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ORIGINAL ARTICLE

Normal, bound and nonbound testosterone levels in normally ageing men: results from the Massachusetts Male Ageing Study

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Summary

Objective There is little consensus on what androgen levels are 'normal' for healthy, ageing men. Using data from the Massachusetts Male Ageing Study (MMAS), we estimated age-specific, normal androgen levels for men aged 40–79 years while accounting for health status and behavioural factors known to influence hormone levels.

Design Prospective, observational study.

Patients Community-based random sample of men aged 40–79 years: $n = 1677$ men studied at T1 (1987–1989), $n = 1031$ at T2 (1995–1997) and $n = 631$ at T3 (2002–2004), for a total of 3339 observations. The average number of years between the T1 and T2 interviews was 8·8 (range 7·1–10·4 years) and 6·4 (range 5·6–7·9 years) between T2 and T3.

Measurements Serum total testosterone (T) and sex hormone-binding globulin (SHBG) were measured on nonfasting blood samples collected within 4 h of subject's awakening. Free and bio-available T were calculated from T and SHBG using the Södergard equation. Trained interviewers administered an in-home questionnaire of health, medication and lifestyle. Participants were considered apparently healthy if all of the following were met: (i) absence of self-reported chronic disease (diabetes, heart disease, high blood pressure, cancer, ulcer); (ii) not on prescription medication believed to affect hormone levels; (iii) body mass index (BMI) not exceeding 29 kg/m^2; (iv) alcohol consumption less than or equal to six drinks/day; and (v) nonsmoking.

Results Chronic disease and high BMI significantly decreased whereas smoking tended to increase total, free and bio-available T concentrations. Apparently healthy men had significantly higher median hormone concentrations at most time points than did not apparently healthy men. Due to the opposite effects of smoking and the other components of the definition, apparently healthy men were compared to nonsmoking, apparently unhealthy men. The former group had significantly higher androgen levels (Wilcoxon rank-sum *P*-values ranged from 0·01 to 0·0001) for all hormones at all interviews. Ninety-five percent of apparently healthy men in their 40s, 50s, 60s and 70s would be expected to have total T in the range (2·5–97·5th percentile): 8·7–31·7, 7·5–30·4, 6·8–29·8 and 5·4–28·4 nM (251–914, 216–876, 196–859, 156–818 ng/dl), respectively.

Conclusions Age, health and lifestyle factors impact androgen levels and should be accounted for in calculations of normal reference ranges. We propose the following age-specific thresholds, below which a man is considered to have an abnormally low total T: 8·7, 7·5, 6·8 and 5·4 nM (251, 216, 196 and 156 ng/dl) for men in their 40s, 50s, 60s and 70s, respectively. These cutoffs correspond to the 2·5th percentile in our data; thus, approximately 2·5% of men aged 40–79 years would have abnormally low T levels based on hormone levels alone.

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Several developments provide motivation for this paper. First is the recent rapid ageing of the US population. Compared to the 3 million Americans aged 65 years and older alive in 1950, there were over 35 million in 2000. The US population aged 65–74 years is projected to grow 74% between 1990 and 2020. Increasingly, mid and older aged male patients are seeking care for a broad range of health problems. Second is the burgeoning interest in male health. Health advice books for ageing men, which are often based on personal anecdotes and generally lack evidence from well-designed research studies, are widely available.[1,2] New professional societies are forming [e.g. The International Society for the Study of the Ageing Male (ISSAM), The Andropause Society, The Society for Gender and Health, the Sexual Medicine Society of North America, Inc. (SMS), among many others], and new medical specialties are emerging (e.g. andrology and sexual medicine). These stakeholders devote considerable attention to the existence of a 'male menopause' or 'andropause', and the pharmacologic management of midlife male symptomatology. Third are changes in the marketing of pharmaceuticals. There has been a dramatic increase in Direct-to-Consumer (DTC) advertising – from only \$791 million in 1996 to \$2·5 billion in 2000.[3] Injectable,

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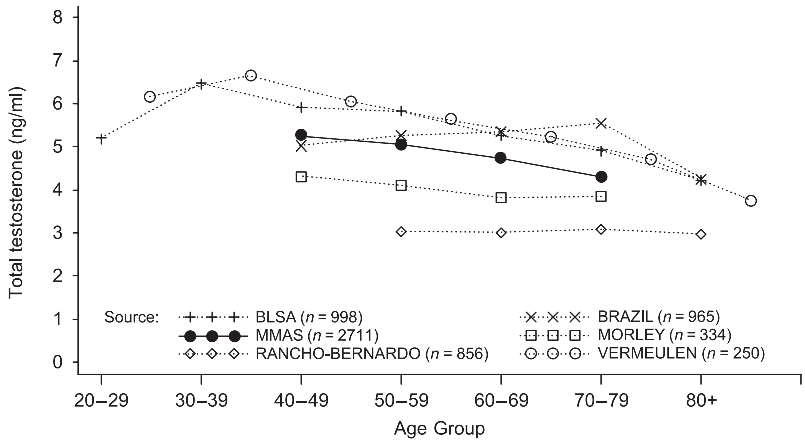

Fig. 1 Total testosterone (ng/ml) by age group: comparison of MMAS with five epidemiologic studies. *BLSA*;[17] *BRAZIL*;[19] *MORLEY*;[15] *RANCHO*;[18] *VERMEULEN*.[13] Reprinted from O'Donnell AB, Araujo AB, McKinlay JB (2004) The health of normally ageing men: The Massachusetts Male Ageing Study (1987–2004), Experimental Gerontology 39, 979, with permission from Elsevier.

transdermal, buccal and oral testosterone (T) preparations are now available (e.g. AndroGel, Testim, Androderm) and widely advertised, exhorting men (who may experience symptoms such as fatigue, insomnia, impaired cognition, depression, irritability and decreased libido) to 'Ask your Doctor about . . . '. Prescription sales of testosterone replacement therapies (TRT) have increased by more than 500% since 1993.[4] Despite the rush to TRT for men, and the historical parallels with HRT and women,[5,6] there are still no large-scale, well-designed clinical trials of the clinical effectiveness and safety of TRT in men.[7–9] Fourth are advances in understanding the epidemiology of male hormone changes. It is now widely accepted that male hormone levels decline gradually with ageing.[10] The Massachusetts Male Ageing Study (MMAS) has reported the average annual decline across the lifespan of the major androgens (longitudinal changes in total T: 1·6%/year; Free T: 2·8%/year; albumin-bound T: 2·5%/year).[11] Several cross-sectional[12–14] and longitudinal studies[15–17] corroborate the MMAS findings. Figure 1 shows that serum total T data from the MMAS is comparable to that reported by other major epidemiologic studies using similar radioimmunoassay techniques.[13–15,17–19] Compared to the other five studies, the MMAS T data fall near the middle of the range for each age group, and the rate of decline in T with age is very similar. Despite the consistency across studies, the clinical significance of age-related changes in T remains uncertain as Snyder[20] points out: 'an essential but still unanswered question is whether this decrease in the testosterone concentration is physiologic, perhaps conveying a benefit, or pathologic, causing harm'.

While chronological ageing appears to be important, co-morbid chronic illnesses and certain medications may contribute to and accentuate the decline in T levels with ageing.[11,13,21–25] Gray found that 10–15% lower levels of total and free T were found in men with chronic illnesses such as diabetes, CAD, hypertension and cancer[22] (see also[11]). Lifestyle issues such as tobacco intake also influence T levels and may alter age-related changes.[13,14,26] Vermeulen[13] showed that among nonobese, otherwise healthy men, smokers had significantly higher total and free T levels than did nonsmokers. It is clearly important to account for chronic illnesses and lifestyle factors when defining the normal ranges of T in men.

Hypogonadism as distinct from 'andropause' or 'male menopause' is a clinical condition marked by low levels of serum T combined with symptoms thought to include decreased libido, erectile dysfunction, reduced muscle mass and bone density and depression.[27] Prevalence estimates obviously vary with the definition used. Using an algorithm that incorporates both signs/symptoms of androgen deficiency and total and calculated free T, Araujo and colleagues found lower prevalences: 7, 12 and 23% for men aged 50–59, 60–69 and 70–79 years, respectively.[28] Harman reported age-specific prevalences of hypogonadism of 12, 19, 28 and 49% for men in their 50s, 60s, 70s and 80s, respectively, where hypogonadism was defined as total T < 11·3 nM (325 ng/dl).[17]

Medical practitioners need normative data to assist in the management of ageing male patients who present with signs and symptoms suggesting endocrinologic conditions such as hypogonadism. Providers frequently ask such questions as: what is a normal T level for a male aged say, 65 years? How is this level affected by major co-morbidities (like diabetes, CHD, hypertension and prostate cancer)? What affect do lifestyles (e.g. physical activity levels, alcohol consumption and cigarette smoking) have on normal levels? Such information is important when initiating pharmacotherapies (e.g. TRT), especially as reversing declining T concentrations may exacerbate the testosterone-dependent diseases to which elderly men are prone, including prostate cancer, benign prostatic hyperplasia, erythrocytosis and sleep apnoea.[9,10,20,29–31]

Using data from the community-based MMAS (and not a convenience sample of ageing male patients) we provide answers to these questions by estimating age-specific normal androgen levels, after accounting for health status and behavioural factors known to influence hormone levels.

Methods

Subjects

The MMAS is a population-based, observational study of ageing and hormones in middle-aged men conducted in three waves (T1: 1987–1989; T2: 1995–1997; T3: 2002–2004). The sampling design has been described previously.[32,33] Briefly, men aged 40–70 years from 11 cities and towns in the Boston, Massachusetts area were randomly selected from annual state census listings at T1. Age-stratified cluster sampling was used to yield a sample with approximately equal percentages of men in each age decade (40–49, 50–59 and 60–69 years). A total of 1709 (52%) of those eligible participated at T1. This response rate is comparable to other epidemiologic field studies requiring early morning phlebotomy and an extensive in-home interview and offering no financial incentive.[34] Comparison of the MMAS baseline figures with data from the Third National Health and Nutrition Examination Survey 1988–1994[35] for men aged 40–70 show that prevalence estimates of general health indicators were similar in the two samples. There was a low representation of racial minorities (4%) in the MMAS, consistent with the population of Massachusetts in 1990.[36]

Of the 1709 men who were recruited at T1, 213 were ineligible for follow-up (180 confirmed deceased, 28 seriously ill, five overseas residents), leaving a total of 1496 eligible men. Of these, 1156 (conditional response rate 77%) were interviewed at T2. The remaining 340 participants were not followed (six suspected deceased, 75 lost and 259 refused). At T3, we did not attempt to follow men who were confirmed deceased at T2 ($n = 180$), who refused at T2 and did not want any further contact with the MMAS ($n = 48$), or who were ineligible (109 confirmed deceased, 21 seriously ill). Of the 1351 eligible men, 853 men (conditional response rate 63%) were interviewed at T3. A total of 498 men were not followed (50 suspected deceased, 206 lost, 242 refused).

The Institutional Review Board at New England Research Institutes approved all protocols including informed consent procedures.

Data collection and coding

Unless noted, data collection methods were the same for all three waves. Trained interviewer/phlebotomists visited the men in their homes and conducted a standardized interview. Physical measurements, including height and weight, were obtained using standard methods.[37] Chronic disease, current cigarette smoking and alcohol use were ascertained by self-report. Daily alcohol intake was calculated by defining 12 g ethanol as one drink and accounting for the amount and frequency of regular and binge drinking.[38]

The interviewer inventoried all prescription and nonprescription medication by copying the medication name from the medication label and asking the reason for use. Afterwards, two pharmacoepidemiologists (M. Barbour and A. Hume, University of Rhode Island, Providence, RI, USA) classified the medications using a system based on the American Hospital Formulary Service.[39] An endocrinologist (A. Guay) reviewed the prescription medications and identified ones that may affect hormone levels.

A participant was considered apparently healthy if he met all of the following criteria:

- absence of self-reported chronic disease (diabetes, heart disease, high blood pressure, ulcer, or cancer)
- not currently taking any prescription medication believed to affect hormone levels
- BMI not exceeding 29 kg/m^2, which is considered 20% above ideal weight[40]
- alcohol consumption not exceeding 600 ml ethanol/week (approximately six drinks/day)
- nonsmoker.

Hormones

Nonfasting blood samples were collected within 4 h of the participant's awakening to control for diurnal variations in hormone levels.[41] Two nonfasting samples were drawn 30 min apart from the antecubital space and pooled in equal aliquots at the time of assay to smooth episodic secretion.[42] The serum was transported in ice-cooled containers and centrifuged within 6 h. The samples were stored in 5-ml scintillation vials at −20 °C until they were shipped to the laboratory on dry ice within 1 week by same-day courier. The serum was stored at −70 °C until the time of assay.

For all three waves, hormone assays were performed at The Endocrine Laboratory, University of Massachusetts Medical School (Worcester, MA, USA) under the supervision of Dr Christopher Longcope. Total T was determined by radioimmunoassay (RIA; Diagnostic Products Corp., Los Angeles, CA, USA). The assay cross-reactivity with DHT was 2·8%. T1 total T was assayed in 1994 on samples stored since baseline, whereas T2 and T3 samples were assayed shortly after blood collection. To assess the stability of stored samples and check for assay drift, 60 T1 and T2 samples were re-assayed in 2000 in the same batch. Results showed negligible change due to storage or assay drift.

At T1 and T2, sex hormone-binding globulin (SHBG) was measured by RIA from kits by the same manufacturer, although distributors changed (T1: Farmos Diagnostica, Farmos Group LTD, Oulunsalo, Finland; T2: Orion Diagnostica, Espoo, Finland). At T3, SHBG was measured by chemiluminescent enzyme immunometric assay with Immulite (Diagnostic Products Corp., Los Angeles, CA, USA). Dr Longcope's laboratory performed validation studies using samples analysed with both the old (RIA) and new (chemiluminescence) assays and found that results from the old assay could be replicated using the new assay. For all three waves, SHBG was assayed soon after blood collection.

The intra-assay coefficients of variation (CV) for T1, T2 and T3 total T were 5·4%, 5·8% and 3·5%, and the interassay CV were 8·0%, 9·0% and 8·3%, respectively. The intra-assay CV for SHBG were 8·0%, 4·5% and 2·0%, and the interassay CV were 10·9%, 7·9% and 3·0%, respectively.

Free T was calculated using the Södergard equation assuming a fixed albumin-bound concentration, and bio-available T was computed as a multiple of free T.[43] The Södergard equation produces estimates for free T and bio-available T which closely approximate those obtained from equilibrium dialysis and ammonium sulphate precipitation, respectively.[44]

Analysis sample

The samples from the three waves were treated as if each contained different men and were combined for analysis. We excluded men who were missing hormone (T1: $n = 22$; T2: $n = 116$; T3: $n = 150$) or apparent health (T1: $n = 10$; T2: $n = 9$; T3: $n = 7$) data. In addition, one T3 observation was removed because the T3 total T concentration was suspect (more than 10 standard deviations above the next highest T3 total T measurement). After removing these exclusions, 64 men aged 80–89 remained, but only five were apparently healthy. Because we report reference ranges for apparently healthy men only, we removed the 80- to 89-year-olds due to scarce data. The resulting subsamples were 1677, 1031 and 631 for T1, T2 and T3, respectively, for a grand total of 3339 observations.

Statistical analysis

The Wilcoxon rank-sum test was used to compare median hormone concentrations by the definition of apparent health and by each of its components. Descriptive analyses revealed that men who had chronic disease, were on medication, or who had high BMI tended to have lower total, free and bio-available T levels, whereas smokers tended to have higher levels. To prevent the effect of smoking from canceling out the effects of the other components, the apparently healthy variable was split into two variables for modelling purposes: (a) met all of conditions 1–4 of the definition and (b) nonsmoker.

We chose to define reference ranges using percentiles rather than the commonly used formula, mean ± 2SD. Our rationale is as follows. When a variable is normally distributed, mean – 2SD and mean + 2SD are approximately equal to the 2·5th and 97·5th percentiles, respectively. Thus, 2·5% of observations will fall below mean – 2SD, and 2·5% will be above mean + 2SD. However, if a variable is skewed, extreme values cause the SD to be inflated. Consequently, the interval (mean – 2SD, mean + 2SD) becomes inflated and may no longer approximate the 2·5–97·5th percentiles. Depending on how skewed the data are, this interval may become unrealistically wide and very few observations, if any, may fall outside the interval. Also, the boundaries of the interval may not be very clinically meaningful cutpoints.

In our sample, the hormone distributions were skewed towards high values. Initially, we log-transformed the data to lessen the impact of the high values, but the log created outliers at the lower end of the distribution. Thus, we decided to define reference ranges using percentiles. Royston and Wright[45–47] have developed methodology to estimate age-specific percentiles from a regression model using maximum likelihood estimation. Their method allows the user to control for covariates and is effective at handling severely non-normal dependent variables. In brief, the method works as follows. The mean and scale parameters are modelled separately using fractional polynomial regression and can be treated as linear, quadratic, or some other power, whichever yields the best model fit. The dependent variable is modelled on the original scale or transformed towards normality using logarithms or other methods. Once an appropriate model is chosen, percentile estimates are computed and, if necessary, back transformed to the original scale.

Using this method, we treated total, free and bio-available T as dependent variables and modelled each separately as a function of age (continuous), apparent health (using the two variables described above) and time (using two dummy variables to indicate interview). In our sample, linear terms for the mean and scale parameters provided the best fit for all three hormones, except for total T, where the scale parameter was not needed. For all three hormones, the modulus-exponential-normal transformation was used to transform the data toward normality.[48]

To improve the precision of our estimates, the full analysis sample ($n = 3339$) was used to fit the models. From these models, we computed normal ranges for apparently healthy men ($n = 791$) only, as we believe that is the appropriate reference population for ageing men.

SAS software was used for the descriptive analyses and bivariate comparisons.[49] All other analyses were conducted using the STATA software ado-files, XRIML and CENTCALC.[50]

Results

Table 1 presents demographic characteristics of the analysis sample by interview. The T1 demographics closely match those of the full T1 cohort of 1709 men (data not shown) and the male population of Massachusetts at the time of recruitment.[36] By design, approximately equal percentages of men aged 40–49, 50–59 and 60–69 years were recruited at T1. Both follow-up samples had higher proportions of participants 60 and older. Mean (SD) age was 55·2 (8·7), 62·7 (8·3) and 66·6 (7·0) years, and age ranged from 40–70, 48–79 and 55–79 at T1, T2 and T3, respectively. At all three interviews, the majority of men were white, married, employed, and most had at least a high school education. The men in the follow-up samples were less likely to be employed and more likely to be in the top income category than those in the T1 sample. The average number of years between T1 and T2 was 8·8 (SD 0·71; range 7·1–10·4 years) and between T2 and T3 was 6·4 (SD 0·34; range 5·6–7·9 years).

For each chronic disease studied, the percentage of men with disease increased across the three interviews (Table 2). The percent with BMI at or below 29 kg/m^2 decreased over time as did the percent free of prescription medication affecting hormone levels. Smoking became less common. At T1, 28% of the men were classified as apparently healthy. At T2 this decreased to 21% and further to 17% at T3.

Table 3 displays median hormone concentrations by interview and apparent health. Both chronic disease and high BMI significantly lowered all three forms of T at all three interviews (*P*-values ranged from 0·03 to 0·0001). Prescription medication usage did not influence androgen levels until T3 where it lowered them. High alcohol intake had little impact. Smokers tended to have higher hormone concentrations than did nonsmokers. Apparently healthy men had higher androgen levels, but not all comparisons were significantly different possibly because smoking raises T and the other components lower T resulting in a canceling out effect. When apparently healthy men were compared to nonsmoking, nonapparently healthy men, the former group had significantly higher androgen levels (*P*-values ranged from 0·01 to 0·0001) for all hormones at all interviews.

In the multivariable models used to estimate the normal reference ranges, the three samples were combined. The above noted relationships between androgens and the smoking and nonsmoking components of the apparently healthy definition were preserved (data not shown). Smoking increased androgen levels ($P < 0·0001$) as did being apparently healthy according to the nonsmoking components (total T: $P < 0·0001$; free T: $P < 0·03$; bio-available T: $P < 0·02$). All three hormones declined with age ($P < 0·0001$).

Normal reference ranges (percentiles) by age decade are presented in Table 4. Only results for apparently healthy men are shown. A typical, apparently healthy man in his 50s would be expected to have a total T of approximately 17·2 nM or 496 ng/dl (median). Half of such men will have a total T above and half below this level. Ninety-five percent of apparently healthy men in their 50s will have total T in the range of 7·5–30·4 nM or 216–876 ng/dl (2·5–97·5th percentiles), and 90% will have values ranging from 9·0 to 27·7 nM or 259–798 ng/dl (5–95th percentiles). Estimates for the other age groups and other hormones can be interpreted in a similar fashion.

Since it is common to define normal reference ranges as mean ± 2SD, we report these results in Table 4 for comparison with

Table 1. Demographic characteristics of analysis sample by interview, Massachusetts Male Ageing Study, 1987–2004

	Interview					
	T1 1987–1989 (*n* = 1677)		T2 1995–1997 (*n* = 1031)		T3 2002–2004 (*n* = 631)	
Characteristic	*n*	(%)	*n*	(%)	*n*	(%)
Age in decades						
40–49	555	(33)	27	(3)	0	(0)
50–59	524	(33)	391	(38)	143	(23)
60–69	546	(33)	381	(37)	259	(41)
70–79	22	(1)	232	(23)	229	(36)
Race						
White	1598	(95)	983	(96)	609	(97)
Black	52	(3)	18	(2)	7	(1)
Other	25	(2)	25	(3)	12	(2)
Marital status						
Never married	157	(9)	80	(8)	56	(9)
Currently married	1259	(75)	792	(77)	473	(75)
Divorced/separated	208	(12)	105	(10)	66	(10)
Widowed	53	(3)	52	(5)	36	(6)
Currently employed for pay	1316	(78)	663	(64)	346	(55)
Education						
Less than high school	190	(11)	90	(9)	40	(6)
High school graduate	289	(17)	153	(15)	96	(15)
More than high school	1198	(71)	781	(76)	495	(78)
Annual household income						
< $40 000	628	(39)	301	(30)	145	(24)
$40 000–79 999	679	(42)	340	(34)	198	(33)
> $79 999	319	(20)	350	(35)	264	(43)

Table 2. Health characteristics of analysis sample by interview, Massachusetts Male Ageing Study, 1987–2004

	Interview					
	T1 1987–1989 (*n* = 1677)		T2 1995–1997 (*n* = 1031)		T3 2002–2004 (*n* = 631)	
Characteristic	*n*	(%)	*n*	(%)	*n*	(%)
Chronic disease*						
Diabetes	131	(8)	91	(9)	79	(13)
Heart disease	212	(13)	168	(16)	134	(21)
High blood pressure	514	(31)	389	(38)	313	(50)
Ulcer	168	(10)	128	(12)	76	(12)
Cancer	105	(6)	176	(17)	144	(23)
Components of apparently healthy definition						
1 No chronic disease†	857	(51)	401	(39)	190	(30)
2 No prescription medication‡	1522	(91)	846	(82)	472	(75)
3 BMI $\leq$ 29 kg/m^2	1217	(73)	682	(68)	390	(63)
4 Alcohol intake $\leq$ 6 drinks/day§	1601	(96)	1007	(98)	617	(98)
5 Nonsmoker	1266	(75)	896	(87)	579	(92)
Apparently healthy¶	462	(28)	219	(21)	110	(17)

*Self-report.
†Absence of self-reported diabetes, heart disease, high blood pressure, cancer, and ulcer.
‡Not on any prescription medications believed to affect hormone levels.
§1 drink is equivalent to 15 ml ethanol (10 oz beer, 4 oz wine, or 1·5 oz spirits).
¶Men were classified as apparently healthy if they met all of 1–5 above.

Table 3. Testosterone (T) concentrations by interview and apparent health. Massachusetts Male Ageing Study, 1987–2004

Characteristic	Median[e] total T (nM[f])			Median[e] free T (nM)			Median[e] bio-available T (nM)		
	T1[g] (*n* = 1677)	T2 (*n* = 1031)	T3 (*n* = 631)	T1 (*n* = 1677)	T2 (*n* = 1031)	T3 (*n* = 631)	T1 (*n* = 1677)	T2 (*n* = 1031)	T3 (*n* = 631)
Components of apparently healthy[a] definition									
(1) Chronic disease[b]									
No	18·0***	15·6**	14·3*	0·463***	0·362***	0·252*	8·75***	6·85***	4·76*
Yes	16·9	14·8	13·3	0·426	0·333	0·234	8·06	6·29	4·43
(2) Medication[c]									
No	17·5	15·0	14·1**	0·445	0·343	0·243***	8·42	6·49	4·60***
Yes	17·5	15·4	12·3	0·424	0·350	0·215	8·02	6·63	4·06
(3) BMI > 29 kg/m^2									
No	18·2***	15·9***	14·7***	0·450**	0·352***	0·250**	8·51**	6·65***	4·73**
Yes	15·7	13·2	11·9	0·419	0·330	0·225	7·92	6·25	4·26
(4) Alcohol intake > 6 drinks/day[d]									
No	17·5	15·0	13·6	0·443	0·344	0·241	8·39	6·51	4·55
Yes	17·3	15·6	11·3	0·441	0·377	0·209	8·35	7·13	3·96
(5) Current smoker									
No	17·1***	14·8***	13·4*	0·431***	0·339***	0·239	8·15***	6·41***	4·51
Yes	18·6	16·3	15·6	0·476	0·385	0·240	9·00	7·27	4·54
Apparently healthy									
No	17·2	14·9**	13·4**	0·441	0·342	0·233*	8·34	6·47	4·41*
Yes	18·0	15·9	15·4	0·454	0·352	0·258	8·58	6·66	4·88
Apparently healthy									
No (non smokers only)	16·6***	14·6***	12·9***	0·417**	0·334*	0·232**	7·89**	6·32*	4·39**
Yes	18·0	15·9	15·4	0·454	0·352	0·258	8·58	6·66	4·88

[a]Men were classified as apparently healthy if all of the following were met: (1) absence of self-reported chronic disease (diabetes, heart disease, high blood pressure, cancer, ulcer); (2) not on prescription medication believed to affect hormone levels; (3) BMI not exceeding 29 kg/m^2; and (4) Alcohol consumption not exceeding approximately 6 drinks/day and (5) nonsmoking.
[b]Self-report.
[c]Not on any prescription medications believed to affect hormone levels.
[d]One drink is equivalent to 15 ml ethanol (10 oz beer, 4 oz wine, or 1·5 oz spirits).
[e]Medians are not adjusted for age or any other variables.
[f]nM may be converted to ng/dl by dividing by 0·0347.
[g]T1: 1987–1989, T2: 1995–1997, T3: 2002–2004.
$*P < 0{\cdot}05$, $**P < 0{\cdot}01$, $***P < 0{\cdot}001$ from Wilcoxon rank-sum test comparing medians of two groups.

other studies. However, as explained in the statistical methods section, we recommend using percentiles as normal ranges for statistical reasons. Note that in our sample mean – 2SD is lower, in some cases substantially lower, than the 2·5th for all hormones and age groups. In contrast, mean + 2SD usually lies between the 95th and 97·5th percentiles.

Discussion

The term 'andropause' refers to the slow decrease in the androgenic hormones, T and DHEA, in men as they age. Many have interpreted this decline as testosterone 'deficiency', or hypogonadism. However, very little useful data exist on what constitutes a 'normal' T level at any particular age. Haren[51] noted that ageing and androgen deficiency share many clinical features. Wespes[52] states that the andropause syndrome is characterized by declines in libido, erectile capacity, bone mineral density, lean body mass, body hair and intellectual activity, plus increases in obesity, fatigue and depression. However, he admits that the degree to which the age-dependent decrease in androgen levels results in actual health problems is still unknown.

There is tremendous confusion among clinicians, researchers and pharmaceutical industry professionals over what level of androgen deficiency requires treatment. Pharmaceutical companies have an obvious conflict of interest in proposing treatment levels. Without normal data, all definitions of 'abnormal' are suspect. Furthermore, there are still no large, long-term studies demonstrating the benefits of TRT.[8] The risks of administering hormones to men (including increase in prostate volume, stimulation of undiagnosed prostate tumours, infertility and erythrocytosis) may outweigh the benefits.[9,10,20,29–31] In a recent editorial, Barrett-Connor and Bhasin[53] pointed out that 'the potential market is huge, which means that the potential benefit and harm are also large'.

In this paper, we present normal reference ranges for total, free and bio-available T levels by age decade in a large, community-based

Table 4. Normal reference ranges (percentiles) for testosterone (T) by age decade. Apparently healthy[a] men only, Massachusetts Male Ageing Study, 1987–2004

						Normal reference ranges percentile[d]						
	n	Mean[c]	SD	Mean − 2SD	Mean + 2SD	2·5%	5%	10%	50%	90%	95%	97·5%
Total T (nM[b])												
40–49	224	18·7	6·1	6·5	30·9	8·7	10·3	12·1	18·5	26·2	29·0	31·7
50–59	285	17·3	6·0	5·2	29·4	7·5	9·0	10·8	17·2	24·9	27·7	30·4
60–69	229	17·4	5·6	6·2	28·5	6·8	8·3	10·1	16·5	24·3	27·1	29·8
70–79	53	14·7	5·4	4·0	25·5	5·4	6·9	8·7	15·1	22·9	25·7	28·4
Free T (nM)												
40–49	224	0·496	0·184	0·128	0·864	0·183	0·234	0·293	0·487	0·724	0·818	0·912
50–59	285	0·411	0·163	0·085	0·737	0·146	0·190	0·240	0·407	0·609	0·698	0·770
60–69	229	0·360	0·130	0·100	0·620	0·128	0·165	0·207	0·348	0·519	0·586	0·654
70–79	53	0·263	0·118	0·027	0·499	0·077	0·107	0·142	0·257	0·398	0·453	0·509
Bio-available T (nM)												
40–49	224	9·4	3·5	2·4	16·3	3·46	4·43	5·54	9·21	13·70	15·47	17·25
50–59	285	7·8	3·1	1·6	14·0	2·77	3·60	4·55	7·69	11·52	13·03	14·55
60–69	229	6·8	2·5	1·9	11·7	2·42	3·12	3·92	6·57	9·81	11·09	12·37
70–79	53	5·0	2·2	0·5	9·4	1·45	2·03	2·68	4·86	7·52	8·57	9·62

[a]A participant was considered apparently healthy if all of the following criteria were met: (1) absence of self-reported chronic disease (diabetes, heart disease, high blood pressure, cancer, ulcer); (2) not on prescription medication believed to affect hormone levels; (3) BMI not exceeding 29 kg/m^2; (4) Alcohol consumption not exceeding approximately 6 drinks/day and (5) nonsmoking.
[b]nM may be converted to ng/dl by dividing by 0·0347.
[c]Unadjusted.
[d]Results were computed from models adjusted for age (continuous), apparent health, and interview.

sample of randomly selected, ageing men, while controlling for various factors believed to impact the relationship between T and ageing (i.e. chronic disease, medications, BMI, lifestyle). We have shown that poor health lowers T, and therefore, normal ranges should be based on healthy men. We propose the following age-specific thresholds, below which a man is considered to have abnormally low total T: 8·7, 7·5, 6·8 and 5·4 nM (251, 216, 196 and 156 ng/dl) for 40–49, 50–59, 60–69 and 70–79-year-old men, respectively. These cut-offs correspond to the 2·5th percentile in our data; thus, approximately 2·5% of men aged 40–79 would have abnormally low T levels based on hormone levels alone. Combining T results with information about symptoms or other clinically important factors may reduce this percentage further. Given the potential risks and currently unproven benefits of TRT, conservative cut-offs are warranted. Note that in our sample we do not recommend applying the common definition of normal reference ranges (e.g. mean ± 2SD) for statistical reasons.[46,47] Because outliers can inflate standard deviations, the mean ± 2SD may sometimes provide an inaccurate representation of the normal range. We reported mean ± 2SD in Table 4 only to allow comparisons with other studies.

Much of the confusion in contrasting normal T levels across studies may stem from ignoring the methodological differences of these studies. First of all, because T levels decline slowly with age[11,13,15,17,22,54] an age appropriate reference population should be used. What is 'normal' for a 20-year-old should not be assumed to be 'normal' for a 60-year-old. Similarly, reference ranges should be presented by age or age group rather than in a one size fits all fashion.[55] Third, as chronic illness, certain medications, BMI and lifestyle issues (e.g. alcohol and smoking) can influence T levels, which we and others have shown[11,13,21–25] these factors should be taken into account. Finally, careful attention should be paid to the statistical methods used to calculate reference intervals since outliers can inflate standard deviations and, thus, distort reference ranges.

Table 5 displays normal reference ranges from four studies of non-clinic based populations. Because the 2·5th and 97·5th percentiles were not available, we quote mean ± 2SD. When not reported explicitly, results were converted to nM. Deslypere[56] sampled 73 healthy, nonsmoking, nonalcoholic men who passed a routine physical exam and were within 10% of ideal body weight. Although he reports results in 20-year rather than 10-year age groups, his reference ranges, as defined by mean ± 2SD, are narrower than ours. It is interesting to note that mean − 2SD for his 40–59 age group (8·0 nM) falls between the 2·5th percentiles for our 40–49 (8·7 nM) and 50–59-year groups (7·5 nM). Similarly, the lower limit for his 60–80-year group (6·7 nM) is in between the 2·5th percentiles for our 60–69-year (6·8 nM) and 70–79-year (5·4 nM) groups. An analogous correspondence exists between his mean + 2SD and our 95th percentiles. Vermeulen's data for 250 healthy, nonobese men are similar to the MMAS data if mean − 2SD from both studies is compared, though he uses different age groups.[13] The mean + 2SD results for his study are higher than ours. Szulc and colleagues[57] calculated T ranges using data from 792 men aged 50–85. The men were randomly selected clients of a large insurance company in France and were recruited for a prospective study of osteoporosis. For total T, the

Table 5. Normal reference ranges for total testosterone (T) by age for selected studies

Study	Age (years)	T (nM)*	T (ng/dl)*	Assay technique	Sample
Deslypere & Vermeulen[56]	20–39	9·6–31·4	278–906	RIA on plasma after chromatographic separation of DHT and 5α-androstane-3α, 17β-diol	n = 73 healthy male volunteers from a suburb of an industrial city. All men were nonsmoking, not alcoholics, not on medication, within 10% of ideal body weight, and were healthy according to a routine physical examination and blood chemistry.
	40–59	8·0–28·8	230–830		
	60–80	6·7–26·0	193–749		
Vermeulen *et al.*[13]	45–54	6·3–35·8	182–1032	RIA on plasma	n = 250 healthy nonobese men living in a semi-industrial area. None were taking medication, and all were healthy according to a clinical exam and routine blood work.
	55–64	6·0–33·0	173–951		
	65–74	4·5–31·8	130–917		
Szulc *et al.*[57]	50–85	3·7–31·7	107–914	Tritiated RIA on serum after diethyl ether extraction	Random sample of 792, 50- to 85-year-old men insured by an insurance company in France.
Schatzl *et al.*[55]	20–29	10·7–28·8	310–830	RIA on serum	n = 133 healthy male volunteers of a health screening project: BMI < 30 kg/m^2, fasting serum cholesterol < 200 g/dl and diastolic blood pressure < 95 mm HG.
	30–39	10·4–28·8	300–830		
	40–49	9·7–24·3	280–700		
	50–59	8·3–21·8	240–630		
	60–69	7·3–18·7	210–540		
	70–89	5·9–17·0	170–490		

*Mean ± 2SD.

lower range of 3·7 nM (107 ng/dl) is the lowest of the four studies and substantially lower than MMAS mean – 2SD. The use of such a broad age range may be why this value is so low. Schatzl published reference ranges for total T in 133 healthy men aged 20–89 who volunteered for a health screening examination.[55] For all comparable age groups, mean – 2SD is substantially higher than our mean – 2SD and mean + 2SD is substantially lower.

The primary strengths of our study are that we used a large, age-appropriate, population-based rather than a clinic-based sample, and we controlled for confounding factors such as chronic illness, BMI, medications and lifestyle. We presented reference ranges by age decade rather than for all ages combined. For all three time points, our hormones were measured by a lab with a good reputation for accuracy (University of Massachusetts Medical School, Worcester, MA, USA), and all assays were conducted by a single technician, thus minimizing technician variability. Finally, our method of measuring T (i.e. DPC RIA) has been shown to perform favourably on adult male sera when compared to the gold standard liquid chromatography-tandem mass spectrometry.[58]

One of the limitations of this study is that we do not have a large percentage of racial minorities in our sample, and thus, could not control for any possible racial differences in hormones. However, our sample is representative of the population of Massachusetts. Another limitation is that since this is a large field study, illness and medication use were based on self-report, and consequently, there may be measurement error in these variables.

In summary, this paper establishes age-specific, normal T ranges using data from apparently healthy, community-based men. Such definitive data are currently lacking and will be needed before clinicians can reach a consensus on the definition of androgen deficiency. We hope that better data regarding normal T levels will promote the appropriate use of TRT.

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