The Effects of Injected Testosterone Dose and Age on the Conversion of Testosterone to Estradiol and Dihydrotestosterone in Young and Older Men

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Background: During testosterone (T) therapy, T is partly converted to 17β-estradiol (E_2) and 5α-dihydrotestosterone (DHT). Effects of age, testosterone dose, and body composition on total and free E_2 and DHT levels are unknown.

Objective: We evaluated age and dose-related differences in E_2 and DHT levels in response to graded doses of testosterone enanthate in young and older men.

Methods: Fifty-one young (aged 19–35 yr) and 52 older (aged 59–75 yr) men completed treatment with monthly injections of a GnRH agonist plus randomly assigned weekly doses of testosterone enanthate (25, 50, 125, 300, or 600 mg) for 5 months.

Results: During testosterone administration, total and free E_2 levels increased dose-dependently (dose effect, $P < 0.001$) in both young and older men. Total and free E_2 levels and E_2:T ratios during T administration were higher in older than young men, but age-related differences in free E_2 and free E_2:T ratios were not significant after adjusting for testosterone levels, percentage fat mass, and SHBG. DHT levels and DHT:T ratios were dose-related but did not differ between young and older men. Mechanistic modeling of free hormone data revealed that the conversions of T to E_2 and DHT were both consistent with saturable Michaelis-Menten kinetics. The *in vivo* K_m values were estimated to be 1.83 nM for aromatase and 3.35 nM for 5α-reductase, independent of age. The V_{max} parameter for E_2 was 40% higher in older men than younger men, but V_{max} for DHT was not significantly different between age groups.

Conclusions: During im testosterone administration, E_2 and DHT levels exhibit saturable increases with dose. The rate of whole body aromatization is higher in older men, partly related to their higher percentage fat mass, SHBG, and testosterone levels. **(*J Clin Endocrinol Metab* 95: 3955–3964, 2010)**

Testosterone, the major circulating androgen in men, serves both as a hormone and as a prohormone. Testosterone is converted in the body to two active metabolites, 17β-estradiol (E_2) and 5α-dihydrotestosterone (DHT), which mediate some actions of testosterone in target tissues. Most of the circulating E_2 is derived from peripheral aromatization of testosterone in adipose tissue, and DHT is derived by 5α-reduction of testosterone (1, 2).

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Abbreviations: BMI, Body mass index; DHT, 5α-dihydrotestosterone; E_2, 17β-estradiol; GAM, generalized additive models; GLM, generalized linear models; LC–MS/MS, liquid chromatography–tandem mass spectrometry; log, logarithm; NS, nonsignificant; TE, testosterone enanthate.

It is generally believed that ratios of E_2 to testosterone (E_2:T) and DHT to testosterone (DHT:T) do not change during therapy when testosterone is administered parenterally by im injections of testosterone esters. Indeed, DHT and E_2 concentrations are low in androgen-deficient men and increase in proportion to the increase in serum testosterone concentrations when these men are treated with testosterone esters (3–7). However, it is not known whether, in the presence of testosterone therapy, E_2:T and DHT:T ratios in response are influenced by age and body composition. Serum E_2 concentrations are higher in obese men than lean men (8–12), but the effects of body composition, age, and testosterone dose on E_2:T ratio during testosterone therapy have not been fully investigated (13).

Using blood samples and data from our previously published testosterone dose-response studies in young and older men (14, 15), we determined the effects of age, body composition, and testosterone dose on E_2:T and DHT:T ratios. We found that the E_2:T ratio, but not the DHT:T ratio, was higher in older men than young men during administration of graded doses of testosterone enanthate (TE) in men whose endogenous testosterone production had been suppressed by administration of a long-acting GnRH agonist. Because older men had higher body mass index (BMI) than young men, we investigated whether age effects on E_2:T ratio during testosterone administration could be explained by differences in BMI in young and older men. We also examined free E_2 and DHT concentrations during testosterone administration to determine whether conversion of testosterone to E_2 and DHT in men conforms to the Michaelis-Menten kinetics and whether these conversions are affected by age.

Subjects and Methods

Human subjects

Blood samples were derived from healthy young men (aged 18–35 yr) and older men (aged 60–75 yr) with normal testosterone levels who were participants in a testosterone dose-response study. The design and main findings of this study have been reported (14, 15). The study protocols were approved by the institutional review boards of Charles Drew University and Harbor-University of California Los Angeles Research and Education Institute. All participants provided informed consent.

Exclusion criteria included history of prostate cancer, prostate-specific antigen above 4 ng/ml, American Urological Association (AUA) lower urinary tract symptom score above 7, hematocrit above 48%, severe sleep apnea, diabetes mellitus, congestive heart failure, myocardial infarction in the preceding 6 months, or participation in moderate to intense exercise training. Men who had used androgens, GH, or any other anabolic therapy in the preceding year were excluded also.

After a 4-wk control period, participants received monthly injections of a GnRH agonist (leuprolide depot, 7.5 mg; TAP Pharmaceuticals, North Chicago, IL) to suppress endogenous testosterone production. Concomitantly, the participants were randomized to receive weekly im injections of TE (Delatestryl, 200 mg/ml; Savient Pharmaceuticals, Iselin, NJ) in one of five doses: 25, 50, 125, 300, or 600 mg (14, 15). Treatment duration was 20 wk. Hormone levels were measured twice during the control period and every month thereafter during the treatment and recovery periods; blood was drawn before receiving the hormone injections 7 d after the previous TE injection. The Data and Safety Monitoring Board stopped the 600-mg TE dose group due to a number of serious adverse events in older men in this dose group. After this point, randomization was limited to one of four TE dose groups: 25, 50, 125, or 300 mg weekly.

Hormone assays

Serum total testosterone levels were measured by a specific RIA that has been validated previously against liquid chromatography–tandem mass spectrometry (LC–MS/MS) (15) and published extensively (14, 16, 17). The intra- and interassay coefficients of variation for total testosterone assay were 8.2 and 13.2%, respectively, and sensitivity was 0.6 ng/dl. The RIA and LC–MS/MS methods were compared by analyzing samples prepared in charcoal stripped serum to which known amounts of testosterone had been added; additionally, serum samples from men were analyzed by both methods. These measurements demonstrated a correlation of 0.99 between the RIA and LC–MS/MS measurement. Serum SHBG levels were measured by an immunofluorometric assay that has a sensitivity of 6.25 nmol/liter. Serum E_2 levels were measured by a previously described RIA that had a sensitivity of 2.5 pg/ml (ICN Pharmaceuticals, Costa Mesa, CA) (18). The cross-reactivity of testosterone, dehydroepiandrosterone, dehydroepiandrosterone sulfate, androstenedione, and progesterone in the estradiol assay was less than 0.1%. The intra- and interassay coefficients of variation for the estradiol assay were 5 and 12%, respectively. Dihydrotestosterone concentrations were measured after extraction of serum samples by hexane-ethyl acetate mixture followed by celite chromatography, as described (19). For measurement of DHT levels, serum samples were extracted and chromatographed through a celite column. DHT concentrations were measured in the eluate from the celite fractions by RIA (20) and corrected for recovery. The mean recovery was greater than 75%. The sensitivity of the DHT assay was 0.1 nmol/liter. Intra- and interassay coefficients of variation were ±9 and ±15%. Based on previous analysis (21), which showed that total and free testosterone levels were steady-state by treatment d 84, all hormones (testosterone, E_2, and DHT) were measured on d 84 and 112 (7 d after the previous TE injection) and averaged. Free testosterone, free E_2, and free DHT were calculated from the total concentrations of testosterone, E_2, and DHT and the measured concentrations of albumin and SHBG using a novel spreadsheet (22) calculator based on the law of mass action (23).

Body composition assessment

Body composition was assessed at baseline and during wk 20 by dual-energy x-ray absorptiometry using a Hologic 4500 densitometer (Hologic, Inc., Waltham, MA). A body composition phantom was used to calibrate the machine before each measurement.

Statistical analysis

Graphical and tabular displays were used to assess normality of variables, the prevalence of outliers and influence points, and preliminary evidence of associations. The relationships between age, dose, and total E_2 and DHT were evaluated using two-way ANOVA. Ratios of DHT and E_2 to testosterone were assessed using the same approach. We conducted a step-wise regression analysis using age group, treatment testosterone levels, treatment SHBG levels, and treatment percentage fat mass to predict the metabolites of testosterone. These analyses were performed using SAS version 9.2 (SAS Institute, Cary, NC).

We employed two models to explore the relationship between testosterone and its metabolites. First, we used an empirical power law to assess the relationship of posttreatment circulating testosterone levels to E_2 levels. We used exploratory plots stratified by age group, with scatterplot smoothing using generalized additive models (GAM) (24); these models apply semiparametric locally weighted smoothing to diagnose nonlinearities in associations. These results were used to motivate the fitting of parametric models of E_2 on total testosterone and covariates using generalized linear models (GLM), assuming a γ distribution for outcomes and a log link function. The overall fit of the parametric models was measured by the Akaike Information Criterion (25)—a penalized likelihood-based statistic of model deviation from the data—for GLM and coefficient of determination (R^2) for linear models. The statistical significance of individual regression coefficients was assessed using Wald tests. To assess the independent contributions of individual covariates to model fit while controlling for the influence of other variables, a forward stepwise fitting procedure was used. Results were considered statistically significant if null hypotheses of no association could be rejected at the 0.05 level. Analyses were performed using R (26) version 2.9.2 (R Foundation, Vienna, Austria).

We also analyzed the data using a mechanistic model in which the four relationships (*i.e.* total E_2 *vs.* total testosterone, total DHT *vs.* total testosterone, free E_2 *vs.* free testosterone, and free DHT *vs.* free testosterone) were modeled using rectangular hyperbolae, *i.e.* $Y = A X/ (B + X)$. Four equations (with corresponding A and B values) were derived for young men and old men, respectively. Based on pharmacokinetic principles and Michaelis-Menten kinetics (see Appendix A, published on The Endocrine Society's Journals Online web site at http://jcem.endojournals.org), the parameter A corresponds to the ratio of V_{max} (the maximal rate of conversion of total or free testosterone to the metabolite) divided by MCR (the metabolic clearance rate of the total or free metabolite); the B parameter corresponds to the K_m value for the enzymatic conversion of total or free testosterone to the metabolite. The model parameter estimation (including the SE of the estimate, SE, and R^2 value) was performed using the 2D Michaelis-Menten equation curve fitting software available online at http://zunzun.com/Equation/2/BioScience/Michaelis-Menten/. Parameter estimates are derived using a downhill simplex method, with initial values derived from a genetic algorithm (personal correspondence from zunzun-.com). The data points were equally weighted in the present analyses. Statistical comparison of the A and B parameters between young and old men were based on unpaired *t* tests. Because the results of the empiric power model and mechanistic model were similar, only the results of the mechanistic model are described in detail.

Results

Subjects

The characteristics of young and older men have been described (17, 18). Fifty-two of 60 randomized older men and 54 of 61 randomized young men completed the study. For the current analysis, data were available on 51 young men who completed the study (11 in the 25-mg, 8 in the 50-mg, 11 in the 125-mg, 10 in the 300-mg, and 11 in the 600-mg group) and 52 older men who completed the study (12 in the 25-mg, 12 in the 50-mg, 11 in the 125-mg, 10 in the 300-mg, and 7 in the 600-mg group).

TABLE 1. Baseline characteristics of young and older men

Variable	Young men	Older men	*P*
n	51	52	
Age (yr)	26.3 ± 4.5	65.6 ± 4.3	
Total T (ng/dl)	581.7 ± 168.6	335.4 ± 93.8	<0.0001
Free T (pg/ml)	138.8 ± 49.5	53.7 ± 17.8	<0.0001
Total E_2 (pg/ml)	20.8 ± 7.3	26.0 ± 5.3	<0.0001
Free E_2 (pg/ml)	0.5 ± 0.2	0.5 ± 0.2	0.5
Total DHT (ng/dl)	54.0 ± 16.8	28.1 ± 14.6	<0.0001
Free DHT (pg/ml)	6.0 ± 2.42	1.9 ± 0.9	<0.0001
Total E_2:total T ratio (%)	0.4 ± 0.1	0.8 ± 0.2	<0.0001
Total DHT:total T ratio (%)	9.5 ± 2.1	8.3 ± 3.5	0.05
Free E_2:free T ratio (%)	0.4 ± 0.1	1.0 ± 0.2	<0.0001
Free DHT:free T ratio (%)	4.3 ± 1.0	3.5 ± 1.4	0.0017
SHBG (nM/liter)	31.4 ± 14.1	52.8 ± 23.8	<0.0001
BMI (kg/m^2)	24.19 ± 3.0	26.8 ± 3.6	0.0002
Lean body mass (kg) (%)	57.7 ± 7.3	57.8 ± 6.4	0.94
Percentage fat mass (%)	18.6 ± 5.4	26.4 ± 5.5	<0.0001
Fat mass (kg)	14.4 ± 5.7	22.3 ± 7.4	<0.0001
Height (cm)	176.5 ± 5.7	176.2 ± 5.7	0.82

Data are expressed as mean ± SD. To convert total testosterone to nanomoles per liter, multiply by 0.03467; to convert free testosterone to picomoles per liter, multiply by 3.467. To convert E_2 concentrations to picomoles per liter, multiply by 3.671. To convert total DHT to nanomoles per liter, multiply by 0.0344; to convert free DHT to picomoles per liter, multiply by 3.44.

Baseline characteristics

Baseline characteristics of the 51 young and 52 older men included in these analyses are shown in Table 1. Young men had higher levels of total testosterone and free testosterone but lower levels of total E_2 than older men. However, free E_2 did not differ between the two groups. Total E_2:total T and free E_2:free T ratios were higher in older men than young men. Total and free DHT levels

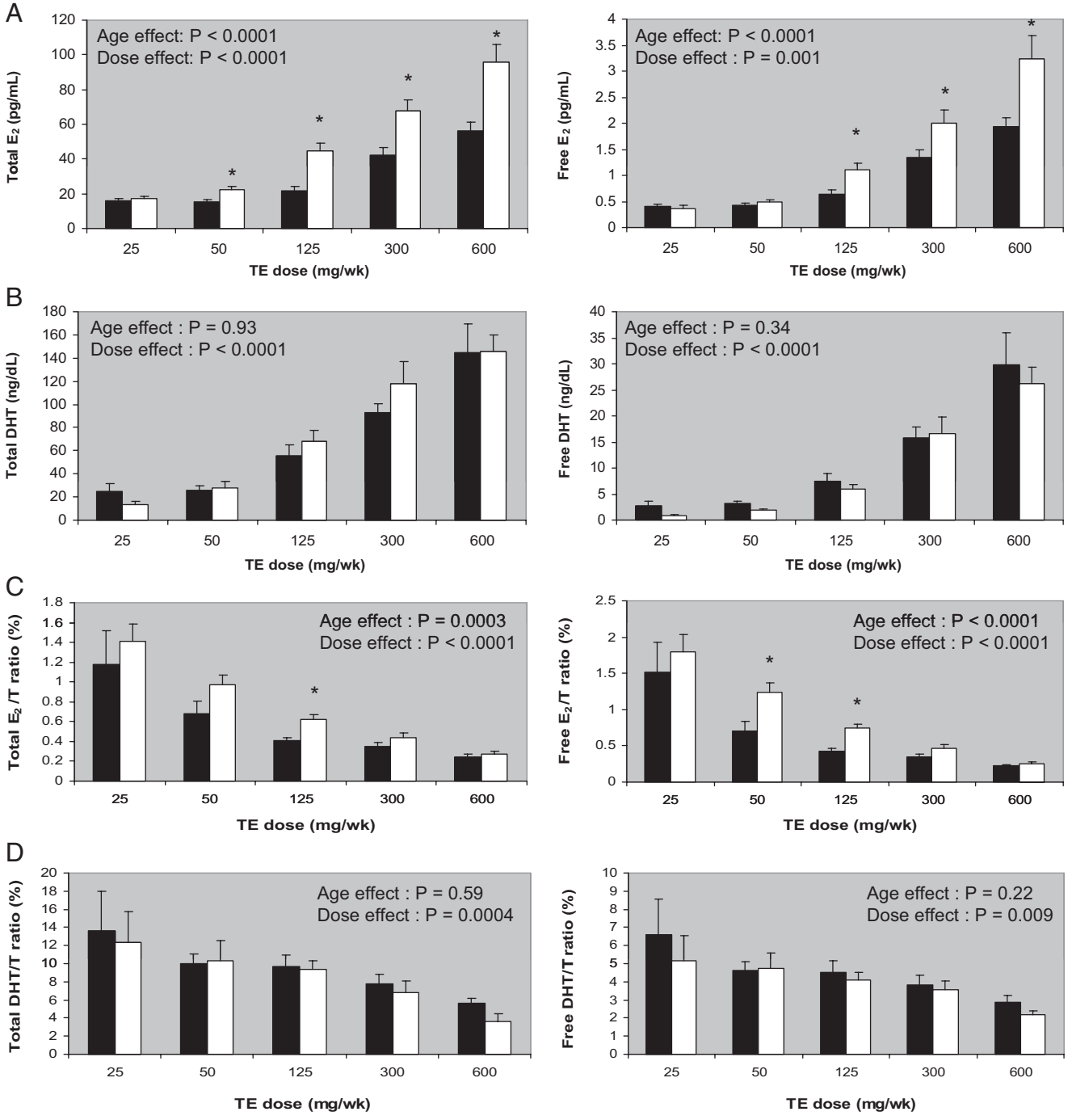

FIG. 1. Serum E_2, DHT, E_2:T ratios, and DHT:T ratios in response to administration of graded doses of TE in young (*black bars*) and older men (*white bars*). If there was a significant age effect, the values for young and older men for each dose were compared using *t* tests. The *asterisk* indicates significant differences between young and older men receiving that dose ($P < 0.05$). A, A dose-dependent increase in serum total E_2 (*left*) and free E_2 (*right*) levels with a significant age and dose effect. B, A dose-dependent increase in serum total DHT (*left*) and free DHT (*right*) levels with a significant dose effect only. C, A dose-dependent decrease in total E_2:total T (*left*) and free E_2:free T (*right*) ratios with a significant age and dose effect. D, A dose-dependent decrease in serum total DHT:total T (*left*) and free DHT:free T ratios (*right*) with a significant dose effect only. To convert total testosterone to nanomoles per liter, multiply by 0.03467; to convert free testosterone to picomoles per liter, multiply by 3.467. To convert estradiol concentrations to picomoles per liter, multiply by 3.671. To convert total DHT to nanomoles per liter, multiply by 0.0344; to convert free DHT to picomoles per liter, multiply by 3.44.

were higher in young men than older men. Total DHT:total T and free DHT:free T ratios were higher in young men than older men. Lean body mass did not differ between the two age groups, but older men had greater mean BMI, fat mass, and percentage fat mass than young men.

Exploratory analyses by age group

Untransformed total testosterone was positively associated with total E_2 ($r = 0.47$; $P < 0.01$) and free E_2 ($r = 0.36$; $P < 0.01$) in young but not older men. Total testosterone was also correlated with total DHT ($r = 0.75$; $P < 0.01$) and free DHT ($r = 0.53$; $P < 0.01$) in young men but only with total DHT in older men ($r = 0.51$, $P < 0.01$). Baseline total E_2, free E_2, total E_2:T ratio, and free E_2:T ratio were not significantly associated with body composition measures such as body weight, BMI, lean body mass, fat mass, or percentage fat mass. Free E_2 was significantly inversely correlated with SHBG in young ($r = -0.47$; $P < 0.001$) and older ($r = -0.55$; $P < 0.001$) men.

Response to treatment (Fig. 1)

Results of two-way ANOVA

As reported previously (15), both total testosterone and free testosterone concentrations increased with TE dose and were significantly higher in older men than young men in the 125-, 300-, and 600-mg dose groups ($P < 0.05$). In the current analysis, total E_2 increased in a dose-dependent manner in both young and older men (dose effect, $P < 0.0001$). Additionally, during testosterone administration, older men had higher total E_2 levels than young men (age effect, $P < 0.0001$) with significant differences in the 50-, 125-, 300-, and 600-mg dose groups ($P < 0.05$). Similarly, free E_2 levels increased dose dependently (dose effect, $P = 0.001$) and were higher in older men (age effect, $P < 0.0001$) than young men with significant differences in the 125-, 300-, and 600-mg dose groups ($P < 0.05$).

Total E_2:total T and free E_2:free T ratios decreased dose dependently in both young and older men (dose effect, $P < 0.0001$ for each). Older men had higher total E_2:total T and higher free E_2:free T ratios than young men (age effects, $P = 0.0003$ and <0.001, respectively).

Total and free DHT levels increased dose dependently in both age groups (dose effect, $P < 0.0001$ for both total and free DHT); however, total and free DHT levels during treatment did not differ significantly between the two groups. Similarly, total DHT:total T and free DHT:free T ratios decreased dose dependently in young and older men (dose effect, $P = 0.0004$ and $P = 0.009$, respectively), but without a statistically significant difference between the two age groups.

Results of step-wise regression

Because E_2 levels might be affected by testosterone levels, percentage fat mass, and SHBG levels that differed in young and older men, we compared E_2 levels after adjusting for percentage fat mass and SHBG, both individually and in combination, in addition to treatment testosterone levels. Differences between old and younger men in total E_2 [46.6 *vs.* 28.9 pg/ml; P (age) < 0.0001] and free E_2 [1.4 *vs.* 0.9 pg/ml; P (age) < 0.001] as well as total E_2:T ratios [0.8 *vs.* 0.5; P (age) $= 0.02$] and free E_2:T ratios [1.0 *vs.* 0.6; P (age) $= 0.03$] persisted after adjusting for SHBG alone. After controlling for percentage fat mass alone, older men had significantly higher total E_2 levels [43.1 *vs.* 32.7 pg/ml; P (age) < 0.001], and higher total E_2:T ratios [0.8 *vs.* 0.6; P (age) $= 0.04$] and free E_2:T ratios [1.0 *vs.* 0.6; P (age) $= 0.01$] than young men; however, free E_2 levels were not significantly different between the two age groups [1.2 *vs.* 1.0 pg/ml; P (age) $= 0.24$]. Finally, in the model containing treatment testosterone levels, percentage fat mass, and SHBG levels, older men continued to have significantly higher total E_2 [45.8 *vs.* 30 pg/ml; P (age) $= 0.001$] and total E_2:T ratios (0.8 *vs.* 0.5; $P = 0.04$) but free E_2 levels [1.3 *vs.* 1.1 pg/ml; P (age) $= 0.24$] and free E_2:T ratios [1.0 *vs.* 0.6; P (age) $= 0.6$] did not differ significantly between the young and older men.

Modeling the relationships between metabolite and testosterone levels

Power-law analysis

Plotting of E_2 *vs.* total testosterone during treatment using GAM indicated a curvilinear relationship (concave downward), so that cross-sectional increases in total testosterone were associated with increases in E_2 of proportionately lesser magnitude. Plots of transformed values (data not shown) indicated a linear association between

TABLE 2. GLM: multiplicative trends in E_2 as a function of cross-sectional differences in total testosterone, age group, and percentage fat mass during treatment

	Effect[a]	95% CI
Total testosterone (100% increase)[b]	1.40	1.35, 1.46
Age group (older *vs.* young men)	1.37	1.19, 1.57
Fat mass (10%)[c]	1.02	0.97, 1.06

CI, Confidence interval.

[a] Multiplicative cross-sectional difference in E_2 per difference in covariate values.

[b] Testosterone was log-transformed in models; back-transformation provides estimate of proportionate change in E_2 per proportionate change in total testosterone. Here a cross-sectional doubling of total testosterone is associated with a 38% increase in E_2.

[c] Multiplicative difference per 10-unit difference in body fat percentage.

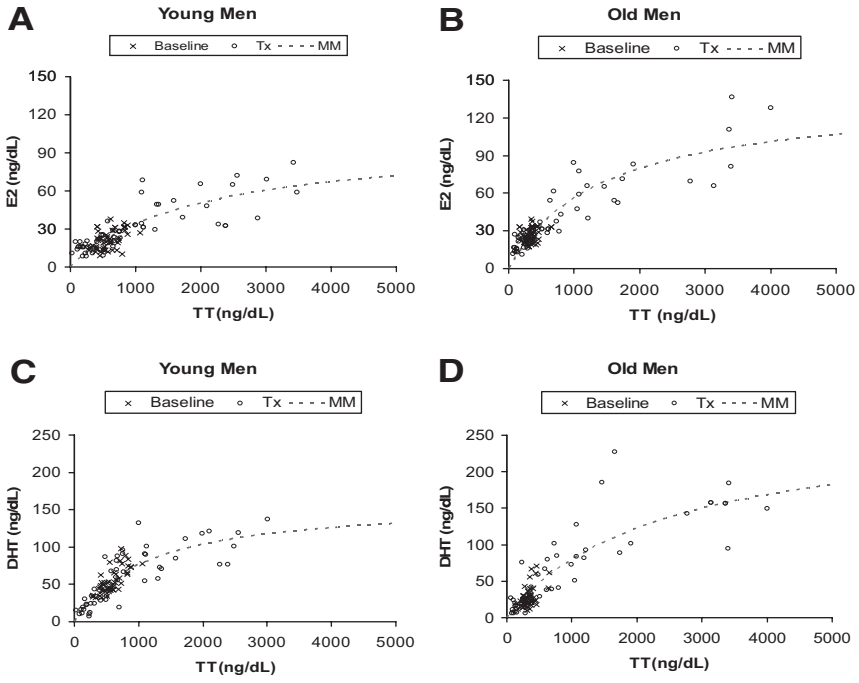

FIG. 2. The relationship of total E_2 and total DHT to total testosterone (TT) concentrations at baseline (symbol X) or during treatment (Tx; symbol ○) with graded doses of testosterone in young men (A and C) and older men (C and D). *Dashed lines* are derived from fitting all data points to a Michaelis-Menten (MM) mechanistic model (see Appendix A for details).

the logarithm (log) of E_2 and the log of total testosterone. These observations motivated fitting a GLM specifying that the log of average E_2 is a linear function of the log of total testosterone, with E_2 values assumed to follow a γ distribution. This model is appropriate for data where the variance increases with the mean (27), as it did for E_2 values, and accounts for the curvilinear relation of E_2 to total testosterone. Total testosterone, age group, and percentage fat mass exhibited statistically significant associations with E_2, controlling for the other factors. Conditional on percentage fat mass and age group, a cross-sectional doubling of total testosterone was associated with an approximate 40% increase in mean E_2 concentrations (Table 2). For a given total testosterone and percentage fat, older subjects had approximately 37% greater E_2 concentrations than their younger counterparts. Comparison of this model with candidate submodels via Akaike Information Criterion indicates preference for the full model.

Mechanistic modeling

Using the combined baseline and on-treatment data for all treatment groups, we graphically explored and mathematically modeled the four relationships (total E_2 *vs.* total testosterone, total DHT *vs.* total testosterone, free E_2 *vs.* free testosterone, and free DHT *vs.* free T) using separate analyses for young and older men. Figures 2, A–D, and 3, A–D, show the data and the fitted curves, corresponding to the total and free hormone concentrations, respectively. In all cases, the combined data exhibited curvilinear relationships that were well described by a rectangular hyperbolae, $Y = A\,X/(B + X)$, consistent with a saturable conversion of testosterone to metabolite governed by Michaelis-Menten kinetics (Appendix A). Table 3 lists the parameter values estimated from the curve fitting.

The total and free E_2 *vs.* testosterone curves in older men (Figs. 2B and 3B) had A values (Table 3) that were approximately 30–40% higher than in young men (Figs. 2A and 3A), consistent with a higher maximal rate of whole body aromatization in older men. In contrast, the B values were not significantly different between age groups (Table 3). Based on the B values from the free E_2 *vs.* free testosterone models (Appendix A), the estimated Michaelis-Menten $K_{m,\,E2}$ values for whole body aromatization were comparable in the two age groups [2.12 ± 0.34 nM in young men and 1.56 ± 0.18 nM in older men; *P* value, nonsignificant (NS)]. The mean value of 1.83 nM characterizes the combined result.

The total and free DHT *vs.* testosterone curves (Figs. 2, C and D, and 3, C and D) exhibited more complex behavior between age groups but were nevertheless consistent with the general predictions given in Appendix A. Based on the total hormone concentrations, the parameters A and B were both significantly smaller for young men than older men. In contrast, the parameters A and B derived from the free hormone concentrations were not significantly different between the age groups (Table 3). The former observation is consistent with the lower SHBG levels in young men compared with the older men (Table 1), which would be expected to reduce the A and B values. The latter observation indicates that the maximal whole body production rate of DHT from testosterone is not affected by age and that the $K_{m,\,DHT}$ value of the 5α-reductase pathway is also the same in young and older men. Based on the B values from free DHT *vs.* free testosterone models (Table 3), the estimated $K_{m,\,DHT}$ values were 2.71 ± 0.53 nM in young men and 3.98 ± 0.82 nM in older men (*P* value, NS). The mean value of 3.35 nM characterizes the combined result.

Discussion

We found that during administration of graded doses of TE, the older men had higher total and free E_2 concentra-

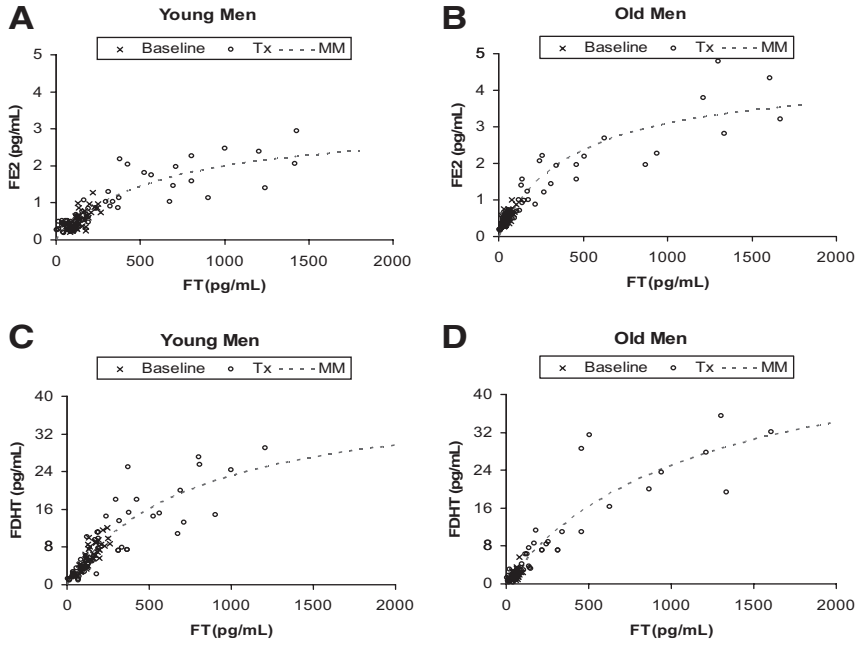

FIG. 3. The relationship of free E_2 (FE2) and free DHT (FDHT) to free testosterone (FT) concentrations at baseline (symbol X) or during treatment (Tx; symbol ○) with graded doses of testosterone in young men (A and C) and older men (C and D). *Dashed lines* are derived from fitting all data points to a Michaelis-Menten (MM) mechanistic model (see Appendix A for details).

tions and higher total and free E_2:T ratios than young men after adjusting for serum testosterone levels. In contrast to E_2 levels, serum DHT levels and DHT:T ratios did not differ between young and older men. Regression modeling revealed that percentage fat mass independently and partly explained the difference in free E_2 levels between young and older men, and jointly with SHBG explained most of the differences in free E_2 levels as well as free E_2:T ratios in the two groups. There also was an apparent effect of testosterone dose on metabolite to testosterone ratios; higher doses of testosterone were associated with seemingly lower metabolite to testosterone ratios in both young and older men. Mechanistic modeling indicated that the whole body conversion of testosterone to E_2 and DHT was consistent with saturable Michaelis-Menten kinetics.

In men in whom endogenous testicular steroidogenesis has been suppressed by administration of GnRH agonist, the steady-state circulating E_2 concentrations during testosterone administration should reflect the ratio of E_2 production from peripheral aromatization of testosterone and plasma E_2 clearance (as discussed in Appendix A). Although differences in SHBG levels may contribute to altered clearance rates, the observation that the free E_2 levels are also higher in older men is consistent with an increase in whole body aromatization.

Modeling the free E_2 *vs.* free testosterone and free DHT *vs.* free testosterone relationships showed that our data are consistent with Michaelis-Menten enzyme kinetics in both cases. From this analysis, we estimated the $K_{m,\ E2}$ value for aromatization to be approximately 1.83 nM; whereas for the 5α-reductase pathway the $K_{m,\ DHT}$ value was approximately 3.35 nM. The maximum free testosterone levels in our study only reached two to three times these values, so that full saturation had not been attained. Within the range of testosterone concentrations observed in this experiment, our GAM and GLM analyses did not find aromatization of testosterone to be dose-limited. However, the data conformed to Michaelis-Menten kinetic model, and we cannot exclude the possibility that the aromatization of testosterone may be saturable at testosterone concentrations that are higher than those observed in our study.

In vitro estimates of $K_{m,\ E2}$ in human cell lines containing aromatase range from 6.7 to 68 nM using androstenedione as a substrate (28–31). There are limited data using testosterone as a substrate [K_m of 41 nM (31)], and it is conceivable that the greater hydrophobicity of testosterone, as reflected in albumin binding (32) would be associated with a lower $K_{m,\ E2}$ than for androstenedione. This might explain the apparent discrepancy between our *in vivo* estimate of $K_{m,\ E2}$ (1.83 nM) and the *in vitro* values for androstenedione.

Two isoforms of 5α-reductase have been found in human tissues with widely divergent values of $K_{m,\ DHT}$ estimated *in vitro*. The type I isoenzyme has a $K_{m,\ DHT}$ value on the order of 2000 nM, whereas the type 2 isoenzyme has a $K_{m,\ DHT}$ value on the order of 12 nM (33, 34). The type 2 isoenzyme is thought to be the more prevalent form in humans (35) and is more consistent with our *in vivo* estimate of $K_{m,\ DHT}$ (3.35 nM). In rat tissue, the type 1 and type 2 isoenzymes have $K_{m,\ DHT}$ values of 2300 and 0.8 nM, respectively (36); the type 2 value again is consistent with our *in vivo* estimate.

Higher levels of total and free E_2 in older men could potentially be due to age-related differences in body composition, circulating testosterone levels, and SHBG levels. Because adipose tissue is an important site of testosterone to E_2 conversion in adult men, older men might be expected to have higher E_2 levels because of higher fat mass. The proinflammatory state associated with adiposity and aging that is believed to promote aromatase activity via IL-6 might be one contributory factor (37). Although the age-related differences in total E_2 levels and total E_2:T ratios during testosterone therapy persisted even after ad-

TABLE 3. Estimation of model parameters based on fit to rectangular hyperbola using combined baseline and on-treatment data

	A	B	R^2
E_2 *vs.* TT			
Young men (n = 50, 50)			
Estimate	100.7[a]	1981.0	0.660
SE	12.4[a]	394.3	
Older men (n = 50, 50)			
Estimate	138.3[a]	1470.1	0.812
SE	9.3[a]	178.1	
P value	0.016	NS	
DHT *vs.* TT			
Young men (n = 48, 47)			
Estimate	161.2	1109.2	0.702
SE	16.0	199.0	
Older men (n = 48, 47)			
Estimate	269.4	2389.6	0.749
SE	35.4	503.7	
P value	0.006	0.020	
FE *vs.* FT			
Young men (n = 49, 50)			
Estimate	3.22[a]	611.8[a]	0.775
SE	0.29[a]	96.7[a]	
Older men (n = 51, 50)			
Estimate	4.49[a]	450.7[a]	0.875
SE	0.25[a]	51.4[a]	
P value	0.001	NS	
FDHT *vs.* FT			
Young men (n = 48, 47)			
Estimate	41.27	780.9	0.786
SE	5.23	151.8	
Older men (n = 48, 47)			
Estimate	53.88	1148.6	0.869
SE	6.72	236.0	
P value	NS	NS	

Data are expressed as nanograms per deciliter, unless otherwise specified. "n" values correspond to baseline and on-treatment data points, respectively. *P* values correspond to the comparison of young and older men. We analyzed the data using a mechanistic model in which the four relationships (*i.e.* total E_2 *vs.* total T, free E_2 *vs.* free T, total DHT *vs.* total T, and free DHT *vs.* free T) were modeled using rectangular hyperbolae, *i.e.* $Y = A X/(B + X)$. Four equations (with corresponding A and B values) were derived for young men and old men, respectively. Based on pharmacokinetic principles and Michaelis-Menten kinetics (see Appendix A), the parameter A corresponds to the ratio of V_{max} (maximal rate of conversion of total or free T to the metabolite) divided by MCR (the metabolic clearance rate of the total or free metabolite); the B parameter corresponds to the K_m value for the enzymatic conversion of total or free T to the metabolite. The model parameter estimation (including the SE of the estimate, and R^2 value) was performed using the 2D Michaelis-Menten equation curve fitting software available online at http://zunzun.com/Equation/2/BioScience/Michaelis-Menten/. TT, Total testosterone; FE, free E_2; FT, free testosterone; FDHT, free DHT.

[a] Data represent picograms per milliliter.

justing for the higher percentage fat mass and SHBG levels in older men, the differences were attenuated after adjustment for these covariates. Furthermore, free E_2 levels and free E_2:T ratios were not significantly different between the two groups, after adjusting for fat mass, testosterone levels, and SHBG levels. Thus, age-related differences in E_2 levels and E_2:T ratios are at least partly related to differences in body composition between the young and older men and partly to differences in testosterone and SHBG levels.

Anecdotally, high doses of testosterone and other androgens have been reported to be associated with gynecomastia. Our data indicate that the occurrence of gynecomastia with high doses of testosterone cannot be explained on the basis of high estradiol to testosterone ratios; however, the high levels of E_2 attained at high doses may be sufficient to account for it.

The clinical consequences of higher E_2 levels and higher E_2:T ratios in older men remain poorly understood. In longitudinal studies in older men, higher E_2 levels have been associated with adverse outcomes such as stroke (38) and cognitive decline (39). In cross-sectional studies, higher E_2 levels have been associated with the increased risk of metabolic syndrome (40) and type 2 diabetes mellitus. Very low E_2 levels have been associated with bone loss and fracture risk (41–43), insulin resistance and premature atherosclerosis (44), and higher risk of mortality in elderly men (45). Hence, too much as well as too little estrogen may be detrimental to the health in men. Further research is needed to elucidate the role of estrogen in men's health, including defining thresholds beyond which E_2 levels might be detrimental.

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