

## Reference Ranges for Serum Dehydroepiandrosterone Sulfate and Testosterone in Adult Men

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**ABSTRACT:** Dehydroepiandrosterone (DHEA) is the main adrenal androgen, which mostly exists in a sulfated version (DHEAS). Both DHEA and DHEAS are metabolic intermediates in the biosynthesis of the male sex hormone testosterone. In men, testosterone is involved in the regulation of fertility, libido, and muscle mass and is valuable for the assessment of gonadal, adrenal, and pituitary function and for the diagnosis of hypogonadism. The objective of the present study was to calculate age-specific reference ranges for serum DHEAS and serum testosterone using 1) linear regression and the mean  $\pm$  1.96 standard deviation concept and 2) quantile regression. From the cross-sectional Study of Health in Pomerania a total of 1078 men aged 20–79 years were included in the analyses. Serum DHEAS and testosterone levels were quantified using IMMULITE 2500 immunoassays. Linear and quantile regression were performed to calculate age-specific reference ranges. Both

statistical methods generated different results: The reference ranges based on linear regression identified 17 men (1.6%) with DHEAS levels and 45 men (4.2%) with serum testosterone levels outside the reference range. Using quantile regression, 54 men (5.0%) and 50 men (4.6%) with serum DHEAS and testosterone levels outside the range were detected, respectively. The present study established age-specific reference ranges for serum DHEAS and testosterone levels for men. Quantile regression should be preferred to calculate reference ranges; a better concordance with original data is possible because no distribution assumption is required and the robustness against outliers is given.

**Key words:** Total testosterone, dehydroepiandrosterone sulfate, reference ranges, quantile regression, Study of Health in Pomerania (SHIP).

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Dehydroepiandrosterone (DHEA) is a key steroid predominantly produced in the adrenal gland. The main fraction of DHEA is sulfated to dehydroepiandrosterone sulfate (DHEAS). Although DHEA levels follow a circadian rhythm with a peak in the morning, DHEAS levels are relatively stable throughout the day (Nieschlag et al, 1973; Rosenfeld et al, 1975) and thereby are used as markers for DHEA levels and adrenal androgen secretion (Korth-Schutz et al, 1976; Lobo et al, 1981). Both DHEA and DHEAS are metabolic intermediates in the biosynthesis of other

steroids like the principal male sex hormone testosterone. A high proportion of testosterone is bound to either albumin or sex hormone-binding globulin (SHBG) and less than 2% exists as free testosterone. In men, testosterone is involved in the regulation of fertility, libido, and muscle mass. Furthermore, testosterone serves as an important marker for gonadal, adrenal, and pituitary function and for the diagnosis of hypogonadism. Additionally, DHEA and DHEAS are precursor steroids not only for testosterone but also for androgen and estrogen in males. For example, one study suggested that the administration of DHEA results in increased estrogen concentration in men (Arlt et al, 1999).

In adulthood, a decline in serum total testosterone with increasing age is well documented (Leifke et al, 2000; Harman et al, 2001; Liu et al, 2007) and has been implicated in a wide variety of physiological changes of the aging male. Several studies showed that low testosterone levels are associated with depression, loss of muscle tone, increased abdominal fat, low bone density, reduced sexual function, Alzheimer disease, and heart disease (Chute et al, 1987; Phillips et al, 1994; Morley, 2001; Tan and Pu, 2003; Carnahan and Perry, 2004; Rucker et al, 2004).

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For the use of serum DHEAS and serum testosterone as diagnostic markers or for monitoring testosterone therapy, age-dependent reference values are necessary. Currently available reference values of serum DHEAS were calculated only for children or young adults (Elmlinger et al, 2002), for very old subjects (Birkenhager-Gillesse et al, 1994), or by using nonrepresentative samples of adult men (Elmlinger et al, 2003; IMMULITE 2500 DHEA-SO<sub>4</sub>, 2004). Hence, the representativity of such studies remains low. Regarding serum testosterone, a wider range of reference values are available, especially for adult men (Elmlinger et al, 2003, 2005; Schatzl et al, 2003; Boyce et al, 2004; IMMULITE 2500 Total Testosterone, 2004; Mohr et al, 2005; Okamura et al, 2005). However, almost all available reference values are not adequately adjusted for age (Boyce et al, 2004; IMMULITE 2500 Total Testosterone, 2004; Okamura et al, 2005) and refer to nonrepresentative samples of men (Schatzl et al, 2003; Elmlinger et al, 2005).

Furthermore, we demonstrated recently that the use of sufficient statistical method has an important influence on the accuracy of reference values (Friedrich et al, 2008). Although the general concept of mean  $\pm$  2 SD assumes a normal Gaussian distribution of the analyte and the calculation of SD is highly sensitive to outliers, a nonparametric approach of quantile regression analysis reaches a better concordance to the data and covers exactly the central 95% range.

The objective of the present study was to calculate age-specific reference values for serum DHEAS and serum testosterone using 1) linear regression and the mean  $\pm$  2 SD concept and 2) the quantile regression method in a population-based sample of German men.

## Material and Methods

### Study Population

The Study of Health in Pomerania (SHIP) is a cross-sectional population-based survey of the adult population, which was conducted in the northeast of Germany (John et al, 2001). From the entire population of 212 157 inhabitants living in the area a sample was selected from the population registration offices, where all German inhabitants are registered. A 2-stage cluster sampling method was adopted from the WHO MONICA Project Augsburg, Germany (Keil et al, 1988). A representative sample of 7008 men and women, aged 20–79 years, with 292 individuals of each gender in each of the twelve 5-year age strata, was drawn. Data collection started in October 1997 and was finished in May 2001. A total of 4310 individuals (2177 men; 2193 women; 68.8% of eligible subjects) participated in this study. All participants were investigated in 1 of 2 health examination centers established for the purpose of the study. All participants gave written informed consent.

The study conformed to the principles of the Declaration of Helsinki as reflected by an a priori approval of the Ethics Committee of the University of Greifswald.

Of the 2117 men, 524 men were excluded because of the presence of at least 1 of the following diseases (overlap exists): diabetes mellitus (n = 186), renal diseases (n = 134), liver diseases (n = 124), chronic obstructive pulmonary disease (n = 135), diseases of the pituitary gland (n = 1), and all subjects older than 45 years with a fractional shortening less than 20% (n = 18). In addition, all medications taken in the last 7 days were recorded and categorized in a standardized fashion using the Anatomical Therapeutic Chemical (ATC) classification index, which classifies drugs on the basis of the target organ or system and on the therapeutic and chemical characteristics of the drug (Fricke and Güntler, 2002). Thirty-one men who received sexual hormones (ATC G03), testosterone 5 $\alpha$  reductase inhibitors (G04CB), sexual hormone antagonists (L02B), ketoconazole (D01AC08, G01AF11, J02AB02), spironolactone (C03DA01, C03EC01, C03EC21, C03EC41, C03ED01), anabolic steroids (A14A), glucocorticoids (H02AB), or opiates (N02A) were identified and therefore excluded. In more detail (overlap exists): testosterone (G03BA03; n = 1), finasteride (G04CB01; n = 2), flutamide (L02BB01, n = 1), spironolactone (C03DA01, C03EC41; n = 1), methylprednisolone (H02AB04; n = 1), prednisolone (H02AB06; n = 6), prednisone (H02AB07; n = 2), triamcinolone (H02AB08, H02AB58; n = 2), morphine (N02AA01, n = 1), codeine (N02AA66, N02AA69, n = 4), tramadole (N02AX02; n = 1), and tilidine (N02AX51; n = 1). None of the subjects reported use of anabolic steroids (A14A) or received chemotherapy (D06B, D06C). Men with a daily alcohol intake higher than 60 g were also excluded (n = 88). Furthermore, we excluded all subjects with a body mass index (BMI) greater than 30 or less than 18 kg/m<sup>2</sup> (n = 328) and with missing data for serum testosterone or DHEAS levels (n = 68). Altogether the final study population comprised 1078 men who were included in the present analyses.

### Measurements

A computer-aided personal interview was used to collect information on medical history, behavioral, and socio-demographic characteristics. BMI was calculated in kg/m<sup>2</sup>. Alcohol consumption was assessed using a drink-specific quantity-frequency measure. Average alcohol consumption (in grams per day) was calculated by multiplying frequency and amount of alcohol from beer, wine, and spirits, respectively, using a standard ethanol content of 4.8% (by volume) in beer, 11% (by volume) in wine and 33% (by volume) in spirits to conversion (Bühninger et al, 2002). The definition of diabetes based on self-reported physician's diagnosis or self-reported use of antidiabetic medication (ATC A10) in the last 7 days. Diseases of the pituitary gland were diagnosed as self-reported intake of pituitary gland or hypothalamus hormones (ATC H01). A blood sample was drawn from the cubital vein in the supine position. Serum aliquots were prepared for immediate analysis and for storage at –80°C for further analysis. From fresh serum, creatinine levels were determined with the Jaffé method (Hitachi 717,

Table 1. Serum testosterone levels by time blood was drawn

	Serum Testosterone, nmol/L	
	Blood Drawn Before Noon (n = 665)	Blood Drawn After Noon (n = 413)
Mean (SD)	18.0 (5.7)	16.5 (5.7)
Percentile		
97.5	31.0	28.9
90	25.2	23.4
75	21.3	19.3
50	17.6	15.8
25	14.0	13.0
10	11.4	10.4
2.5	8.9	7.6

Roche Diagnostics GmbH, Mannheim, Germany). Creatinine clearance (CrCl) was estimated using the Cockcroft-Gault formula. Renal diseases were defined on self-reported renal diseases or a CrCl of less than 50. Gamma-glutamyl transferase (GGT), aspartate aminotransferase (ASAT), and alanine aminotransferase (ALAT) levels were measured photometrically (Hitachi 717). The definition of liver diseases was based on self-reported liver cirrhosis or atrophy of the liver. Additionally, all subjects with ASAT, ALAT, or GGT levels greater than the population mean + 2 SD were classified as subjects with liver diseases. Chronic obstructive pulmonary disease was defined as productive cough for at least 3 consecutive months during the past 12 months.

DHEAS and total testosterone levels were measured from frozen serum aliquots using competitive chemiluminescent enzyme immunoassays on an IMMULITE 2500 analyzer (Siemens IMMULITE 2500 DHEA-SO<sub>4</sub>, ref L5KDS, lot 106, and Siemens IMMULITE 2500 Total Testosterone, ref L5KWT, lot 110; Siemens Healthcare Medical Diagnostics, Bad Nauheim, Germany). Measurement was carried out from December 2005 to January 2006. An aliquot of 2 alternating levels of a third-party commercial control material (Bio-Rad Lymphochek Immunoassay Plus Control, lots 40151 and 40152; Bio-Rad, Munich, Germany) was included in each series in single determination. During the course of the study the interassay coefficient of variation was 14.0% with a systematic deviation of +0.21% at the 48 µg/dL level and 8.4% with a systematic deviation of -5.0% at the 128 µg/dL level in the DHEAS assay. In the total testosterone assay the interassay coefficient of variation was 13.2% with a systematic deviation of +2.3% at the 3.2 nmol/L level, and 8.9% with a systematic deviation of +0.24% at the 22.5 nmol/L level. All assays were performed according to the manufacturers' recommendations by skilled technical personnel. In men who had blood drawn before noon, serum testosterone levels were on average 1.4 nmol/L higher than in men with blood drawn after noon (Table 1).

### Statistical Analyses

Continuous data are expressed as median (25th; 75th percentiles). For the evaluation of reference values of serum DHEAS and testosterone levels we performed 2 statistical

approaches: 1) linear regression and the mean  $\pm$  1.96 SD concept and 2) quantile regression, a statistical method for estimating models for the conditional median function and other conditional quantile functions (Koenker, 2005). Unlike the nonparametric quantile regression, linear regression is a parametric approach and assumes that the reference distribution follows a Gaussian distribution. For this reason an initial transformation of the serum DHEAS levels has been carried out. Transformations (log, powers between 0.2 and 0.8) were compared to determine the best conformance to the normal distribution of our DHEAS data. The best conformance was found for the log transformation and was constant over different age groups in both sexes. For serum testosterone levels no transformation was necessary. In both statistical approaches restricted cubic splines (Stone and Koo, 1985) were used to detect a possible nonlinear dependency of serum DHEAS and testosterone levels on age. Three knots were prespecified, located at the 10th, 50th, and 90th percentiles as recommended by Stone and Koo (1985), resulting in 1 component of the spline function: age'. In linear regression, reference curves for back-transformed DHEAS and testosterone data were calculated as  $exp[f_{DHEAS}(age) \pm 1.96SD]$  and  $f_T(age) \pm 1.96SD$  based on the SD of the transformed DHEAS and original testosterone data, respectively. In quantile regression, the 2.5th, 50th, and 97.5th percentiles of the serum DHEAS and testosterone levels were fitted.

All data were weighted to adjust for nonresponse and to reflect age-sex distribution of the European adult population (Gesundheitsberichterstattung des Bundes, 2006). PROC REG and PROC QUANTREG in SAS were used for statistical analyses (SAS version 9.1.3; SAS Institute, Inc, Cary, North Carolina).

## Results

For both serum DHEAS and serum testosterone levels an age-related decline was found (Table 2). In men aged 20–24 years, the DHEAS and testosterone levels were 286 µg/dL (226; 361 µg/dL) and 19.2 nmol/L (15.8; 24.2 nmol/L), and these levels decreased to 90 µg/dL (53; 125 µg/dL) and 15.5 nmol/L (12.5; 19.0 nmol/L), respectively, in men older than 74 years.

Given these results, we calculated age-dependent reference values for DHEAS and testosterone. Linear regression models for mean serum testosterone levels and after initial transformation for mean serum DHEAS levels were fitted (Table 3). The reference curves (Figure) corresponded to mean  $\pm$  1.96 SD. Quantile regression yielded for each of the 2.5th, 50th, and 97.5th percentiles 1 fitted model (Table 4). The calculated reference curves are also displayed in the Figure. Serum DHEAS and testosterone reference values for selected ages resulting from both statistical methods are given in Table 5. Both methods reflect the age-related decline in DHEAS and testosterone levels.

Table 2. Serum dehydroepiandrosterone sulfate and testosterone distribution by age groups

Age Group, y	n	Serum DHEAS, $\mu\text{g/dL}^a$	Serum Testosterone, nmol/L <sup>a</sup>
20–24	91	286 (226; 361)	19.2 (15.8; 24.2)
25–29	106	287 (217; 394)	18.5 (14.6; 21.7)
30–34	106	260 (195; 321)	18.6 (14.5; 22.0)
35–39	113	229 (174; 293)	17.2 (14.3; 20.5)
40–44	88	218 (157; 272)	15.9 (13.2; 19.3)
45–49	96	186 (135; 276)	16.5 (12.6; 20.0)
50–54	81	151 (109; 208)	16.1 (12.9; 20.1)
55–59	101	137 (103; 179)	15.3 (13.3; 18.7)
60–64	91	114 (77; 164)	16.0 (13.0; 20.8)
65–69	91	88 (58; 133)	16.7 (13.5; 20.5)
70–74	64	89 (62; 125)	16.4 (12.7; 19.9)
>74	50	90 (53; 125)	15.5 (12.5; 19.0)
Overall	1078	200 (129; 282)	17.1 (13.7; 21.0)

Abbreviation: DHEAS, dehydroepiandrosterone sulfate.

<sup>a</sup> Continuous data are given as median (25th percentile; 75th percentile) and were poststratified. To convert the values for DHEAS to  $\mu\text{mol/L}$ , multiply by 0.02714; to convert the values for testosterone to ng/dL, multiply by 28.82.

Comparison Between Linear and Quantile Regression

With respect to serum DHEAS levels (Figure) the upper reference limit based on linear regression was above and the lower reference limit below those that resulted from quantile regression. Thus, the reference range based on linear regression was wider over the full age range compared to the reference range calculated by quantile regression. Using the reference range derived from linear regression, 17 subjects had serum DHEAS levels outside the reference range (7 above and 10 below the reference range), whereas 54 subjects were diagnosed to have serum DHEAS levels outside the reference range (27 above and 27 below the reference range) using quantile regression.

The widths of the reference ranges based on linear and quantile regression for serum testosterone levels were comparable. However, both upper and lower reference limits calculated by linear regression were below those of quantile regression. Based on linear regression, 45

individuals had serum testosterone levels outside the reference range (37 above and 8 below the reference range), whereas the reference subjects data calculated by quantile regression detected 50 subjects who had levels outside

Table 3. Parameter estimates with standard error for linear regression models (for details, see "Material and Methods")

Independent Variable	Parameter Estimate	Standard Error	P
<b>Serum DHEAS</b>			
Intercept	6.1087	0.0781	<.01
Age	-0.0181	0.0023	<.01
Age'	$-4.02 \times 10^{-6}$	$1.24 \times 10^{-6}$	<.01
SD (95% CI)	0.601 (0.576; 0.627)		
<b>Serum testosterone</b>			
Intercept	23.1530	0.9774	<.01
Age	-0.1485	0.0286	<.01
Age'	$4.84 \times 10^{-5}$	$1.55 \times 10^{-5}$	<.01
SD (95% CI)	5.771 (5.537; 6.026)		

Abbreviations: CI, confidence interval; DHEAS, dehydroepiandrosterone sulfate; SD, standard deviation.

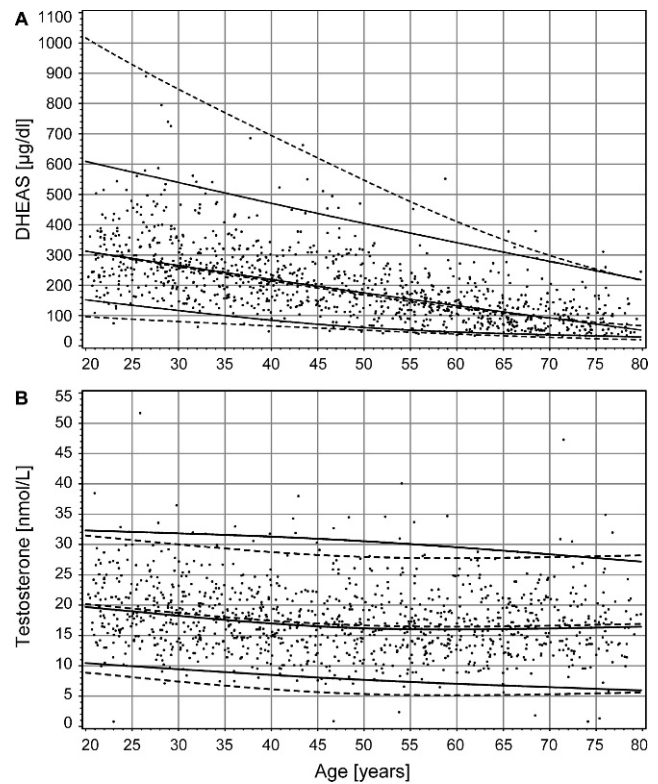


Figure. (A) Serum dehydroepiandrosterone sulfate (DHEAS) and (B) testosterone reference limits in men. Individual values (•) of 1078 men are displayed. Reference curves based on linear regression (---) for the fitted mean, mean  $\pm$  1.96 SD and on quantile regression (—) for the median, 2.5th percentile, and 97.5th percentile. To convert the values for DHEAS from  $\mu\text{g/dL}$  to  $\mu\text{mol/L}$ , multiply by 0.02714; to convert the values of testosterone from nmol/L to ng/dL, multiply by 28.82.

Table 4. Parameter estimates with 95% confidence interval for quantile regression models (for details, see "Material and Methods")

Percentile	Independent Variable	Parameter Estimate	95% Confidence Limits	
			Lower	Upper
<b>Serum DHEAS</b>				
2.5	Intercept	222.6565	181.0386	255.7550
	Age	-3.5340	-4.4495	-2.4627
	Age'	0.0008	0.0001	0.0010
50	Intercept	406.7163	372.9070	449.2625
	Age	-4.7039	-5.9423	-3.7989
	Age'	0.0002	-0.0002	0.0007
97.5	Intercept	748.2267	535.4029	1351.6610
	Age	-6.9575	-15.1482	-0.2341
	Age'	0.0002	-0.0032	0.0025
<b>Serum testosterone</b>				
2.5	Intercept	12.4415	7.5953	14.5206
	Age	-0.1001	-0.1857	-0.0178
	Age'	$1.31 \times 10^{-5}$	$-6.98 \times 10^{-5}$	$6.68 \times 10^{-5}$
50	Intercept	22.6711	19.9773	26.1437
	Age	-0.1478	-0.2440	-0.0724
	Age'	$4.81 \times 10^{-5}$	$8.46 \times 10^{-6}$	$8.77 \times 10^{-5}$
97.5	Intercept	33.2269	26.0972	48.6674
	Age	-0.0456	-0.3952	0.1631
	Age'	$-2.07 \times 10^{-5}$	$-1.26 \times 10^{-4}$	$1.63 \times 10^{-4}$

Abbreviation: DHEAS, dehydroepiandrosterone sulfate.

the reference range (25 above and 25 below the reference range; Figure).

## Discussion

In the present study, we established age-specific reference ranges for serum DHEAS and serum testosterone levels in a population-based sample of 1078 German men aged 20–79 years. For the calculation of the reference ranges we used 2 different statistical approaches.

### Serum DHEAS

Both methods reflect the documented age-related decline in serum DHEAS levels (Morley, 2001; Feldman et al, 2002; Elmlinger et al, 2003). However, the reference range based on linear regression is wider for young men and converges to the width provided by quantile regression in older men. Reference values for adult men are currently provided by the manufacturers (IMMULITE 2500 DHEA-SO<sub>4</sub>, 2004), who quote only the 5th and 95th percentiles, and a German study (Elmlinger et al, 2003), which also measured serum DHEAS levels with the IMMULITE system and determined the central 95% range. The reference limits based on quantile regression are well comparable to the reference limits of the German study (Elmlinger et

al, 2003). However, in younger subjects the upper reference limit calculated by linear regression exceeds the values of the German study by 5–13 µg/dL (Elmlinger et al, 2003).

### Serum Testosterone

Also for testosterone the results of both statistical methods show the known age trend (Leifke et al, 2000; Harman et al, 2001; Liu et al, 2007). The lower limits of the age-dependent reference ranges for serum testosterone levels based on quantile regression presented here are in good agreement with other studies (Elmlinger et al, 2003, 2005; Schatzl et al, 2003; Mohr et al, 2005) (Table 6). The upper reference limits in men older than 39 years based on quantile regression, however, were up to 10 nmol/L higher compared to these provided by the majority of these studies (Elmlinger et al, 2003, 2005; Schatzl et al, 2003). Nevertheless, 1 study (Mohr et al, 2005) provided similar upper reference limits to our results. Some of the studies (Elmlinger et al, 2003, 2005) calculated the central 95% range only for 10-year groups and not as a continuous function of age or using the mean ± 2 SD concept (Schatzl et al, 2003). The differences in reference ranges might be because of the use of nonrepresentative studies, different exclusion criteria, and relatively small sample sizes (Elmlinger et al, 2003, 2005; Schatzl et al, 2003).

Table 5. Serum dehydroepiandrosterone sulfate and reference ranges for men. Values were calculated on the basis of linear and quantile regression (for details, see "Material and Methods" and Figure)<sup>a</sup>

Selected Age	Linear Regression		Quantile Regression	
	-1.96 SD	+1.96 SD	2.5%	97.5%
<b>Serum DHEAS, µg/dL</b>				
20	96	1017	152	609
25	88	929	134	574
30	80	847	117	540
35	73	770	100	505
40	66	695	85	471
45	59	620	72	437
50	52	547	61	404
55	45	476	53	372
60	39	410	47	340
65	33	351	43	308
70	28	299	39	277
75	24	253	36	246
<b>Serum testosterone, nmol/L</b>				
20	8.9	31.5	10.4	32.3
25	8.1	30.8	9.9	32.1
30	7.4	30.0	9.4	31.9
35	6.7	29.3	9.0	31.6
40	6.1	28.8	8.5	31.3
45	5.7	28.3	8.1	31.0
50	5.3	28.0	7.7	30.6
55	5.2	27.8	7.3	30.1
60	5.1	27.8	7.0	29.5
65	5.2	27.8	6.7	29.0
70	5.3	27.9	6.5	28.4
75	5.4	28.1	6.2	27.8

Abbreviations: DHEAS, dehydroepiandrosterone sulfate; SD, standard deviation.

<sup>a</sup> To convert the values for DHEAS to µmol/L, multiply by 0.02714; to convert the values for testosterone to ng/dL, multiply by 28.82.

Gaussian vs Quantile Reference Limits

As previously noted (Friedrich et al, 2008), quantile regression has some statistical advantages (Buchinsky, 1998; Gannoun et al, 2002; Koenker, 2005) over linear regression, including 1) the nonparametric approach, by which no initial transformation is necessary; 2) the estimation of conditional quantile functions and the independence of a global distribution parameter like the SD; and 3) the robustness to outlier observations in the response variable. Our study reflects the latter point particularly for the upper reference limit of DHEAS (Figure). In young men, outliers led to an up to 400 µg/dL higher upper reference limit in linear regression compared to the limit calculated by quantile regression.

Reference ranges should cover the central 95% range of distribution, which denotes that 5% of subjects lie outside the reference range, with 2.5% above and 2.5% below. The reference ranges based on quantile regression fulfill this condition and detected 54 (5.0%) subjects (above: 27 [2.5%]; below: 27 [2.5%]) and 50 (4.6%) subjects (above: 25 [2.3%]; below: 25 [2.3%]) with serum DHEAS and serum testosterone levels outside the reference range, respectively. In contrast, linear regression revealed 17 (1.6%) subjects (above: 7 [0.6%]; below: 10 [0.9%]) and 45 (4.2%) subjects (above: 37 [3.4%]; below: 8 [0.7%]) who had serum DHEAS and serum testosterone levels outside the reference range, respectively.

The major strength of our study is the use of data from a large population-based sample of adult men, compared with former studies which used non-population-based or clinical human samples (Elmlinger et al, 2003, 2005; Schatzl et al, 2003; IMMULITE 2500

Table 6. Age-dependent reference ranges for serum testosterone levels in men provided by different international studies

	Mohr et al, 2005	Elmlinger et al, 2003	Elmlinger et al, 2005	Schatzl et al, 2003	Present Study <sup>a</sup>
Country	USA	Germany	Germany	Austria	Germany
n	3339	300	446	133	1078
Method	RIA and CLEIA	CLEIA	CLEIA	CLEIA	CLEIA
Statistic	Regression analyses (central 95% range)	Descriptive (central 95% range)	Descriptive (central 95% range)	Descriptive (mean ± 2 SD)	Quantile regression (central 95% range)
Serum Testosterone Reference Range, nmol/L <sup>b</sup>					
Age, y					
20–29		11.3–34.7	9.7–29.6	10.7–28.8	9.9–32.1
30–39		10.5–31.3	8.7–26.0	10.4–28.8	9.0–31.6
40–49	8.7–31.7	7.5–19.9	7.8–22.0	9.7–24.3	8.1–31.0
50–59	7.5–30.4	5.0–20.2	8.8–21.6	8.3–21.9	7.3–30.1
60–69	6.8–29.8	5.3–19.6	5.8–22.7	7.3–18.7	6.7–29.0
70–79	5.4–28.4	4.4–21.2 <sup>c</sup>	0.8–22.0 <sup>d</sup>	5.9–17.0 <sup>e</sup>	6.2–27.8

Abbreviations: CLEIA, chemiluminescent enzyme immunoassay; RIA, radioimmunoassay; SD, standard deviation.

<sup>a</sup> 25, 35, 45, 55, 65, and 75 years.

<sup>b</sup> To convert the values for testosterone to ng/dL, multiply by 28.82.

<sup>c</sup> 70–86 years.

<sup>d</sup> 70–99 years.

<sup>e</sup> 70–89 years.

DHEA-SO<sub>4</sub>, 2004; IMMULITE 2500 Total Testosterone, 2004). Furthermore, we were able to exclude subjects with disorders known to affect DHEAS and testosterone levels not only on the basis of self-report questionnaires as in other studies (Elmlinger et al, 2003, 2005; Schatzl et al, 2003; Okamura et al, 2005) but also based on clinical and laboratory examinations as well as medication use. A further strength is the measurement of the hormones used. All assays were conducted by a single technician, thus minimizing technician variability. Our study is limited by the lack of free testosterone, SHBG, or albumin for calculation of bioavailable testosterone. Furthermore, it is well known that current assay technology for testosterone lacks a certain degree of precision and accuracy. Mass spectrometry is the gold standard method for measuring testosterone, as previously demonstrated (Wang et al, 2004). Nevertheless, the comparison of the immunoassays used with mass spectrometry demonstrated that automated immunoassays are capable of distinguishing eugonadal from hypogonadal males. However, the same authors stated that reference ranges have been established in each individual laboratory for adult men (Wang et al, 2004). Our reference data for testosterone was appraised on the IMMULITE 2500 platform, which is declared to be technically identical to IMMULITE 1000 and IMMULITE 2000 by the manufacturer. The data should not be assigned to other measurement platforms in an uncritical way, which means without a method comparison in a sufficient population.

In conclusion, the present study established age-specific reference ranges for serum DHEAS and testosterone values standardized for the European age distribution using data from apparently healthy men. The advantages of the quantile regression led to a better adaptation of reference limits to the original data. This statistical approach might be preferable for the calculation of reference ranges in particular by nonnormal distributed variables. Our data might help clinicians reach a consensus on the definition of androgen deficiency.

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## References

Arlt W, Haas J, Callies F, Reincke M, Hubler D, Oettel M, Ernst M, Schulte HM, Allolio B. Biotransformation of oral dehydroepiandrosterone in elderly men: significant increase in circulating estrogens. *J Clin Endocrinol Metab.* 1999;84:2170–2176.

Birkenhager-Gillesse EG, Derksen J, Lagaay AM. Dehydroepiandrosterone sulphate (DHEAS) in the oldest old, aged 85 and over. *Ann N Y Acad Sci.* 1994;719:543–552.

Boyce MJ, Baisley KJ, Clark EV, Warrington SJ. Are published normal ranges of serum testosterone too high? Results of a cross-sectional survey of serum testosterone and luteinizing hormone in healthy men. *BJU Int.* 2004;94:881–885.

Buchinsky M. Recent advances in quantile regression models: a practical guideline for empirical research. *J Hum Resour.* 1998;33:88–12.

Bühringer G, Augustin R, Bergmann E, Bloomfield K, Funk W, Junge B, Kraus L, Merfert-Diete C, Rumpf HJ, Simon R, Töppich J. *Alkoholkonsum und Alkoholbezogene Störungen in Deutschland [Alcohol Consumption and Alcohol-Related Problems in Germany]*. Baden-Baden, Germany: Nomos Verlagsgesellschaft; 2002.

Carnahan RM, Perry PJ. Depression in aging men: the role of testosterone. *Drugs Aging.* 2004;21:361–376.

Chute CG, Baron JA, Plymate SR, Kiel DP, Pavia AT, Lozner EC, O'Keefe T, MacDonald GJ. Sex hormones and coronary artery disease. *Am J Med.* 1987;83:853–859.

Elmlinger MW, Dengler T, Weinstock C, Kuehnel W. Endocrine alterations in the aging male. *Clin Chem Lab Med.* 2003;41:934–941.

Elmlinger MW, Kuehnel W, Ranke MB. Reference ranges for serum concentrations of luteinizing hormone (LH), follicle-stimulating hormone (FSH), estradiol (E<sub>2</sub>), prolactin, progesterone, sex hormone-binding globulin (SHBG), dehydroepiandrosterone sulfate (DHEAS), cortisol and ferritin in neonates, children and young adults. *Clin Chem Lab Med.* 2002;40:1151–1160.

Elmlinger MW, Kuehnel W, Wormstall H, Doller PC. Reference intervals for testosterone, androstenedione and SHBG levels in healthy females and males from birth until old age. *Clin Lab.* 2005;51:625–632.

Feldman HA, Longcope C, Derby CA, Johannes CB, Araujo AB, Coviello AD, Bremner WJ, McKinlay JB. Age trends in the level of serum testosterone and other hormones in middle-aged men: longitudinal results from the Massachusetts male aging study. *J Clin Endocrinol Metab.* 2002;87:589–598.

Fricke U, Güntler J. Anatomisch-therapeutisch-chemisch klassifikation mid Tagesdosen für den deutschen Arzneimittel masht [Anatomical Therapeutic Chemical (ATC) classification index with defined daily dose (DDD)]. Bonn, Germany: Wissenschaftliches Institut der AOK; 2002.

Friedrich N, Alte D, Volzke H, Spilcke-Liss E, Ludemann J, Lerch MM, Kohlmann T, Nauck M, Wallaschofski H. Reference ranges of serum IGF-1 and IGFBP-3 levels in a general adult population: Results of the Study of Health in Pomerania (SHIP). *Growth Horm IGF Res.* 2008;18:228–237.

Gannoun A, Girard S, Guinot C, Saracco J. Reference curves based on non-parametric quantile regression. *Stat Med.* 2002;21:3119–3135. Gesundheitsberichterstattung des Bundes. <http://www.gbe-bund.de/>. Accessed October 10, 2006.

Harman SM, Metter EJ, Tobin JD, Pearson J, Blackman MR. Longitudinal effects of aging on serum total and free testosterone levels in healthy men. Baltimore Longitudinal Study of Aging. *J Clin Endocrinol Metab.* 2001;86:724–731.

IMMULITE 2500 DHEA-SO<sub>4</sub> [manufacturer's instructions]. PIL5KDS-2. Los Angeles, CA: Siemens Healthcare Medical Diagnostics; 2004.

IMMULITE 2500 Total Testosterone [manufacturer's instructions]. PIL5KTW-2. Los Angeles, CA: Siemens Healthcare Medical Diagnostics; 2004.

John U, Greiner B, Hensel E, Ludemann J, Piek M, Sauer S, Adam C, Born G, Alte D, Greiser E, Haertel U, Hense HW, Haerting J, Willich S, Kessler C. Study of Health in Pomerania (SHIP): a health examination survey in an east German region: objectives and design. *Soz Präventivmed.* 2001;46:186–194.

- Keil U, Stieber J, Doring A, Chambless L, Hartel U, Filipiak B, Hense HW, Tietze M, Gostomzyk JG. The cardiovascular risk factor profile in the study area Augsburg. Results from the first MONICA survey 1984/85. *Acta Med Scand Suppl.* 1988;728:119–128.
- Koenker R. *Quantile Regression*. New York, NY: Cambridge University Press; 2005.
- Korth-Schutz S, Levine LS, New MI. Dehydroepiandrosterone sulfate (DS) levels, a rapid test for abnormal adrenal androgen secretion. *J Clin Endocrinol Metab.* 1976;42:1005–1013.
- Leifke E, Gorenai V, Wichers C, Von Zur Muhlen A, Von Buren E, Brabant G. Age-related changes of serum sex hormones, insulin-like growth factor-1 and sex-hormone binding globulin levels in men: cross-sectional data from a healthy male cohort. *Clin Endocrinol (Oxf)*. 2000;53:689–695.
- Liu PY, Beilin J, Meier C, Nguyen TV, Center JR, Leedman PJ, Seibel MJ, Eisman JA, Handelsman DJ. Age-related changes in serum testosterone and sex hormone binding globulin in Australian men: longitudinal analyses of two geographically separate regional cohorts. *J Clin Endocrinol Metab.* 2007;92:3599–3603.
- Lobo RA, Paul WL, Goebelsmann U. Dehydroepiandrosterone sulfate as an indicator of adrenal androgen function. *Obstet Gynecol.* 1981;57:69–73.
- Mohr BA, Guay AT, O'Donnell AB, McKinlay JB. Normal, bound and nonbound testosterone levels in normally ageing men: results from the Massachusetts Male Ageing Study. *Clin Endocrinol (Oxf)*. 2005;62:64–73.
- Morley JE. Androgens and aging. *Maturitas.* 2001;38:61–71; discussion 71–73.
- Nieschlag E, Loriaux DL, Ruder HJ, Zucker IR, Kirschner MA, Lipsett MB. The secretion of dehydroepiandrosterone and dehydroepiandrosterone sulphate in man. *J Endocrinol.* 1973;57:123–134.
- Okamura K, Ando F, Shimokata H. Serum total and free testosterone level of Japanese men: a population-based study. *Int J Urol.* 2005;12:810–814.
- Phillips GB, Pinkernell BH, Jing TY. The association of hypotestosteronemia with coronary artery disease in men. *Arterioscler Thromb.* 1994;14:701–706.
- Rosenfeld RS, Rosenberg BJ, Fukushima DK, Hellman L. 24-hour secretory pattern of dehydroisoandrosterone and dehydroisoandrosterone sulfate. *J Clin Endocrinol Metab.* 1975;40:850–855.
- Rucker D, Ezzat S, Diamandi A, Khosravi J, Hanley DA. IGF-I and testosterone levels as predictors of bone mineral density in healthy, community-dwelling men. *Clin Endocrinol (Oxf)*. 2004;60:491–499.
- Schatzl G, Madersbacher S, Temml C, Krenn-Schinkel K, Nader A, Sregi G, Lapin A, Hermann M, Berger P, Marberger M. Serum androgen levels in men: impact of health status and age. *Urology.* 2003;61:629–633.
- Stone CJ, Koo C. Additive spline in statistics. In: *Proceedings of the Statistical Computing Section of the American Statistical Association*. Washington, DC: American Statistical Association; 1985:45–48.
- Tan RS, Pu SJ. A pilot study on the effects of testosterone in hypogonadal aging male patients with Alzheimer's disease. *Ageing Male.* 2003;6:13–17.
- Wang C, Catlin DH, Demers LM, Starcevic B, Swerdloff RS. Measurement of total serum testosterone in adult men: comparison of current laboratory methods versus liquid chromatography-tandem mass spectrometry. *J Clin Endocrinol Metab.* 2004;89:534–543.