intervals to estimate the rapid-phase T half-life with greater precision. To the extent that several doses in the current paradigm of pulsatile T delivery mimic episodic T secretion in healthy men, the outcomes presented should

apply to the kinetics of T pulses generated endogenously in healthy men of similar age, body composition and SHBG concentrations.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Figure 1A.

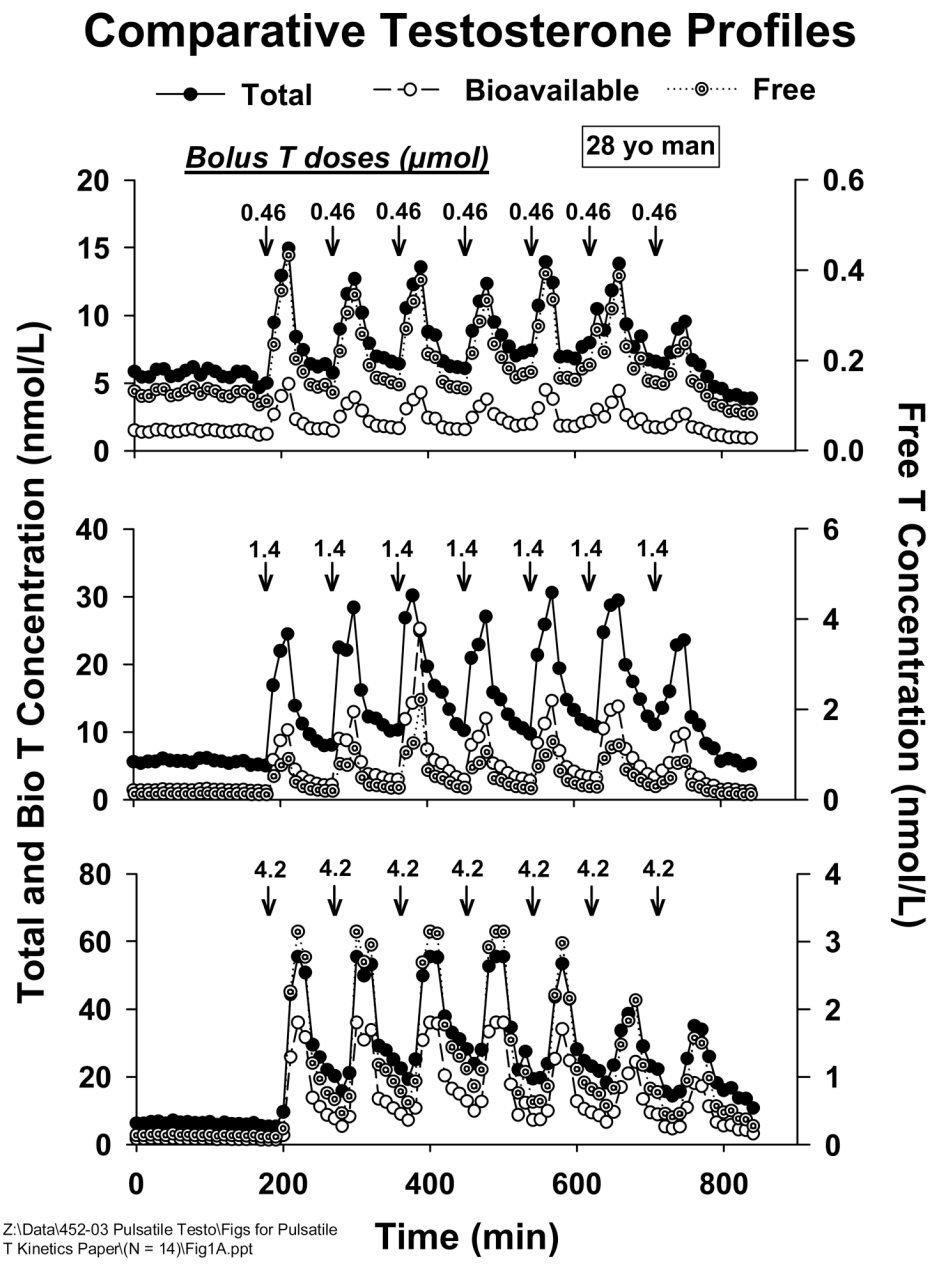


Figure 1B.

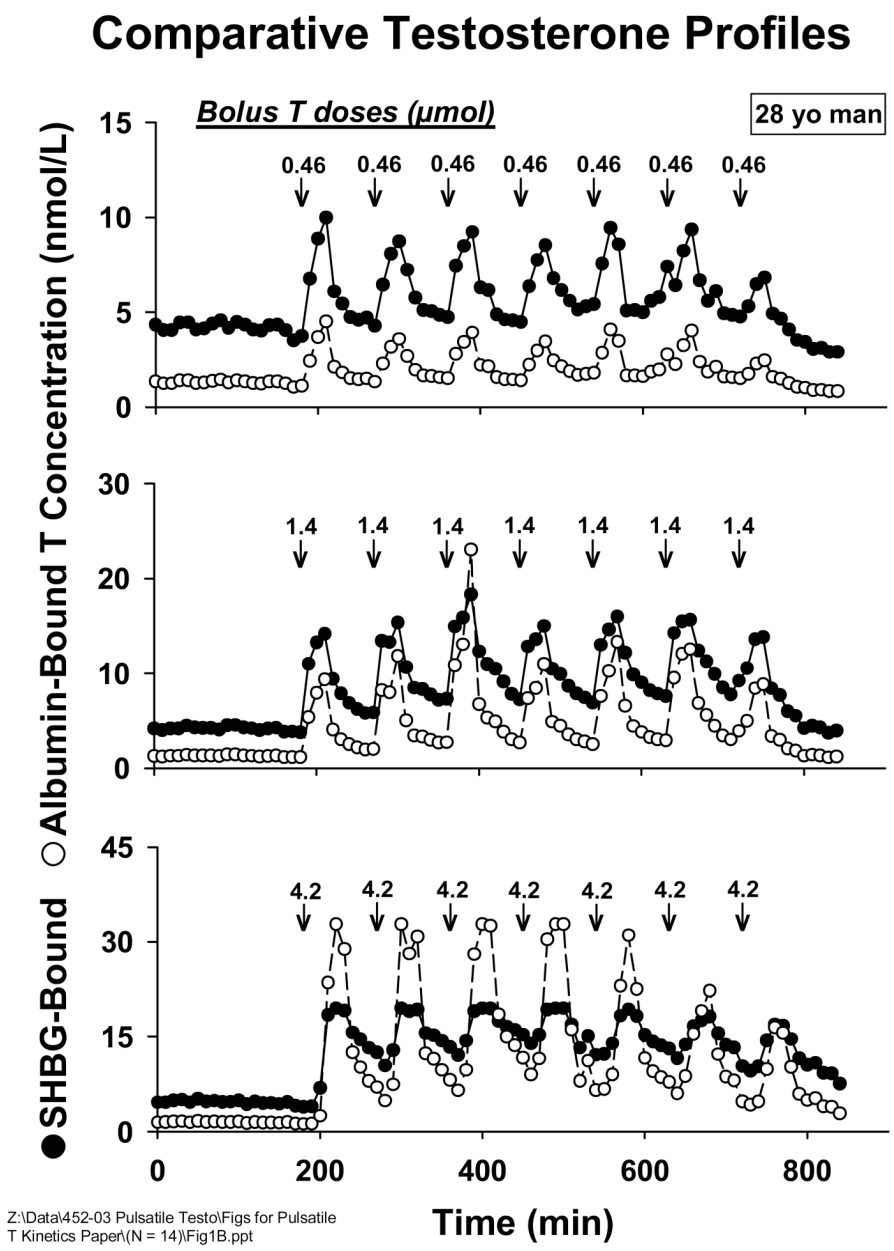
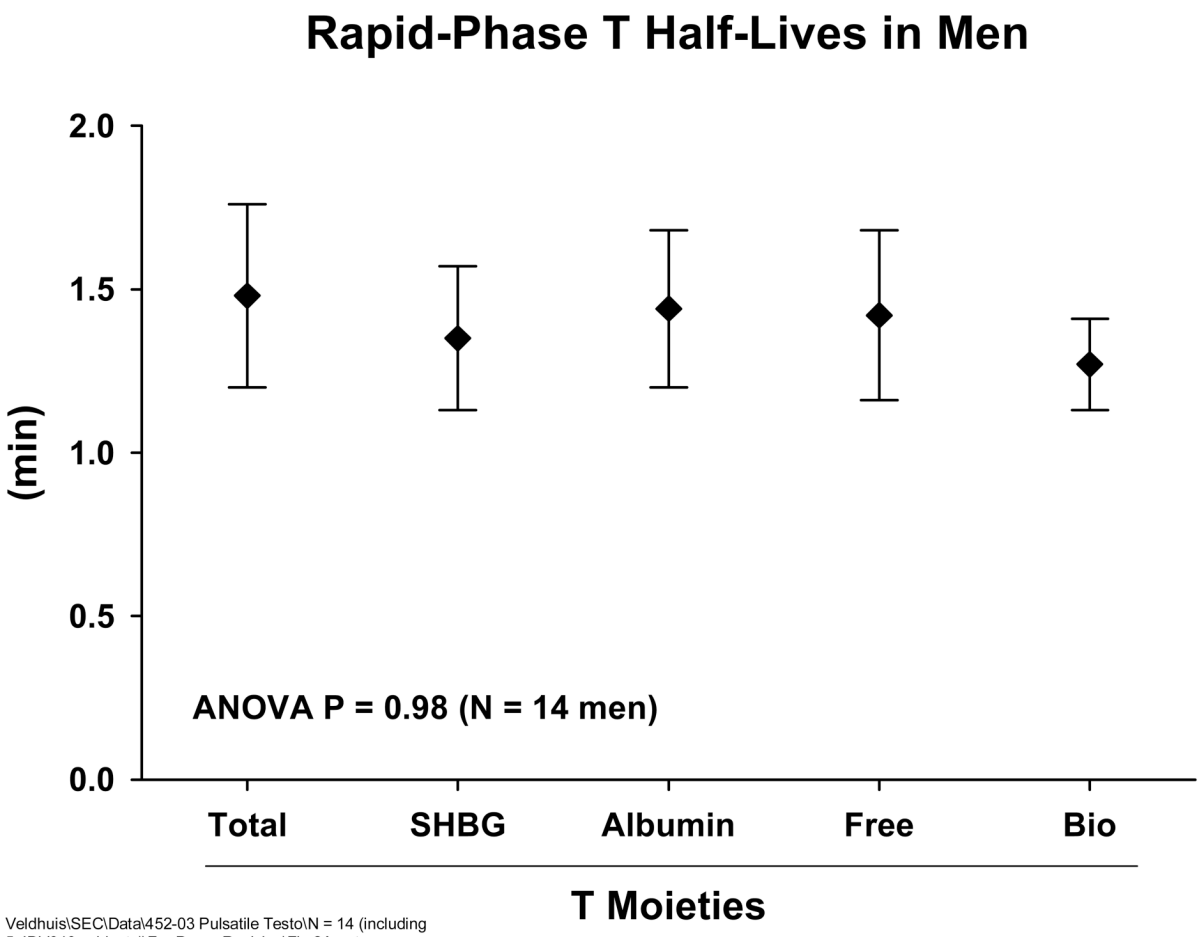


Figure 1.

Illustrative pulsatile T-concentration profiles in a 28-year-old subject administered ketoconazole and glucocorticoid replacement to deplete endogenous T followed by 7 consecutive 30-min i.v. pulses of 0.46, 1.4 or 4.2 μmol T (*top-to-bottom*), one every 90 min superimposed upon a basal T infusion, following a 3-hr baseline interval. *Panel A.* Profiles of three T moieties (total, bioavailable and free) estimated in each 10-min serum sample from mean SHBG and albumin concentrations measured every hr (*Methods*). *Panel B.* Profiles of SHBG- and albumin-bound T in the same subject.

Figure 2A.



Veldhuis\SEC\Data\452-03 Pulsatile Testo\N = 14 (including 5 JDV040 subjects)\For Paper Revision\Fig 2A.ppt

Figure 2B.

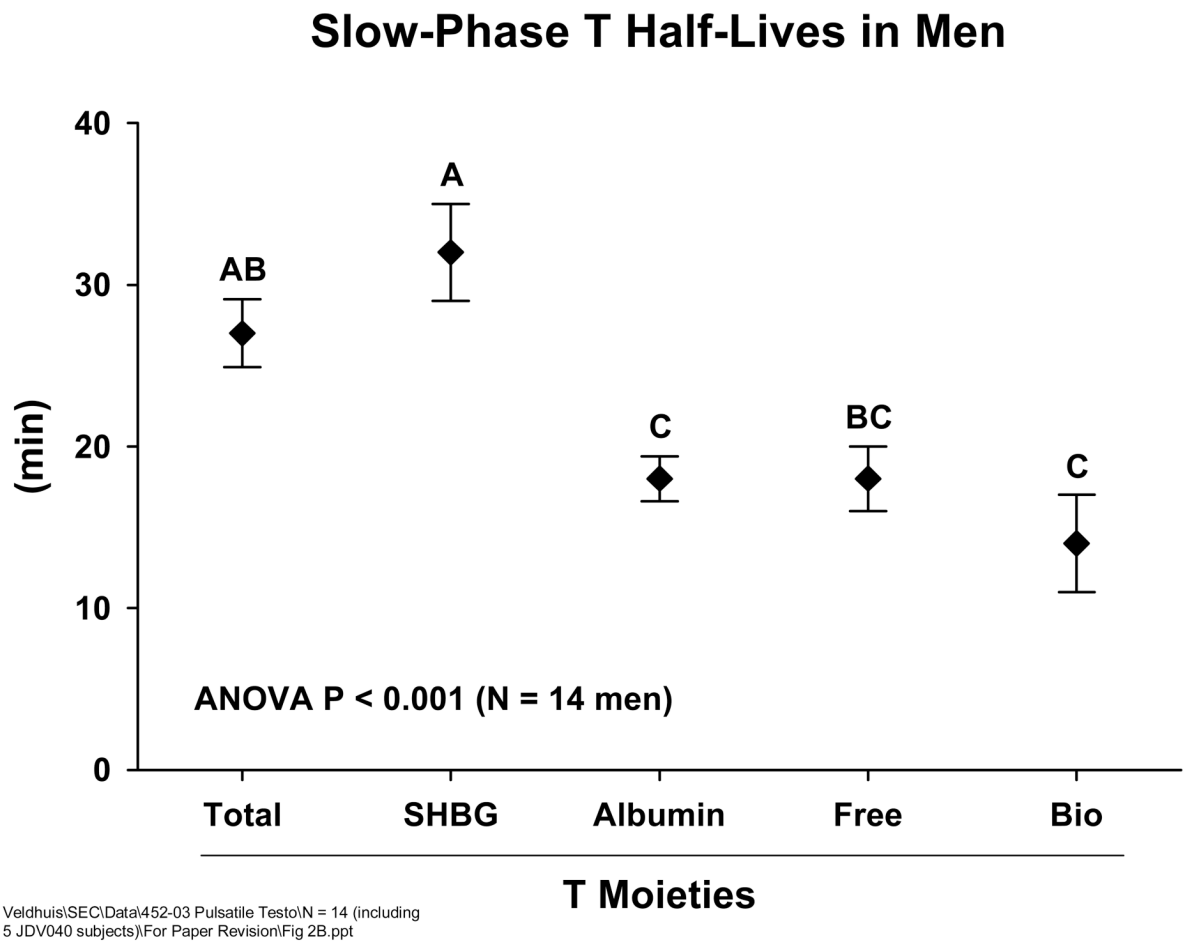


Figure 2.

Deconvolution-estimated biexponential half-lives of moiety-specific T pulses averaged across 4 T doses in 14 men. Data are the arithmetic mean ± SEM (of geometic means for the individual 4 T doses in **Tables 1 and 2**) for the rapid (*Panel A*) and the slow (*Panel B*) phases of T disappearance. P values were estimated by ANOVA. Different means are denoted by unique (unshared) alphabetic characters (*Panel B*).

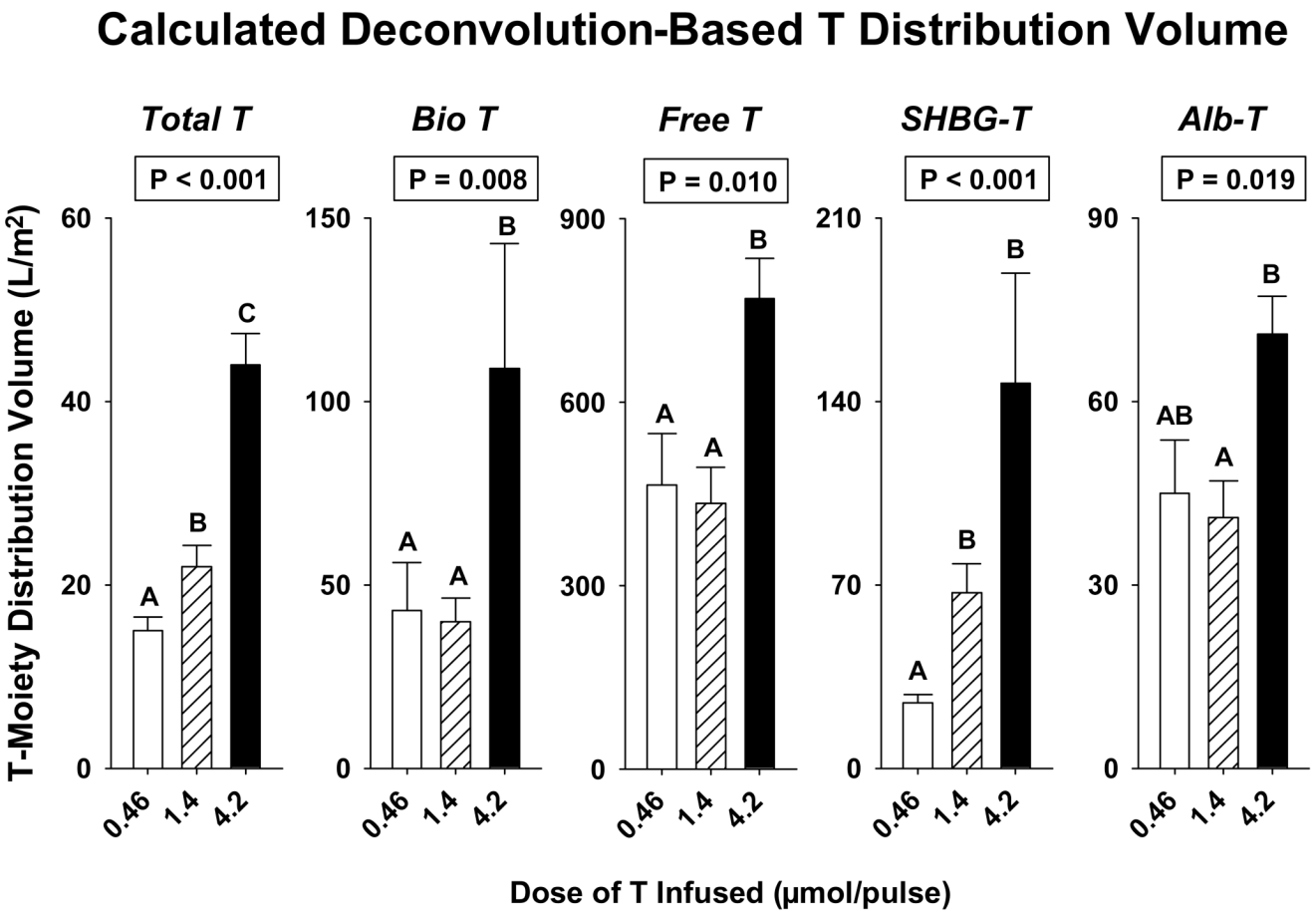


Figure 3.

Distribution volumes (L/m^2) of total, SHBG-bound, albumin-bound, free and bioavailable T in 9 healthy young and middle-aged men given 3 different i.v. doses of T as 30-min pulses. To obtain Vd, the injected T dose was divided by the deconvolution-estimated pulse mass (rather than the peak T concentration).