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AGE AND SEASONAL VARIATION IN SERUM TESTOSTERONE CONCENTRATION AMONG MEN

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Abstract—Serum testosterone concentrations from 4,462 military veterans, ages 32–44, were examined for age and seasonal variation. Testosterone concentrations were assayed from a single serum sample from each subject. All samples were drawn before breakfast, at about 8:00 a.m., from subjects recruited over a 16-month study period. Mean levels declined with age ($P < .001$), from 864 ng/dl at age 32 to 602 ng/dl at age 44. Mean levels also varied with month of testing ($P < .01$), with a seasonal peak in December (the seasonal peak was in November for men in their early 30s). The age effect was greater than the seasonal effect. Both effects may bear upon behaviour and should be treated as possible sources of error in studies of testosterone.

Key words—Testosterone, age, cycle, seasonal, circannual.

Introduction

Testosterone concentrations in human males change across the life span and across periods of time ranging from hours to months (1–13). A better understanding of these changes would help control for error when individual subjects were monitored over time or groups of subjects were measured at different times. It would also help relate changes in testosterone to psychological and behavioural processes, especially involving aggression and reproduction, that change over time.

Males testosterone concentrations vary from infancy to old age. Concentrations are about the same in newborn male infants as in female adults (1). They drop within a few days of birth and rise back to their initial levels around 1–4 months, before dropping again and remaining relatively low until puberty (1). They rise during puberty to a peak around 15–16 years (2) and then begin a slow decline to old age. Read and Walker (3) reported a linear decline with age ($r = -.62$) from ages 28–85. Neischlag (4), studying healthy older men, reported a less consistent decline with age from the 20s to the 60s and beyond ($r = -.22$).

Testosterone concentrations also fluctuate with pulses about every 90 min (5), and they vary across the day (6–8) and across the year (9–13). Circadian rhythms have been widely reported, with mean testosterone generally declining from a high around awakening to a low in late evening (6–8). Annual cycles have been studied less thoroughly than circadian ones, and with less consistent findings. Reinberg and Lagoguey (9) found testosterone highest in the fall in a sample of five males, and Smals *et al.* (10) found testosterone highest in the summer and fall in a sample of 15 males. Bellastella *et al.* (11) found testosterone highest in the summer among 72 pre-pubertal boys, and Nicolau *et al.* (12–13) found it highest in the summer among 63 elderly men. Age might reduce the amplitude of the annual cycle, as it does in the case of the circadian cycle (14–15). Phase and amplitude of the circadian rhythm have also been reported to change with time of year (9), and these changes in turn might vary with men of different ages.

While age and seasonal effects add variability to testosterone measurements, the exact nature of this variability is unknown. Sample sizes have been rather small, and statistical power has consequently been low. Data are needed representing a large number of subjects from a large range of ages. In the present study, data from military veterans were examined for age and

seasonal variation in the morning testosterone level.

Method

The study examined archival data from 4,462 military veterans. The veterans, half of whom had served in Vietnam during 1965–1971, were studied by the Centers for Disease Control beginning in 1985 to assess .possible long-term effects of the Vietnam military experience (16). Their mean age was 37 years, and most were within the age range of 32–44. They came from all parts of the United States and were representative of the United States population with regard to race, education, income and occupation. They were interviewed by telephone and underwent extensive medical, physical, psychological, neuropsychological and laboratory examinations at the Lovelace Medical Foundation (Albuquerque, NM), and were presumably diurnally active. Their testosterone concentrations were assayed from serum samples collected in the morning at 0800 before eating. The assay was conducted using a standard double-antibody radioimmunoassay method; the assay coefficient of variation (CV) among quality control samples was less than 10% (17). Different subjects provided serum samples each day over the course of the study period, which lasted from June 1985 to September 1986. Each subject was classified according to age and month in which his sample was collected. The nominal 32 and 44 year old groups included 17 subjects younger than 32 and 20 subjects older than 44. In December, subjects were tested only during the first two weeks of the month.

Data were grouped according to age of subject and month of testing. Trends across age and month of testing were first analyzed using two-way analysis of variance and then examined in more detail using regression and cosinor analysis. Age effects were examined by regression of yearly age means onto the best-fitting set of linear and quadratic weights, which would describe a downward trend with a curvilinear component. Month of testing effects were examined by regression of monthly testing means onto the best-fitting set of linear, quadratic, cubic and quartic weights, which would describe a trend with three inflection points, such as a curve that completes slightly more than one annual sinusoidal cycle in a 16-month testing period. Cosinor analysis (18) was employed to find the year-long cosine curve that fits the data best and to quantify this curve in terms of acrophase (seasonal peak time), amplitude (half the peak–trough difference) and mesor (rhythm-adjusted time series mean).

Results

Overall mean testosterone concentration was 680 ng/dl, SD = 235. Table 1 shows the number

Table 1. Numbers of subjects and mean testosterone concentrations (ng/dl) in each age group and each month of the study period

Age	Number	Testo. (ng/dl)	Month	Number	Testo. (ng/dl)
32	61	846	Jun 85	311	652
33	109	775	Jul 85	267	677
34	241	761	Aug 85	344	678
35	372	714	Sep 85	284	684
36	528	715	Oct 85	402	708
37	662	684	Nov 85	288	746
38	789	661	Dec 85	278	679
39	662	646	Jan 86	371	725
40	402	639	Feb 86	346	727
41	313	640	Mar 86	316	690
42	194	609	Apr 86	293	675
43	93	616	May 86	199	665
44	76	602	Jun 86	239	673
			Jul 86	231	643
			Aug 86	163	641
			Sep 86	130	699

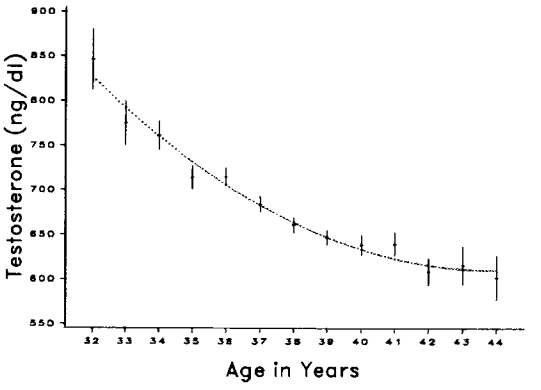

Figure 1. Age changes in testosterone: Mean serum testosterone concentrations in subgroups of men (total N = 4,462) ranging from 32 to 44 years of age. Error bars show the standard error of each mean. The curve represents the best-fitting model for the data, from regression analysis (see text).

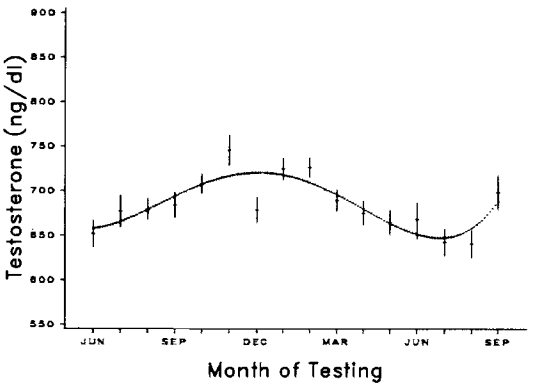

Figure 2. Seasonal changes in testosterone: Mean serum testosterone concentrations in subgroups of men (total N = 4,462) tested in different months across a 16-month period. Error bars show the standard error of each mean. The curve represents the best-fitting model for the data, from regression analysis (see text).

of subjects and mean testosterone in each age group and each month of testing. Mean testosterone concentrations varied significantly with age [F (12, 4256) = 14.61, $P < .001$] and month of testing [F(15, 4256) = 5.43, $P < .001$]. The interaction between age and month of testing was also significant [F(178, 4256) = 1.36, $P < .01$], but the effect was small and examination of the means revealed no readily interpretable pattern. Least squares means, also known as marginal or population means, were computed to adjust age effects for differences in month of testing, and month of testing effects for differences in age (19). It is these least square means that are shown in Table 1 and Figures 1 and 2.

Figure 1 shows a mean decline in testosterone from age 32 to 44, with a slight leveling off as age increases. The curve in Figure 1 was generated by regression of yearly age means onto the best-fitting set of linear and quadratic weights. This model fitted the data well, accounting for 97% of the variance in the pattern of age-specific means.

Figure 2 shows variation in testosterone across the 16-month study period. The means follow a seasonal pattern, with highest ones in early winter. The curve in Figure 2 was generated by regression of monthly means onto the best-fitting set of linear, quadratic, cubic, and quartic weights. This model accounted for 70% of the variance in the pattern of means. Cosinor analysis revealed a circannual rhythm with the amplitude significantly greater than zero (F (2, 13) = 10.64, $P < .01$). The acrophase was 23 December (95% confidence interval 26 November–21 January); mesor 691 ng/dl (95% confidence interval 679–702); and amplitude 33 ng/dl (95% confidence interval 16–48). This model accounted for 62% of the variance in the pattern of means. The regression and cosinor analyses agree in placing peak testosterone levels in early winter, although Figure 2 shows the observed value for December was well below its predicted level. This low value may reflect transient effects of the pre-holiday season—samples were drawn only during the first half of December—but there is no way of knowing exactly why it was low.

Given the strong age effect, it seemed appropriate to examine the relationship between seasonal pattern and age. Subjects were divided into four groups, based upon age at nearest birthday, and separate cosinor analyses were performed on each group. The results are summarized in Table 2. The mesor declined with age, and the acrophase was in late fall rather than early winter for the youngest group.

Discussion

Testosterone concentrations were higher in younger than older men and higher in late fall

Table 2. Cosinor analysis showing seasonal pattern by age (95% confidence intervals in parentheses). Mesor and amplitude units are ng/dl

Age	N	Acrophase	Mesor	Amplitude	*F*-ratio	*P*
32–34	575	30 Nov ± 42 days	766 ± 27	52 ± 38	4.14	0.04
35–37	1785	6 Jan ± 34 days	698 ± 14	33 ± 36	6.76	0.01
38–40	1599	20 Jan ± 43 days	651 ± 14	26 ± 20	4.35	0.04
41–44	503	20 Jan ± 50 days	620 ± 26	41 ± 36	3.29	0.07

and early winter than at other times of the year. As can be seen from Figures 1 and 2, age produced about twice as much variation as month of testing. For investigators studying group differences or treatment conditions affecting testosterone, age may produce more error than time of year when data are collected.

The decline in testosterone concentration with age was striking, even within the relatively short 15-year period. The decline was largely linear with a curvilinear component, reflecting a faster drop in testosterone in the early years. A question arises as to whether this pattern would be different across other ages. Nicolau *et al.* (13) reported a mean testosterone concentration of 476 ng/dl among 36 men in their 70s, and Bremner *et al.* (14) reported a mean of 508 ng/dl among 12 men in their 70s. It is difficult to compare across studies, due to differences in laboratory technique and materials, but it is clear that the decline in testosterone in the decade of the 30s is large.

It is possible that a seasonal pattern other than that observed here was masked by phase shifts in the circadian pattern, such that daily peaks did not always occur before breakfast. Reinberg and Lagoguey (9) reported circadian peaks in mid-morning in spring and midafternoon in fall. However, Reinberg and Lagoguey observed only five subjects, and morning testosterone concentrations have generally been found to be high (8).

The behavioural significance of age and seasonal variations in testosterone could be far-reaching. Testosterone, in both animals and human beings, is related to sexual and aggressive behaviour (20). A large drop in testosterone by the end of the fourth decade of life could be related to a decline in criminal behavior at this time (21) and a shift from the striving achievement of early adulthood to the quieter time of middle adulthood (22). High testosterone levels during the autumn could be related to reported heightened sexual aggression at this time of year (22) and elevated higher birth rates nine months later (24). The definitive study must wait until data are collected from a wide age range of subjects and assayed in a single laboratory, providing a detailed picture of changes across the life span.

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References

1. Amano T. The study of serum testosterone level during childhood: I. Growth and circadian rhythm. *J Kyoto Prefect Med Univer* **94**, 1325–1339, 1985.
2. Swift A. D. Salivary testosterone in the male adolescent. In: Ferguson D. B., ed. *Steroid Hormones in Saliva*. Karger, Basel, pp. 69–79, 1984.
3. Read G. F. and Walker R. F. Variation of salivary testosterone with age in men. In: Read G. F., Riad-Fahmy D., Walker R. F. and Griffiths K., eds. *Immunoassays of Steroids in Saliva*. Alpha Omega, Cardiff, Wales, 1984.
4. Neischlag E. Comment on Read and Walker. In: Read G. F., Riad-Fahmy D., Walker R. F. and Griffiths D., eds. *Immunoassays of Steroids in Saliva*. Alpha Omega, Cardiff, Wales, 1984.
5. Veldhuis, J. D., King J. C., Urban R. J., Rogol A. D., Evans W. S., Kolp S. A. and Johnson M. L. Operating characteristics of the male hypothalomo-pituitary-gonadal axis: Pulsatile release of testoterone and follicle-

stimulating hormone and their temporal coupling with luteinizing hormone. *J. Clin Endocrinol Metab* **65**, 929–941, 1987.
6. Lincoln G. A., Rowe P. H. and Racey R. A. The circadian rhythm in plasma testosterone concentration in man. In: Aschoff J., Ceresa F. and Halberg F., eds. *Chronobiological Aspects of Endocrinology*. Schattauer, Stuttgart, pp. 137–149, 1974.
7. Lejeune-Lenain C., Van Cauter E., Desir D., Beyloos M. and Franckson F. R. M. Control of circadian and episodic variations of adrenal androgens secretion in man. *J. Endocrinol Invest* **10**, 267–276, 1987.
8. Dabbs J. M. Jr. Salivary testosterone measurements: Reliability across hours, days and weeks. *Physiol Behav* **48**, 1990. (to appear).
9. Reinberg A. and Lagoguey M. Circadian and circannual rhythms of sexual activity and plasma hormones (FSH, LH, testosterone) of five human males. *Arch Sex Behav* **7**, 13–30, 1978.
10. Smals A. G. H., Kloppenbort P. W. C. and Benraad T. H. Circannual cycle in plasma testosterone levels in man. *J. Clin Endocrinol Metab* **42**, 979–982, 1976.
11. Bellastella A., Esposito V., Mango A. and D'Alessandro B. Temporal relationship between circannual levels of luteinizing hormone and testosterone in prepubertal boys with constitutional short stature. *Chronobiologia* **9**, 123–125, 1982.
12. Nicolau G. Y., Lakatua D. J. and Haus E. Circadian and circannual rhythms of hormonal variables in elderly men and women. *Chronobiol Int* **1**, 301–319, 1984.
13. Nicolau G. Y., Haus E., Lakatua D. J., Bogdan C., Sackett-Lundeen L., Popescu M., Berg H., Petrescu E. and Robu E. Circadian and circannual variations of FSH, LH, testosterone, dehydroepiandrosterone-sulfate (DHEA-S), and 17-hydroxy progesterone (17 OH-PROG) in elderly men and women. *Roumanian J Med* **23**, 223–246, 1985.
14. Bremner W. J., Vitiello M. V. and Prinz P. N. Loss of circadian rhythmicity in blood testosterone levels with aging in normal men. *J. Clin Endocrinol Metabol* **56**, 1278–1281, 1983.
15. Lakatua D. J., Nicolau G. Y., Bogdan C., Petrescu E., Sackett-Lundeen L. L., Irvine P. W. and Haus E. Circadian endocrine time structure in humans above 80 years of age. *J. Gerontol* **39**, 648–654, 1984.
16. Centers for Disease Control. Health status and Vietnam veterans. *J. Am Med Ass* **259**, 2701–2719, 1988.
17. Centers for Disease Control. *Health Status of Vietnam Veterans*. Author, Atlanta, GA, 1989.
18. Halberg F., Nelson W., Runge W. and Sothern R. Autorhythmometry procedures for physiologic self-measurements and their analysis. *Physiol. Teacher* **1**, 1–11, 1972.
19. SAS Institute. *SAS User's Guide: Statistics, Version 5 edition*. Author, Carey, NC, 1985.
20. Svare B. B. *Hormones and Aggressive Behavior*. Plenum, New York, 1983.
21. Blumstein A., Cohen J., Roth J. A. and Visher C. A. *Criminal Careers and "Career Criminals," Vol. 1*, National Academy Press, Washington, DC, 1986.
22. Levinson D. J. *The Seasons of Man's Life*. Ballatine, New York, 1978.
23. Bicakova-Rocher A., Smolensky M., Reinberg A. and De Prins J. Seasonal variations in socially and legally unacceptable sexual behaviour. *Chronobiol Int* **3**, 203–208, 1985.
24. Randall W. A Statistical analysis of the annual pattern in births in the USA, 1967 to 19786. *J. Interdiscip Cycle Res* **1**, 179–191, 1987.