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BRIEF REPORT

Age-Related Changes in Serum Testosterone and Sex Hormone Binding Globulin in Australian Men: Longitudinal Analyses of Two Geographically Separate Regional Cohorts

Peter Y. Liu, Jonathan Beilin, Christian Meier, Tuan V. Nguyen, Jacqueline R. Center, Peter J. Leedman, Markus J. Seibel, John A. Eisman, and David J. Handelsman

Department of Andrology (P.Y.L., D.J.H.), Concord Hospital and ANZAC Research Institute, University of Sydney, Sydney, New South Wales 2139, Australia; Department of Endocrinology and Diabetes (J.B., P.J.L.), Royal Perth Hospital, Perth 6847, Western Australia, Australia; Bone Research Program (C.M., M.J.S.), ANZAC Research Institute, University of Sydney, Sydney 2006, Australia; Division of Endocrinology, Diabetes, and Clinical Nutrition (C.M.), University Hospital Basel, 4031 Basel, Switzerland; Bone and Mineral Research Program (T.V.N., J.R.C., J.A.E.), Garvan Institute of Medical Research, St. Vincent's Hospital and University of New South Wales, Sydney 2010, Australia; and Laboratory for Cancer Medicine (P.J.L.), Centre for Medical Research, School of Medicine and Pharmacology, University of Western Australia and the Western Australian Institute for Medical Research, Perth 6000, Australia

Background: Cross-sectional studies from different populations show a variable decline in blood testosterone concentrations as men age. Few population representative cohorts have been followed up over time.

Objective: The objective of the study was to quantify longitudinally the change in serum testosterone and SHBG concentrations with age in two well-defined, representative but geographically widely separated regional Australian cohorts.

Subjects and Setting: The Busselton cohort comprises individuals aged 18–90 yr residing in Western Australia assessed prospectively since 1981. Sera were assayed from 910 men, from whom further samples were available 14 yr later in 480. The Dubbo cohort involves individuals aged 61–90 yr living in Eastern Australia. Baseline sera were collected from 610 men and additional sera on a second (n = 370) and third (n = 200) occasion from 1989 to 2004. Men from both cohorts are community dwelling and of predominately European origin.

Results: Longitudinal analyses show the following: 1) total testosterone declines comparably ($P > 0.9$) by 1.3% (Busselton) and 0.9% (Dubbo) per annum with the same rates of decline when analyses were restricted to men older than 60 yr of age; 2) annual changes in SHBG were also very similar in age-restricted analyses (2.3% *vs.* 2.5%, $P = 0.48$); and 3) the annual increase in SHBG was steeper in middle-aged and older men ($P < 10^{-3}$ *vs.* young men). These longitudinal changes were all up to 4-fold greater in magnitude, compared with cross-sectional analyses of baseline data.

Conclusion: In two separate regional Australian populations, blood testosterone fell and SHBG increased comparably with age. Age-related changes in blood testosterone and SHBG previously described in urban-dwelling men are the same in men who reside in smaller regional cities of another continent. **(*J Clin Endocrinol Metab* 92: 3599–3603, 2007)**

INCREASING POPULATION longevity creates a need to foster methods to promote healthy, independent, and enjoyable living. Reduced testosterone production could contribute to aging-associated increases in frailty, falls, and fractures and decreases in bone strength, muscle mass and strength, and physical performance (1). Whether the age-related decline in systemic testosterone exposure, described thus far in longitudinal studies from the United States (2–6) and Europe (7), occurs at a similar rate in other populations is not yet known. This would support the extrapolation of findings from any large-scale, randomized, controlled trials to test a testosterone intervention to different populations (1).

Representative population-based cross-sectional studies of ethnically comparable but geographically distinct cohorts have generally shown that total testosterone (TT) declines in adults with each year of age (8–11), with few exceptions (12). Furthermore, the quasilongitudinal effect size estimates obtained have varied by as much as 10-fold from 0.1% per annum (10) to 1.6% per annum (13). This large variation in effect size estimates suggest that the age-associated changes in systemic testosterone exposure may differ among ethnically comparable but geographically distinct cohorts. Longitudinal studies are needed to resolve this. However, longitudinal studies are limited in number (2–7); all examined men residing in urban populations, two involved less than 100 men (5, 6), and another two examined extremes of adult age (2, 7). Potential geographical differences in the longitu-

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Abbreviations: ANCOVA, Analysis of covariance; BMI, body mass index; TT, total testosterone.

dinal rate of these declines and whether such changes even occur in regional or rural locations have not been investigated previously. Theoretically, this could occur due to putative differences in nutrition or obesity in urban compared with regional centers. This is pertinent because obesity is the most important identified confounder of the age-related decline in testosterone because it amplifies (2, 3) but does not fully explain this phenomenon. Furthermore, geographical differences in the age-related decline of testosterone have been reported (14), although small sample size and assessment of salivary rather than blood testosterone are important limitations. Additionally, whether the fall in testosterone occurs consistently among young, middle-aged, or older men is underexplored, and this information may be important for the timing of potential interventions.

The present study therefore aimed to determine whether: 1) blood testosterone concentrations decline with age in two separate regional city cohorts located at opposite sides of the Australian continent; 2) the magnitude of these changes might differ by geographical region; 3) the rate of decline in testosterone varies among young, middle-aged, or older men; and 4) age-related changes are due to obesity.

Subjects and Methods

Subjects

Participants were recruited from two population-based regional Australian centers from the west and east coast of the continent. The Busselton Population Survey is an ongoing representative and comprehensive survey of the cardiovascular and respiratory health of residents in the Shire of Busselton in regional southwestern Western Australia (15). Of the total adult male population of 2770 in 1981, 1458 subjects completed the survey. The present analysis was restricted to men for whom blood testosterone concentrations were measured from blood stored in 1981 (n = 910), which represents approximately 35% of the total adult male population in the Busselton shire. Of these men, 53% (n = 480) were reassessed in 1994–1995.

The participants from Dubbo are part of the ongoing Dubbo Osteoporosis Epidemiology Study (16). Briefly, since 1989, all men and women 60 yr old or older living in Dubbo, a regional inland city of 32,000 people in the state of New South Wales, Australia, were invited to participate. By July 2004, 862 men were participating in the study. The present analysis was restricted to men who had a baseline serum sample available (n = 610, 70.6%, defined as the first visit at which serum was collected). Thereafter men were followed up at approximately 2-yr intervals until 2004. About 60% (n = 370), 30% (n = 200), and 5% (n = 30) had a second, third or fourth blood sample available for analysis.

Each study was approved by its respective ethics review committees, and all subjects provided written informed consent. Consent included approval to store serum for future hormone or other measurements.

Laboratory measurements

For the Busselton cohort, all baseline blood samples were obtained between 0710 and 1240 h. All subsequent blood samples were collected early in the morning. Serum samples were stored at −20 C. TT and SHBG were measured by commercial chemiluminescent immunoassay (Immulite 2000; Diagnostic Products Corp., Los Angeles, CA.) in 2001 (for initial blood samples collected in 1981) and 2003 (for subsequent blood samples collected in 1994–1995). All samples were measured on the same platform by the same technician. Prior reports suggest that blood SHBG or testosterone concentrations do not change appreciably after samples are frozen at −20 C for decades (4). Coefficients of variation averaged 15.5 (TT) and 2.9% (SHBG).

For the Dubbo cohort, blood samples were collected since 1989 at a convenient rather than a fixed time of day, but most were collected in the morning. Serum samples were stored at −80 C until analysis in late 2004. TT and SHBG were measured by commercial immunoassays (DELFIA; Wallac Oy, Turku, Finland). Coefficients of variation averaged 10.3 (TT) and 7.9% (SHBG). All samples were assayed at one time.

For both cohorts, free testosterone and bioavailable testosterone were calculated (17, 18).

Statistical analysis

To minimize the effect of outliers, nonparametric methods were used. Kruskal-Wallis and signed-rank tests and Spearman's rank-order correlation were performed as appropriate. $P < 0.05$ was construed as significant, although recognizing the multiple testing may violate nominal significance levels. Data are median (interquartile range).

Longitudinal analyses

For each individual with serial data, a slope term was calculated by linear regression as an annual change in that analyte. Both the absolute and relative (percentage) annual changes were calculated and separately analyzed. Within each cohort, significant annualized change in each analyte was evaluated by signed-rank test of nonzero slope. A difference in the relative change in testosterone or SHBG between the Busselton and Dubbo cohorts was assessed by Mann-Whitney *U* test. The main approach was to evaluate relative changes in these dimensionless slope parameters to minimize the impact of using different assay methods.

The effect of age on these relationships was examined by dividing men from the Busselton cohort into three groups aged 18–40, 40–60, and older than 60 yr. Examining differences among the three epochs of age was assessed by Kruskal-Wallis test without excluding outliers. Analysis of covariance (ANCOVA) was used to simultaneously assess the effects of continuous [age, body mass index (BMI)] and categorical (geographical location) variables. Because ANCOVA can be heavily influenced by outliers, 25 men with baseline TT less than 5 nM were excluded beforehand (<5% of all data).

Cross-sectional analyses

Cross-sectional analyses were also performed to allow comparison with previous publications. Linear regression was performed to ascertain the magnitude of the relationship between biochemical or anthropometric parameters with age. The slope is the annual change in that parameter or the change per year of age. This was converted to a relative (percentage) change per year of age by dividing the slope by the corresponding cohort median.

Results

Subjects

Compared with the Dubbo (n = 610) cohort, men in the Busselton (n = 910) cohort were younger [51 (29) *vs.* 72 (8) years, median (interquartile range), $P < 0.001$], with a wider age range (18–90 *vs.* 61–90 yr), slightly taller [174 (9) *vs.* 172 (9) cm, $P < 0.001$] and had been followed for longer [12.4 (13) *vs.* 4.0 (6.1) yr, $P < 0.001$]. Weight [77 (14) *vs.* 77 (18) kg] and BMI [25.7 (4.4) *vs.* 26.0 (5.0) kg/m^2] did not significantly differ.

Longitudinal analyses

Significant relative (and absolute) decreases in TT were evident in the Busselton cohort and increases in SHBG and BMI were evident in both cohorts: Table 1. The relative decrease in TT in the Dubbo cohort was not significant ($P = 0.07$).

The relative fall in TT was comparable between the two cohorts when analysis was restricted to men older than 60 yr ($P = 0.62$) and in the two cohorts as a whole ($P = 0.95$) (Table 1). In contrast, discordant results were obtained with and without restricting the analysis to men aged older than 60 yr for derived testosterone parameters and SHBG (Table 1). The

TABLE 1. Longitudinal (median and interquartile range) analyses among testosterone, SHBG, and BMI with age

Longitudinal (n = 850)	Busselton, n = 480 (all ages)			Busselton, n = 80 (age > 60 yr)			Dubbo, n = 370			Busselton *vs.* Dubbo	
	Annual change[c]	% annual change[c]	*P* value[d]	Annual change[c]	% annual change[c]	*P* value[d]	Annual change[c]	% annual change[c]	*P* value[d]	*P* value (age-restricted)[a]	*P* value (all)[b]
TT (nmol/liter)	−0.2 (0.6)		<0.0001	−0.2 (0.6)		<0.0001	−0.1 (1)		0.016		
		−1.3 (3.3)	<0.0001		−1.3 (3.3)	0.0001		−0.9 (8.1)	0.07	0.62	0.95
Calculated free testosterone (pmol/liter)	−4.9 (10.8)		<0.0001	−5.0 (12.1)		<0.0001	−3.1 (18.1)		<0.0001		
		−1.8 (3.6)	<0.0001		−2.1 (3.5)	<0.0001		−2.2 (10.2)	0.0002	0.67	0.02
Calculated non-SHBG bound testosterone (pmol/liter)	−161 (339)		<0.0001	−194 (276.7)		<0.0001	−95 (389)		<0.0001		
		−1.9 (3.3)	<0.0001		−2.4 (2.7)	<0.0001		−2.2 (8.0)	<0.0001	0.87	0.01
SHBG (nmol/liter)	0.3 (1.0)		<0.0001	0.7 (1.6)		<0.0001	1.0 (3.5)		<0.0001		
		1.2 (3.9)	<0.0001		2.3 (6.2)	<0.0001		2.5 (7.8)	<0.0001	0.48	0.003
BMI (kg/m^2)	0.1 (0.2)		<0.0001	0.01 (0.15)		0.59	0.1 (0.4)		<0.0001		
		0.3 (0.7)	<0.0001		0.0 (0.6)	0.91		0.4 (1.4)	<0.0001	<0.0001	0.30

[a] *P* value between Busselton and Dubbo cohorts (age restricted).
[b] *P* value between Busselton and Dubbo cohorts (all).
[c] Median (interquartile range).
[d] Upper *P* value for annual change, lower *P* value for percent annual change.

percent changes in TT and SHBG were comparable in men over the age of 60 yr from both cohorts, so these data were pooled. This showed that TT fell by 1.0% (6%) per year and SHBG increased by 2.4% (7.5%) per year in these men.

In the three age groups from the Busselton cohort (Table 2), TT and calculated free testosterone did not differ by age group. However, the relative increase in SHBG in the Busselton cohort rose more steeply with age ($P < 10^{-4}$, Kruskal-Wallis test). Calculated bioavailable but not free testosterone also differed by age ($P = 0.038$ and 0.13, respectively, Kruskal-Wallis test). Bonferroni-adjusted *post hoc* analyses showed that this was due to significant ($P < 0.05$) differences between the youngest (aged 18–40 yr) and oldest (aged older than 60 yr) groups for both SHBG and calculated bioavailable testosterone. These findings were confirmed by two-way ANCOVA using a model to examine simultaneously the effect of age, BMI, and geographical location (data not shown). This analysis also showed that obesity (BMI) did not explain any of these relationships ($P > 0.4$ for BMI term).

Cross-sectional analyses

TT fell ($-0.32 \pm 0.07\%$, mean ± SEM, $P < 0.001$) and SHBG increased ($0.46 \pm 0.11\%$, $P < 0.001$) per year of age in men from Busselton. TT exhibited a similar but not significant decline ($-0.37 \pm 0.37\%$, $P = 0.08$) per year of age in men from Dubbo whereas SHBG increased ($1.10 \pm 0.34\%$, $P < 0.001$). Analogous relationships were obtained with cross-sectional analysis of data from the next visit after baseline samples (not shown). The magnitude of longitudinal hormonal estimates exceeded those obtained from the cross-sectional analyses of the baseline data by as much as 4-fold (compared with relative changes obtained from longitudinal analysis shown in Table 1).

Discussion

We show in two longitudinal population-based representative cohorts of men from different regional city cohorts at opposite geographical areas of Australia that blood testosterone falls and SHBG increases with age. Separately, we show in one of the few direct comparisons between separate cohorts that the magnitude of these changes are similar, even after adjustment for obesity (1–3). Obesity was examined as a confounder because of its immediate relevance due to the escalating global obesity epidemic and because it was prospectively determined using a standardized method in both cohorts. Such findings underscore the consistency between populations and geographical areas of the declining blood testosterone concentrations in older men, a topic of growing interest (1). Our findings in two Australian cities extend previous longitudinal studies to regional centers in another continent and also replicate the findings among men of predominantly European origin (2–7). Our pooled findings of approximately 1% per annum decline in TT in men over the age of 60 yr, consistently throughout adult life are only marginally lower, compared with these previous longitudinal studies.

One important caveat to these findings is the different assays used by the two cohorts. This makes it difficult to compare directly the absolute concentrations such as the

TABLE 2. Longitudinal annual percent changes in testosterone and SHBG according to age tertiles in the Busselton cohort

	Age 18–40 yr (n = 200)		Age 40–60 yr (n = 200)		Age over 60 yr (n = 80)		*P* value[a]
	Annual % change	*P* value[b]	Annual % change	*P* value[b]	Annual % change	P value[b]	
TT	−1.4 (3.2)	<0.0001	−1.1 (3.6)	0.002	−1.3 (3.3)	<0.0001	0.24
Calculated free testosterone	−1.8 (3.6)	<0.0001	−1.5 (4)	0.0002	−1.8 (3.6)	<0.0001	0.13
Calculated non-SHBG bound testosterone	−1.6 (3.5)^{A,B}	<0.0001	−1.7 (3.5)^{B,C}	<0.0001	−1.9 (3.3)^{C}	<0.0001	0.038
SHBG	0.2 (4.1)^{A}	0.005	1.3 (3.4)^{B}	<0.0001	1.2 (3.9)^{B}	<0.0001	<0.0001
BMI	0.5 (0.7)^{A}	<0.0001	0.2 (0.7)^{B}	<0.0001	0.03 (0.6)^{C}	0.6	<0.0001

Values are expressed as median (interquartile range). *Different uppercase superscripts* indicate the *post hoc* Bonferroni-adjusted pairwise differences assessed by Mann-Whitney *U* test.

[a] *P* values (Kruskal-Wallis) show whether annual changes differ across the three age groups.

[b] *P* values (signed-rank) for nonzero slope.

apparently higher blood testosterone and lower SHBG in men from Busselton at baseline (under cross-sectional analysis). For this reason, we used an analytical strategy of examining within-individual (and within method) relative (percentage) annual changes in testosterone or SHBG by linear regression for the longitudinal analyses to reduce potential systematic differences due to assay method. The consistency in the magnitude of the changes in testosterone and SHBG between these two cohorts, particularly after age is controlled for, supports the reliability of these findings and this analytical approach.

Within each cohort, we found that longitudinal exceeded cross-sectional estimates of change substantially (up to 4-fold greater) and additionally steeper falls in calculated free and non-SHBG testosterone, compared with measured TT with either analytical method. These findings are also consistent with other large studies (3, 4).

The significance of the steeper declines in free and non-SHBG bound testosterone is unclear because: 1) the importance of these derived testosterone measures, abstractions calculated as a nonlinear function of TT and SHBG (17), have not been directly tested (19) and 2) these measures overlook putative physiological processes actively mediated by SHBG. Our finding of steeper increases in SHBG with increasing baseline age is therefore intriguing. Increases in SHBG with age have previously been described longitudinally (2, 3); however, our findings additionally show that these rates accelerate with aging, and are independent of obesity, which lowers SHBG.

This study had some limitations. First, as with all observational studies, direct causality cannot be firmly attributed to the relationship between circulating testosterone and age. Second, subjects were older men of mainly European descent living in regional Australia, so generalization to other populations should be cautious. Third, serum was not collected consistently in the morning particularly in the Dubbo cohort, so that circadian rhythms could have contributed to the variability. However, because circadian rhythmicity of blood testosterone concentrations is blunted in older men, diurnal variability is unlikely to have significant impact on our results (20).

We conclude that the age-related decline in serum testosterone and increase in SHBG are comparable across two geographically separated regional Australian communities and also that longitudinal analyses of blood testosterone and SHBG may show up to 4-fold higher changes than cross-sectional analyses, suggesting that longitudinal data are necessary to properly characterize age-related changes. These data extend longitudinal data previously restricted to U.S. and European populations to predominantly Caucasian Australian men.

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Address all correspondence and requests for reprints to: David J. Handelsman, MBBS, Ph.D., FRACP, Department of Andrology, Concord Hospital, ANZAC Research Institute, University of Sydney, Sydney, New South Wales 2139, Australia. E-mail: djh@anzac.edu.au.

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