Age-specific population centiles for androgen status in men

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Abstract

Aim: The age-specific population profiles in men of circulating testosterone and its two bioactive metabolites dihydrotestosterone (DHT) and estradiol (E₂) across the adult lifespan and its determinants are not well described.

Objective: Our objective was to deduce smoothed age-specific centiles of circulating testosterone, DHT, and E₂ in men using pooled data from population-based studies in three Australian cities from liquid chromatography–mass spectrometry steroid measurements in a single laboratory.

Design, setting, and participants: We pooled data of 10 904 serum samples (serum testosterone, DHT, E₂, age, height, and weight) from observational population-based studies in three major cities across Australia.

Main outcome measures: Age-specific smoothed centiles for serum testosterone, DHT, and E₂ in men aged 35–100 years were deduced by large sample data analysis methods.

Results: We found that serum testosterone, DHT, and E₂ decline gradually from ages 35 onwards with a more marked decline after 80 years of age. Higher weight, BMI, and body surface area as well as shorter stature are associated with reduced serum testosterone, DHT, and E₂.

Conclusions: Among Australian men, there is a gradual progressive population-wide decline in androgen status during male aging until the age of 80 years after which there is a more marked decline. Obesity and short stature are associated with reduced androgen status. Research into the age-related decline in androgen status should focus on the progressive accumulation of age-related comorbidities to better inform optimal clinical trial design.

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Introduction

Male reproductive health including spermatogenesis, fertility, virilization, and sexual function as well as androgen effects on non-reproductive target tissues are determined by the effects of testosterone together with its two bioactive metabolites dihydrotestosterone (DHT) and estradiol (E₂) (1). In men, virtually all testosterone is secreted by the Leydig cells of the testis into the circulation with subsequently ~4% converted to DHT, a more potent, pure androgen by a 5 α -steroid reductase enzyme, and another 0.1% to E₂ by aromatase. These

bioactive steroids are transported via the bloodstream to reach their remote target tissues where testosterone and DHT both activate the androgen receptor (AR) and E₂, the estrogen receptor. Hence, a full appraisal of systemic androgen status in men requires concurrent consideration of all three bioactive sex steroids (2). Yet the age-specific population profile of circulating testosterone and its two bioactive metabolites across the lifespan is not well described through suitably large population-based studies using mass spectrometry (MS)-based steroid

measurements. On this background, using a well-validated steroid profile by liquid chromatography (LC)–MS in a single laboratory and employing statistical methods suitable for large datasets, we deduced age-specific smoothed centiles from early adulthood until advanced old age using pooled data of over 10 000 samples arising from population-based studies of men in three cities spanning across the Australian continent.

Subjects and methods

Samples

Early morning serum samples obtained from the following population-based studies of community-dwelling men, which have had their design, primary outcomes, and ethical review described in detail elsewhere, were analyzed for this study. In Perth, the Health in Men Study (HIMS) comprised a population-based cohort study of community-dwelling men (3). From the original cohort of 12 203 men (wave 1, 1996–1999) randomly selected from the electoral roll, the second wave (4248 men, 2001–2004), then aged 70–89 years and over 95% of European Caucasian descent, provided blood samples and were studied (4). In Adelaide, the Men, Androgens, Inflammation, Lifestyle, Environment and Stress (MAILES) study combined the harmonized populations of the Florey Adelaide Male Aging Study (FAMAS) (5), with blood samples collected between 2002 and 2005, and the male population of the North West Adelaide Health Study (NWAHS) (6), with blood samples collected between 2007 and 2010, both of which are prospective longitudinal cohort studies of randomly selected, community-dwelling adults, including men aged 35–86 years with over 96% of European Caucasian descent, in metropolitan Adelaide, Australia. Samples were obtained from two waves of the MAILES study with a median of 5 years between samples. In Sydney, the Concord Health and Aging in Men Project (CHAMP) comprised a population-based study of men of predominantly (>93%) European Caucasian descent aged 70 years and over (range 70–99 years) living in a defined geographical region around Concord Hospital. The sampling frame was the New South Wales Electoral Roll noting that registration is compulsory in Australia (7). Blood samples were obtained from two waves of the study, between 2005 and 2007 and then again between 2007 and 2009, with a median of 2 years between samples. The population of these three cities, comprising 35% of the national population, is similar and representative of the national population in demographic characteristics

(see www.stat.abs.gov.au/itt/r.jsp?databyregion#/), which supports the validity pooling the data for this analysis. In each city, the next most common ancestry was Asian with about half of Chinese descent.

Sex steroid assays

Serum samples had the same sex steroid profile measuring testosterone, DHT, and E_2 in batch per study using a LC–MS method as described (8). The steroid measurements were calibrated against certified reference materials for testosterone and DHT (National Measurement Institute, North Ryde, NSW, Australia) and E_2 (European Commission's Institute for Reference Materials and Measurements) and had between-run coefficients of variation at three levels (low, medium, and high) of quality control (QC) specimens of 1.9–4.5% (testosterone), 3.8–7.6% (DHT), and 2.9–13.6% (E_2) over 184 runs that spanned the period of the samples included in this study. Overlapping QC samples were used continuously to span all of the batches measured in separate batches. Serum samples were stored frozen at -80°C for up to 8 years prior to analysis, making it unlikely there was any influence of duration of storage on serum steroid measurements, which are stable in frozen storage for decades (9, 10, 11).

Statistical analysis

Data were analyzed to deduce smoothed centiles using the General Additive Model for Location, Scale and Shape (GAMLSS), which adopts the LMS approach originally developed by Cole for pediatric growth charts (12, 13) and is related to the method of quantile regression developed for econometrics (14), using an implementation in R Software by Rigby & Stasinopoulos (15, 16). For this analysis, the sample rather than the person was the primary unit of analysis. Where the same person was sampled in two different waves of a study (sampled at a median 4.2 years, range 2–6.3 years, between samples), each sample entered the analysis separately with its own specific age and blood hormone result at the time of sampling and was considered an independent sample for this analysis. Using the raw data, smoothed centiles (1, 2.5, 5, 10, 25, 50, 75, 90, 95, 97.5, and 99) for the age-specific distribution of the serum, testosterone, DHT, and E_2 were deduced. To better illustrate net androgen effects considering testosterone together with its more potent metabolite DHT as used previously, we defined a new variable serum androgen as the sum of serum testosterone plus five times serum DHT concentrations

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Table 1 Descriptive data of study population.

Variable	n	Mean (s.d.)	Minimum	Q1	Q2	Q3	Maximum
Age (years)	10 904	68.7 (11.2)	35.1	65.5	70.9	75.9	99.2
Height (cm)	10 876	172 (7.2)	147.5	167	172	177	208
Weight (kg)	10 879	82.0 (13.8)	42.0	72.8	80.6	89.6	167
BSA (kg/m ²)	10 869	1.99 (0.19)	1.38	1.86	1.98	2.10	2.95
BMI (m ²)	10 869	27.7 (4.0)	15.5	25.0	27.3	29.8	60.4
Serum testosterone (nmol/l)	10 902	14.6 (6.3)	0	10.7	14.0	18.0	91.6
Serum DHT (nmol/l)	10 466	1.47 (0.79)	0	0.96	1.37	1.86	18.9
Serum A ^a (nmol/l)	10 902	16.3 (6.7)	0	12.1	15.7	20.0	102
Serum E ₂ (pmol/l)	10 070	84 (38)	0	61	80	103	1324
DHT/testosterone ratio	10 460	0.12 (0.76)	0	0.08	0.10	0.12	72.5
E ₂ /testosterone ratio	10 064	9.4 (32.2)	0	4.5	5.8	7.5	1163

Q1, first quartile; Q2, second quartile (median); Q3, third quartile.

^aAndrogen is defined as the sum of serum testosterone plus five times the serum DHT reflecting the higher androgenic bioactivity of DHT. For details, see text.

(testosterone + 5 × DHT) with the multiplier reflecting the fact that DHT has two- to tenfold higher intrinsic androgenic potency than testosterone (17, 18) due to its higher affinity and slower dissociation from the AR (19). For that definition where DHT was undetectable, a value of 0 was assigned. To reduce any impact of biological or analytical outliers, smoothed centiles of distributions were estimated using data trimmed (two-sided) by 1, 2, and 5%. Preliminary analysis and sensitivity estimates (data not shown) resulted in defining the 2% trim as optimal for further robust analyses and was used for smoothed age-specific centile analyses. This means that a total of 4% samples were deleted including the highest 2% and the lowest 2% of samples prior to analysis. BMI was defined as weight/height² and body surface area (BSA) by the Gehan–George formula (20).

Results

A summary of the descriptive data of all variables is presented in Table 1 and the age distribution of the study population is presented in Table 2. The raw data of serum testosterone was the same in the complete data ($n=10\,897$ samples from 7921 men) and the data from first visit only ($n=7921$ samples) showing a wide scatter that was substantially reduced by adjustment for height and weight (Fig. 1). Both also show a near identical distribution (Table 3). Both show a wide scatter of raw data, which was substantially reduced by adjustment for height and weight. All subsequent analyses used the complete data. A summary of the estimated centiles of serum testosterone for men under 65, 65–75, 75–85, and over 85 years is presented in Table 4.

The smoothed age-specific centiles for serum testosterone, DHT, androgen, and E₂ are shown in Fig. 2. The smoothed centiles for serum testosterone according to height, weight, BMI, and BSA are shown in Fig. 3. Using linear regression (for comparison with previous studies), there was an age-related decrease in serum testosterone concentrations (0.11 nmol/l or 0.5%/year) and serum DHT (0.01 nmol/l or 0.5%/year) and to a lesser extent serum E₂ (0.2 pmol/l or 0.2%/year). Increasing weight, BMI, and BSA and shorter height were associated with reduced serum testosterone. For each anthropometric measurement, the same pattern persisted after age-adjustment.

Discussion

A comprehensive evaluation of androgen status in men requires an appraisal of serum testosterone together with its bioactive metabolites DHT and E₂ (2). Such appraisal first became practically feasible with MS-based multi-analyte steroid profiling to provide a sensitive and specific

Table 2 Age distribution of study population.

Age (years)	n
<40	176
40–45	399
45–50	487
50–55	489
55–60	558
60–65	497
65–70	2310
70–75	2828
75–80	1956
80–85	841
85–90	270
>90	86

Figure 1

Serum testosterone concentrations measured by LC–MS in a single laboratory plotted as raw data (black dots), adjusted for height and weight (red dots), and a smoothed median (green line) from 10 897 serum samples from 7921 men aged over 35 years from population-based studies in three Australian

cities. The left panel displays the complete data from 10 897 samples while the right panel uses only the first samples from 7921 men. A full colour version of this figure is available at <http://dx.doi.org/10.1530/EJE-15-0380>.

simultaneous measurement of serum testosterone, DHT, and E_2 . The present study overcomes that limitation using a large, single dataset of samples (> 10 000) from pooling population-based studies from three major cities in a demographically homogenous national population all measured with the same LC–MS method. This method also reduces analytical variability by measuring both androgens and estrogens within a single run despite their different ionization optima. Additionally, we introduce large sample methods to deduce smoothed age-specific centiles from the fourth decade to advanced old age as well as to trim the dataset to eliminate potentially influential but uncharacterized outliers. The present approach is an advance on the largest previous population-based studies (21, 22), which pool data from different countries involving greater between-center heterogeneity in ethnicity, sociocultural and demographic factors than used herein by the pooling of centers with similar demography within a single country. Furthermore, serum DHT was rarely measured in population-based studies as few in-house DHT immunoassays were available and none in multiplex or valid single-tube formats (23). Similarly, serum E_2 was usually measured by direct immunoassays, which are inaccurate at the low circulating E_2 levels prevailing in men of all ages (24, 25, 26). Finally, few studies reported measuring serum testosterone, DHT, and E_2 in studies of thousands of participants (21, 22).

Distinctive findings from this study include that men's serum testosterone, DHT, and E_2 decline gradually during aging. These findings differ from a compilation of data from 13 different studies showing a minimal decline but increased variability in serum testosterone until the men are in their late 1980s (27). The increased variability may reflect a limitation that all but two studies used direct (non-extraction) testosterone immunoassays and interpolated an estimate of MS-equivalent serum testosterone

Table 3 Distribution of serum testosterone in complete and first visit data.

Variable	Complete data	First visit
<i>n</i>	10 428	7653
Mean	14.1	14.0
S.E.M.	0.1	0.1
S.D.	5.5	5.4
Minimum	0.7	0.7
Maximum	28.8	28.8
Percentiles		
1	1.5	1.7
5	4.9	5.7
10	7.5	7.7
25	10.6	10.4
50	13.8	13.6
75	17.7	17.4
90	21.6	21.3
95	24.0	23.7
99	27.5	27.5

Table 4 Estimated centiles for men aged <65, 65–75, 75–85, and >85 years of age.

	Testosterone (nmol/l)				DHT (nmol/l)				Androgen ^a (nmol/l)				Estradiol (pmol/l)			
	<65	65–75	75–85	>85	<65	65–75	75–85	>85	<65	65–75	75–85	>85	<65	65–75	75–85	>85
<i>n</i>	2606	5141	2797	358	2401	4982	2725	358	2606	5141	2797	358	2034	4945	2733	358
1%	6.1	0.2	0.1	0.1	0.7	0.2	0	0	6.9	0.4	0	0	32	12	13	17
2.5%	7.6	2.1	0.3	0.1	0.8	0.3	0	0	8.9	2.2	0.2	0	38	23	22	22
5%	8.7	5.5	3.4	0.3	0.8	0.4	0.3	0	10.9	3.8	2.0	0.1	47	33	34	32
25%	12.9	10.2	10.1	8.1	1.2	0.9	0.9	0.7	18.6	10.0	5.4	3.7	68	58	60	60
50%	16.5	13.1	13.6	13.3	1.6	1.3	1.3	1.2	24.1	17.5	8.2	5.5	88	77	81	85
75%	20.6	16.8	17.5	17.9	2.1	1.8	1.8	1.7	30.3	23.7	16.9	7.7	112	98	104	111
95%	27.9	23.8	25.5	23.6	3.0	2.8	2.7	2.6	41.3	34.1	30.7	10.6	168	138	148	160
97.5%	30.7	26.5	28.6	25.4	3.5	3.2	3.1	3.0	46.4	38.1	35.1	11.4	196	154	166	174
99%	34.7	29.2	32.6	30.9	4.2	3.8	3.5	3.5	52.1	45.7	41.1	18.1	228	176	198	207

^aAndrogen is defined as the sum of serum testosterone plus five times the serum DHT reflecting the higher androgenic bioactivity of DHT. For details, see text.

levels assuming a linear correlation. However, as serum testosterone concentrations measured by direct immunoassay deviate non-linearly from MS measurements, especially at lower serum testosterone concentrations characteristic of older men (8), the increased variability may be due to the reliance on inaccurate re-calibration of

serum testosterone concentrations. In the present study the decline in serum testosterone and DHT are ~0.5%/year, which is comparable but a little lower than previous estimates usually in the range of 0.8–2%/year including those using MS-based methods (reviewed in (28, 29, 30)). Longitudinal estimates for rate of decline

Figure 2

Plot of smoothed age-specific population centiles (97.5, 95, 75, 50, 25, 5, and 2.5%) for serum testosterone (nmol/l), DHT (nmol/l), androgen (nmol/l), and E₂ (pmol/l) in 10 897 samples from men aged over 35 years from population-based studies in

three Australian cities (Adelaide, Perth, and Sydney) using LC–MS steroid measurements from a single lab with data analysis using GAMLSS modeling. A full colour version of this figure is available at <http://dx.doi.org/10.1530/EJE-15-0380>.

Figure 3

Plots of smoothed centiles (97.5, 95, 75, 50, 25, 5, and 2.5%) of serum testosterone according to height (cm), weight (kg), BMI (kg/m^2), and body surface area (BSA, m^2) deduced by GAMLSS modeling from serum testosterone concentrations in 10 897

sera from men aged >35 years from population-based studies in three Australian cities. A full colour version of this figure is available at <http://dx.doi.org/10.1530/EJE-15-0380>.

are reportedly similar (31, 32) or higher (33) than cross-sectional annual estimates. The latter may reflect the population-wide temporal downward trend in serum testosterone (by immunoassay) reported in Denmark (34) and in one (35) of two USA studies (36) – trends that persist despite attempts to eliminate temporal changes in technical assay methodology and/or to increase population prevalence of obesity (37). An important interpretation of our findings is that an age-invariant reference range for sex steroids in men is very wide. An alternative is to define age-specific reference intervals for androgen status as outlined in this study.

No comparable estimates of rates of age-related changes appear to be available for serum DHT or E_2 . In our study, age-specific serum DHT centiles showed a minimal decline before, but a marked decline after,

65 years of age. Previous studies of serum DHT in men have reported no change (38, 39, 40, 41), an increase (33) or decrease (42) by age group in male aging using conventional RIAs or no change when measured by gas chromatography (GC)–MS (43).

Similarly, our finding of minimal changes in serum E_2 is consistent with a comparable trend in serum E_2 with age in the community-based Framingham Offspring Study (44) and our Healthy Man Study (45). Other previous studies have reported stable serum E_2 levels with regard to male aging using conventional RIAs (38, 39, 40, 46) or GC–MS (43, 47, 48), whereas studies using direct immunoassays reported no change (49, 50) or a decline (51). Some of these discrepancies are likely due to the unreliability of E_2 immunoassays at the low circulating levels prevailing in men (24, 25, 26, 52).

The mechanism and significance of the biochemical decline in androgen status affecting aging men cannot be determined from the present observational study. Nevertheless, an important and plausible explanation is that this decline reflects the impact of the co-morbidities accumulating during aging on an otherwise healthy reproductive system. This is consistent with the findings that androgen status does not decline with aging among older men who remain in excellent (asymptomatic) health (45) although there is a decline associated with the co-morbidities of aging such as obesity, depression or cardiovascular disease. This is also consistent with the conclusions of other longitudinal studies (31, 32). Nevertheless, our cross-sectional study cannot fully exclude that age itself, apart from co-morbidities, might also contribute to declines in age-related serum testosterone, DHT, and E_2 concentrations.

In this study, we utilize an illustrative response metameter to define net androgen status (53), defined as the sum of testosterone and DHT concentrations, using a weighting for DHT to reflect its higher androgenic potency. DHT is between two and ten times more potent than testosterone as an androgen, as measured by various mammalian and yeast AR bioassays (17, 18) and reflecting its higher affinity for, and greater stability of, AR binding with a slower dissociation rate compared with testosterone (19). The similarity of this index of androgen status with the temporal profile of testosterone and of DHT is consistent with the relative stability of the DHT/testosterone ratio (median 0.10) across a large population of men. Nevertheless, although DHT is usually converted from testosterone by 5α reductase, the independent backdoor pathway that synthesizes DHT bypassing testosterone (54) allows for independent regulation of serum DHT levels with potentially differential effects on health outcomes such as cardiovascular disease and mortality (55, 56). Hence, this novel response metameter, incorporating simultaneous measurements of both testosterone and DHT, may provide a more comprehensive metric to characterize androgen status for individual men and male populations where the impact on the two major androgens may diverge.

The study provided insight into the anthropometric determinants of serum testosterone, DHT, androgen, and E_2 . As expected, increasing weight, BMI, and BSA are associated with lower serum testosterone. A surprising finding is that short stature is associated with lower serum testosterone, an effect that was not due to confounding by age. A consequence of this finding is that height

differences may be an unrecognized confounder of apparent racial differences in androgen status (21, 57).

Despite the strengths of this large, population-based study cohort using a single MS-based measurement and large sample data analysis methods, it has limitations. One is that the national population was predominantly Caucasian so the findings may not apply directly to other ethnic groups. Nevertheless, the small ethnic/racial differences reported in androgen status (57) are likely to be predominantly environmental rather than genetic (58, 59) so are likely minimized in this study. Like all comparable studies, another limitation is that it is notoriously difficult to distinguish age from a potentially confounding period or cohort effects, which are inevitably collinear with age (60).

We conclude that circulating testosterone, DHT, and E_2 decline gradually during male aging. This age-associated decline may be due in part to the progressive impact of co-morbidities that accumulate during aging on a functionally intact reproductive system. Future research directed toward evaluating the mechanisms of the age-related decline in androgen status should focus on the impact of co-morbidities of aging to better inform clinical trial design aiming to ameliorate late life somatic effects associated with aging.

Declaration of interest

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research reported.

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Author contribution statement

D J Handelsman conceived, designed, directed the data analysis, and wrote the manuscript, L P Ly performed the data analysis, and all authors contributed to providing and interpreting the data and drafting the manuscript.

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