Nationally Representative Estimates of Serum Testosterone Concentration in Never-Smoking, Lean Men Without Aging-Associated Comorbidities

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Context: Testosterone deficiency prevalence increases with age, comorbidities, and obesity.

Objective: To inform clinical guidelines for testosterone deficiency management and development of targets for nonpharmacologic intervention trials for these men, we determined serum testosterone in never-smoking, lean men without select comorbidities in nationally representative surveys.

Design, Setting, Participants: We used cross-sectional data for never-smoking, lean men ≥20 years without diabetes, myocardial infarction, congestive heart failure, stroke, or cancer, without use of hormone-influencing medications, and participated in morning sessions of National Health and Nutrition Examination Survey (NHANES) III (phase I 1988–1991) or continuous NHANES (1999–2004). By age, we determined median total testosterone (ng/mL) measured previously by a Food and Drug Administration-approved immunoassay and median estimated free testosterone concentration.

Results: In NHANES III, in never-smoking, lean men without comorbidities, median (25th, 75th percentile) testosterone was 4% to 9% higher than all men—20 to 39 years: 6.24 (5.16, 7.51), 40 to 59: 5.37 (3.83, 6.49), and ≥60: 4.61 (4.01, 5.18). In continuous NHANES, in never-smoking, lean men without comorbidities, levels were 13% to 24% higher than all men—20 to 39 years: 6.26 (5.32, 7.27), 40 to 59: 5.86 (4.91, 6.55), and ≥60: 4.22 (3.74, 5.73). In never-smoking, lean men without comorbidities, median estimated free testosterone was similar to (NHANES III) or slightly higher than (continuous NHANES) in all men.

Conclusions: These nationally representative data document testosterone levels (immunoassay) in never-smoking, lean men without select comorbidities 30 and 15 to 20 years ago. This information can be incorporated into guidelines for testosterone deficiency management and used to develop targets for nonpharmacologic intervention trials for testosterone deficiency.

Abbreviations: FDA, Food and Drug Administration; BMI, body mass index; NHANES, National Health and Nutrition Examination Survey; CV, coefficients of variation; Q1, first quartile; Q3, third quartile.

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The prevalence of testosterone deficiency, also known as low testosterone, increases with age [1], and testosterone concentration is lower in men who are obese [2, 3], and who have aging-associated comorbidities such as diabetes [4]. We previously estimated that 8.4 million US men aged 40 years old and older may have low total testosterone [1]. In that study, we used testosterone <3.00 ng/mL (<10.4 nmol/L) to define low testosterone, although cutpoints to define low testosterone in aging men and men with certain comorbidities associated with aging and obesity have not been agreed upon by authoritative bodies. Nevertheless, a large number of men may experience the symptoms of low testosterone and be at risk for adverse health consequences [4–6] and premature death [7–12].

Strategies to increase testosterone levels may include pharmacologic and non-pharmacologic interventions. Testosterone (supplementation) therapy is widely available in the United States, and manufacturers advertise it to consumers for alleviating the symptoms of "low T" in aging men. Professional societies in the United States and Australia generally recommend measuring serum testosterone concentration in men with signs or symptoms of testosterone deficiency, but do not recommend screening asymptomatic men for low testosterone [13–15]. The diagnosis of testosterone deficiency is usually made based on the presence of signs and symptoms and consistently low testosterone levels. One guideline recommends against routine testosterone therapy for older men with low testosterone and instead recommends individualized decision-making for men 65 years and older who are symptomatic and have documented low testosterone [13]. Another guideline indicates that data do not currently justify testosterone therapy for older men, often with chronic diseases, who have low circulating testosterone levels but who do not have hypothalamic, pituitary, or testicular diseases [14]. In 2018, the US Food and Drug Administration (FDA) required updates to testosterone product labeling to "inform of possible increased risk of heart attack and stroke with use" and cautioned against the unapproved use of testosterone to treat low levels due to aging, even in symptomatic men [16].

Although observational studies suggest that men who are leaner, physically active, and do not have diabetes have higher serum testosterone [2, 4, 17, 18], whether non-pharmacologic strategies such as decreasing body fat, increasing lean mass, increasing physical activity, and controlling diabetes are effective in raising serum testosterone in older men and men with comorbidities and obesity have not been tested in large, phase 3 intervention trials.

Optimal circulating testosterone concentration for aging men, whether for use of testosterone therapy or for defining outcome in nonpharmacologic intervention trials aiming to raise testosterone, has not been established. Clinical guidelines for men with hypogonadism due to conditions of the hypothalamus, pituitary, and testes, typically indicate that the target level during testosterone therapy is the age-specific lower range for eugonadal men [14]. Given the possibility of adverse effects of testosterone therapy, targets for aging men are needed.

Thus, to inform clinical guidelines for the diagnosis of laboratory testosterone deficiency and its pharmacologic treatment, and to inform the development of target testosterone levels for nonpharmacologic intervention trials for low testosterone in aging men, men with aging-associated comorbidities, and obese men, we determined typical serum total testosterone concentrations measured by immunoassay in never-smoking, lean men without select aging-associated comorbidities by age in the US nationally representative National Health and Nutrition Examination Surveys (NHANES) cross-sectionally at two calendar times ~30 and 15 to 20 years ago.

1. Materials and Methods

A. Study Design and Population

We used previously measured serum testosterone concentrations and other data for men aged 20 years and older who participated in the morning sessions of NHANES III (1988–1991) or continuous NHANES (1999–2004). Both were cross-sectional surveys that used multistage stratified, clustered probability samples of the US population (civilian, noninstitutionalized); no individual was intentionally sampled twice. Details on the sampling of participants from NHANES III and from continuous NHANES for the measurement of serum testosterone were previously published [19, 20]. Testosterone data were available for 1625 men in NHANES III and 1508 men in continuous NHANES. We excluded men who were <20 years old (NHANES III, N = 164; continuous NHANES, N = 523); taking drugs (androgens, anabolic steroids, 5-α-reductase inhibitors, and antigonadotropic agents) that affect serum concentration of hormones (NHANES III, N = 2; continuous NHANES, N = 5); or who had implausible testosterone values [continuous NHANES, N = 2 (−0.2, 50 ng/mL)]. In total, we included 672 non-Hispanic white, 359 non-Hispanic black, 372 Mexican-American men, and 56 men other race/ethnicity in NHANES III, and 506 non-Hispanic white, 188 non-Hispanic black, 216 Mexican-American men, and 68 men of other race/ethnicity in continuous NHANES. In stratified analyses, we also excluded men with missing information on weight, height, or waist circumference (NHANES III, N = 49; continuous NHANES, N = 34).

B. Serum Testosterone Measurement

Serum samples from both surveys were previously assayed for testosterone concentration using an FDA-approved competitive electrochemiluminescence immunoassay on the 2010 Elecsys autoanalyzer (Roche Diagnostics, Indianapolis, IN) in the laboratory of Dr. Nader Rifai at Children's Hospital in Boston, Massachusetts. The limit of detection of the assay is 0.02 ng/mL, and the functional sensitivity is 0.12 ng/mL [21]. The coefficients of variation (CVs) were 5.9% and 5.8% at 2.5 and 5.5 ng/mL in quality control specimens in NHANES III and was 4.8% for 21 duplicate specimens in continuous NHANES. We estimated free testosterone concentration [22] from measured testosterone, SHBG, and albumin (available in the NHANES III and continuous NHANES public use databases); in general, free testosterone may be the better measure of androgen effect in individuals with abnormal SHBG concentrations.

C. Other Variables

As part of the NHANES protocols, participants were interviewed, during which age, race, cigarette smoking, and history of chronic diseases—diabetes, myocardial infarction, stroke, congestive heart failure, or cancer—were assessed, and underwent a physical examination, during which height, weight, and waist circumference were measured. Medications used in the last 30 days were ascertained during the interview, and when available, confirmed by review of the participants' medication containers. We calculated body mass index (BMI) as weight (kilograms) divided by the square of height (meters). We defined never smokers as men who smoked <100 cigarettes over their lifetimes.

D. Statistical Analysis

We applied sampling weights and determined the mean and SE (Taylor series robust variance estimation), and the 10th, 25th (first quartile), 50th (median), 75th (third quartile), and 90th percentiles of total and free testosterone concentration among men 20 to 39 years, 40 to 59 years, and ≥60 years old overall and among lean men (BMI < 25 kg/m^2 and WC < 102 cm), men without select major aging-associated comorbidities (diabetes, heart attack, congestive heart failure, stroke, cancer), and men who were lean and did not have select major aging-associated

comorbidities. We repeated these analyses in men who were never smokers (in NHANES III, current smokers had higher and former smokers had lower serum testosterone than never smokers after multivariable adjustment [17]). Finally, we repeated all of these analyses separately among non-Hispanic white, non-Hispanic black, and Mexican-American men.

We also modeled total testosterone concentrations by weighted LOESS (locally estimated scatterplot smoothing) linear regression, so that typical concentration could be determined for any given adult age.

2. Results

A. Total Testosterone

Median (25th, 75th percentile) testosterone concentrations (ng/mL) in men ages 20 to 39, 40 to 59, and ≥60 years old were 5.99 (4.84, 7.31), 4.86 (3.79, 6.18), and 4.35 (3.25, 5.36), respectively, in NHANES III [medians in Table 1; means, SEs, and percentiles in Supplemental Table 1 (supplemental tables are deposited in [23])], and 5.42 (4.34, 6.85), 4.45 (3.39, 5.90), and 3.92 (2.74, 5.14), respectively, in continuous NHANES (Table 1; Supplemental Table 2 [23]). In lean men without comorbidities, across all three age groups, median serum testosterone concentrations were higher compared with all men in both NHANES III (Table 1; Supplemental Table 1 [23]) and continuous NHANES (Table 1; Supplemental Table 2 [23]). After restricting to never-smoking men, those who were lean and without comorbidities had median serum testosterone concentrations that were higher (4% to 9% in NHANES III and 13% to 24% in continuous NHANES) than in all men, but generally lower than when including ever smokers; concentrations were 6.24 (5.16, 7.51), 5.37 (3.83, 6.49), and 4.61 (4.01, 5.18), respectively, in NHANES III (Table 1; Supplemental Table 3 [23]), and 6.26 (5.32, 7.27), 5.86 (4.91, 6.55), and 4.22 (3.74, 5.73), respectively, in continuous NHANES (Table 1; Supplemental Table 4 [23]). Modeled distributions of total testosterone concentration by age among each subpopulation are shown in Figs. 1 (NHANES III) and 2 (continuous NHANES).

Table 1. Median Total Testosterone Concentration (ng/mL) by Age Among All Men and Among Men Without Health States That Influence Testosterone Level in NHANES III (1988–1994) and in Continuous NHANES (1999–2004)

	Age Group, y		
	20–39	**40–59**	**60+**
NHANES III			
All men	5.99	4.86	4.35
Lean men[a]	6.75	5.75	4.78
Men without comorbidities[b]	6.00	4.93	4.45
Lean men without comorbidities	6.77	5.85	4.92
Never-smoking men	5.52	4.78	4.44
Never-smoking lean men	6.24	5.36	4.36
Never-smoking men without comorbidities	5.51	4.79	4.48
Never-smoking lean men without comorbidities	6.24	5.37	4.61
Continuous NHANES			
All men	5.42	4.45	3.92
Lean men[a]	6.55	5.76	4.92
Men without comorbidities[b]	5.45	4.61	4.09
Lean men without comorbidities	6.57	5.85	5.06
Never-smoking men	5.19	4.43	4.08
Never-smoking lean men	6.27	5.84	4.37
Never-smoking men without comorbidities	5.19	4.48	4.15
Never-smoking lean men without comorbidities	6.26	5.86	4.22

To convert testosterone to nmol/L, multiple values by 3.4672.
[a]BMI < 25 kg/m^2 and waist circumference < 102 cm.
[b]No diabetes, myocardial infarction, congestive heart failure, stroke, or cancer.

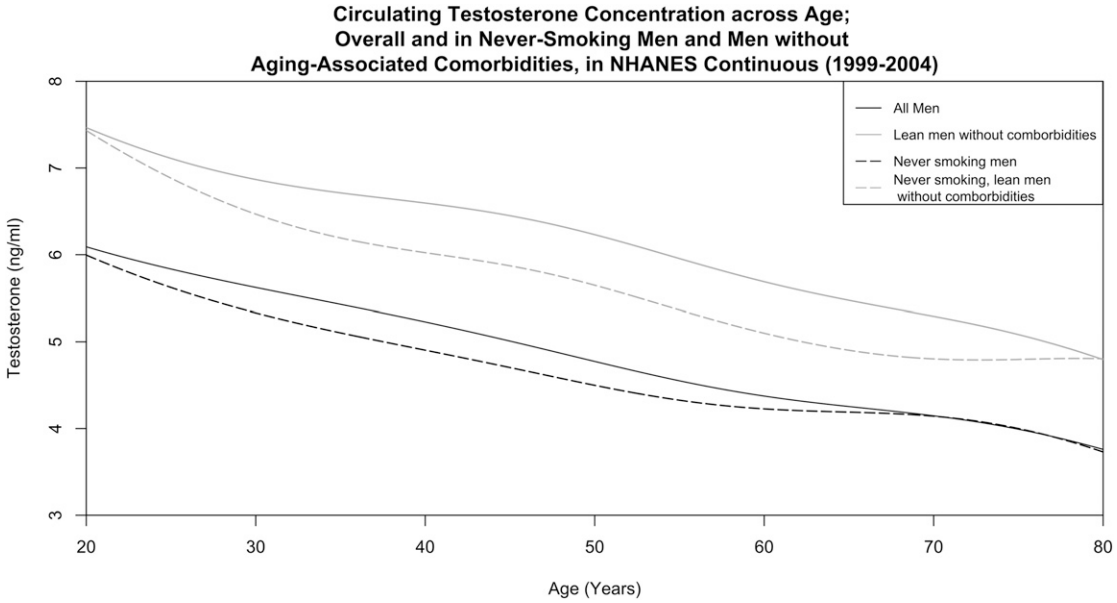

Figure 1. Circulating testosterone concentration across age: overall and in never-smoking men and men without aging-associated comorbidities, in NHANES III (1988–1991).

B. Free Testosterone

Median (25th, 75th percentile) free testosterone concentrations (ng/mL) in men ages 20 to 39, 40 to 59, and ≥60 years old were 0.13 (0.11, 0.15), 0.10 (0.08, 0.12), and 0.07 (0.05, 0.09), respectively, in NHANES III (Table 2; Supplemental Table 5 [23]), and 0.12 (0.10, 0.14), 0.09 (0.07, 0.11), and 0.06 (0.05, 0.08), respectively, in continuous NHANES (Table 2; Supplemental Table 6 [23]). In lean men without comorbidities, across all three age groups, median free testosterone concentrations were similar to all men in NHANES III (Table 2; Supplemental Table 5) but higher than in all men in continuous NHANES (Table 2; Supplemental

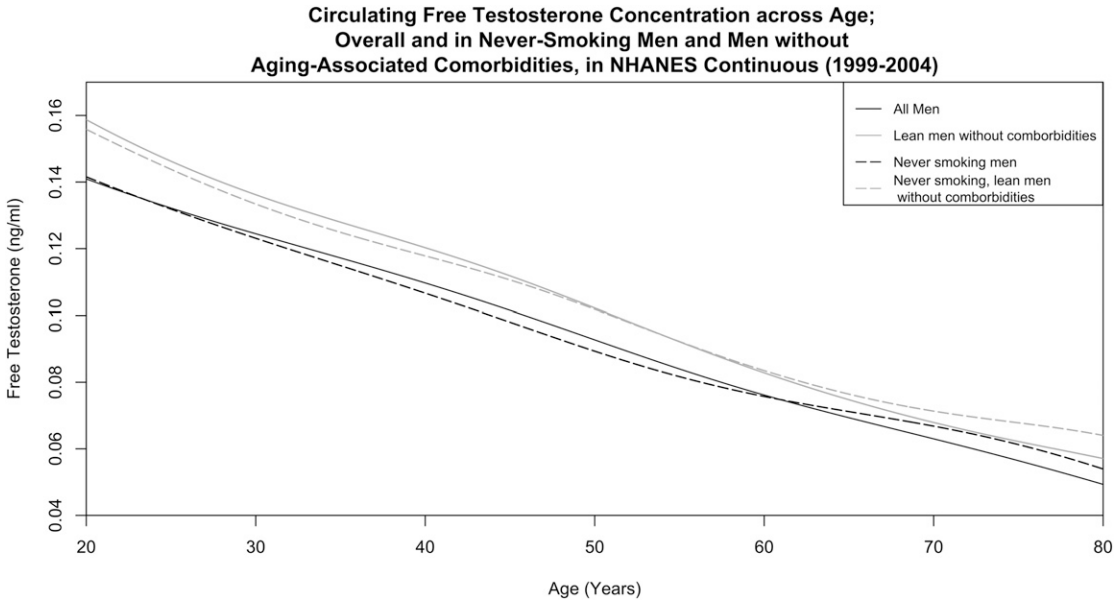

Figure 2. Circulating testosterone concentration across age: overall and in never-smoking men and men without aging-associated comorbidities, in NHANES continuous (1999–2004).

Table 2. Median Free Testosterone Concentration (ng/mL) by Age Among All Men and Among Men Without Health States That Influence Testosterone Level in NHANES III (1988–1994) and in Continuous NHANES (1999–2004)

	Age Group, y		
	20–39	40–59	60+
NHANES III			
All men	0.13	0.10	0.07
Lean men[a]	0.14	0.13	0.11
Men without comorbidities[b]	0.13	0.10	0.07
Lean men without comorbidities	0.13	0.11	0.07
Never-smoking men	0.12	0.10	0.07
Never-smoking lean men	0.13	0.11	0.07
Never-smoking men without comorbidities	0.12	0.10	0.07
Never-smoking lean men without comorbidities	0.13	0.11	0.07
Continuous NHANES			
All men	0.12	0.09	0.06
Lean men[a]	0.13	0.10	0.06
Men without comorbidities[b]	0.12	0.09	0.06
Lean men without comorbidities	0.13	0.10	0.07
Never-smoking men	0.12	0.09	0.07
Never-smoking lean men	0.13	0.11	0.08
Never-smoking men without comorbidities	0.12	0.09	0.07
Never-smoking lean men without comorbidities	0.13	0.11	0.07

To convert free testosterone to nmol/L, multiple values by 3.4672.
[a]BMI < 25 kg/m^2 and waist circumference < 102 cm.
[b]No diabetes, myocardial infarction, congestive heart failure, stroke, or cancer.

Table 6 [23]). After restricting to never-smoking men, in NHANES III, those who were lean and without comorbidities had medians that were similar to all men and when including ever smokers; free testosterone concentrations were 0.13 (0.10, 0.14), 0.11 (0.08, 0.13), and 0.07 (0.06, 0.08), respectively (Table 2; Supplemental Table 7 [23]). After restricting to never-smoking men, in continuous NHANES, those who were lean and without comorbidities had medians that were higher compared with all men, but similar to when including ever smokers; free testosterone concentrations were 0.13 (0.11, 0.16), 0.11 (0.09, 0.12), and 0.07 (0.05, 0.09), respectively (Table 2; Supplemental Table 8 [23]). Modeled distributions of free testosterone concentration by age among each subpopulation are shown in Figs. 3 (NHANES III) and 4 (continuous NHANES).

C. By Race/Ethnicity

C-1. Total testosterone

Across all three age groups, patterns for total testosterone were similar by race/ethnicity with concentrations generally being higher in lean men without comorbidities than in all men in both NHANES III (medians in Table 3; distributions in Supplemental Table 9 [23]) and continuous NHANES (Table 3; Supplemental Table 10 [23]) and generally being higher in never-smoking lean men without comorbidities than in all men in both NHANES III (Table 3; Supplemental Table 11 [23]) and continuous NHANES (Table 3; Supplemental Table 12 [23]).

C-2. Free testosterone

Across all three age groups, in NHANES III, patterns for free testosterone were similar by race/ethnicity with concentrations in lean men without comorbidities generally being equal to

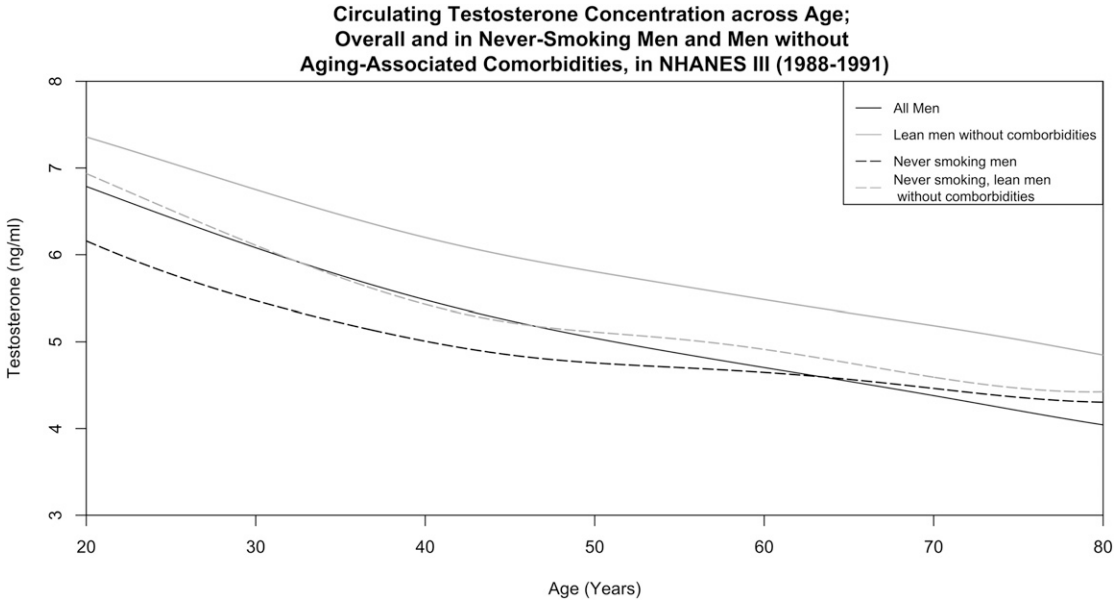

Figure 3. Circulating free testosterone concentration across age: overall and in never-smoking men and men without aging-associated comorbidities, in NHANES III (1988–1991).

all men (Table 4; Supplemental Table 13 [23]). In contrast, in continuous NHANES, patterns for free testosterone differed by race/ethnicity with concentrations in lean men without comorbidities being higher than in all men among non-Hispanic white men and being the same as in all men among Mexican-American men; no discernable pattern was present in non-Hispanic black men (Table 4; Supplemental Table 14 [23]). No patterns for free testosterone were discernable by racial/ethnic group in never-smoking lean men without comorbidities compared with all men in either NHANES III (Table 4; Supplemental Table 15 [23]) or continuous NHANES (Table 4; Supplemental Table 16 [23]).

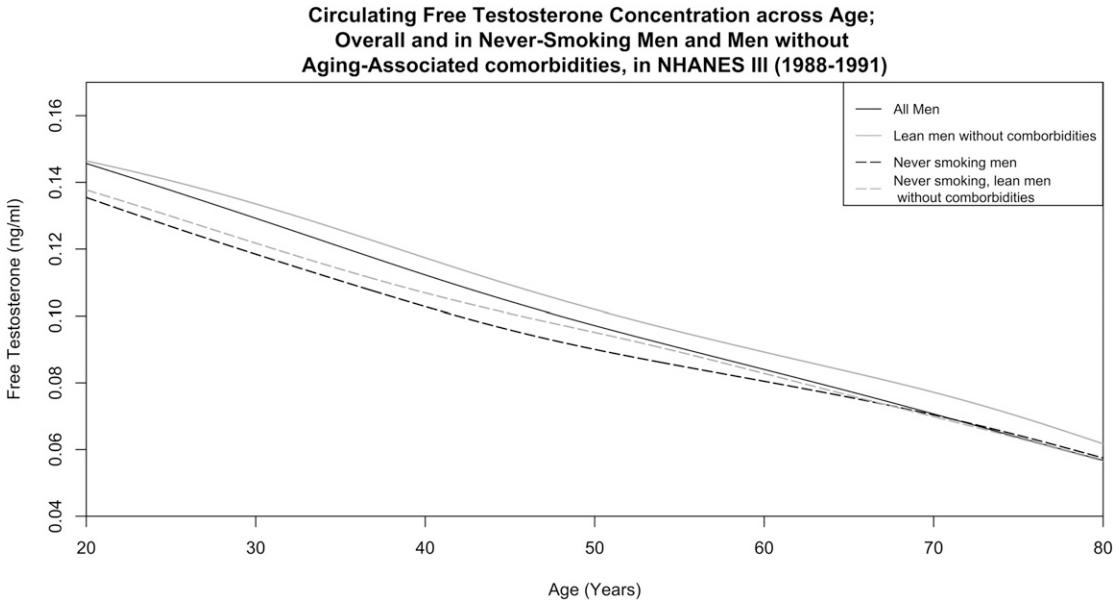

Figure 4. Circulating free testosterone concentration across age: overall and in never-smoking men and men without aging-associated comorbidities, in NHANES continuous (1999–2004).

Table 3. Distribution of Total Testosterone Concentration (ng/mL) by Age and Race Among All Men and Among Men Without Health States That Influence Testosterone Level in NHANES III (1988–1994) and in Continuous NHANES (1999–2004)

	Age Group (y)		
	20–39	40–59	60+
NHANES III			
Non-Hispanic white			
All men	6.04	4.81	4.43
Lean men without comorbidities[a]	6.93	5.72	5.13
Never-smoking men	5.38	4.79	4.69
Never-smoking lean men without comorbidities	5.93	5.11	4.79
Non-Hispanic black			
All men	6.23	5.75	4.29
Lean men without comorbidities[a]	7.05	5.94	4.88
Never-smoking men	5.80	5.30	3.44
Never-smoking lean men without comorbidities	7.06	6.23	3.37
Mexican-American			
All men	5.84	4.31	4.20
Lean men without comorbidities[a]	6.73	6.01	3.92
Never-smoking men	5.85	4.21	4.14
Never-smoking lean men without comorbidities	6.72	5.40	4.16
Continuous NHANES			
Non-Hispanic white			
All men	5.20	4.38	3.85
Lean men without comorbidities[a]	6.45	5.74	5.11
Never-smoking men	4.86	4.34	4.06
Never-smoking lean men without comorbidities	5.96	5.83	4.19
Non-Hispanic black			
All men	6.44	5.83	4.26
Lean men without comorbidities[a]	7.93	6.44	4.93
Never-smoking men	6.24	5.72	3.91
Never-smoking lean men without comorbidities	8.57	4.31	5.69
Mexican-American			
All men	5.45	4.33	3.98
Lean men without comorbidities[a]	6.22	5.95	3.77
Never-smoking men	5.24	4.26	3.48
Never-smoking lean men without comorbidities	5.86	5.85	5.02

To convert testosterone to nmol/L, multiple values by 3.4672.
[a]BMI < 25 kg/m^2 and waist circumference < 102 cm and no diabetes, myocardial infarction, congestive heart failure, stroke, or cancer.

3. Discussion

Irrespective of whether aging men, men with aging-associated comorbidities, or obese men with low testosterone are managed via testosterone supplementation, weight reduction/lifestyle modifications, or other strategies, definitions of low testosterone and target testosterone levels are needed. Here, we provided needed information for these guidelines and targets on typical serum total and free testosterone concentrations that were measured by immunoassay in normal weight, never-smoking men without comorbidities from US nationally representative surveys conducted 30 and 15 to 20 years ago. Given the decline in testosterone with age, whether the target level for an older man should be the same as for a younger man is unclear. Thus, we also provided levels among younger, middle-aged, and older men, as well as modeled curves, so that typical concentrations in healthy men could be determined at any adult age. We also provided levels by race/ethnicity, given reported racial/ethnic differences in concentrations [19, 20, 24].

The risks and benefits of testosterone therapy have been reviewed comprehensively in clinical guidelines [13, 15]. From the public health perspective, use of nonpharmacologic

Table 4. Distribution of Free Testosterone Concentration (ng/mL) by Age and Race Among All Men and Among Men Without Health States That Influence Testosterone Level in NHANES III (1988–1994) and in Continuous NHANES (1999–2004)

	Age Group (y)		
	20–39	40–59	60+
NHANES III			
Non-Hispanic white			
All men	0.13	0.10	0.07
Lean men without comorbidities[a]	0.13	0.10	0.07
Never-smoking men	0.11	0.10	0.07
Never-smoking lean men without comorbidities	0.12	0.11	0.07
Non-Hispanic black			
All men	0.13	0.10	0.07
Lean men without comorbidities[a]	0.14	0.10	0.07
Never-smoking men	0.13	0.10	0.06
Never-smoking lean men without comorbidities	0.15	0.10	0.05
Mexican-American			
All men	0.13	0.09	0.07
Lean men without comorbidities[a]	0.13	0.11	0.07
Never-smoking men	0.13	0.09	0.07
Never-smoking lean men without comorbidities	0.13	0.11	0.07
Continuous NHANES			
Non-Hispanic white			
All men	0.12	0.09	0.06
Lean men without comorbidities[a]	0.13	0.10	0.07
Never-smoking men	0.12	0.09	0.06
Never-smoking lean men without comorbidities	0.12	0.10	0.06
Non-Hispanic Black			
All men	0.14	0.10	0.07
Lean men without comorbidities[a]	0.16	0.10	0.08
Never-smoking men	0.13	0.11	0.06
Never-smoking lean men without comorbidities	0.17	0.07	0.09
Mexican-American			
All men	0.12	0.09	0.07
Lean men without comorbidities[a]	0.12	0.10	0.07
Never-smoking men	0.12	0.08	0.06
Never-smoking lean men without comorbidities	0.12	0.09	0.07

To convert free testosterone to nmol/L, multiple values by 3.4672.
[a]BMI < 25 kg/m^2 and waist circumference < 102 cm and no diabetes, myocardial infarction, congestive heart failure, stroke, or cancer.

strategies to normalize testosterone levels in older men with comorbidities may be preferable to testosterone supplementation because such strategies may benefit overall health and not be associated with adverse effects. Such randomized intervention trials have not been conducted on a large scale, so it remains unclear whether the published data indicating that leaner men who have higher serum testosterone than men with more body fat and less lean mass would translate into increases in testosterone if men with more fat and less lean mass were to lose fat and gain lean mass [25].

We provided distributions of total and free testosterone for men participating only in the morning sessions of phase 1 of NHANES in 1988 to 1991 or continuous NHANES in 1999 to 2004 given the diurnal variation in testosterone in men [26]. The CVs for the NHANES III samples that were assayed at both time points were 6.3% for testosterone, and 5.3% for SHBG, which was used in the calculation of free testosterone. Despite being measured at different times, the mean concentrations among all men at the two time points from our prior work (without the exclusions in the current work) were similar at 5.37 and 5.34 ($P = 0.75$) for testosterone and 0.099 and 0.097 ($P = 0.67$) for free testosterone in NHANES III and

continuous NHANES, respectively [20]. The low CVs and similar means at the two time points suggest the comparability of the assay despite being performed at different times for the two surveys. In the current analysis, in lean men without select comorbidities, mean (median) total testosterone concentrations were within 5% between NHANES III and continuous NHANES: −2.2% (+3.0%), −4.6% (0.0%), and −2.7% (−2.8%) in men 20 to 39, 40 to 49, and 60+ years old. Thus, any differences in testosterone levels between the two surveys after excluding men who had excess adiposity, comorbidities, and ever smoked (the prevalences of which have changed over calendar time) are unlikely solely due to assay variability over time, and more likely due to secular differences in prevalences in other factors that influence testosterone or due to chance variability.

The major strengths of this analysis are that we used nationally representative data, enhancing the generalizability of the estimated testosterone concentrations to US men, and the data were from established surveys that have a wealth of data, allowing us to restrict to subpopulations so that we could obtain what could be considered typical testosterone concentrations in healthy men. Although other studies have previously reported on age-specific testosterone levels in healthy men, these were not representative general populations of healthy men (*e.g.*, blood donors, sports club members [27]).

Several aspects of this study warrant discussion. First, *a priori*, we considered never smoking, lean men without histories of select, common aging-associated comorbidities to be those with typical testosterone levels for healthy men. We did not exclude men with less common, nonaging comorbidities, such as men with HIV who are immunosuppressed and more likely to have lower testosterone [28]. Second, we noted small numbers of men in the two analytic populations who had serum testosterone concentrations that were near the limit of detection of 0.02 ng/mL (*e.g.*, testosterone $<$ 0.1 ng/mL: NHANES III, N = 5; continuous NHANES, N = 4); the number of these men was lower when restricting to normal weight men without comorbidities (NHANES III, N = 1; continuous NHANES, N = 0). Although the reasons for these low levels—congenital or acquired or laboratory error—are not known, we presume them to be representative of population-level sampling. Whether these men with such low testosterone would benefit from interventions directed at increasing testosterone via lifestyle modification is not known. Third, for quantitating testosterone concentration, we used the Elecsys Testosterone Immunoassay (Roche Diagnostics), which was FDA approved and in wide clinical use. The method was standardized by isotope dilution gas chromatography mass spectrometry [21]. The functional sensitivity (limit of quantitation) was well below the often used cutpoint for low testosterone of 3.0 ng/mL. Although mass spectrometry may produce more accurate quantitation of sex steroid hormones and is considered to be the gold standard, that assay method is not currently FDA approved for clinical use [29]. The values we report may be assay method-dependent. Fourth, given that the biologically available fraction of serum testosterone is free testosterone, rather than SHBG or albumin-bound testosterone, we also determined typical free testosterone in never-smoking, lean men without comorbidities. SHBG is well recognized to be lower in obese men, which helps maintain free testosterone level. Thus, men with low total testosterone may have normal range free testosterone. We estimated the free fraction using each man's measured SHBG and albumin. Fifth, although we used nationally representative data, we were only able to study the prevalent racial/ethnic groups at the time of the two surveys. Sixth, because we used existing data, sample sizes were small in some subgroups. Finally, although the data we provide could be used to define population-based low levels by age and/or age and race distributions, such cutpoints would not necessarily map to the extent and severity of health states influenced by testosterone. In this study, we did not evaluate whether the levels of total and free testosterone in the never-smoking, lean men without comorbidities are associated with a lower likelihood of symptoms associated with low testosterone, such as fatigue, lower libido, and erectile dysfunction, information also needed for revising clinical guidelines and establishing target testosterone levels.

In summary, we determined typical serum testosterone concentrations that were measured by immunoassay in healthy, never-smoking normal weight men representative of the US

population cross-sectionally at two calendar times 30 and 15 to 20 years ago. We expect that these data provide some of the information that is needed to: (i) refine the definition of testosterone deficiency in older men and to inform its pharmacologic treatment, and (ii) establish target total serum testosterone levels for nonpharmacologic intervention trials for men with testosterone deficiency associated with aging, aging-associated comorbidities, and obesity.

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Data Availability: NHANES data are publicly available. Data generated or analyzed during this study are included in this published article or in the data repositories listed in the references.

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