ORIGINAL ARTICLE

Sex steroids and sex steroid-binding globulin levels amongst middle-aged and elderly men and women from general population

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Abstract

Background and Aims: Availability of age- and sex-specific reference values for sex steroids and sex steroid-binding globulin (SHBG) levels allows for appropriate interpretation of research findings and their clinical applications. We report the sex-specific distribution and reference levels of sex steroids, including total estradiol, total testosterone and (calculated) free androgen index (cFAI), SHBG and other androgens dehydroepiandrosterone (DHEA), dehydroepiandrosterone sulphate (DHEAS) and androstenedione across age.

Methods: Using data from 3291 participants from the prospective population-based Rotterdam Study (2006–2008), we visualised the distribution of sex steroids and SHBG levels by calculating and depicting the 5th, 25th, 50th, 75th and 95th percentiles per year and per age-year across 5-year age bands to provide reference value ranges in men and women. Total estradiol and SHBG were measured using automated immunoassay and androgens using liquid chromatography-mass spectrometry (LC–MS/MS).

Result: Mean age was 56.8 (range 45.6–79.9) years in men and 56.9 (range 45.7–79.9) years in women. Amongst men, total estradiol and SHBG showed an increasing trend from 45 years onwards. In women, total estradiol and SHBG showed a decreasing trend from 45 years until the age of 60. From 60 years onwards, SHBG showed an increasing trend. For total testosterone, a clear declining trend was observed amongst men but not women. Other androgens showed a similar decreasing trend in both sexes from 45 years onwards.

Discussion and Conclusion: Our study underlines sex-specific trends in sex steroids and SHBG levels with ageing. This warrants taking into account sex- and age-specific reference values for sex steroids and SHBG when investigating their impact on health outcomes to prevent controversial results and allow for their appropriate clinical application.

KEYWORDS

epidemiology, reference values, sex steroid-binding globulin, sex steroids

1 | INTRODUCTION

Increasing body of evidence has shown the pivotal role of sex steroids and its main binding protein the sex steroid-binding globulin (SHBG) for overall health outcomes and well-being, as well as comorbid conditions.[1,2] Sex steroids influence, amongst others, tissue and bone mass,[3,4] cognitive functioning,[5] brain health[6–8] and cardiometabolic health[9] via various pathways. Moreover, besides the impact on somatic disorders and symptoms, prior studies have suggested sex steroids may also play an important role in mental health and pathophysiology of mental disorders.[10,11]

Despite the wide range of health effects of sex steroids, current results regarding the association of sex steroids with various outcomes, in particular with cardiometabolic outcomes and changes in cardiovascular risk, are not conclusive.

Previous studies have suggested age-related variations in serum concentrations and health-related effects of sex steroids.[12–14] The impact of sex steroids is dependent on free available steroid concentrations, and therefore, SHBG levels are also important to be taken into consideration when studying sex steroids. It is known that the interplay between sex steroids and SHBG is complex (a simplified overview of the sex steroidal pathway is provided in Figure 1). Failing to take into account such complexities might, at least partly, account for the discrepancies in the results of studies regarding the associations of sex steroids with various outcomes.

The availability of age-specific reference values for sex steroids and SHBG levels is crucial to allow for appropriate interpretation of the studies and their clinical applications. However, current literature on age-specific reference values is scarce[15–18] and limited to investigation of only one specific hormone or focussing on only one sex. Therefore, studies reporting age-specific reference values for a broad range of sex steroids and SHBG in a wide age range of both men and women are lacking. For this purpose, more robust epidemiological data, based on large population-based cohort studies, comprehensively
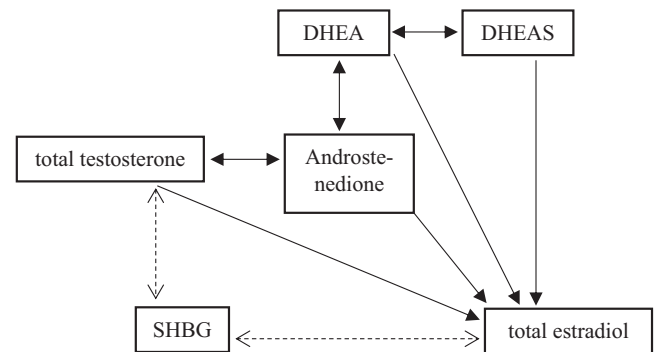
FIGURE 1 Simplified overview of the sex steroidal pathway. DHEA; dehydroepiandrosterone; DHEAS; dehydroepiandrosterone sulphfate; SHBG; sex steroid-binding globulin.

investigating oestrogens, androgens and SHBG in both sexes are required. We, therefore, report the distribution and concentrations of sex steroids, including total estradiol, total testosterone and (calculated) free androgen index (cFAI), SHBG and other androgens dehydroepiandrosterone (DHEA), dehydroepiandrosterone sulphate (DHEAS) and androstenedione across age. Using data from the large prospective Rotterdam Study cohort, we provide age- and sex-specific reference values for sex steroids and SHBG amongst middle-aged and elderly men and women from general population.

2 | MATERIALS AND METHODS

2.1 | Study population

This study was embedded within the Rotterdam Study, an ongoing prospective, population-based cohort study amongst individuals of 55 years and older living in the Ommoord district of Rotterdam, the Netherlands. The rationale and study design have been described in detail elsewhere.[19] The baseline examination of the Rotterdam Study included 7983 individuals between 1989 and 1993 (Rotterdam Study-I). Rotterdam Study has been extended

twice (Rotterdam Study-II: 2000–2001, Rotterdam Study III: 2006–2008) to include participants who were 45 years or older or who had moved to the study research area. The overall response for all three Rotterdam Study cycles at baseline was 72.0% (14.926 of 20.744). This study included participants from the extended cohort Rotterdam Study III-1 (2006–2008). Data for any sex steroids and SHBG were available in 3449 participants. We excluded 115 women and three men with current use of sex-steroid medication, defined as use before 90 days of blood collection. Next, we also excluded 43 participants aged >80 years. This resulted in 3291 participants with available sex steroids and SHBG measurements. (Figure 2).

2.2 | Assessment of sex steroids and SHBG

All blood samples were drawn in the morning (≤11 am) and were fasting. Sex steroids and SHBG were measured using automated immunoassay or liquid chromatography-mass spectrometry (LC–MS/MS).

The same method (per measure) was used throughout the entire Rotterdam Study to measure sex steroids and SHBG. The choice of the method was based on sensitivity, specifically in the lower ranges, given also the epidemiologic approach in this study. Total estradiol and SHBG were measured using automated immunoassay and androgens using liquid chromatography-mass spectrometry. Total estradiol levels were measured with COBAS 8000 Modular Analyser (Roche Diagnostics GmbH), serum levels of total testosterone with liquid chromatography–tandem mass spectrometry (LC–MS/MS), and sex steroid-binding globulin (SHBG) was measured as with the Immulite platform (Diagnostics Products Corporation Breda). The corresponding interassay coefficients of variations for total estradiol, total testosterone and SHBG were <7%, <5% and <5%. The minimum detection limit

for estradiol was 18.35 pmol/L. Undetectable estradiol was scored as 18.35. The (calculated) free androgen index was calculated in women as (total testosterone/SHBG)*100.[20] DHEA, DHEAS and androstenedione were measured on a Waters XEVO-TQ-S system (Waters) using the CHS™ MSMS Steroids Kit (Perkin Elmer). The interassay coefficients of variation of DHEA, DHEAS and androstenedione were <6.5%. Serum was immediately frozen (within 2 h) at −150°C and afterwards stored at −80°C.

2.3 | Other measurements (provided as Appendix S1)

An interview was performed to obtain information on current health status, medical history, medication use (including hormones), menopausal status and smoking. Blood pressure was measured in the sitting position on the right upper arm with a random-zero sphygmomanometer. The retrospective data on self-reported use of antihypertensive were collected by a questionnaire during the home interview. Smoking status was assessed by asking participants whether they were current smokers of cigarettes, cigars or pipe. Postmenopausal women were defined as women who reported the absence of menstrual periods for 12 months. The retrospective data on self-reported use of antihypertensive were collected by a questionnaire during the home interview. Smoking status was assessed asking participants whether they were current smokers of cigarettes, cigars or pipe. Body mass index was calculated as weight (kg) divided by height squared (m^2). Waist circumference was measured in standing position, without heavy outer garments, midway between the lower rib and iliac crest. Waist–hip ratio was calculated. All biochemical parameters were assessed in fasting serum. Total cholesterol, high-density lipoprotein cholesterol (HDL) and C-reactive protein were measured on the COBAS 8000 Modular Analyser (Roche Diagnostics GmbH). The corresponding
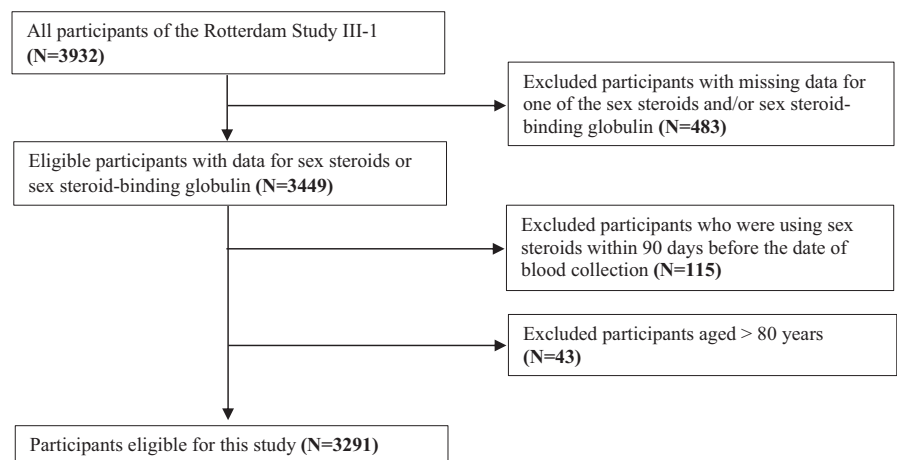

FIGURE 2 Flow chart of the study population. *N*, number.

interassay coefficients of variations are the following: lipids <2.1% and C-reactive protein <16.9%. Prevalent CHD was defined as a history of myocardial infarction or coronary revascularization including PCI or CABG. Diabetes mellitus type 2 diagnosis was considered present if a participant used glucose-lowering drugs, in case a nonfasting random serum glucose level was found to be ≥11 mmol/L or use of antidiabetic medication.

2.4 | Statistical analysis

Characteristics of women and men were presented as mean (standard deviation; SD) or median (25th -75th quartile) for continuous variables and number (percentage) for categorical variables.

All analyses were performed separately for men and women. First, we visualised the distribution of sex steroids and SHBG across age by calculating and depicting the 25th, 50th and 75th percentiles per age-year. Smoothed percentile lines were plotted to visualise the data. We also calculated the 5th, 25th, 50th, 50th and 95th percentiles for 5-year age bands to provide reference value ranges. The following age categories were constructed: 45–49 years, 50–54 years, 55–59 years, 60–64 years, 65–69 years, 70–74 years and 75–79 years.

As a sensitivity analysis, we further assessed the trend of sex steroids and SHBG across age amongst overweight and nonoverweight participants separately and after restricting the population to postmenopausal women only.

Statistical analysis was performed with IBM SPSS statistics software version 24 and R version 3.4.4. R package 'ggplot2' was used to construct the plots. Generalised additive mode smoothing (GAM) method was used to visualise the data using smoothed lines.

3 | RESULTS

3.1 | Characteristics of the study population

Table 1 depicts the characteristics of the study population. The mean age in men was 56.8 (range 45.6–79.9 years) and in women 56.9 (range 45.7–79.9 years).

Figure 3 depicts the relationship between age and concentrations of total estradiol, total testosterone and SHBG. Figure S1 depicts the relationship between age and levels of DHEA, DHEAS and androstenedione. Age- and sex-specific percentile values for sex steroids and SHBG are provided in Table 2.

3.2 | Total estradiol

Amongst men, total estradiol levels increased from the age of 45 years onwards. A steady increase was observed until the age of 75 years and a decline in the eldest age group 75–79 years. In women, total estradiol levels declined starting from 45 years until approximately 60 years. Due to the lower assay detection limit of 18.35 pmol/L, further decline was not detectable in this study.

3.3 | Total testosterone and cFAI

In men, both total testosterone and cFAI declined with increasing age. Whilst a more prominent decline was observed after the age of approximately 65 years for total testosterone, cFAI showed a steady constant decline from 45 years onwards.

Amongst women, after an initial decrease, a slight increase was observed for total testosterone after 60 years. However, cFAI in women showed first a slight increase from the age of 45 to approximately 65 years, reflecting the trend of SHBG with increasing age. Afterwards, a declining trend was observed.

3.4 | SHBG

Serum SHBG levels decreased from the age of 45 years onwards in men. Amongst women, a U-shaped trend was observed. SHBG levels decreased until the age of 60, followed by a steady and stronger increase until older ages.

3.5 | DHEA, DHEAS and androstenedione

In both sexes, DHEA, DHEAS and androstenedione declined with a constant slope from 45 years onwards.

Whilst in men, the slope of decline for DHEA slowed down after the age of 60 years; in women, this occurred approximately after the age of 70 years. However, the slope of decline for DHEAS in men occurred approximately at the age of 70 years, Whilst in women, this occurred around the age of 65 years.

The pattern of decline for androstenedione was similar amongst men and women; the decline slowed down after the age of 60 years.

TABLE 1 Characteristics of the study population

	Men (N = 1490)	Women (N = 1801)
Age, years	56.75 (6.18)	56.87 (6.09)
Body mass index, kg/m^2	27.88 (3.90)	27.68 (5.09)
Waist–hip ratio	0.93 (0.07)	0.83 (0.07)
Current smoking, N (%)	426 (31.2)	401 (23.1)
Diastolic blood pressure, mmHg	83.38 (10.67)	82.09 (11.05)
Systolic blood pressure, mmHg	135.44 (17.76)	130.20 (19.37)
Antihypertensive therapy, N (%)	420 (28.5)	447 (25.0)
Total cholesterol, mg/dl	5.43 (1.02)	5.76 (1.06)
High-density lipoprotein cholesterol, mmol/L	1.24 (0.35)	1.58 (0.44)
Lipid lowering medication, N (%)	383 (25.9)	357 (19.9)
C-reactive protein, mg/L	1.10 [0.50, 2.50]	1.30 [0.60, 3.00]
Prevalent diabetes, N (%)	194 (13.0)	131 (7.3)
Prevalent coronary heart disease, N (%)	100 (6.8)	24 (1.3)
Sex steroids		
Total estradiol, pmol/L	94.07 [73.78, 118.60]	30.96 [18.35, 66.54]
Total testosterone, nmol/L	16.85 [13.38, 21.28]	0.79 [0.57, 1.08]
Calculated free androgen index	0.42 [0.35, 0.50]	0.01 [0.01, 0.02]
Sex steroid-binding globulin, nmol/L	40.37 [30.83, 50.92]	56.76 [40.88, 78.32]
Dehydroepiandrosterone, nmol/L	13.10 [8.37, 19.20]	13.36 [8.68, 19.90]
Dehydroepiandrosterone sulphate, nmol/L	3619.85 [2352.41, 5082.61]	2316.89 [1448.54, 3454.40]
Androstenedione, nmol/L	3.32 [2.60, 4.40]	2.65 [1.92, 3.70]
Female-specific variables		
Age at menopause, years	NA	48.11 (6.30)
Time since menopause, years	NA	10.53 (7.76)
Natural menopause, N (%)	NA	1028 (75.9)

Note: Values are reported as number (percentage) for categorical variables and mean (SD) or median [25th–75th quartile] for continuous variables.

Abbreviations: *N*; number, NA; not applicable.

3.6 | Sensitivity analysis

After stratifying for overweight and nonoverweight participants, similar trend of changes was observed in the two groups in both men and women. (Figure S2) However, in both sexes, SHBG levels were lower amongst overweight individuals. Amongst men, total testosterone levels were also lower in overweight individuals. In women, higher cFAI levels amongst overweight individuals were observed across all age categories.

After restricting the population to postmenopausal women, a similar trend, as in the total population of women, was observed for total estradiol and SHBG. (Figure S3) A different pattern was observed for total testosterone, and other androgens (DHEA, DHEAS and androstenedione), showing an increasing trend from 45 years onwards to approximately 55 years in postmenopausal women. However, after the age of 55 years, similar decreasing trends were observed.

4 | DISCUSSION

In this large population-based cohort study, we describe the distribution of sex steroids and SHBG and provide age- and sex-specific reference values for middle-aged and elderly men and women from general population. Our study underlines sex-specific trends in the levels of sex steroids and SHBG with ageing and shows reference ranges differ by age category.
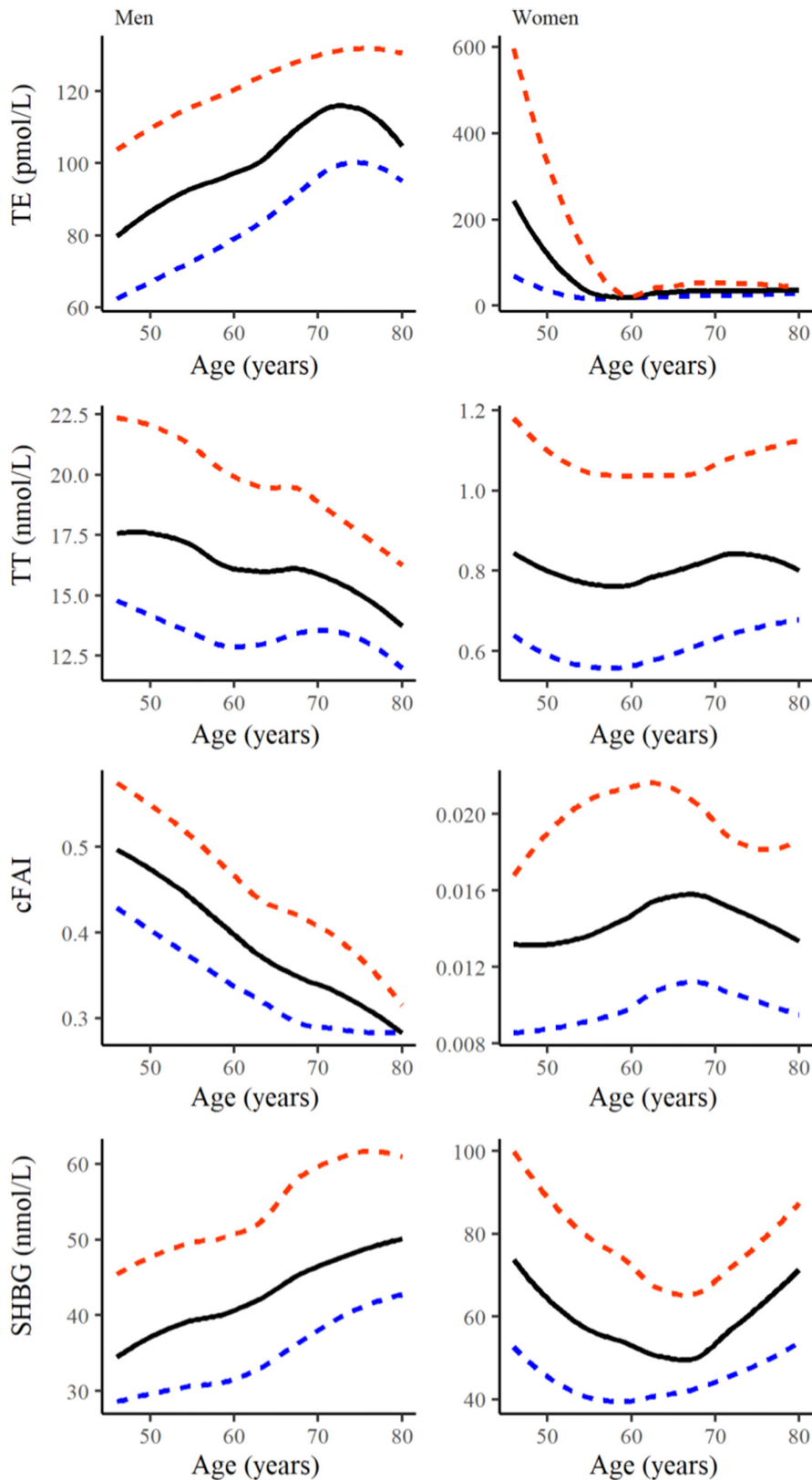
FIGURE 3 Levels of total estradiol, total testosterone, cFAI and SHBG across age. Smoothed percentile plots of total estradiol, total testosterone, cFAI, and SHBG for each age-year in 1490 men (left) and 1801 women (right). The median is a solid line, 25th and 75th percentile are depicted as dashed lines. cFAI, calculated free androgen index; SHBG, sex steroid-binding globulin; TE, total estradiol; TT, total testosterone.

4.1 | Total estradiol

We observed a sex-specific trend for total estradiol levels with increasing age. Whilst estradiol levels declined in women with ageing, we observed an opposite trend in men. Although the age-related decline of total estradiol in women is known to be related to the perimenopausal period, the underlying mechanisms for the observed age-related increase in men remain unknown. Nevertheless, we speculate that these changes may reflect, partly, age-related

TABLE 2 Age- and sex-specific percentile values for serum sex steroids and sex steroid-binding globulin

(A) Total estradiol (pmol/L)

Men

Age-groups	N	5th	25th	50th	75th	95th
45–49 year	212	41.93	63.70	86.92	109.43	155.55
50–54 year	391	46.90	71.62	90.78	115.80	154.95
55–59 year	471	45.09	74.06	94.47	115.70	160.10
60–64 year	300	54.02	80.51	99.78	125.73	161.30
65–69 year	49	64.69	83.17	104.40	129.30	165.16
70–74 year	39	64.79	95.28	123.20	131.90	155.29
75–79 year	23	51.11	75.21	114.40	125.45	161.81

Women

Age-groups	N	5th	25th	50th	75th	95th
45–49 year	245	18.35	32.37	165.30	512.50	1719.00
50–54 year	456	18.35	18.35	40.75	129.98	800.20
55–59 year	582	18.35	18.35	18.49	43.60	85.84
60–64 year	387	18.35	18.35	27.80	45.30	79.32
65–69 year	51	18.35	18.35	32.59	50.32	110.60
70–74 year	41	18.35	24.28	38.43	51.62	138.20
75–79 year	29	18.35	18.96	34.40	44.02	68.68

(B) Total testosterone (nmol/L)

Men

Age-groups	N	5th	25th	50th	75th	95th
45–49 year	212	10.05	14.43	17.98	22.97	28.00
50–54 year	391	9.97	13.97	17.27	21.79	27.63
55–59 year	471	9.60	13.05	16.67	20.82	27.70
60–64 year	298	9.25	12.78	16.31	20.43	26.95
65–69 year	49	9.55	12.46	17.26	20.30	27.82
70–74 year	39	9.61	12.15	15.77	19.43	24.08
75–79 year	23	10.34	12.01	13.46	18.00	22.84

Women

Age-groups	N	5th	25th	50th	75th	95th
45–49 year	245	0.37	0.59	0.80	1.06	1.66
50–54 year	452	0.38	0.57	0.80	1.10	1.63
55–59 year	580	0.34	0.55	0.73	1.01	1.70
60–64 year	386	0.38	0.59	0.82	1.12	1.68
65–69 year	51	0.36	0.59	0.86	1.11	1.53
70–74 year	41	0.30	0.55	0.81	1.13	1.61
75–79 year	28	0.31	0.60	0.78	1.14	2.74

(C) cFAI

Men

Age-groups	N	5th	25th	50th	75th	95th
45–49 year	212	0.33	0.40	0.49	0.57	0.71
50–54 year	391	0.30	0.39	0.45	0.53	0.70
55–59 year	471	0.25	0.35	0.41	0.49	0.62
60–64 year	298	0.26	0.33	0.40	0.47	0.60
65–69 year	49	0.23	0.29	0.35	0.43	0.55
70–74 year	39	0.19	0.25	0.31	0.40	0.52
75–79 year	23	0.24	0.27	0.28	0.34	0.37

Women

Age-groups	N	5th	25th	50th	75th	95th
45–49 year	245	0.004	0.009	0.013	0.018	0.028
50–54 year	452	0.004	0.009	0.013	0.021	0.034
55–59 year	580	0.005	0.009	0.014	0.020	0.039
60–64 year	386	0.006	0.011	0.016	0.023	0.037
65–69 year	51	0.006	0.011	0.014	0.022	0.032
70–74 year	41	0.005	0.009	0.017	0.021	0.038
75–79 year	28	0.005	0.007	0.012	0.018	0.035

(Continues)

TABLE 2 (Continued)

(D) SHBG (nmol/L)

Men

Age-groups	N	5th	25th	50th	75th	95th
45–49year	212	18.92	28.57	37.85	47.68	66.01
50–54year	391	19.21	29.53	38.61	50.11	65.40
55–59year	471	21.61	31.68	41.12	50.75	70.68
60–64year	300	20.40	31.76	41.73	52.78	76.27
65–69year	49	25.36	35.48	43.48	58.55	90.36
70–74year	39	24.00	38.45	47.32	63.30	91.73
75–79year	23	32.03	40.48	49.14	64.64	89.16

Women

Age-groups	N	5th	25th	50th	75th	95th
45–49year	245	26.18	45.27	67.08	92.76	171.94
50–54year	456	25.38	42.26	59.41	82.89	128.95
55–59year	582	24.70	39.53	54.89	76.49	115.51
60–64year	387	25.79	39.61	52.31	71.97	109.15
65–69year	51	32.66	41.40	53.65	69.00	96.64
70–74year	41	27.57	38.93	49.29	75.93	110.80
75–79year	29	34.23	52.26	74.90	86.62	132.14

(E) DHEA (nmol/L)

Men

Age-groups	N	5th	25th	50th	75th	95th
45–49year	209	6.85	12.67	17.77	25.32	46.77
50–54year	389	6.10	9.45	14.42	20.84	38.67
55–59year	459	5.07	8.51	12.69	18.19	30.93
60–64year	294	4.25	7.23	10.15	16.40	27.64
65–69year	49	3.75	6.28	9.12	12.62	23.47
70–74year	39	2.74	3.91	6.42	10.05	19.62
75–79year	23	2.47	3.39	4.61	9.72	14.45

Women

Age-groups	N	5th	25th	50th	75th	95th
45–49year	241	5.12	10.14	14.71	23.24	45.23
50–54year	450	4.83	10.17	14.94	22.03	38.70
55–59year	573	4.54	8.67	13.24	19.18	32.92
60–64year	384	4.28	8.52	12.92	19.34	34.77
65–69year	51	3.08	7.34	9.19	13.22	23.78
70–74year	41	2.63	4.13	7.28	11.98	22.34
75–79year	27	2.20	3.15	5.60	8.88	16.88

Men

Age-groups	N	5th	25th	50th	75th	95th
45–49year	193	2027.79	3487.16	4835.29	6171.53	7458.33
50–54year	360	1613.14	2839.77	4055.80	5367.02	7030.06
55–59year	455	1175.83	2472.76	3612.80	5043.47	6815.23
60–64year	286	946.81	1872.69	2913.95	4264.78	7004.31
65–69year	49	788.18	1470.75	2393.82	3464.91	5564.23
70–74year	39	668.93	1057.61	1508.26	2525.77	4943.30
75–79year	23	558.24	970.90	1491.19	2573.66	4170.95

Women

Age-groups	N	5th	25th	50th	75th	95th
45–49year	242	786.85	1878.14	3067.44	4197.49	6546.83
50–54year	447	866.30	1662.73	2545.95	3771.91	5684.64
55–59year	577	660.39	1408.54	2323.50	3317.62	5200.07
60–64year	386	649.74	1313.61	2016.49	2923.86	5072.82
65–69year	51	522.88	1034.04	1592.58	2745.01	4593.76
70–74year	41	251.97	862.73	1494.59	2077.82	3038.15
75–79year	29	136.07	541.30	1065.05	1588.92	3108.19

TABLE 2 (Continued)

(G) Androstenedione (nmol/L)

Men							Women						
Age-groups	N	5th	25th	50th	75th	95th	Age-groups	N	5th	25th	50th	75th	95th
45–49year	211	2.00	3.04	3.86	4.75	8.01	45–49year	245	1.32	2.30	3.33	4.39	7.33
50–54year	391	2.02	2.81	3.48	4.49	6.55	50–54year	451	1.26	2.15	2.97	3.98	6.17
55–59year	469	1.79	2.55	3.19	4.30	6.18	55–59year	577	1.17	1.80	2.49	3.30	5.57
60–64year	298	1.73	2.43	3.10	4.14	5.87	60–64year	386	1.12	1.84	2.53	3.39	5.41
65–69year	49	1.47	2.07	2.73	3.67	5.85	65–69year	50	1.18	1.65	2.28	3.01	4.50
70–74year	39	1.25	1.76	2.60	3.56	4.74	70–74year	41	0.94	1.57	1.79	2.64	4.64
75–79year	23	1.28	1.93	2.18	3.03	4.20	75–79year	29	0.78	1.37	1.80	2.42	3.60

Abbreviations: N; number, cFAI; calculated free androgen index, SHBG; sex steroid-binding globulin, DHEA; dehydroepiandrosterone, DHEAS; dehydroepiandrosterone sulfate.

changes in body weight and/or increase in comorbid conditions. A prior study similarly reported an increasing trend of total estradiol levels in middle-aged men.[21]

4.2 | SHBG

Evident sex differences in the trend of SHBG levels with ageing were observed. Whilst SHBG levels increased from the age of 45 years onwards amongst men, a U-shaped trend was observed amongst women. However, the lowest and highest reference ranges were different for age categories in both men and women. Importantly, the U-shaped trend in women was also reflected in the reference ranges. A previous study has also showed a similar U-shaped trend for SHBG amongst women.[12] Another study showed similarly a decreasing trend from SHBG during the menopausal transition.[22]

Whilst the decrease of SHBG in women after the age of 45 years mirrors the parallel decrease of estradiol, the observed increase from the age of 60 years onwards merits further investigations. Similarly, the underlying mechanisms for the observed trend in men also remain to be established. Previous studies have suggested estradiol levels may increase and androgens may decrease SHBG levels.[23,24] However, the exact role of sex steroids and their interplay with SHBG, which is known to be complex for the regulation of SHBG levels and may be bidirectional, remains also to be elucidated.

4.3 | Total testosterone and cFAI

In line with previous reports, amongst Danish,[25] European,[26] Australian[13] and American[27] population, we observed a decrease in total testosterone levels with ageing in men. Although it was previously suggested that testosterone levels decrease in women from adolescence until menopause,[13] we observed increasing levels with ageing from 60 years onwards similar to other studies.[13,14]

We observed similar decreasing trend for total testosterone and cFAI amongst men. However, total testosterone and cFAI showed different patterns with ageing amongst women. This could be a reflection of the differences in the trend of SHBG with increasing age in men and women (constant increasing trend in men vs. U-shaped trend in women).

4.4 | DHEA, DHEAS and androstenedione

Our study showed a declining trend for DHEA, DHEAS and androstenedione after the age of 45 years, which

is consistent with previous studies in men[28] and in women.[14] The approximate age at which the slope of the trend changed was similar in both men and women for androstenedione, but differed by sex for DHEA and DHEAS.

An increasing trend for testosterone and other androgens was observed amongst the younger postmenopausal women aged 45 until 55 years, which is the average age of 5 years since menopause.

We speculate that this could be explained by the late perimenopausal and early menopausal androgen production in the ovaries in response to stimulation by gonadotropins (luteinizing hormone and follicle-stimulating hormone) produced in the pituitary, which disappears later in the menopause.[29]

4.5 | Age-specific reference ranges

Our results show that normal reference ranges (i.e., P5-P95 or P25-P75) are inconsistent for sex steroids and SHB across different age categories in both genders. This further highlights the clinical importance of taking into account age-related changes. Our findings also, at least partly, explain the inconsistencies in proposed reference values by various studies.[30–32] The age-specific reference values (for women, both pre- and postmenopausal) could also, in part, be responsible for discrepancies in the reported associations between sex steroids and SHBG with health outcomes in various studies.[9,33–36] Our findings warrant both clinicians and researchers to account for age-related changes in sex steroids when diagnosing or investigating hypo- and hyperandrogenism. Consideration of age-specific reference values for sex steroids and SHBG levels will allow for appropriate interpretation of the studies and their clinical applications.

4.6 | Strength and limitations

The major strengths of this study include the large community-dwelling study sample of men and women from a prospective cohort study. Moreover, a broad range of sex steroids and SHBG was simultaneously available in both women and men. Finally, total testosterone was measured using the golden standard method. Limitations of this study include the cross-sectional design that does not allow for trajectories of longitudinal changes within the same individuals. In this study, we used only single sex steroid measurements, whilst sex steroids levels may fluctuate due to diurnal variation. However, in this study, blood was drawn in the morning in all participants

to minimise influence of diurnal rhythm. Moreover, at older ages and in postmenopausal women, sex steroids levels are more stable over time. Finally, the immunoassay to measure estradiol had a minimum detection limit of 18.35 pmol/L. We did not exclude participants with co-morbid conditions, including CHD, diabetes and obesity, whilst previous studies have suggested these conditions are associated with sex steroids and SHBG. Additionally, no separate subgroup analysis was performed for participants with endocrine disorders. However, this was not the aim of this study as we aimed to provide reference values for a general population, which can be used for future clinical purposes and settings. The use of different laboratory assays may lead to variation in measurements of sex steroids and SHBG values, and reference ranges may vary amongst different laboratories. As such, this should be taken into account when extrapolating the data for use of measurements performed by other assays than those used in this study. Although this may affect the absolute sex steroids or SHBG levels, it does not affect observed age- and sex-specific trends. Additionally, the majority of the Rotterdam Study participants were of Euroancestry. Therefore, ethnicity-specific reference values were not available.

In our study, serum albumin levels were not available. As sex steroids are partly bound to albumin, future studies should also investigate age-specific reference values for serum albumin levels.

5 | CONCLUSIONS

In this large population-based cohort study, we describe the distribution of sex steroids and SHBG and provide age- and sex-specific reference values for middle-aged and elderly men and women from general population. Our study underlines sex-specific trends in the levels of sex steroids and SHBG with ageing, which was also reflected in the reference ranges across different age categories. This warrants taking into account sex- and age-specific reference values for sex steroids and SHBG when investigating their impact on health outcomes to prevent controversial results. Consideration of age-specific reference values (for women both pre- and postmenopausal) for sex steroids and SHBG levels will allow for appropriate interpretation of the studies and their clinical applications. Future studies assessing longitudinal within-person changes are warranted.

AUTHOR CONTRIBUTIONS
EA, JERL, YBR and MK contributed to the conception or design of the work. EA, JERL, YBR, JSEL, MAI, RPP and MK contributed to the acquisition, analysis or

interpretation of data for the work. EA drafted the manuscript. All critically revised the manuscript and gave final approval and agree to be accountable for all aspects of work ensuring integrity and accuracy.

CONFLICT OF INTEREST
EA, JERL, YBR, MAI, RPP and MK have nothing to disclose. JSEL reports grants from fAstellas (Tokyo, Japan), Dutch Heart Association (Utrecht, the Netherlands), ZonMw (Amsterdam, the Netherlands), personal fees from Titus Healthcare (Hoofddorp, the Netherlands) and grants and personal fees from Ferring (Hoofddorp, the Netherlands), Ansh Labs (Webster, Tx, USA), outside the submitted work.

INFORMED CONSENT
Informed consent was obtained from all individual participants included in the study.

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