Temporal Trend in Androgen Status and Androgen-Sensitive Outcomes in Older Men

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Context: Although androgen status decreases with aging in unselected men, the contemporaneous relationship over time between circulating hormones and androgen-sensitive outcomes has not been reported.

Objectives: To investigate the temporal relationships between age-specific androgen status and muscle (mass, strength), hemoglobin, and prostate-specific antigen (PSA).

Design, Setting and Participants: Men aged 70 years and older from the Concord Health and Ageing in Men Project study were assessed at baseline (2005–2007; n = 1705) and at 2-year (n = 1367) and 5-year follow-up (n = 958).

Main Outcomes and Measures: At all assessments, serum T, dihydrotestosterone (DHT), estradiol (E2), and estrone (E1) were measured by liquid chromatography-tandem mass spectrometry, and serum SHBG, LH, and FSH were measured by immunoassay together with calculation of free T (cFT). Muscle mass, strength of upper (hand grip) and lower (walking speed) limbs, hemoglobin, and prostate size (serum PSA) were measured.

Results: Serum hormones showed longitudinal, within-man decreases in serum T (−2.6%/y), DHT (−2.6%/y), E1 (−3.2%/y), and cFT (−2.8%/y) but increases in serum E2 (2.6%/y), SHBG (1.3%/y), LH (1.9%/y), and FSH (1.8%/y). Significant positive correlation was observed between changes in serum T with muscle mass, strength, and hemoglobin but not with PSA across the three time-points. Changes in serum DHT, cFT, and E1 had significant correlation with muscle mass, strength, and hemoglobin, but not with PSA.

Conclusions: These extended observational data are consistent with the impact of reduced androgen status on some somatic features of male aging. However, they do not exclude reverse causality or independent effects of aging on both androgen status and androgen-sensitive outcomes. **(*J Clin Endocrinol Metab* 101: 1836–1846, 2016)**

Among unselected men in population-based studies, serum T declines with age, with the decline contributed to or accelerated by concomitant systemic disease, accumulation of comorbidities, and medications (1). Age-related decline in circulating dihydrotestosterone (DHT) and estrone (E1) is also associated with poor health outcomes in older men (2–6). The relationship of this progressive age-related decline in androgen status with an-

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Abbreviations: ALM, appendicular lean mass; 17B-HSD, 17 β-hydroxysteroid dehydrogenase; BMI, body mass index; cFT, calculations of free T; CI, confidence interval; CV, coefficient of variation; DHT, dihydrotestosterone; E1, estrone; E2, estradiol; GEE, generalized estimating equation; LC-MS/MS, liquid chromatography-tandem mass spectrometry; PSA, prostate-specific antigen; QC, quality control.

drogen-sensitive endpoints such as muscle mass and strength, hemoglobin, and the prostate size were previously investigated in studies with a sole focus on serum T (7–12), but little is known of their relationships with other circulating reproductive hormones such as DHT, estradiol (E2), E1, SHBG, LH, and FSH that change concomitantly during male aging.

To our knowledge, there are very few population-based studies reporting dynamic longitudinal changes in serum T and related reproductive hormones by measuring circulating concentrations across several time-points and none that have related this contemporaneously with changes in androgen-sensitive outcomes. Very few studies have examined this relationship with a comprehensive focus on androgen (T, DHT) and estrogen (E2, E1) status using serum steroids measured by mass spectrometry, which is not only more accurate and specific than direct steroid immunoassays (13) but also features multianalyte capabilities necessary for comprehensive evaluation of androgen status (14).

Age-related trends for E2 in older men, mostly measured by inaccurate direct immunoassays for male serum samples (15), remain unclear and conflicting (2, 16–20). Furthermore, few studies have investigated the age trend and any biological role of serum E1, possibly underestimating the role of this low potency, proestrogen as a possible biomarker. In addition, it is important to examine serum DHT because of its higher potency as an androgen arising from its higher binding affinity to, and slower dissociation from, the androgen receptor (21–23).

The objectives of our study were: 1) to examine the longitudinal, within-man, trends over 5 years in reproductive hormones in older men by measuring serum androgen (T, DHT) and estrogen (E2, E1) levels using liquid chromatography-tandem mass spectrometry (LC-MS/MS) as well as serum SHBG, FSH, and LH by immunoassays and calculations of "free" T (cFT); and 2) to examine the longitudinal association between change in serum hormone levels with changes in a range of androgen-sensitive endpoints (lean mass, grip strength, walking speed, serum hemoglobin and prostate-specific antigen [PSA] concentrations) across three time-points over 5 years of follow-up.

Subjects and Methods

Study participants

The Concord Health and Ageing in Men Project (CHAMP) is a longitudinal, population-based observational study of the epidemiology of male aging conducted among men living around Concord Hospital in Sydney, New South Wales, Australia, as described in detail previously (24). Potential subjects were community-dwelling men aged at least 70 years with no other inclusion or exclusion criteria. Invitational letters were sent to 3627 men with 2815 eligible men successfully contacted to yield a cohort of 1705 participants. Baseline measurements were conducted between January 2005 and June 2007 using self-reported and interviewer-administered questionnaires and a wide range of clinical assessments. Follow-up assessments were conducted between January 2007 and October 2009 for the 2-year follow-up and between August 2010 and July 2013 for the 5-year follow-up, with identical measurements as at baseline.

Reproductive hormone measurement

Participants had an early morning fasting blood sample taken at all three time-points, and serum was stored at −80°C until assay. Measurements of serum T, DHT, E2, and E1 were by liquid chromatography-tandem mass spectrometry (LC-MS/MS) as originally described (25). Changes to improve assay performance by substituting ultra-pressure for high-pressure liquid chromatography with corresponding changes in extraction methodology were validated according to U.S. Food and Drug Administration (FDA) criteria (see supplementary methods in Ref. 26). The steroid measurements were calibrated against certified reference materials for T and DHT (National Measurement Institute), E2 (European Commission's Institute for Reference Materials and Measurements), and E1 (Cerilliant Corporation). The assays had between-run coefficients of variation (CVs) at three levels (low, medium, high) of quality control (QC) specimens of 1.9–4.5, 3.8–7.6, 2.9–13.6, and 5.7–8.7% in respect to T, DHT, E2, and E1 over 224 runs, including all samples from the three waves of this study. Overlapping QC samples were routinely run at the start, middle, and end of every run, with each new QC control run multiple times for calibration before use. There was no evidence of assay drift in Levey-Jennings QC plots (see QC plots in Supplemental Figure 1). Steroid profiles were measured in separate batches for the baseline, 2-year, and 5-year follow-up samples, with all samples from one study period run together. The change to ultra-pressure liquid chromatography was introduced between 2- and 5-year follow-up samplings after rigorous confirmation that it did not change accuracy or precision for any QC samples. The steroid assays had limits of quantification (defined by FDA/European Medicines Evaluation Agency as the lowest detectable measurement with CV $< 20\%$) of 0.025 ng/mL (T), 0.10 ng/mL (DHT), 5 pg/mL (E2) and 3 pg/mL (E1). Serum LH, FSH, and SHBG were measured by automated immunoassays (Roche Diagnostics Australia) subject to ongoing external QC program calibration with between-assay CV for three levels of QC specimens in each run of 2.1–2.2% for LH, 2.7–3.0% for FSH, and 2.0–2.8% (two QC levels only) SHBG. cFT levels were computed using an empirical formula validated in two large data sets consisting of more than 6000 blood samples (27, 28).

Lean mass measurement

Lean mass was measured using dual-energy x-ray absorptiometry (Discovery-W scanner; Hologic). The appendicular lean mass (ALM) was calculated as the sum of the lean mass of arms and legs (kilograms) (29). The ALM was standardized by body mass index (BMI) (ALM_{BMI}) to take into account the body size of participants (30).

Grip strength measurement

Handgrip strength was measured with a Jamar dynamometer (Promedics). Participants performed two trials on each hand, and the mean value of the two trials was obtained. The maximum strength in either hand was used as the handgrip strength for analyses.

Walking speed measurement

Walking speed was measured at the participants' usual pace (31). Trained staff used a stopwatch to record the time taken by the men to walk 6 m. The fastest time from two trials was used.

Biochemistry

Fasting blood samples were obtained at each visit for biochemistry tests, including hemoglobin (absorption spectrometry) and serum PSA (ElecsysE170; Roche Diagnostics GmbH), performed at the accredited clinical pathology department of Concord Hospital.

Potential confounder measurement

Tobacco usage status (current, ex-, or never smoker) and the years of education were by self-reported questionnaires. BMI was calculated from clinic measurements of height (Harpenden stadiometer) and weight. A comorbidity score was calculated as the sum of all conditions reported from the 19 disorders listed in the questionnaire. The self-rated health data were assessed using the 12-item Short Form Health Survey (SF-12) (32). Physical activity was measured using the Physical Activity Scale for the Elderly (PASE) (33).

Data analyses

Hormone levels were natural logarithm-transformed to remove mild positive skew. Linear mixed model was used with random subject-level intercepts and slopes to estimate longitudinal trend (34) and an autoregressive covariance structure allowing for differences between study time-points.

The concurrent longitudinal associations between changes in reproductive hormones and temporal changes in muscle mass, grip strength, walking speed, and hemoglobin and serum PSA across baseline, 2-year follow-up, and 5-year follow-up were evaluated by generalized estimating equation (GEE) analyses (35). GEE takes into account the time-varying nature of both the outcome and the exposure so that the association between two longitudinally measured variables can be studied using all longitudinal data simultaneously and adjusting for within-person correlations caused by repeated measurement on each participant using robust estimation of the variances of the regression coefficients. GEE longitudinal analysis included a term for the study follow-up period (baseline, 2-year and 5-year follow-up) that served as a covariate parameter to adjust for between study periods (eg, in assay or other methodology). Beta coefficients were reported as the slope of the change in the studied androgen-sensitive endpoints for changes in serum hormone levels across baseline and 2- and 5-year follow-up.

The effect of each studied hormone is addressed independently, on the basis of laboratory measurements (which are entirely free from cross-reactivity), as well as statistically in that the impact of each hormone (including consideration of interaction and curvature terms) was in no way influenced by another hormone. The GEE analysis treats each of the hormone measurements as a distinct and independent variable, with the observed beta coefficients providing its relative impact on the studied endpoints at a population level after adjustment for covariates. Nevertheless, hormones such as T and DHT, and E1 and E2, are in precursor-product relationships so that, biologically, they cannot be considered as completely independent pairwise of each other.

The model building for all longitudinal analyses included adjustment for known covariates, notably BMI, smoking status, physical activity, comorbidity, and self-rated health. Nonlinearity of variables with age was evaluated by the addition into models of quadratic (curvature) terms, and statistical interaction between the studied hormones and covariates was also examined. Post hoc analysis, Bonferroni corrections were performed to account for multiple comparisons involved in evaluating nine hormones in three models (= 27 comparisons) for each independent endpoint. This modified the conventional 0.05 level of significance to a threshold of 0.002 (0.05/27). Models were fitted using SPSS software version 20 (IBM Corp), SAS software 9.3 (SAS Institute Inc), and STATA version 12 (Stata Corp).

Ethics approval

The CHAMP study has approval from the Concord Hospital Human Research Ethics Committee, and study participants provided written informed consent for all procedures.

Results

The descriptive statistics at the baseline and 2- and 5-year follow-up and those lost to follow-up are shown in Table 1. The men lost to follow-up did not differ from those seen at follow-up except for being older (79 vs 77 years) and having lower PASE scores (103 vs 124) and higher serum SHBG (53.8 vs 50.1 nmol/L) at baseline. The cross-sectional age-related trends in reproductive hormones at the three follow-up time-points are shown in Table 2 and Figure 1. The mean hormone levels plotted in relation to the three study periods stratified by age are shown in Supplemental Figure 2. In the cross-sectional analyses, there were declines in serum T and cFT per year of age and increases in serum SHBG, LH, and FSH. For each hormonal variable, the annualized rate was similar at each of the three time-points (Table 2). There were no statistically significant age trends observed in serum DHT, E2, and E1 in cross-sectional analyses at any of the three studied time-points. These cross-sectional findings were not significantly changed when curvature terms for age were introduced.

The longitudinal age-related annual change in reproductive hormones across three time-points over 5 years (Table 3) showed declines for serum T, DHT, cFT, and E1 with increases for serum E2, FSH, LH, and SHBG. After covariable adjustment for health and lifestyle characteristics, the longitudinal changes for all hormones remained quantitatively similar (Table 3). The annualized changes

Table 1. Descriptive Statistics by CHAMP Study Period

	Baseline	2-Year Follow-Up	5-Year Follow-Up	Loss to Follow-Up at 2 Years[a]	Loss to Follow-Up at 5 Years[a]
n	1659	1291	910	394	775
Age, y	76.9 (5.5)	78.5 (5.2)	81.3 (4.6)	78.9 (6.3)[b]	78.6 (6.0)[b]
BMI, kg/m^2	27.8 (4.0)	27.8 (3.8)	27.5 (3.9)	27.5 (4.8)	27.7 (4.3)
No. of comorbidities	2.5 (1.7)	2.5 (1.7)	2.5 (1.6)	2.7 (1.9)	2.6 (1.9)
Physical activity (PASE)	124.5 (62.2)	119.8 (59.7)	117.4 (63.2)	102.5 (64.8)[b]	108.4 (60.8)[b]
Grip strength, kg	34.3 (7.5)	34.7 (8.0)	32.6 (8.3)	33.1 (7.4)	33.5 (7.5)
ALM, kg	0.8 (0.1)	0.8 (0.1)	0.8 (0.1)	0.8 (0.1)	0.8 (0.1)
Walking speed, m/s	0.9 (0.2)	0.9 (0.2)	0.9 (0.2)	0.8 (0.2)	0.8 (0.2
Hemoglobin, g/dL	14.3 (1.4)	14.2 (1.3)	14.1 (1.4)	14.0 (1.7)	14.0 (1.6)
PSA, ng/mL	3.2 (10.6)	3.5 (6.0)	3.6 (6.8)	3.3 (5.4)	3.4 (4.1)
Current smoker, n (%)	100 (6)	49 (4)	33 (4)	33 (8)	50 (6)
Low self-rated health, n (%)	488 (30)	366 (29)	236 (26)	119 (30)	254 (33)
T, ng/mL	4.3 (1.9)	4.2 (1.9)	3.4 (1.8)	4.1 (2.0)	4.1 (2.0)
DHT, ng/mL	0.4 (0.2)	0.4 (0.2)	0.3 (0.2)	0.4 (0.2)	0.4 (0.2)
cFT, pg/mL	59.6 (22.5)	58.2 (22.6)	46.9 (22.5)	56.6 (24.5)	56.7 (25.1)
SHBG, nmol/L	50.1 (20.7)	52.0 (21.0)	56.3 (23.8)	53.8 (23.2)[b]	52.9 (22.3)[b]
E2, pg/mL	25.3 (12.4)	24.1 (9.6)	36.0 (14.9)	25.2 (11.1)	24.9 (10.4)
E1, pg/mL	40.4 (16.3)	39.8 (15.8)	28.5 (11.8)	41.2 (19.4)	40.0 (18.1)
FSH, IU/L	14.7 (14.9)	15.3 (14.9)	17.3 (16.4)	16.3 (17.2)	16.5 (17.1)
LH, IU/L	9.6 (8.8)	10.1 (8.3)	11.3 (8.9)	11.0 (11.9)	10.9 (11.0)

Data are expressed as mean (SD), unless specified otherwise.

[a] The data for loss to follow-up at 2 years and 5 years were based on the baseline descriptive data.

[b] Significantly different from baseline ($P < .05$)

were in each case more pronounced in longitudinal than in cross-sectional changes for the same analyte.

The contemporaneous associations between longitudinal changes in reproductive hormones and androgen-sensitive outcomes across baseline and 2- and 5-year follow-up are shown in Table 4 (muscle mass, upper and lower limb strength) and Table 5 (hemoglobin, serum PSA). Univariate analyses revealed statistically significant positive correlation between changes in T with changes in lean mass, grip strength, walking speed, and hemoglobin and negative correlation with PSA over time. Similar significant correlations remained across all androgen-sensitive endpoints with changes in serum T, DHT, and cFT in multivariate-adjusted models. Longitudinal changes in serum E2 were only correlated with hemoglobin, whereas serum E1 was positively correlated with lean mass, walking speed, and hemoglobin, but not grip strength or PSA. All significant findings in Tables 4 and 5 remained significant after Bonferroni correction for multiple comparisons, except for between serum E1 and FSH in muscle mass, serum E1 and LH in walking speed, and serum T, cFT, FSH, and LH with serum PSA, which became nonsignificant. The term for study period (baseline, 2-year, and 5-year follow-up) as a nuisance parameter in these analyses to adjust for any unidentified systematic differences between study periods was in each instance nonsignificant. Statistically significant interaction was observed between serum T or E2 and age with walking speed and

Table 2. Cross-Sectional Age Trend in Reproductive Hormones at Baseline, 2-Year Follow-Up, and 5-Year Follow-Up

	Baseline (n = 1659)		2 Years (n = 1291)		5 Years (n = 910)		Pooled (n = 3860)[a]	
	Mean Decline	*P* Value	Mean Decline	*P* Value	Mean Decline	*P* Value	Mean Decline	*P* Value
T	−0.6	.002	−0.9	.001	−1.0	.02	−1.0	<.001
DHT	−0.6	.2	−0.5	.1	−0.7	.3	−0.6	.05
cFT	−1.1	<.001	−1.3	<.001	−1.4	<.001	−1.3	<.001
SHBG	2.3	<.001	2.1	<.001	2.2	<.001	3.3	<.001
E2	0.07	.8	0.1	.6	−0.07	.8	0.05	.6
E1	−0.2	.4	0.1	.6	0.2	.5	0.1	.5
FSH	6.2	<.001	6.4	<.001	6.8	<.001	6.2	<.001
LH	5.5	<.001	5.4	<.001	6.4	<.001	5.7	<.001

Mean decline is expressed as percentage per year.

[a] Pooled baseline, 2-year, and 5-year data for examining per person analysis.

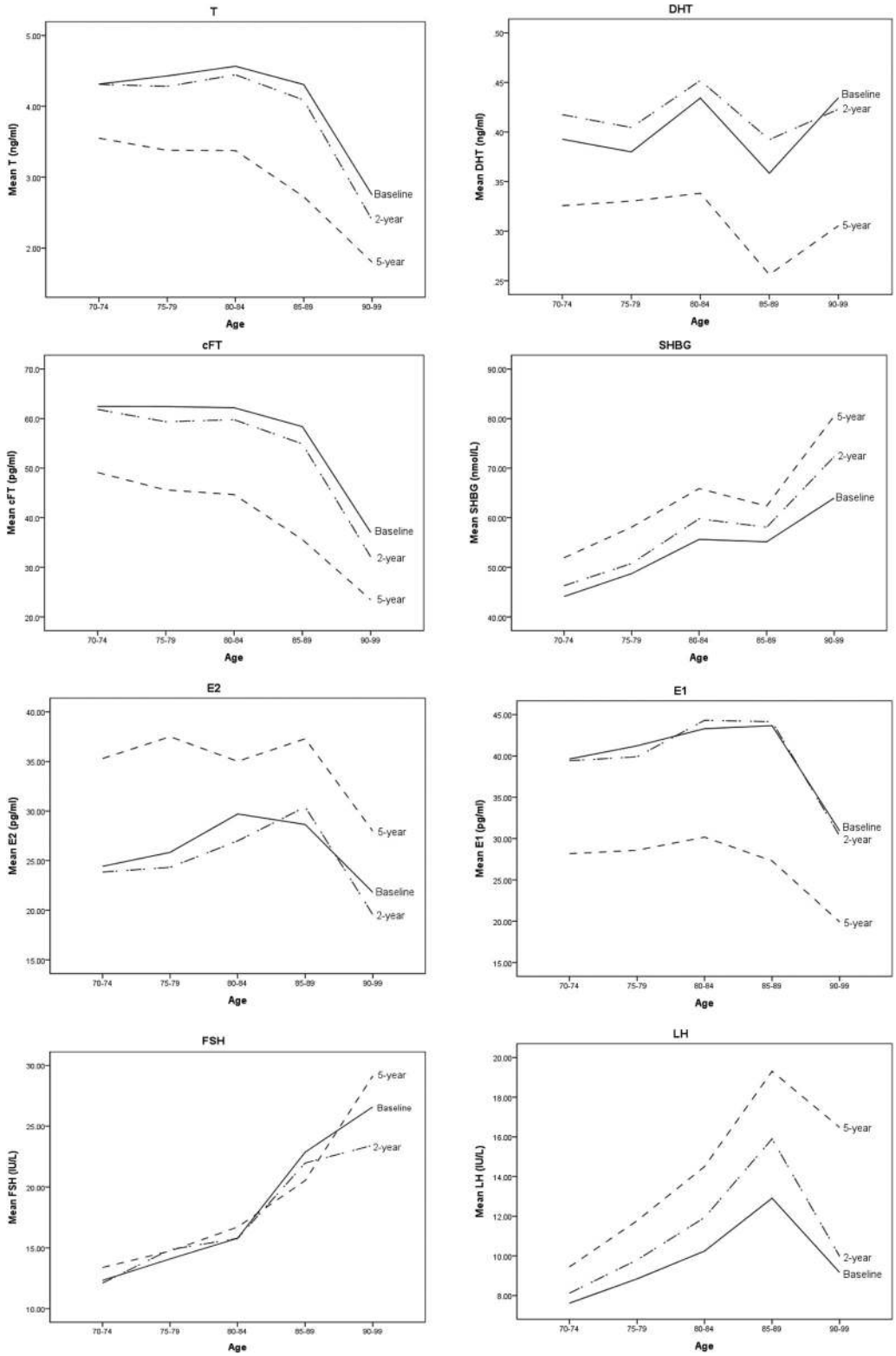

Figure 1. Mean hormone levels plotted in relation to age and with stratification by study period (baseline, 2-year follow-up, and 5-year follow-up).

hemoglobin, but not with muscle mass, strength, and PSA. No other significant interactions were observed between the studied hormones and with other androgen-sensitive outcomes.

Sensitivity analyses involving additional models that adjusted for specific major chronic diseases (diabetes, myocardial infarction, stroke, dementia, and depression), rather than number of comorbidities, con-

Table 3. Longitudinal Population Change in Reproductive Hormones Across Three Time-Points Over 5-Year Follow-Up Period

	Unadjusted		Multivariable Adjusted[a]	
	Annual Change	*P* Value	Annual Change	*P* Value
T	−2.6	<.001	−2.1	<.001
DHT	−2.6	<.001	−2.1	<.001
cFT	−2.8	<.001	−2.3	<.001
SHBG	1.3	<.001	1.1	.01
E2	2.6	<.001	2.3	<.001
E1	−3.2	<.001	−2.8	<.001
FSH	1.8	<.001	1.5	.01
LH	1.9	<.001	1.7	<.001

Annual change is expressed as percentage per year.

[a] Adjusted for BMI, smoking status, physical activity, self-rated health, and number of comorbidities.

firmed and reinforced our original analysis (data not shown).

Discussion

This study provides new insights into the androgen-dependent features of male aging through an analysis of longitudinal age-related changes in major reproductive hormones and their contemporaneous association with key androgen-sensitive outcome measures in older men. By using steroid mass spectrometry-based analysis, this study provides a comprehensive and accurate appraisal of androgen status in men, with concurrent evaluation of circulating levels of potent androgens T and DHT as well as E2 and E1 together with serum LH, FSH, and SHBG. Serum T, DHT, and cFT show declines in both cross-sectional and longitudinal analyses but with 2- to 4-fold greater magnitude in longitudinal within-man changes compared with cross-sectional between-man changes. On the contrary, age-related increases in serum SHBG, LH, and FSH were smaller in longitudinal than in cross-sectional changes. Surprisingly, longitudinal age-related changes in opposite directions were observed for the potent estrogen E2, which increased by nearly 3% per year, compared with the proestrogen E1, which decreased by a similar magnitude. The absence of similar changes in the baseline cross-sectional analysis indicates that these variables feature much greater within-individual changes in a subpopulation compared with the relatively wide change between subject variability in those variables.

Comparable longitudinal changes in serum T related to increasing age from this study (−2%/y) have been reported from the Massachusetts Male Aging Study (−1.6%/y) and pooled data from two other Australian centers (−1%/y) (36, 37). Similarly, other longitudinal population-based cohort studies report steeper age-related decline in serum T levels compared to cross-sectional changes (37, 38). This suggests that poor health might accelerate the age-related decline in serum T, DHT, and cFT over time. However, this explanation does not explain the greater cross-sectional than longitudinal changes in serum SHBG, LH, or FSH. An alternative explanation may involve complex collinearity of age-period-cohort effects (39). Several independent studies in the United States and Europe have reported very similar significant temporal downward trends in serum T, independent of apparent concurrent effects of age or other known covariables such as obesity (37, 38, 40, 41). Although age often appears as an explanatory variable in such observational analyses, its significance is not well understood. Age, solely a measure of elapsed time, is neither a specific biological mechanism nor a clinical diagnosis. Rather, it can be understood as a surrogate for undefined physicochemical mechanisms in models where most variance remains unexplained by the optimal mathematical models for nonrandomized comparisons. Indeed, as a temporal variable, age may have limited or no explanatory power in its own right. For example, a 95% confidence interval (CI) for serum T of 2.0–8.1 ng/mL deduced from a cohort of men deemed to be in excellent health (42) may be compared with cohorts of young men who were unselected (43) or selected for normal reproductive function (44) and had analogous 95% CI values for serum T of 2.3–8.8 and 3.3–9.5 ng/mL, respectively. Hence, the remaining differences between the older and younger cohorts, often attributed to age, may represent residual uncontrolled confounding of undiagnosed cardiovascular disease, reproductive health disorder, and other chronic diseases. Furthermore, in a mixed longitudinal and cross-sectional study of men over 40 years of age recruited for self-reported excellent, asymptomatic health found no decrease in serum T, DHT, or E2 associated with age, a study design powerful enough to detect effects of obesity, smoking, and previously unrecognized impact of fasting (45).

We previously reported statistically significant cross-sectional associations between serum T and lean muscle mass in arm and leg and also predicted subsequent functional decline (46) consistent with other longitudinal studies (7, 10). We now extend these observations by showing that men with decreasing serum T as well as DHT and cFT and with increased LH over time were more likely to exhibit decreasing lean mass and weaker upper and lower extremity strength. However, based on our findings, the cohort average decrease in muscle mass per unit change in serum T and DHT over the three study phases across 5

Table 4. Longitudinal Associations Between Change in Reproductive Hormones and Muscle Mass, Upper and Lower Extremity Strength Over Time Across Baseline, 2 Years, and 5 Years Expressed as β Coefficients Comprising the Slope of the Relationship Between the Endpoint and the Reproductive Hormones

	Unadjusted		Age Adjusted		Multivariate Adjusted[a]	
	β Coefficient (95% CI)	P Value	β Coefficient (95% CI)	P Value	β Coefficient (95% CI)	P Value
Muscle mass (ALM_{BMI}), kg[b,c]						
T, ng/mL	10 (4, 70)	<.001	10 (4, 70)	<.001	10 (4, 70)	<.001
DHT, ng/mL	20 (10, 30)	<.001	20 (10, 30)	<.001	20 (10, 30)	<.001
cFT, pg/mL	0.4 (0.3, 0.5)	<.001	0.4 (0.3, 0.5)	<.001	0.2 (0.1, 0.3)	<.001
SHBG, nmol/L	0.4 (0.2, 0.5)	<.001	0.4 (0.3, 0.6)	<.001	0.4 (0.3, 0.6)	<.001
E2, pg/mL	0.04 (−0.5, 0.2)	.6	0.04 (−0.1, 0.2)	.6	0.06 (−0.1, 0.2)	.4
E1, pg/mL	0.2 (0.2, 0.3)	.02	0.2 (0.02, 0.3)	.02	0.2 (0.04, 0.3)	.02
FSH, IU/L	−0.2 (−0.4, −0.02)	<.001	−0.2 (−0.4, −0.02)	.03	−0.2 (−0.4, −0.001)	.04
LH, IU/L	−1 (−1, −0.5)	<.001	−1 (−1, −0.3)	<.001	−1 (−1, −1)	<.001
Upper-extremity strength (grip strength), kg						
T, ng/mL	0.34 (0.21, 0.46)	<.001	0.27 (0.14, 0.39)	<.001	0.25 (0.13, 0.37)	<.001
DHT, ng/mL	0.90 (0.06, 1.74)	.04	0.72 (−0.09, 1.54)	.10	0.88 (0.06, 1.71)	.04
cFT, pg/mL	0.03 (0.02, 0.04)	<.001	0.02 (0.01, 0.03)	<.001	0.02 (0.01, 0.03)	<.001
SHBG, nmol/L	−0.03 (−0.04, −0.01)	<.001	−0.004 (−0.02, 0.01)	.48	0.002 (−0.01, 0.01)	.75
E2, pg/mL	0.01 (−0.003, 0.03)	.14	0.01 (−0.005, 0.02)	.19	0.01 (−0.01, 0.02)	.99
E1, pg/mL	−0.001 (−0.02, 0.01)	.87	−0.004 (−0.02, 0.01)	.57	−0.005 (−0.02, 0.01)	.47
FSH, IU/L	−0.04 (−0.10, −0.02)	<.001	−0.01 (−0.03, 0.004)	.13	−0.01 (−0.03, 0.01)	.24
LH, IU/L	−0.11 (−0.14, −0.10)	<.001	−0.06 (−0.09, −0.03)	<.001	−0.05 (−0.08, −0.02)	.001
Lower-extremity strength (walking speed), m/s[c]						
T, ng/mL	10 (10, 20)	<.001	10 (10, 20)	<.001	10 (2, 10)	.001
DHT, ng/mL	60 (30, 90)	<.001	50 (30, 80)	<.001	40 (10, 70)	<.001
cFT, pg/mL	1 (1, 2)	<.001	1 (1, 1)	<.001	1 (0.2, 1)	.001
SHBG, nmol/L	−1 (−1, −0.2)	.002	0.2 (−0.2, 0.5)	.4	−0.2 (−0.5, 0.2)	.4
E2, pg/mL	0.3 (−0.2, 1)	.2	0.3 (−0.2, 1)	.3	0.3 (−0.2, 1)	.2
E1, pg/mL	1 (0.1, 1)	.01	1 (0.04, 1)	.04	1 (0.1, 1)	.02
FSH, IU/L	−2 (−2, −1)	<.001	−1 (−1, −0.1)	.02	−1 (−1, −0.1)	.06
LH, IU/L	−3 (−4, −2)	<.001	−2 (−2, −1)	.001	−1 (−2, −0.3)	.01

Bonferroni adjustments for multiple comparisons for each independent endpoint lower the conventional *P* threshold from .05 to .002 based on nine hormones investigated in three models.

[a] Adjusted for age, BMI, smoking status, physical activity, self-rated health and number of comorbidity.

[b] The multivariate model for appendicular lean mass standardized to BMI was adjusted for the above covariables except for BMI.

[c] The β coefficients were multiplied by 1000.

years was very small at 10 and 20 g, respectively. Likewise, the cohort average decrease in grip strength minimally declined per unit change in serum T and DHT at 250 and 880 g, and walking speed changed at 0.1 and 0.4 m/s, respectively. Nevertheless, although our observational data cannot deduce causality, interventional studies have shown that T treatment of older men with low serum T increases muscle mass function (47). It remains unclear how such changes in physical activity and its determinants relate to decreased serum T and reduced muscle mass and function. Whether they are cause or effect, and are therefore rectifiable contributions to morbidity or instead serve as informative biomarkers, respectively, can only be determined through relevant interventional studies.

The examination of DHT in this study was important to fully evaluate androgen status in men. DHT is a pure androgen with 2- to 10-fold higher intrinsic androgenic potency than T (21) due to its higher affinity (22) and slower dissociation from (23) the androgen receptor with its circulating levels, typically around 10% of circulating T (48), originating mainly from nonprostatic tissues (49). Recent studies have shown that serum DHT may have selective impact on some health outcomes independent from those of serum T (3, 50–53). The present studies show consistent relationships between serum DHT with muscle mass and strength as well as hemoglobin, but not serum PSA. Further studies are warranted to investigate the distinctive and contributory roles of DHT in male aging. The positive correlation between serum T and DHT with changes in hemoglobin are consistent with previous studies reporting that older men with low T have a higher risk of anemia (11) and that androgens increase erythroid mass (54).

Previous cross-sectional findings on age-related changes of serum E2 in men, measured by immunoassays,

Table 5. Longitudinal Associations Between Change in Reproductive Hormones and Hemoglobin and PSA Over Time Across Baseline, 2 Years, and 5 Years Expressed as β Coefficients Comprising the Slope of the Relationship Between the Endpoint and the Reproductive Hormones

	Unadjusted		Age-Adjusted		Multivariate-Adjusted[a]	
	β Coefficient (95% CI)	P Value	β Coefficient (95% CI)	P Value	β Coefficient (95% CI)	P Value
Hemoglobin, g/dL[b]						
T, ng/mL	0.17 (0.15, 0.20)	<.001	0.16 (0.14, 0.19)	<.001	0.17 (0.15, 0.20)	<.001
DHT, ng/mL	0.97 (0.80, 1.14)	<.001	0.96 (0.78, 1.12)	<.001	1.02 (0.85, 1.19)	<.001
cFT, pg/mL	0.01 (0.01, 0.02)	<.001	0.01 (0.01, 0.02)	<.001	0.01 (0.01, 0.02)	<.001
SHBG, nmol/L	0.001 (−0.001, 0.03)	.5	0.004 (0.002, 0.01)	<.001	0.01 (0.003, 0.01)	<.001
E2, pg/mL	0.01 (0.01, 0.02)	<.001	0.02 (0.01, 0.02)	<.001	0.02 (0.01, 0.02)	<.001
E1, pg/mL	0.01 (0.02, 0.02)	<.001	0.01 (0.02, 0.02)	<.001	0.02 (0.01, 0.02)	<.001
FSH, IU/L	−0.01 (−0.01, −0.01)	<.001	−0.01 (−0.02, −0.01)	<.001	−0.02 (−0.02, −0.01)	<.001
LH, IU/L	−0.03 (−0.04, −0.03)	<.001	−0.03 (−0.04, −0.03)	<.001	−0.03 (−0.03, −0.02)	<.001
PSA, ng/mL[b]						
T, ng/mL	−0.22 (−0.37, −0.06)	.01	−0.19 (−0.35, −0.03)	.02	−0.19 (−0.36, −0.03)	.02
DHT, ng/mL	−0.67 (−1.85, 0.51)	.3	−0.59 (−1.77, 0.59)	.3	−0.65 (−1.88, 0.59)	.3
cFT, pg/mL	−0.02 (−0.03, −0.01)	.001	−0.02 (−0.03, −0.004)	.01	−0.02 (−0.03, −0.004)	.01
SHBG, nmol/L	0.02 (0.002, 0.03)	.03	0.01 (−0.006, 0.02)	.3	0.01 (−0.005, 0.02)	.2
E2, pg/mL	−0.02 (−0.04, 0.01)	.1	−0.02 (−0.04, 0.01)	.1	−0.02 (−0.04, 0.003)	.1
E1, pg/mL	−0.01 (−0.03, 0.01)	.4	−0.01 (−0.03, 0.01)	.4	−0.01 (−0.03, 0.01)	.5
FSH, IU/L	−0.01 (−0.03, 0.01)	.4	−0.02 (−0.04, 0.0002)	.05	−0.02 (−0.04, −0.004)	.02
LH, IU/L	−0.02 (−0.05, 0.01)	.2	−0.04 (−0.08, −0.01)	.02	−0.05 (−0.08, −0.01)	.01

Bonferroni adjustments for multiple comparisons for each independent endpoint lower the conventional *P* threshold from .05 to .002 based on nine hormones analyzed in three models.

[a] Adjusted for age, BMI, smoking status, physical activity, self-rated health, and number of comorbidities.

[b] Reference range is 13.5–17.5 g/dL for hemoglobin and <4.0 ng/mL for PSA.

have shown age-related declines (16–18, 55, 56), no change (19, 57), or increases (20) with age. Our findings agree with the Framingham study, which reported a significant age-related increase in serum E2 by LC-MS/MS (2). Our present findings of a longitudinal increase in serum E2 of nearly 3% per year and a positive relationship with changes in hemoglobin are consistent with recent findings from the Swedish MrOS study and the Busselton Health Survey, which also measured E2 by mass spectrometry (50, 58).

Limited studies of serum E1 have provided conflicting data cross-sectional studies showing decline or increase (55, 59) with age. We observed no significant cross-sectional changes but a longitudinal age-related decrease of 3% per year for serum E1 measured by LC-MS/MS together with an annual increase in serum E2 of similar magnitude. This dichotomy draws attention to the enzyme 17 β-hydroxysteroid dehydrogenase (17B-HSD), which mediates the enzymatic conversion between E1 and E2 (60). However, the rate, extent, and tissue location of the relevant 17B-HSD isozymes responsible for the interconversion of circulating E1 to E2 remains unclear. Experimental studies have reported that interconversion rates between E1 and E2 differ between tissues (61). Aromatization may also have a significant role in the age-related changes in serum E1 and E2 (62). The observed contrary changes of serum E1 and E2 in older men suggest possible specific changes in 17B-HSD or in aromatase activity, but further studies are warranted to explain the mechanisms and significance of these age trends.

A major strength of our study is the use of longitudinal data to investigate age trends of a comprehensive profile of androgen status over multiple follow-up time-points in conjunction with key androgen-sensitive outcomes. To our knowledge, this is the first study to examine longitudinal age-related changes in serum E2 and E1 in men across multiple time-points. Another strength is the use of the LC-MS/MS, the current "gold standard" for steroid assays, providing multianalyte steroid profiling as well as the use of a more accurate formula for cFT. Direct immunoassay methods that do not include extraction and chromatography have poor accuracy in measuring low levels of sex steroids, which is particularly problematic for measuring circulating T, DHT, E2, and E1 in older men (13, 63). A further strength of CHAMP is that it includes a large and representative group of older Australian men, as demonstrated by similar sociodemographic and health characteristics compared to older men in the nationally representative MATeS study (64).

A significant limitation of our study is the impact of survivor bias that applies to men eligible to enter the study

who were however less healthy and may have already died before reaching the age of 70 years. In addition, survivor bias may also apply to the men who are lost to follow-up in the study (20% by 2 years, another 30% by 5 years), whether due to death or other reasons. However, loss to follow-up in cohort studies of older people is inevitable because of the high mortality rate, which accounted for nearly 35% of the loss to follow-up in our cohort. On the other hand, this older cohort will provide a more complete view of the causes and determinants of mortality among older men. Diurnal variation in hormone concentrations could potentially have influenced our results, but we obtained single morning fasting blood samples to minimize any possible variations.

In conclusion, androgen status, whether represented by serum T, DHT, or cFT, declined progressively over time in older men, and these changes were associated significantly with corresponding changes in androgen-sensitive endpoints; positively with lean muscle mass, grip strength, walking speed, and hemoglobin; but not with serum PSA. A novel finding of this study is that serum E2 increased progressively as men grew older, whereas serum levels of the proestrogen E1 declined. The opposite age-related changes in serum E2 and E1 among older men warrant further investigation for their biological and biomarker implications. Whether hormone therapy has any role in these studied endpoints of older men remains to be evaluated by randomized, placebo-controlled trials of adequate size and duration.

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