Age-Specific Serum Total and Free Estradiol Concentrations in Healthy Men in US Nationally Representative Samples

Susan Chadid,[1] John R. Barber,[1] Sabine Rohrmann,[2] William G. Nelson,[3,4] James D. Yager,[5] Norma F. Kanarek,[5] Gary Bradwin,[6] Adrian S. Dobs,[7] Katherine A. McGlynn,[8] and Elizabeth A. Platz[1]

[1]Department of Epidemiology, Johns Hopkins Bloomberg School of Public Health, Baltimore, Maryland 21205; [2]Division of Chronic Disease Epidemiology, Epidemiology, Biostatistics and Prevention Institute, University of Zurich, 8001 Zurich, Switzerland; [3]Sidney Kimmel Comprehensive Cancer Center at Johns Hopkins, Baltimore, Maryland 21205; [4]Department of Urology and the James Buchanan Brady Urological Institute, Baltimore, Maryland 21205; [5]Department of Environmental Health and Engineering, Johns Hopkins Bloomberg School of Public Health, Baltimore, Maryland 21205; [6]Department of Laboratory Medicine, Harvard Medical School and Children's Hospital, Boston, Massachusetts 02115; [7]Division of Endocrinology, Diabetes, and Metabolism, Department of Medicine, Johns Hopkins University School of Medicine, Baltimore, Maryland 21287; and [8]Division of Cancer Epidemiology and Genetics, National Cancer Institute, Bethesda, Maryland 20892

ORCiD numbers: 0000-0003-4650-1073 (S. Chadid); 0000-0003-3676-8954 (E. A. Platz).

Purpose: To report age-specific serum estradiol concentration in nonsmoking, lean US men without comorbidities. We provide concentrations from 30 and 15 to 20 years ago given previously described declines in serum estradiol in US men over time.

Methods: We used data from the Third National Health and Nutrition Examination Survey (NHANES III; 1988 to 1991) and continuous NHANES (1999 to 2004). Serum estradiol and SHBG were previously measured by competitive electrochemiluminescence immunoassays. Free estradiol was estimated from estradiol, SHBG, and albumin. By age, we calculated median concentrations overall and for nonsmoking, lean (body mass index <25 kg/m^2 and waist <102 cm) men without diabetes, cardiovascular disease, or cancer.

Results: Overall, respective total estradiol medians for men ages 20 to 39, 40 to 59, and ≥ 60 years old were 37.0, 33.9, and 33.5 pg/mL in NHANES III and 31.3, 30.5, and 27.0 pg/mL in continuous NHANES. In nonsmoking, lean men without comorbidities, respective total estradiol medians were 32.0, 32.1, and 32.0 pg/mL in NHANES III and 29.1, 22.7, and 26.1 pg/mL in continuous NHANES. Overall, respective free estradiol medians were 0.82, 0.72, and 0.64 pg/mL in NHANES III and 0.67, 0.61, and 0.47 pg/mL in continuous NHANES. In nonsmoking, lean men without comorbidities, respective free estradiol medians were 0.64, 0.67, and 0.62 pg/mL in NHANES III and 0.58, 0.42, and 0.40 pg/mL continuous NHANES.

Conclusion: We report US nationally representative serum estradiol concentrations in healthy men, which could be used for targeting estradiol during testosterone supplementation and for general good health.

Freeform/Key Words: estradiol, age-specific, healthy men

Abbreviations: AUA, American Urological Association; BMI, body mass index; CV, coefficient of variation; NHANES III, Third National Health and Nutrition Examination Survey; WC, waist circumference.

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Symptoms accompanying testosterone deficiency are often treated with testosterone supplementation. Such treatment could lead to higher estradiol levels via peripheral aromatization of testosterone to estradiol in adipose tissue [1], especially in men with excess body fat. Increases in circulating estradiol as a result of testosterone supplementation may be clinically important for at least two reasons. First, gynecomastia, which has a prevalence of one- to two-thirds of men overall [2] and ≤70% in men 50 to 69 years old [3], can be exacerbated by replacement testosterone. Indeed, the recent American Urological Association (AUA) Clinical Practice Guidelines for the Evaluation and Management of Testosterone Deficiency recommended that estradiol levels be measured in men with breast symptoms or gynecomastia before supplemental testosterone therapy or during therapy if breast symptoms or gynecomastia developed [4]. However, neither the AUA [4] nor the Endocrine Society [5] recommends measuring estradiol levels before prescribing testosterone for other men being considered for supplementation. Second, elevated circulating estradiol levels have been associated with altered inflammatory and immune responses in men [6], including higher concentrations of circulating proinflammatory markers [7].

The typical laboratory reference range for serum estradiol in men is 10 to 40 pg/mL [8]. However, serum estradiol ranges have not been established for optimal health for older men, such as those without common chronic diseases, who are not overweight or obese, and who do not smoke, all factors that can influence these levels. Age-specific estradiol ranges for healthy men could be useful as clinical targets for men receiving testosterone supplementation. In addition, age-specific estradiol ranges, both low and high, could be used as targets for overall well-being among men, given that estradiol has beneficial effects in men as they age, including reducing the risk of osteopenia [9].

To support the implementation of clinical guidelines for serum estradiol concentrations for men with symptomatic testosterone deficiency who are candidates for testosterone supplementation, and for men in general for good health, we report total and estimated free estradiol concentrations in nationally representative samples of younger, middle-aged, and older nonsmoking, lean men without common chronic diseases. Given the known racial and ethnic differences in estradiol concentration in men [10], we also report estradiol concentrations by race and ethnicity. We provide information at two time points, about 30 years and 15 to 20 years ago, given the previously reported but unexplained decline in serum estradiol concentration in men in the United States [11].

1. Methods

A. Study Population

We used data from The Third National Health and Nutrition Examination Survey (NHANES III) and three continuous NHANES surveys. NHANES III, conducted between 1988 and 1994, examined 30,818 participants; NHANES 1999 to 2000 examined 9282 participants; NHANES 2001 to 2002 examined 10,122 participants; and NHANES 2003 to 2004 examined 9643 participants. Each survey is a cross-sectional study designed to assess the health and nutritional status of the US population [12, 13]. In each survey, participants were sampled by multistage probability sampling. Evaluations involved home interviews, standard physical examinations, and laboratory tests.

We used serum total estradiol data previously measured in men who had their blood drawn in the morning session (NHANES III phase I only, N = 1462 [14]; NHANES, 1999 to 2004 N = 978 [11]). We included men ≥20 years old with no missing data on smoking, weight, height, or waist circumference (WC). *A priori* we excluded men taking medications that influence sex steroid hormone levels, specifically androgens, anabolic steroids, 5-α-reductase inhibitors, and antigonadotropic agents. After exclusions, we included in the total estradiol analysis 673 non-Hispanic white, 360 non-Hispanic black, and 373 Mexican American men from NHANES III and 507 non-Hispanic white, 187 non-Hispanic black, and 216 Mexican American men from NHANES 1999 to 2004.

B. Serum Estradiol

We used data on serum estradiol and SHBG, which were previously measured [11, 14] at Boston Children's Hospital with a competitive electrochemiluminescence immunoassay on the 2010 Elecsys autoanalyzer (Roche Diagnostics, Indianapolis, IN). Because the NHANES III and NHANES 1999 to 2004 specimens were assayed at different times, a comparability study was previously conducted; the coefficient of variation (CV) for estradiol concentrations for a subset of the NHANES III samples compared the original measurement and the measurement of the same NHANES III samples assayed at the time as the continuous NHANES sample were assayed. The CV was 15.3% [11]. Albumin was measured as part of the NHANES protocol with a Beckman Synchron LX20. Free estradiol was calculated by mass action with each man's estradiol, SHBG, and albumin concentrations [15].

C. Covariates

Covariates were obtained from the NHANES examinations and interviews. Age, race, and cigarette smoking status were self-reported. We maintained the same definitions for race as in the NHANES III and the NHANES 1999 to 2004. We defined nonsmokers as never smokers (smoked <100 cigarettes over the lifetime) and former smokers. Body mass index (BMI) was calculated by dividing weight in kilograms by the square of height (measured to the nearest 0.1 cm) in meters. WC was measured along the iliac crest to the nearest 0.1 cm at minimal respiration. Common chronic diseases were self-reported in response to questions on whether a doctor ever told them they had heart failure or heart attack, diabetes, or cancer. Use of prescription medications in the last 30 days was determined by interview along with physical container confirmation, where possible.

D. Statistical Analysis

After sampling weights were applied, separately for NHANES III and NHANES 1999 to 2004 and by younger (20 to 39 years), middle (40 to 59 years), and older (≥60 years) adult age, the mean and SE (calculated via the Taylor series linearization method), 10th percentile, 25th percentile, median, 75th percentile, and 90th percentile of total and estimated free estradiol were calculated overall and among men categorized as follows: men with both BMI <25 kg/m^2 and WC <102 cm [16]; men without a physician diagnosis of congestive heart failure, heart attack, stroke, diabetes, or cancer; and men with both BMI <25 kg/m^2 and WC <102 cm and without congestive heart failure, heart attack, stroke, diabetes, or cancer. Next, we repeated these analyses excluding current smokers, because in our previous work in NHANES III, current smokers had higher estradiol concentrations than both never and former smokers, who had similar mean total and free estradiol [17]. Finally, we repeated all these analyses stratified by race and ethnicity.

2. Results

In the NHANES III study population, 39.0% were 20 to 39 years, 29.8% were 40 to 59 years, and 31.2% were ≥60 years old; 46.0% were non-Hispanic white, 24.6% were non-Hispanic black, and 25.5% were Mexican American; 68.5% were nonsmokers; 41.5% had a BMI <25 kg/m^2; and 69.4% had a WC <102 cm (Table 1). In the NHANES 1999 to 2004 study population, 36.2% were 20 to 39 years, 31.6% were 40 to 59 years, and 32.2% were ≥60 years old; 51.8% were non-Hispanic white, 19.1% were non-Hispanic black, and 22.1% were Mexican American; 73.3% were nonsmokers; 30.9% had a BMI <25 kg/m^2; and 58.2% had a WC <102 cm (Table 1).

A. Total Estradiol

Median total estradiol concentrations in both NHANES III and NHANES 1999 to 2004 are listed in Table 2, including median concentrations for nonsmokers alone. All results stated

Table 1. Characteristics of Men Who Participated in NHANES III (1988–1994) and Continuous NHANES (1999– 2004)

	NHANES III	NHANES 1999–2004
N	1462	978
Age group, N (%)		
20–39 y	570 (39.0%)	354 (36.2%)
40–59 y	436 (29.8%)	309 (31.6%)
≥60 y	456 (31.2%)	315 (32.2%)
Race and ethnicity, N (%)		
Non-Hispanic white	673 (46.0%)	507 (51.8%)
Non-Hispanic black	360 (24.6%)	187 (19.1%)
Mexican American	373 (25.5%)	216 (22.1%)
Other	56 (3.8%)	68 (7.0%)
BMI <25 kg/m^2, N (%)	607 (41.5%)	302 (30.9%)
WC <102 cm, N(%)	1014 (69.4%)	569 (58.2%)
Nonsmokers,[a] N (%)	1001 (68.5%)	717 (73.3%)
No comorbidities,[b] N (%)	1200 (82.1%)	743 (76.0%)

[a]Nonsmokers are never and former smokers.
[b]No congestive heart failure, heart attack, stroke, diabetes, or cancer.

below refer to Table 2, and unstated full distributions are listed in the supplemental tables [18]. For all men, the respective median total estradiol concentrations among men 20 to 39, 40 to 59, and ≥60 years old were 37.0, 33.9, and 33.5 pg/mL in NHANES III and 31.3, 30.5, and 27.0 pg/mL in NHANES 1999 to 2004 [18]. For lean men without comorbidities, medians were similar to those for all men in both NHANES III and NHANES 1999 to 2004: respective median total estradiol concentrations were 37.5, 33.2, and 34.5 pg/mL in NHANES III and 32.4, 29.6, and 29.8 pg/mL in NHANES 1999 to 2004. For nonsmoking men, total estradiol concentrations were generally slightly lower than for all men: the respective median total

Table 2. Median Total Estradiol by Age Among All Men and Among Men Without Health States That Influence Estradiol, NHANES III (1988–1994) and NHANES 1999–2004

	Median Total Estradiol (pg/mL)		
	20–39 y	40–59 y	60+ y
NHANES III (1988–1994)			
All men	37.0	33.9	33.5
Lean men[a]	37.5	33.2	32.6
Men without comorbidities[b]	37.0	33.8	32.7
Lean men without comorbidities	37.5	33.2	34.5
Nonsmoking men[c]	33.8	32.2	32.6
Nonsmoking lean men	32.1	32.1	31.9
Nonsmoking men without comorbidities	33.7	32.2	31.9
Nonsmoking lean men without comorbidities	32.0	32.1	32.0
NHANES 1999–2004			
All men	31.3	30.5	27.0
Lean men	32.4	29.5	27.2
Men without comorbidities	31.4	30.1	26.8
Lean men without comorbidities	32.4	29.6	29.8
Nonsmoking men	29.7	26.5	26.6
Nonsmoking lean men	29.0	24.1	23.4
Nonsmoking men without comorbidities	29.7	26.4	25.7
Nonsmoking lean men without comorbidities	29.1	22.7	26.1

[a]BMI <25 kg/m^2 and WC <102 cm.
[b]Without congestive heart failure, heart attack, stroke, diabetes, or cancer.
[c]Never or former smokers.

estradiol concentrations were 33.8, 32.2, and 32.6 pg/mL in NHANES III and 29.7, 26.5, and 26.6 pg/mL in NHANES 1999 to 2004 [18]. For nonsmoking, lean men without comorbidities in NHANES III, median total estradiol concentrations were similar to those for all men except 20- to 39-year-old men, whereas in NHANES 1999 to 2004, medians were lower for middle-aged and older men, although the median appeared similar for men 20 to 29 years old: the respective median total estradiol concentrations were 32.0, 32.1, and 32.0 pg/mL in NHANES III and 29.1, 22.7, and 26.1 pg/mL in NHANES 1999 to 2004.

B. Estimated Free Estradiol

Median free estradiol concentrations in both NHANES III and NHANES 1999 to 2004 are listed in Table 3, including median concentrations in nonsmokers alone. All results stated below refer to Table 3, and unstated full distributions are listed in the supplemental tables [18]. The analysis for estimated free estradiol was based on the 1445 men (98.8%) in NHANES III and 973 men (99.8%) in NHANES 1999 to 2004 who additionally had SHBG concentrations available. For all men, the respective median free estradiol concentrations among men 20 to 39, 40 to 59, and ≥60 years old were 0.82, 0.72, and 0.64 pg/mL in NHANES III and 0.67, 0.61, and 0.47 pg/mL in NHANES 1999 to 2004 [18]. For lean men without comorbidities, free estradiol concentrations were slightly lower than for all men in both NHANES III and NHANES 1999 to 2004: the respective medians were 0.78, 0.67, and 0.65 pg/mL in NHANES III and 0.64, 0.54, and 0.48 pg/mL in NHANES 1999 to 2004. For nonsmoking men, free estradiol concentrations were generally lower than concentrations for all younger and middle-aged men in NHANES III and middle-aged men in NHANES 1999 to 2004, whereas for older men free estradiol concentrations were similar in both NHANES III and NHANES 1999 to 2004: the respective medians were 0.75, 0.70, and 0.62 pg/mL in NHANES III and 0.68, 0.57, and 0.46 pg/mL in NHANES 1999 to 2004 [18]. For nonsmoking, lean men without comorbidities, median free estradiol concentrations were lower than for all men in both NHANES III and NHANES 1999 to 2004, except for older men in NHANES III,

Table 3. Median Free Estradiol by Age Among All Men and Among Men Without Health States That Influence Estradiol, NHANES III (1988–1994) and NHANES 1999–2004

	Median Free Estradiol (pg/mL)		
	20–39 y	**40–59 y**	**60+ y**
NHANES III (1988–1994)			
All men	0.82	0.72	0.64
Lean men[a]	0.78	0.68	0.63
Men without comorbidities[b]	0.82	0.72	0.64
Lean men without comorbidities	0.78	0.67	0.65
Nonsmoking men[c]	0.75	0.70	0.62
Nonsmoking lean men	0.64	0.67	0.60
Nonsmoking men without comorbidities	0.75	0.69	0.62
Nonsmoking lean men without comorbidities	0.64	0.67	0.62
NHANES 1999–2004			
All men	0.67	0.61	0.47
Lean men	0.64	0.54	0.41
Men without comorbidities	0.67	0.61	0.47
Lean men without comorbidities	0.64	0.54	0.48
Nonsmoking men	0.68	0.57	0.46
Nonsmoking lean men	0.58	0.44	0.35
Nonsmoking men without comorbidities	0.67	0.57	0.44
Nonsmoking lean men without comorbidities	0.58	0.42	0.40

[a]BMI <25 kg/m^2 and WC <102 cm.
[b]Without congestive heart failure, heart attack, stroke, diabetes, or cancer.
[c]Never or former smokers.

whose concentrations were similar: respective medians were 0.64, 0.67, and 0.62 pg/mL in NHANES III and 0.58, 0.42, and 0.40 pg/mL in NHANES 1999 to 2004. Given that free estradiol was calculated with SHBG, we also provide distributions for SHBG in an online repository [18].

C. Race and Ethnicity

C-1. Estimated total estradiol

Median total estradiol concentrations in both NHANES III and NHANES 1999 to 2004 by race and ethnicity are listed in Table 4, including median concentrations in nonsmokers alone (unstated full distributions are listed in an online repository [18]). For lean men without comorbidities, median total estradiol concentrations were not consistently different from those for all men within their respective race and ethnicity group (Table 4). For nonsmoking, lean men without comorbidities, median total estradiol concentrations were generally lower than for all men within their respective race and ethnicity group, except among non-Hispanic black men in NHANES 1999 to 2004 (Table 4).

Table 4. Median Total Estradiol by Age and Race and Ethnicity Among All Men and Among Men Without Health States That Influence Estradiol, NHANES III (1988–1994) and NHANES 1999–2004

	Median Total Estradiol (pg/mL)		
	20–39 y	**40–59 y**	**60+ y**
NHANES III (1988–1994)			
Non-Hispanic white			
All men	36.5	33.6	34.0
Lean men[a] without comorbidities[b]	36.4	32.7	35.3
Nonsmoking men[c]	33.2	32.3	33.2
Nonsmoking lean men without comorbidities	31.5	32.1	32.8
Non-Hispanic black			
All men	45.5	39.0	38.8
Lean men without comorbidities	44.8	38.2	31.9
Nonsmoking men[c]	44.8	35.7	36.5
Nonsmoking lean men without comorbidities	44.8	35.3	28.1
Mexican American			
All men	33.7	32.4	32.8
Lean men without comorbidities	33.8	35.9	34.5
Nonsmoking men[c]	32.3	30.8	32.8
Nonsmoking lean men without comorbidities	31.7	31.6	35.0
NHANES 1999–2004			
Non-Hispanic white			
All men	28.1	30.9	26.7
Lean men without comorbidities	29.7	29.1	26.5
Nonsmoking men[c]	28.3	25.8	25.6
Nonsmoking lean men without comorbidities	27.9	20.1	21.5
Non-Hispanic black			
All men	36.5	36.9	33.3
Lean men without comorbidities	41.3	35.3	30.7
Nonsmoking men[c]	35.0	35.7	34.0
Nonsmoking lean men without comorbidities	38.6	19.5	38.1
Mexican American			
All men	28.1	25.2	22.3
Lean men without comorbidities	28.9	20.5	8.6
Nonsmoking men[c]	27.2	23.3	22.3
Nonsmoking lean men without comorbidities	27.4	17.3	5.9

[a]BMI < 25 kg/m^2 and WC < 102 cm.
[b]Without congestive heart failure, heart attack, stroke, diabetes, or cancer.
[c]Never or former smokers.

C-2. Estimated free estradiol

Median free estradiol concentrations in both NHANES III and NHANES 1999 to 2004 by race and ethnicity are listed in Table 5, including median concentrations in nonsmokers alone (unstated full distributions are listed in an online repository [18]). For lean men without comorbidities, median free estradiol concentrations were lower than for all men within their respective race and ethnicity group, except in NHANES III among men 60+ years old who are non-Hispanic white and Mexican American (Table 5). The same patterns were present for nonsmoking, lean men without comorbidities (Table 5).

3. Discussion

Using data from two US nationally representative surveys conducted about 30 and 15 to 20 years ago, we reported serum total and estimated free estradiol concentrations in younger, middle-age, and older healthy men. Because of the recognized differences in estradiol concentration between racial and ethnic groups [10], including in NHANES III and continuous

Table 5. Median Free Estradiol by Age and Race and Ethnicity Among All Men and Among Men Without Health States That Influence Estradiol, NHANES III (1988–1994) and NHANES 1999–2004

	Median Free Estradiol (pg/mL)		
	20–39 y	**40–59 y**	**60+ y**
NHANES III (1988–1994)			
Non-Hispanic white			
All men	0.80	0.72	0.64
Lean men[a] without comorbidities[b]	0.75	0.67	0.65
Nonsmoking men[c]	0.73	0.70	0.63
Nonsmoking lean men without comorbidities	0.62	0.67	0.63
Non-Hispanic black			
All men	0.95	0.78	0.72
Lean men without comorbidities	0.90	0.73	0.54
Nonsmoking men[c]	0.93	0.75	0.64
Nonsmoking lean men without comorbidities	0.92	0.68	0.49
Mexican American			
All men	0.75	0.69	0.63
Lean men without comorbidities	0.69	0.61	0.66
Nonsmoking men[c]	0.70	0.68	0.63
Nonsmoking lean men without comorbidities	0.66	0.58	0.69
NHANES 1999–2004			
Non-Hispanic white			
All men	0.66	0.61	0.45
Lean men without comorbidities	0.63	0.50	0.40
Nonsmoking men[c]	0.68	0.55	0.44
Nonsmoking lean men without comorbidities	0.59	0.38	0.33
Non-Hispanic black			
All men	0.82	0.74	0.61
Lean men without comorbidities	0.74	0.66	0.52
Nonsmoking men[c]	0.79	0.74	0.63
Nonsmoking lean men without comorbidities	0.71	0.36	0.64
Mexican American			
All men	0.61	0.51	0.44
Lean men without comorbidities	0.60	0.34	0.16
Nonsmoking men[c]	0.59	0.51	0.43
Nonsmoking lean men without comorbidities	0.57	0.31	0.11

[a]BMI < 25 kg/m^2 and WC < 102 cm.
[b]Without congestive heart failure, heart attack, stroke, diabetes, or cancer.
[c]Never or former smokers.

NHANES 1999 to 2004 [11, 14], we also reported age-specific serum total and free estradiol concentrations among healthy men by racial and ethnic groups (non-Hispanic white, non-Hispanic black, and Mexican American). The context for this work was to provide information that could be used in monitoring estradiol levels during testosterone supplementation and for good health in general.

One purpose of our report is to inform the monitoring of serum estrogen in men receiving testosterone supplementation. Although the literature on the influence of testosterone supplementation on estrogen levels is not extensive, the influence of testosterone supplementation on estradiol levels is supported by the fact that some men who receive testosterone therapy develop estrogen-related symptoms such as gynecomastia and by direct measurements of their serum estradiol concentrations. For example, in a 2015 study by Tan *et al.* [19], of 34,016 men treated for low testosterone across 35 US sites, 20.2% had high estradiol levels, defined as >42.6 pg/mL as measured by electrochemiluminescence immunoassay.

Risks of supplemental testosterone, including estrogen-associated symptoms, have been discussed extensively in the literature and summarized in consensus practice guidelines [5]. However, other possible consequences of increased estrogen levels, whether caused by testosterone supplementation or other factors, have been not been discussed in the same depth. Some studies have reported that estrogen influences carcinogenesis via genomic and nongenomic processes. For example, estrogens can produce DNA damage after being metabolized to catechols [20, 21]. Of particular interest for older men, evidence from animal studies suggests a synergistic role for estrogens in prostate carcinogenesis [6, 22]. In rats, although prostate cancer can be induced by androgens alone, a higher chance of inducing prostate cancer occurs when the combination of androgens and estrogen is administered [6, 22]. However, a large, pooled analysis of epidemiologic studies on circulating estrogens measured once in middle or older age and prostate cancer risk does not support a link [23]. Nevertheless, elevated estrogens, irrespective of the cause, could generate chronic, systemic inflammation; men with higher estrogens have higher white blood cell counts and C-reactive protein concentrations and consequent adverse effects on health in general [7]. Although it remains unknown whether circulating estradiol levels influence intraprostatic inflammation in men, as has been observed in rodents [24], such a possibility is concerning given that intraprostatic inflammation has been associated with an elevated risk of prostate cancer [25, 26].

We reported medians and distributions of estradiol concentrations in healthy men who participated in the morning sessions of phase I of NHANES in 1988 to 1991 or in continuous NHANES in 1999 to 2004. Although the medians were different, patterns of total and free estradiol concentrations were similar at these two time points. We reported data separately by time point because members of our team previously published that estradiol concentrations, adjusted for BMI, WC, and smoking, decreased between the years of these two surveys among non-Hispanic white and Mexican American men [11]. These declines could be the result of secular changes in the prevalences of factors that directly or indirectly influence serum estradiol, although we could not rule out time differences. However, the samples were assayed in the same laboratory by the same method. As noted above, a small number of the NHANES III samples were reanalyzed when the NHANES 1999 to 2004 samples were assayed, and there was a CV of 15.3%, which was not unexpected for this analyte, which has a low concentration in men [11].

To capture estradiol levels in healthy men, we made several restrictions. First, we restricted the analysis to men who were lean. We have previously published in NHANES III and NHANES 1999 to 2004 on factors associated with estradiol levels, including adiposity [27, 28]. Thus, to capture estradiol levels in men, we restricted the analysis to men who were the most likely to be lean, based on having both a normal BMI and a normal WC. We also restricted the analysis to men who were more likely to be healthy, specifically those without diagnoses of cardiovascular disease, diabetes, and cancer. Finally, we restricted the analysis to men who did not currently smoke, given that male smokers tend to have higher total and free estradiol, including men in NHANES III [17].

Estrogen levels, especially free estradiol, generally decrease with age in men. We previously reported decreases cross-sectionally across age in NHANES III [29] and in NHANES 1999 to 2004 [11]. Age-dependent decreases in estradiol may be partially due to age-related decreases in testosterone and increases in SHBG [30–32]. However, these estrogen-reducing events may be countered by the increasing prevalence of proestrogenic states with age, especially age-dependent increases in body fat and in the prevalence of common chronic diseases such as cardiovascular disease and cancer. Up to 80% of estrogen is produced outside the testes through the aromatization of testosterone in adipose cells; thus, the production of estradiol depends on the amount of body fat in men [33].

In the United States, the prevalence of conditions that decrease testosterone levels [27, 34] continues to rise, and thus the target group of men for testosterone supplementation is expanding. For example, according to the National Center for Health Statistics, the prevalence of diabetes (diagnosed and undiagnosed) in adult men increased from 8.6% in 1988 to 1994 to 13.2% in 2011 to 2014, and the prevalence of obesity in men ages ≥20 years increased from 19.5% in 1988 to 1994 to 34.5% in 2013 to 2014 [35]. Therefore, as chronic diseases that influence the likelihood of testosterone insufficiency become more prevalent in adult men, it may be important to consider the possible implications of any increase in the prescription of testosterone supplementation on the resultant increases in estradiol in older men.

Given the age-estrogen decline, an open question is whether older adult men should have the same estradiol levels as younger men for optimal overall and metabolic health, which tends to deteriorate with age. *A priori,* we decided to present total and free estradiol concentrations stratified by age irrespective of whether the optimal target turns out to be levels in younger men (higher concentration) or older men (lower concentration).

This study reports on typical estrogen levels in men without common proestrogenic, proandrogenic, or antiandrogenic states in a nationally representative sample of men overall and by race and ethnicity. Other studies have reported estrogen levels by age but not specifically in healthy men. The most extensive of these reports is a cohort of >10,000 men of predominantly European ancestry ages 35 to 100 years old, pooled from three population-based studies of community-dwelling men in three Australian cities [36]. In that study, median total estradiol concentrations of men aged <65, 65 to <75, 75 to <85, and ≥85 years old were 88 pmol/L (24 pg/mL), 77 pmol/L (21 pg/mL), 81 pmol/L (22 pg/mL), and 85 pmol/L (23 pg/mL), respectively. In a study of 572 German men who were blood donors and older sports club members with BMI <30 kg/m^2 and without chronic illness, mean total estradiol levels (mean ± SD) varied by age decade (20 to 29, 30 to 39, 40 to 49, 50 to 59, 60 to 69, and 70 to 80 years), and were 102.9 ± 23.43 (28.03 ± 6.38 pg/mL), 94.17 ± 18.01 (25.65 ± 4.91 pg/mL), 90.76 ± 18.33 (24.72 ± 4.99 pg/mL), 81.02 ± 15.33 (22.07 ± 4.12 pg/mL), 78.82 ± 16.33 (21.47 ± 4.45 pg/mL), and 80.37 ± 17.14 (21.89 ± 4.67 pg/mL) pmol/L, respectively [37]. The medians and means in all men and in nonsmoking lean men in NHANES III and in NHANES 1999 to 2004, which measured estradiol by electrochemiluminescence immunoassays, were notably higher than the medians in the Australian study, which measured estradiol by using liquid chromatography–mass spectrometry [36], and the means in the German study, which measured estradiol via radioimmunoassay [37].

The major strength of our study is the use of NHANES data. Both NHANES III and NHANES 1999 to 2004 used stringent sampling designs, and we applied sampling weights so that the findings can be generalized to the US population. Because of the wealth of information available in NHANES and because factors influencing estradiol had been previously studied in NHANES, to generate typical medians and distributions for healthy men, we were able to make exclusions based on factors observed to influence estradiol. Nevertheless, our study has several aspects that warrant discussion. Although we used data on serum estradiol measured in the same laboratory with the same assay method, mass spectrometry is the current standard method for research. Nevertheless, the laboratory method in the current study is commonly used clinically, and the kits that were used are approved by the US Food and Drug Administration for clinical use. Measurement of serum total estradiol tends to be less precise than that of other hormones because of its low

concentration in men. Although we estimated free estradiol, the estimation method used each man's measured concentration of SHBG and albumin. Because we used existing data, sample sizes were fixed, which led to small counts for some analyses when stratified by race and ethnicity. We were limited to the most prevalent racial and ethnic groups in the NHANES surveys of 1998 to 1991 and 1999 to 2004; thus, we were not able to provide information relevant to Asian men or American Indian/Alaska Native men.

In conclusion, we assessed the age-specific distributions of total and free estradiol in NHANES III and 1999 to 2004. Our goal was to report typical estradiol levels in a nationally representative population of healthy men to support the implementation of clinical guidelines for serum estradiol concentrations for men with symptomatic testosterone deficiency who are candidates for testosterone supplementation, and for men in general for good health. With respect to the former use, we envision that consensus guideline developers might need this information. With respect to the latter use, clinical consensus would be needed to define the array of estrogen-associated health states that should be considered and how their relative harms and benefits should be included when optimizing target serum estradiol levels for good health in general based on never-smoking, lean men without comorbidities. Given the recognized racial and ethnic variability in circulating estradiol levels, more work is needed, including by future measurement of estradiol concentrations in existing samples from more recent NHANES surveys, to report with precision population-specific estradiol levels.

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Additonal Information

Correspondence: Elizabeth A. Platz, ScD, MPH, Department of Epidemiology, Johns Hopkins Bloomberg School of Public Health, 615 North Wolfe Street, Room E6132, Baltimore, Maryland 21205. E-mail: eplatz1@jhu.edu.

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Data Availability: NHANES data are publicly available.

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