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Testosterone and estrone increase from the age of 70 years; findings from the Sex Hormones in Older Women Study

Susan R Davis MBBS FRACP PhD FAHMS, Robin J Bell MBBS (Hons), PhD, MPH, FAFPHM, Penelope J Robinson M Biostat, David J Handelsman MBBS, FRACP, PhD, FAHMS, Tom Gilbert BSc (Hons), James Phung BMedSci (Pharm Sci), Reena Desai MSc, Jessica E Lockery MBBS, Robyn L Woods PhD, Rory S Wolfe PhD, Christopher M Reid PhD, MPH, Mark R Nelson MBBS, PhD, Anne M Murray MD, MSc, John J McNeil MBBS, FRACP, PhD, FAFPHM, FAHMS

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Testosterone and estrone increase in older women

Testosterone and estrone increase from the age of 70 years; findings from the Sex Hormones in Older Women Study

Susan R Davis MBBS FRACP PhD FAHMS[1]

Robin J Bell MBBS (Hons), PhD, MPH, FAFPHM [1]

Penelope J Robinson M Biostat[1]

David J Handelsman MBBS, FRACP, PhD, FAHMS [2]

Tom Gilbert BSc (Hons)[3]

James Phung BMedSci (Pharm Sci)[3]

Reena Desai MSc[2]

Jessica E Lockery MBBS[3]

Robyn L Woods PhD[3]

Rory S Wolfe PhD[3]

Christopher M Reid PhD, MPH[2,4]

Mark R Nelson MBBS, PhD[3,5]

Anne M Murray MD, MSc[6]

John J McNeil MBBS, FRACP, PhD, FAFPHM, FAHMS [3]

1 Women's Health Research Program, School of Public Health and Preventive Medicine, Monash University, Melbourne, Victoria, Australia.

2. ANZAC Research Institute, University of Sydney, New South Wales, Australia

3. Department of Epidemiology and Preventive Medicine, Monash University, Melbourne, Victoria, Australia.

4. School of Public Health, Curtin University, Perth, Western Australia, Australia

5.Menzies Institute for Medical Research, University of Tasmania, Hobart, Australia.

6. Berman Center for Outcomes and Clinical Research, Hennepin Healthcare Research Institute, Hennepin Healthcare and the Division of Geriatrics, Department of Medicine, University of Minnesota, USA.

ORCiD numbers:

0000-0002-2955-0415

Davis

Susan R

On behalf of the ASPREE Investigator Group*

* ASPREE Investigator Group are listed on www.aspree.org

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Importance There is a lack of understanding of what is normal in terms of sex steroid levels in older women.
Objective To determine whether sex steroid levels vary with age in and establish reference ranges for women beyond 70 years.
Design and setting Cross-sectional, community based, study
Participants 6392 women, aged 70 years and older.
Main outcome measures Sex steroids measured by liquid chromatography-tandem mass spectrometry. A reference group, to establish sex steroid age-specific reference ranges, excluded women using systemic or topical sex steroid, anti-androgen or glucocorticoid therapy or an anti-glycaemic agent.
Results The reference group of 5326 women had a mean age of 75·1 (± 4·2) years, range 70-94·7 years. Median values (range) were estrone (E_1)181·2 pmol/L (3·7-5768·9), T 0·38 nmol/L (0·035-8·56), dehydroepiandrosterone (DHEA) 2·60 nmol/L (0·07-46·85) and SHBG 41·6 nmol/L (2·4-176·6). Estradiol and dihydrotestosterone were below method sensitivity in 66·1% and 72·7% of the samples, respectively. Compared with women aged 70-74 years, women aged 85+ years had higher median levels of E_1 (11·7%, p=0·01), T (11·3%, p=0.02) and SHBG (22·7%, p<0·001) and lower DHEA (30% less, p<0·001). Overweight and obese women had higher E_1 (p<0·001) and T (p<0·03), and lower SHBG (p<0·001) than women with normal body mass index. Smokers had 17·2% higher median T levels (p=0·005).
Conclusion From the age of 70 years, T and E_1 increase with age, despite a steady decline in DHEA. Whether E_1 and T are biomarkers for longevity, or contribute to healthy aging merits investigation.

This study shows estrone and testosterone increase in women after 70 years and that testosterone levels in women aged 70 plus are similar to levels measured in premenopausal women.

Introduction

With the steady increase in the life expectancy of older women over the past 4 decades (1), understanding the factors that keep women healthy as they age is imperative to reduce the number of years lived with disability. Sex hormones are implicated as having a critical role in the development and evolution of age-associated disease, including cardiovascular disease and cancer, with the focus mainly on oestrogens in women. Counter-intuitively, testosterone (T), which circulates in higher concentrations than estrogens in women of all ages, may be as, or even more, important than estrogens in determining disease risk in elderly women (2).

The first step in advancing the understanding of androgens in older women's health is establishing normal levels in a community-based sample of women. Until now, the available data have been limited by small sample sizes and /or the use of direct immunoassays which lack sensitivity and specificity for the measurement of testosterone (T) at the concentrations occurring in women, compared with the higher levels seen in men (3-6).

The ASPREE (ASPirin in Reducing Events in the Elderly) Study was a placebo-controlled randomised clinical trial (RCT) of daily low dose aspirin versus placebo in older people, free of cardiovascular disease (CVD) events, with unimpaired cognition at recruitment. The large sample of women in this cohort has enabled us to determine whether sex steroids vary beyond the age of 70 years and, for the first time, establish age-specific reference ranges for each of the main sex steroids for community-dwelling women aged 70 years and older (70+), using liquid chromatography-tandem mass spectrometry [LC-MS/MS])(7).

Methods

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Study participants

ASPREE was an RCT of aspirin (100 mg enteric coated daily) versus placebo in healthy older people. Details of the trial design have been published elsewhere (7,8). In brief, Australian recruitment to ASPREE was achieved through partnerships with over 2500 general practitioners across the southern states of Victoria, South Australia and Tasmania. Australian participants were aged at least 70 years. Exclusion criteria included any chronic illnesses likely to limit survival to less than 5 years, documented CVD or cerebrovascular disease, dementia or a score of <78 on Modified Mini-Mental State Examination (9), disability (severe difficulty or inability to perform any of the 6 Katz activities of daily living(10)), any condition associated with a high current or recurrent risk of bleeding, anaemia or uncontrolled high blood pressure (systolic ≥ 180 mmHg and/or diastolic ≥ 105 mmHg).

Clinical parameters

Date of birth, all concomitant medication use, and smoking and alcohol consumption were documented at randomisation. Clinical measurements included weight, height, waist circumference, and systolic and diastolic blood pressure.

Sex Steroid measurement

Blood samples were drawn at recruitment (or within 12 months) and plasma stored under nitrogen vapour. Sex steroids and SHBG were measured in a single sample of plasma by LC-MS/MS at the ANZAC Research Institute, University of Sydney. T, dihydrotestosterone (DHT), DHEA, estradiol (E_2), and E_1 were quantified within a single run without derivatization as previously described (11) and with modifications (12).

Briefly, plasma (200 µL) spiked with 50 µL of internal standard (d3-T, d4-E2, d3-DHT, d2-DHEA) were extracted with 1 mL of methyl tert-butyl ether, separated by freezing to allow removal of the organic layer. After evaporation, the extract was reconstituted in 75 µL of 20% methanol so that 50 µL was injected into the LC-MS/MS system. Extracts of samples, standards and quality controls were injected into a Shimadzu Nexera ultra-high pressure liquid chromatography system comprising a Phenomenex Kinetex 1.7µXB C18 100A (50x2.1mm) column with a Phenomenex C18 guard cartridge. The elution solvents were water (A) and methanol (B). A gradient elution was performed at a flow rate of 0.5 mL/min with 25% B (0–0.10 min), 52–62% B (0.11–4.30 min), 100% B (4.31–5.45 min), 25% B (5.46–7.00 min). The column temperature and autosampler were set at 40°C and 4°C, respectively. An AB Sciex API 5000 triple quad mass spectrometer was used with Photospray (APPI) ion source in positive (androgens) and negative (estrogens) polarity. Toluene was used as dopant, delivered at 0.05 mL/min. The ion source, curtain, and collision gas was nitrogen. Multiple reaction monitoring was employed with both quadrupoles at unit resolution. Two mass transitions were monitored for each analyte (13). Certified reference materials were used for assay standards for T, DHT and DHEA (National Measurement Institute (NMI), Sydney) and E_2 and E_1 (Cerillant). Internal standards used were stable isotopes - d3-T, d3-DHT and d2-DHEA (NMI), d4-E_2 (Cambridge Isotopes). For E_1 the internal standard d4-E_2 was used for quantitation purpose.

The assay limits of detection, limits of quantification and within-run and between-run coefficient of variations (CV) are T (35 pmol/L, 0·09 nmol/L, 2·0%, 3·9-6·5%), DHT (0.17 nmol/L, 0.34 nmol/L, 8·1%, 6·7-13·4%), E_2 (11 pmol/L, 18 pmol/L, 6·6%, 4·8-8·6%), E_1 (3·7 pmol/L, 11 pmol/L, 4·7%, 4·6-7·5%) and DHEA (0·07 nmol/L, 0·17 nmol/L, <10%, <10%) (14). SHBG was measured in batches by automated immunoassay (Roche Diagnostics, Australia) with a CV of 1·0-2·0%. The median (range) for T and DHEA for menstruating, premenopausal women, aged 18-39 years (n=602)(15) performed using the same assay are 0·34 nmol/L (0·04-1·0) and DHEA 4·91 nmol/L (0·08-23·51), respectively.

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This study was approved by the Monash Human Research Ethics Committee (CF16/10 - 2016000001) and the Alfred Hospital Human Research Ethics Committee (616/15). All participants provided written informed consent to contribute biospecimens to the ASPREE Healthy Ageing Biobank.

Sample size and statistical analysis

We defined a reference group of women from within the study population to establish normative sex steroid values by age. ASPREE participants were excluded from the reference group if they were using any of the following at recruitment: any form of systemic or topical sex steroid therapy (oestrogen, progestogen, tibolone, dehydroepiandrosterone (DHEA) or testosterone), tamoxifen, or other selective oestrogen receptor modulator, aromatase inhibitors, anti-androgen therapy (spironolactone or cyproterone acetate), glucocorticoid therapy or any anti-glycaemic agent.

It was anticipated that the distribution of women with recruitment sex steroid values measured in the age groups 70 to 74 years, 75 to 79 years, 80 to 84 years and 85 years and older would be approximately n=3787, 1840, 824, 268 respectively. The 80 to 84 year strata would have adequate precision to estimate sex steroid ranges with the mean estimated with 95% CI width of ± 0·07 standard deviations (SD) and the $2{\cdot}5^{th}$ and $97{\cdot}5^{th}$ percentiles will be estimated with approximate 95% CI widths of ± 0·12SD. Means, (SD), medians and 10th to 90th percentile ranges are reported for each sex steroid in each of the age strata 70 to 74, 75 to 79, 80 to 84 and 85 years or more.

The Kruskal Wallis test was used for comparison of the distribution of continuous variables across groups and the chi-square test for categorical variables. Linear regression for each of E_1, T, DHEA and SHBG was done for the ln(analyte) with age, body mass index (BMI) and smoking as factors in the model and coefficients exponentiated to have interpretation as ratios of adjusted geometric means (with a geometric mean being similar in value to a median).

Results

Recruitment to ASPREE commenced in 2010 and was completed by December 2014. Of the 16,703 Australian participants, 9180 (55%) were women. 6392 women had Biobank samples available for measurement of sex steroids and SHBG (Figure 1). The characteristics of these women and the subset of 5326 women in the reference group are shown in Table 1. The included and excluded participants were similar in age, ethnicity, smoking status and blood pressure. The included women were aged 70 to 94·7 years and 98·9% were of European ancestry. They had a lower mean BMI (28·0 ± 5·0 vs 28·6 ± 5·0 kg/m^2, p=0·001) and smaller mean waist circumference than the excluded women (92·6 ± 12·5 vs 94·4 ± 13·2 cm, p=0·001).

For the 5326 women included in the reference group, E_2 and DHT were below the sensitivity of the assay method in 3522 (66·1%) and 3873 (72·7%) respectively (Table 2). The proportion of women with serum E_2 below the measurement limit increased with age from 64·8% in 70-74 years to 72·3% in 85+ aged women ($p<0{\cdot}001$). The proportion of women with serum DHT below the assay sensitivity declined with age from 73·8% in 70-74 year old women to 58·2% in 85+ age group ($p<0{\cdot}001$). For the 1083 women with measurable E_2 the median value was 22·03 pmol/L (range 11·0 to 1373·0). Because of the relatively small numbers of women with values above the assay sensitivity for E_2 and DHT, these steroid levels were not analysed further.

Details for E_1, total T, DHEA and SHBG by age groups are provided in Table 3, and shown graphically in Figure 2. Median steroid values (range) for the total reference group were E_1 181·2 pmol/L (3·7-5768·9), total T 0·38 nmol/L (0·035-8·56), DHEA 2·60 nmol/L

(0·069-46·85) and SHBG 41·6 nmol/L (2·4-176·6). As seen in Figure 2, there were a number of outliers for each steroid in each age group. We examined whether any of the measured variables (BMI, weight, waist circumference, smoking) and any reported medication use, predicted extreme outliers for any of the steroids, but could not identify a common explanation for the outliers.

The following multivariable analysis results are derived from the ratios of adjusted geometric means, as described in the methods. Compared with women aged 70-74 years, women aged 80-84 and 85+ had higher E_1 levels (respectively higher by 9·2%, p=0·001, and 11·7%, p=0·01). Overweight and obese women had higher E_1 levels than normal weight women (respectively higher by 14·6% and 34·1%, p<0·001). T levels were significantly higher in women 80-84 years and 85+ years, than women 70-74 years (higher, respectively by 9·3%, p=0·004 and 11·3%, p=0.02) (Table 4). Overweight and obese women also had higher T levels (5·2%, p=0·03 and 5·5% respectively, p=0·03). Current smokers had 17·2% higher T levels than non-smokers (p=0·005). SHBG increased with age from 70-74 years, being 5·6% higher in women aged 75-79 years, 13·6% higher in 80-84 years and 22·7% higher in 85+ years (p<0·001 for all). Being overweight was associated with a 15% lower SHBG and being obese a 27% lower SHBG level (p<0·001). Current smokers had SHBG levels 7·6% higher than non-smokers (p=0·023). In multivariable linear regression, E_1 was not independently associated with SHBG, when BMI and age were taken into account. T was independently, positively associated with SHBG (p<0.001) when BMI, age, and smoking were included in the model (data not shown).

DHEA levels showed a steady decline with age such that women aged 85+years had 30% less DHEA than women aged 70-74 years (p<0·001). DHEA levels were significantly higher in underweight (25% higher, p=0·036) and overweight women (5·2% higher, p=0·032).

When compared with the reference group, current glucocorticoid users (n=188) had significantly lower levels of DHEA (median 1·04 nmol/L, range 0·07-8·61, p=0·0001), T (0·024 nmol/L,0·03-1·84, p=0·0001) and E_1 (107·1, 3·7-6212·6, p=0·0001). Glucocorticoid users had a lower median SHBG of borderline statistical significance (39·2 nmol/L, 1·4-237·6, p=0·055).

Discussion

This large, cross-sectional study of circulating sex steroid levels in older women free of severe illness demonstrates small, but steady, increases in circulating T and E_1 in women from the age of 70 years, despite a steady decline in DHEA and that circulating T levels in women aged 70+ years are similar to levels in healthy premenopausal women measured by the same assay. This study also highlights the importance of E_1 as the major circulating oestrogen in older, postmenopausal women and possibly a detrimental impact of glucocorticoid use.

The T levels in the women in this study did not differ from levels seen in premenopausal women, measured by the same LC-MS/MS assay, and exhibited a small, but statistically significant, difference across age groups. We previously observed, in a study of women aged 18 to 75 years, a nadir for total T, measured by sensitive immunoassay, for women between 62 and 63 years of age, followed by a small increase beyond that in women up to age 75 (3). Laughlin et al observed a similar, statistically significant, positive association between T and age in their study of women with a mean age of 73.8 years(16). In contrast, an apparent decline in total T, measured by immunoassay, in women between 65 and 80 years was reported in a sample of 347 women, mean age of 74 years (6). Having not included women younger than 70 years, the present study neither supports nor refutes our prior observation of a nadir in total T in women in their early sixties. It does however establish that T levels in older women are maintained in the setting of a 30% decline in T's primary precursor, DHEA.

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As in younger women, circulating levels of DHEA are several fold greater than that of T (4) indicating that biosynthesis of T is dependent on enzyme activity, not precursor availability. The determinants of extra-gonadal T biosynthesis by conversion of circulating precursors of adrenal origin in women are not known. However, as in our previous study (3), and as has been reported for men (17), tobacco smokers had significantly higher T levels than non-smokers. Whether this is due to an effect of smoking on T biosynthesis, secretion or metabolism is not known.

In postmenopausal women, DHEA and its derivative androstenedione, are the major source of circulating E_1 through aromatisation of androstenedione in extra-gonadal tissues, primarily adipose (18). Adipose aromatase gene expression increases with age in women (19,20). Therefore, the capacity for adipose tissue to biosynthesise E_1 increases with age. The steady increase in E_1 levels with increasingly older groups of women in the present study, independent of BMI and SHBG is a new finding that indicates aromatase activity continues to increase with age even in elderly women. With the loss of ovarian E_2 production, in postmenopausal women E_1 is an important precursor for peripheral E_2 biosynthesis, and T is further converted to DHT, with both E_2 and DHT being further metabolised intra-cellularly (18,21). Hence, in the majority of women concentrations of E_2 and DHT were below the limit of detection, consistent with the intracrine production and metabolism of both hormones occurring within the tissues in which they act, with serum levels arising from unregulated spill-over from tissues into the circulation (18).

The increasing proportion of women with unmeasurable E_2 in the older groups most likely reflects different effects of age on the enzymatic pathways essential for the biosynthesis of these hormones. Regardless, a key message from the findings is that studies investigating the association between oestrogens and diseases of ageing in postmenopausal women, must measure E_1 in order to provide meaningful findings.

The positive association between age and SHBG seen in the present study has previously been reported in a study of SHBG across the lifespan (22). As in the present study, we have previously reported a positive, independent association between SHBG and T in postmenopausal women (23). This most likely reflects the high proportion of circulating T that is SHBG bound. SHBG appears to be metabolically important, with low SHBG identified as an independent marker of insulin resistance and type 2 diabetes risk (24). SHBG has also been implicated in the pathogenesis of type 2 diabetes and CVD (24,25). We have previously demonstrated strong, independent and highly statistically significant inverse associations between both insulin resistance, estimated by the homeostasis model of insulin resistance, and SHBG, and between BMI and SHBG (23). These associations were independent of sex steroids. The greater SHBG levels in older women may reflect a metabolic survivorship advantage of women who have higher SHBG.

Agreeing with previous reports, we found glucocorticoid-users had significantly lower levels of T (6), as well as E_1 and DHEA than non-users. The low DHEA in glucocorticoid-users reflects adrenal suppression, but might also be a consequence of their underlying disease. This finding does however support the importance of DHEA for E_1 and T biosynthesis.

Strengths of this study include the large community-based study sample providing high statistical precision for estimating sex steroid ranges. This cohort is likely to be as representative as any other healthy volunteer-based study. Measurement of sex steroids by gold standard LC-MS/MS is an important study strength. Sensitivity for E_2 was a limitation. Although a more sensitive method to measure E_2 was available, the time and labor-demands of the more sensitive technique, it was not feasible for a study of this magnitude. The LC-MS/MS method used for this study does not allow for androstenedione (analysed in positive polarity) and estradiol (analysed in negative polarity) to be measured in the same run as the

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two steroids elute at virtually the same retention time. Our method includes fast polarity switching but cannot cope with co-eluting compounds with opposite polarity. However, in postmenopausal women virtually all androstenedione is of adrenal origin. Therefore, its parent steroid DHEA provides a strong overall index of adrenal androgen production.

Our earlier study(3) and that of Cappola et al(6) reported much higher T levels in older women than measured with LC-MS/MS in the present study, consistent with known issues of cross-reactivity and less specificity of the older RIAs used previously (26,27). To explore the physiology of ageing, we excluded women who reported use of medications that would influence sex steroid measurement or metabolism. We excluded women taking anti-glycaemic medications in order to rule out women with type 2 diabetes requiring treatment, in order to minimise the effects of insulin resistance on sex steroid levels through effects on SHBG. A number of women in the reference group had sex steroid levels suggestive of exogenous hormone use. We examined all medication use in the outliers for each steroid to exclude any unexpected cross-re-activity in assays and found none. Although the higher sex steroid levels found in a few women did not fit our expectations, without evidence of exogenous steroid use, we could not justify their exclusion from the analysis. This decision is supported by prior smaller studies that have reported unexpectedly high estrone and androgen levels in otherwise well postmenopausal women (3,6,28-30). The high levels observed in some women in our study re-affirm the wide range of 'normality' within a community-based population, and support the representativeness of our study sample. A limitation of our study is that we are inferring the association between hormone levels and age from cross-sectional data. Whereas the optimal study design would be longitudinal, it would be prohibitively difficult and vulnerable to attrition bias. Our sample being mostly of European ancestry matches that of the Australian population of this age (31). However, our findings cannot be extrapolated to women of non-European ancestries. Information about past hysterectomy or oophorectomy was not collected, such that the impact of oophorectomy on hormone levels in older women could not be examined.

In summary, we have successfully established normal ranges for sex steroids in women aged 70 years and above. Important observations are that in women aged 70+ years circulating T levels do not differ meaningfully from those of premenopausal women, and together with E_1, appear to increase from the age of 70 years. Concurrently DHEA levels exhibit a progressive decline from the age of 70 years. E_1, T and SHBG may be biomarkers for longevity, or contribute to healthy ageing.

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Author contributions
Literature search: Susan Davis; figures Susan Davis, Penelope Robinson; study design, Susan Davis, Robin Bell, Chris Reid, Mark Nelson, John McNeil, Robyn Woods, Anne Murray, Rory Wolfe, Jessica Lockery; data collection and biobank management: Tom Gilbert, James Phung, Robyn Woods, Jessica Lockery; biochemical analysis: D Handelsman, Reena Desai; data analysis: Penelope Robinson, Robin bell, Rory Wolfe: data interpretation: Susan Davis, Robin Bell, D Handelsman, Robyn Woods; writing: Susan Davis, Penelope Robinson, Robin Bell; manuscript review: all co-authors.

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Correspondence: Professor Susan Davis, Women's Health Research Program, School of Public Health and Preventive Medicine, Monash University, 553 St Kilda Rd, Melbourne 3004, Australia. Tel: +61 3 9030684; Email: susan.davis@monash.edu

Potential conflicts of interest
Dr Davis reports having received honoraria from Besins Healthcare and Pfizer Australia and has been a consultant to Mayne Pharmaceuticals, Lawley Pharmaceuticals and Que Oncology. Dr Handelsman has received institutional grant funding (but no personal income) for

Data Availability
Restrictions apply to the availability of data generated or analyzed during this study to preserve patient confidentiality or because they were used under license. The corresponding author will on request detail the restrictions and any conditions under which access to some data may be provided.

investigator-initiated clinical testosterone pharmacology studies (Lawley, Besins Healthcare) and has provided expert testimony to anti-doping and professional standards tribunals and testosterone litigation. Dr Nelson reports receiving travel support from Bayer and fees for serving on an advisory board from Sanofi. Dr Murray has received travel funds from Bayer Pharmaceuticals. No other potential conflict of interest relevant to this article are reported.

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Figure 1. Flow chart of study participants. Footnote. *not including women who reported estrogen use

Figure 2 Relationship between age and individual sex steroids for the reference group. Footnote. In the box and whisker plots (outliers not included), the box represents the interquartile range (IQR), the line in the box is the median. The whiskers extend to the upper and lower adjacent values. The upper adjacent value is defined as the largest data point <= the 75th percentile + 1·5 X IQR. The lower adjacent value is defined as the smallest data point >= the 25th percentile -1·5 X IQR. Outliers are any values beyond the whiskers. The

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raw data are represented as scatter-graphs with fitted LOWESS curves on a log (base 10) scale. DHEAS, dehydroepiandrosterone-sulphate; SHBG, sex hormone binding globulin.

Table 1: Descriptive statistics of women included in, and excluded from the reference group.

	Included in analysis (n= 5326, 83.3 %)	**Excluded from analysis** (n= 1066, 16.7%)	**p value**
Age (years)			
Mean (SD), range	75·1 (4·2), 70- 94·7	75·0 (4·2), 70·2 -91·7	
70-74, n (%)	3122 (58·6%)	654 (61·45)	0·25
75-79, n (%)	1442 (27·1%)	257 (24·1%)	
80-84, n (%)	592 (11·1%)	121 (11·4%)	
≥ 85, n (%)	170 (3·2%)	34 (3·2%)	
Ethnicity, n (%)			0·61
European	5267 (98·9%)	1053 (98·8%)	
Asian	30 (0·6%)	8 (0·7%)	
Aboriginal/Torres Strait Islander	5 (0·1%)	2 (0·2%)	
Other	24 (0·4%)	3 (0·3%)	
Smoking status, n (%)			0·37
Current	156 (2·93%)	23 (2·16%)	
Former	1683 (31·60%)	344 (32·27%)	
Never	3487 (65·47%)	699 (65·57%)	
Weight (kg), mean (SD)	71·0 (13·3)	72·6 (13·9)	0·0005
Height (cm) mean (SD)	1·59 (0·061)	1·59 (0·058)	0·77
Body mass index (kg/m^2), mean (SD)	28·0 (5·0)	28·6 (5·3)	0·001*
< 18.5 , n (%)	46 (0·87%)	7 (0·66%)	0·005#
18.5 to <25, n (%)	1533 (28·91%)	277 (26·01%)	
25 to <30, n (%)	2128 (40·13%)	402 (37·75%)	
≥ 30, n (%)	1596 (30·10%)	379 (35·59%)	
Waist circumference (cm) mean (SD)	92·6 (12·5)	94·4 (13·2)	<0·001
Systolic blood pressure (mmHg), mean (SD)	141 (18)	141 (18)	0·71
Diastolic blood pressure (mmHg), mean (SD)	78 (11)	77 (11)	0·18

*Kruskal Wallis ; # χ^2.

Table 2. Proportion of women with measurable sex steroid levels by age

Age grouping (years)	**70 to 74**	**75 to 79**	**80 to 85**	**≥ 85**	**Total**
Estrone					
Result available	3093 (99·1%)	1425 (98·8%)	589 (99·5%)	170 (100·0%)	5277 (99·1%)
Below LOD	29 (0·9%)	17 (1·2%)	3 (0·5%)	0 (0·0%)	49 (0·9%)
Estradiol					
Result available	1097 (35·2%)	480 (33·3%)	179 (30·2%)	47 (27·7%)	1803 (33·9%)
Below LOD	2024 (64·8%)	962 (66·7%)	413 (69·8%)	123 (72·3%)	3522 (66·1%)
Total testosterone					
Result available:	3073 (98·4%)	1417 (98·3%)	588 (99·3%)	170 (100·0%)	5248 (98· ·5%)
Below LOD	49 (1·6%)	25 (1·7%)	4 (0·7%)	0 (0·0%)	78 (1·5%)
DHT					
Result available	817 (26·2%)	390 (27·1%)	175 (29·6%)	71 (41·8%)	1453 (27·3%)
Below LOD	2305 (73·8%)	1052 (72·9%)	417 (70·4%)	99 (58·2%)	3873 (72·7%)
DHEA					
Result available	3107 (99·5%)	1424 (98·8%)	590 (99·7%)	170 (100·0%)	5291 (99·3%)
Not detected	15 (0·5%)	18 (1·2%)	2 (0·3%)	0 (0·0%)	35 (0·7%)
SHBG					
Result available	3113 (99·8%)	1441 (99·9%)	592 (100·0%)	169 (100·0%)	5315 (99·9%)
Below LOD	5 (0·2%)	1 (0·1%)	0 (0·0%)	0 (0·0%)	6 (0·1%)

LOD, Limit of detection; DHT, dihydrotestosterone; DHEA, dehydroepiandrosterone; SHBG, sex hormone binding globulin. # 1 sample missing; ## 5 missing samples.

Table 3· Sex steroids by age group in the reference group of women

Age (years)	**70 to 74**	**75 to 79**	**80 to 85**	**≥ 85**	**Total**
Estrone, n (pmol/L)*	3093	1425	589	170	5277
Mean (SD)	210·8 (214·2)	213·7 (194·3)	220·1 (114·9)	223·4 (121·5)	213·0 (197·6)
Median	181·2	181·2	196·0	205·2	181·2
Minimum	3·7	3·7	3·7	14·8	3·7

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10th centile	88·7	85·0	99·8	83·2	88·7
90th centile	340·2	351·3	362·4	369·8	347·6
Maximum	5768·9	4733·4	894·9	717·4	5768·9
Testosterone, n (nmol/L)†	3073	1417	588	170	5248
Mean (SD)	0·45 (0·45)	0·46 (0·37)	0·49 (0·44)	0·52 (0·50)	0·47 (0·43)
Median	0·35	0·35	0·38	0·38	0·38
Minimum	0·03	0·03	0·03	0·03	0·03
10th centile	0·17	0·17	0·17	0·17	0·17
90th centile	0·76	0·87	0·90	1·11	0·83
Maximum	8·56	4·75	5·37	4·16	8·56
DHEA, n (nmol/L) †	3107	1424	590	170	5291
Mean (SD)	3·25 (2·19)	3·02 (2·20)	2·87 (1·99)	2·35 (1·61)	3·11 (2·16)
Median	2·74	2·50	2·32	2·05	2·60
Minimum	0·07	0·07	0·07	0·07	0·07
10th centile	1·11	0·94	0·97	0·82	1·04
90th centile	6·11	5·73	6·07	4·15	6·00
Maximum	46·85	27·38	10·03	9·82	46·85
SHBG, n (nmol/L)	3113	1441	592	169	5315
Mean (SD)	42·9 (18·2)	45·5 (19·3)	49·3 (19·5)	54·6 (21·9)	44·7 (19·0)
Median	39·8	42·8	46·3	51·5	41·6
Minimum	2·8	2·4	13·4	16·6	2·4
10th centile	23·5	24·3	27·2	29·4	24·2
90th centile	66·2	70·0	75·2	83·2	68·9
Maximum	167·6	151·2	155·3	124·2	167·6

†To convert nmol/L to ng/dL or pmol/L to pg/dL divide by 0·0347; ††to convert nmol/L to ng/dL divide by 0·0344; *to convert pmol/L to pg/ml, divide by 3·67
DHT, dihydrotesosterone; DHEA, dehydroepiandrosterone; SHBG, sex hormone binding globulin; SD, standard deviation·

Table 4. Factors contributing to sex steroid levels

Determinants evaluated	**Estrone Univariable analysis**			**Estrone Multivariable analysis**			**Testosterone Univariable analysis**			**Testosterone Multivariable analysis**		
	Effect of determinant	**95% CI**	**p-value**	**Effect of determinant**	**95% CI**	**p-value**	**Effect of determinant**	**95% CI**	**p-value**	**Effect of determinant**	**95% CI**	**p-value**
Age (years)												
70 – 74	1·00						1·00			1·00		
75 – 79	1·0008	0·965 to 1·038	0·96	0·999	0·964 to 1·036	0·99	1·016	0·973 to 1·061	0·48	1·014	0·971 to 1·059	0·527
80 – 84	1·070	1·017 to 1·127	0·010	1·092	1·038 to 1·148	0·001	1·085	1·021 to 1·153	0·008	1·093	1·029 to 1·162	0·004
85+	1·072	0·980 to 1·174	0·13	1·117	1·022 to 1·220	0·014	1·119	1·007 to 1·244	0·037	1·133	1·019 to 1·260	0·021
BMI kg/m^2												
< 18·5	1·015	0·856 to 1·202	0·87	1·002	0·846 to 1·188	0·98	0·941	0·769 to 1·150	0·553	0·925	0·757 to 1·131	0·446
18·5- <25	1·00			1·00			1·00			1·00		
25 - <30	1·142	1·100 to 1·186	< 0·001	1·146	1·103 to 1·190	< 0·001	1·048	1·001 to 1·096	0·045	1·052	1·006 to 1·101	0·028
30+	1·331	1·279 to 1·386	< 0·001	1·341	1·288 to 1·396	< 0·001	1·045	0·996 to 1·097	0·072	1·055	1·005 to 1·108	0·029
Current smoker												
Yes	1·017	0·926 to 1·11	0·722	1·058	0·964 to 1·16	0·233	1·153	1·032 to 1·28	0·012	1·172	1·049 to 1·30	0·005

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		8			0			7			9	
No	1·00						1·00			1·00		
	DHEA Univariable analysis			**DHEA Multivariable analysis**			**SHBG Univariable analysis**			**SHBG Multivariable analysis**		
	Effect of determinant	**95% CI**	**p-value**	**Effect of determinant**	**95% CI**	**p-value**	**Effect of determinant**	**95% CI**	**p-value**	**Effect of determinant**	**95% CI**	**p-value**
Age (years)												
70 – 74	1·00			1·00			1·00			1·00		
75 – 79	0·897	0·858 to 0·937	< 0·001	0·894	0·856 to 0·935	< 0·001	1·056	1·029 to 1·084	< 0·001	1·056	1·030 to 1·082	< 0·001
80 – 84	0·858	0·807 to 0·912	< 0·001	0·859	0·808 to 0·914	< 0·001	1·161	1·119 to 1·204	< 0·001	1·136	1·097 to 1·177	< 0·001
85+	0·706	0·633 to 0·786	< 0·001	0·705	0·633 to 0·786	< 0·001	1·277	1·198 to 1·362	< 0·001	1·227	1·154 to 1·305	< 0·001
BMI kg/m^2												
< 18·5	1·221	0·990 to 1·504	0·061	1·249	1·015 to 1·538	0·036	1·094	0·973 to 1·231	0·133	1·073	0·955 to 1·206	0·238
18·5-<25	1·00			1·00			1·00			1·00		
25 -<30	1·055	1·007 to 1·105	0·024	1·052	1·004 to 1·101	0·032	0·844	0·823 to 0·867	< 0·001	0·848	0·826 to 0·870	< 0·001
30+	1·039	0·989 to 1·092	0·127	1·027	0·978 to 1·079	0·289	0·722	0·702 to 0·743	< 0·001	0·730	0·710 to 0·751	< 0·001
Current smoker												
Yes	1·048	0·936 to 1·174	0·418	1·028	0·918 to 1·151	0·630	1·103	1·032 to 1·179	0·004	1·076	1·010 to 1·147	0·023
No	1·00									1·00		

BMI, body mass index; DHEA, dehydroepiandrosterone, SHBG, sex hormone binding globulin.

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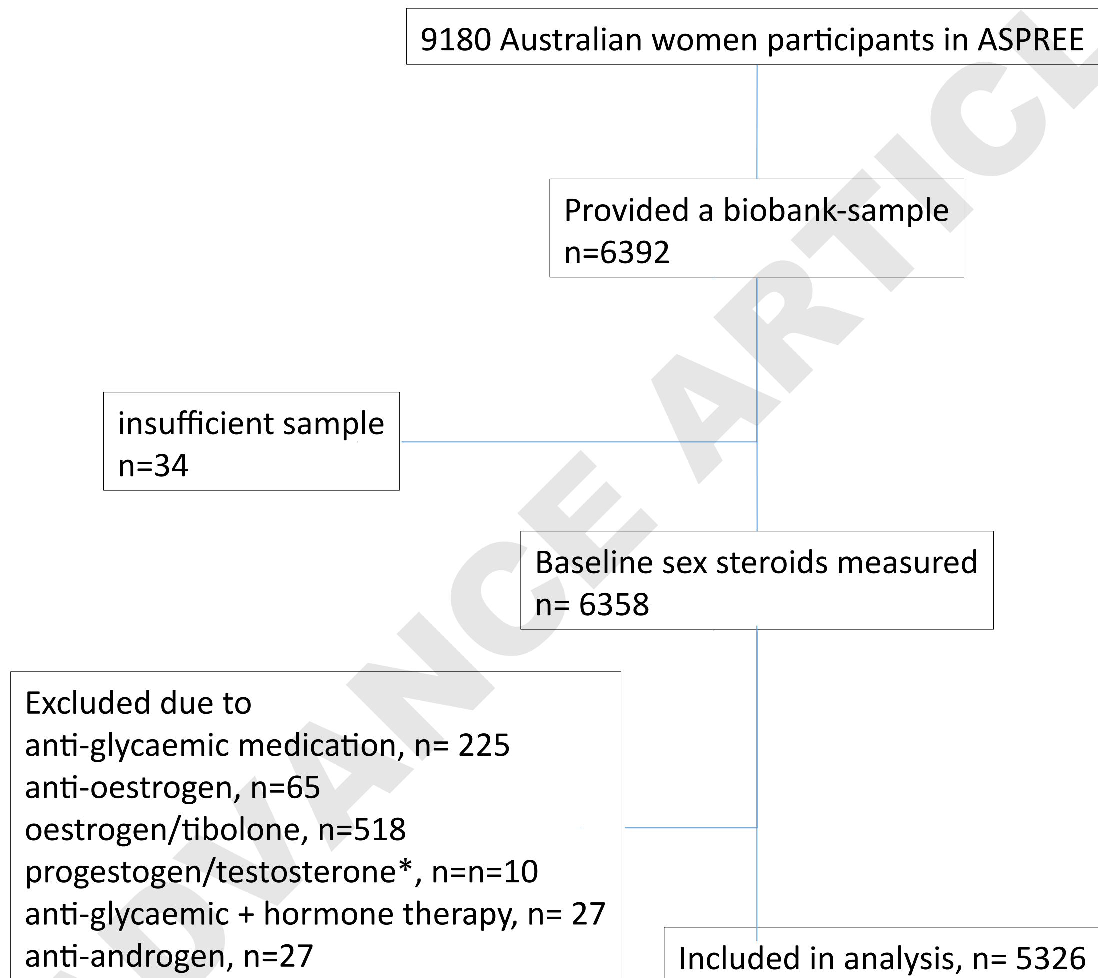

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