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Age Trends in the Level of Serum Testosterone and Other Hormones in Middle-Aged Men: Longitudinal Results from the Massachusetts Male Aging Study

HENRY A. FELDMAN, CHRISTOPHER LONGCOPE, CAROL A. DERBY, CATHERINE B. JOHANNES, ANDRE B. ARAUJO, ANDREA D. COVIELLO, WILLIAM J. BREMNER, AND JOHN B. MCKINLAY

New England Research Institutes (H.A.F., C.A.D., C.B.J., A.B.A., J.B.M.), Watertown, Massachusetts 02472; University of Massachusetts Medical School (C.L.), Worcester, Massachusetts 01655; and University of Washington Medical School (A.D.C., W.J.B.), Seattle, Washington 98195

We used longitudinal data from the Massachusetts Male Aging Study, a large population-based random-sample cohort of men aged 40–70 yr at baseline, to establish normative age trends for serum level of T and related hormones in middle-aged men and to test whether general health status affected the age trends. Of 1,709 men enrolled in 1987–1989, 1,156 were followed up 7–10 yr afterward. By repeated-measures statistical analysis, we estimated simultaneously the cross-sectional age trend of each hormone between subjects within the baseline data, the cross-sectional trend between subjects within the follow-up data, and the longitudinal trend within subjects between baseline and follow-up.

Total T declined cross-sectionally at 0.8%/yr of age within the follow-up data, whereas both free and albumin-bound T declined at about 2%/yr, all significantly more steeply than within the baseline data. Sex hormone-binding globulin increased cross-sectionally at 1.6%/yr in the follow-up data, similarly to baseline. The longitudinal decline within subjects between baseline and follow-up was considerably steeper than the cross-sectional trend within measurement times for total T (1.6%/yr) and bioavailable T (2–3%/yr). Dehydroepiandrosterone, dehydroepiandrosterone sulfate, cortisol, and estrone showed significant longitudinal declines, whereas dihydrotestosterone, pituitary gonadotropins, and PRL rose longitudinally.

Apparent good health, defined as absence of chronic illness, prescription medication, obesity, or excessive drinking, added 10–15% to the level of several androgens and attenuated the cross-sectional trends in T and LH but did not otherwise affect longitudinal or cross-sectional trends.

The paradoxical finding that longitudinal age trends were steeper than cross-sectional trends suggests that incident poor health may accelerate the age-related decline in androgen levels. (*J Clin Endocrinol Metab* 87: 589–598, 2002)

THE NORMAL AGE course of sex hormone levels in middle-aged men has drawn interest in recent years because of the advent of hormone replacement with steroids such as T and dehydroepiandrosterone (DHEA). T is increasingly administered by transdermal patch as well as injection (1). DHEA is touted as an anti-aging diet supplement and is widely available over the counter. The purported benefit of such therapy should be assessed in relation to a definitive set of benchmarks for normal hormone levels at a given age.

Little consensus exists among clinicians as to what constitutes a normal sex hormone profile for an aging male (2). Agreement upon norms is impeded by the complex interrelations of the sex hormones with other hormone systems, with common chronic diseases of aging such as cancer, cardiovascular disease, diabetes, depression, hyperlipidemia, and arthritis, and with associated conditions and behavior such as obesity, sedentariness, nutritional deficiency, impotence, and frailty. The fact that men lack a major, identifiable displacement in hormonal status, comparable to menopause in women, makes the characterization of age-normal male endocrine status particularly difficult.

Abbreviations: AAG, Androstanediol glucuronide; DHEA, dehydroepiandrosterone; DHEAS, DHEA sulfate; DHT, dihydrotestosterone; MMAS, Massachusetts Male Aging Study; SHBG, sex hormone-binding globulin.

What is well established to date is that several important sex hormone levels, although not all, undergo a gradual shift in men after age 40. T and DHEA decline with age, whereas LH, FSH, and sex hormone-binding globulin (SHBG) rise with age (3–9). At least one important T metabolite, dihydrotestosterone (DHT), apparently remains constant despite the decline of its precursor (9–12).

The present report is an analytical summary of longitudinal sex-hormone data from the Massachusetts Male Aging Study (MMAS), a 10-yr prospective observational survey of health and aging in middle-aged men. MMAS was methodologically unique in providing a large community-based random sample of middle-aged men interviewed in their homes (n = 1,709), rather than a clinic-based sample. The participants provided comprehensive health information in interviewer-administered questionnaires as well as a blood sample for hormone analysis in a federally certified laboratory. Sample retention at follow-up was high (n = 1,156), allowing a detailed statistical analysis that addressed age trends both cross-sectionally (between subjects) and longitudinally (within subjects). MMAS thus provided a cohort in which to track the age course of androgen and other hormone levels in the context of a comprehensive picture of health and aging.

Cross-sectional trends of 17 hormones and metabolites in the MMAS were published after the baseline survey (9). That report included an assessment of the influence of general

good health on the hormone levels and trends. In the present report, we add data from the follow-up study and extend the characterization of hormone trends to include (1) a comparison of the levels and cross-sectional trend at follow-up, when the men were aged 50–80, with what we observed a decade earlier; (2) estimation of the within-subject longitudinal trend, which is newly available from the follow-up data; and (3) an assessment of the influence of general health on both the cross-sectional and the longitudinal trends.

Subjects and Methods

Study sample

The baseline phase of MMAS was conducted in 1987–89. A two-stage, age-stratified cluster sample was drawn from Massachusetts' statutory annual street listing in such a way that within each age stratum (40–49, 50–59, 60–69 yr) every male resident of the state had an equal probability of selection. Details of sampling and the in-home data collection protocol are published elsewhere (9, 13–16). Baseline data were obtained from 1,709 respondents, 52% of those sampled and eligible. The response rate was similar to those of comparable field studies of large, randomly selected population-based samples, requiring phlebotomy and an extensive early morning in-home protocol.

Characteristics of the sample are shown in Table 1 and hormone levels in Table 2, separately for baseline and follow-up. Age at baseline was uniformly distributed between 40–70 yr by design. The cohort was predominantly Caucasian, married, employed, and college-educated. The low fraction of racial minorities (5%) was representative of the Massachusetts population in 1987. Anthropometric and physiological parameters closely matched those of the second National Health and Nutrition Examination Survey. Randomly selected nonrespondents, interviewed by telephone, proved similar to respondents in general health and prevalence of chronic diseases (15).

Follow-up data were obtained in 1995–97. Of the eligible baseline cohort of 1,709 men, 1,156 were reinterviewed, whereas 180 were confirmed deceased, and 373 were unavailable for follow-up for other reasons (5 were abroad, 28 too ill to respond, 75 lost, 6 suspected deceased, 259 refused). The retention rate was 76% of those still living. The median interval for reinterview was 8.9 yr, the range was 7.1–10.4 yr. The rate of follow-up was significantly greater for men who were Caucasian, married, employed at baseline, or more highly educated ($P < 0.001$). The reinterviewed men had been slightly younger at baseline (mean age, 54.1 *vs.* 57.4 yr; $P = 0.05$), more physically active (67 *vs.* 58% reporting >200 kcal/d; $P = 0.08$), and more often apparently healthy (29 *vs.* 20%; $P = 0.11$), but did not differ in baseline serum T, DHT, or SHBG levels ($P > 0.30$).

Data collection and coding

All protocols and procedures were approved by the Institutional Review Board of New England Research Institutes.

A trained interviewer-phlebotomist visited each subject in his home between 0800 and 1000 h and obtained written informed consent. Height and weight were measured by standardized methods developed for large-scale field work (17). Health status and current treatment were ascertained by prompted self-report, using a list of nine medical conditions including heart disease, diabetes, and high blood pressure.

The interviewer took inventory of all current prescription and nonprescription medications, noting the subject's stated reason for use of each. Medications were coded afterward by two pharmacoepidemiology consultants (M. Barbour and A. Hume, University of Rhode Island, Providence, RI) using a classification similar to that of the American Hospital Formulary Service (18). Men taking androgens, estrogen, or bromocriptine at baseline were not eligible for the study. Five men taking exogenous T at follow-up were excluded from analysis.

Customary alcohol intake was estimated from self-report of beer, wine, and liquor consumption, counting 12 g ethanol as one drink and accounting for frequency, quantity, and binge drinking according to the Khavari formula (19).

A participant was rated in apparent good health if he met all of the following criteria: 1) no self-report of chronic illness (diabetes, high blood pressure, heart disease, ulcer, or cancer); 2) no current prescription medication; 3) body mass index not exceeding 29 kg/m^2, corresponding to 20% over ideal weight (20); and 4) alcohol consumption not exceeding 600 ml ethanol per week, corresponding to approximately six drinks per day or about five times the U.S. average, one drink being 15 ml ethanol (10 oz beer, 4 oz wine, or 1.5 oz spirits). Men not meeting all four criteria were classified as not in apparent good health.

TABLE 1. Cohort characteristics at baseline and follow-up, MMAS

Variable	Subgroup	Baseline (1987–89)	Follow-up (1995–97)
Number interviewed		1,709	1,156
Married (%)		75	76
Employed (%)		78	62
Race (%)	White	95	96
	Black	3	2
	Other	2	2
Education (%)	Less than high school	11	9
	High school	17	15
	Some college	29	28
	Bachelor's degree	12	12
	Beyond college	30	37
Apparent good health (%)	(a) No chronic disease	51	45
	(b) No prescription medication	51	35
	(c) No excessive body mass	71	67
	(d) No excessive alcohol intake	95	97
	(a), (b), (c), and (d)	26	18
Diabetes (%)		5	7
Heart disease (%)		7	11
Hypertension (%)		16	25
Current smoking (%)		24	13
Physical activity (%)	None	8	10
	Under 200 kcal/d	28	24
	200 kcal/d or more	64	66
Erectile dysfunction (%)	None	56	49
	Minimal	23	22
	Moderate	8	10
	Complete	12	19

Nonfasting blood samples were drawn from the antecubital space within 4 h of the subject's awakening to control for diurnal variation. One tube of blood was taken for lipid assays and two tubes for hormone assays. The two hormone samples were drawn 30 min apart and pooled in equal aliquots at the time of assay to smooth out episodic secretion (21). Blood was kept in an ice-cooled container for transport and centrifuged within 6 h. Serum was stored in 5-ml scintillation vials at −20 C, shipped to the laboratory on dry ice within 1 wk by same-day courier, and stored at −70 C until the time of assay. Assay methods and precision are detailed in Table 3.

Data analysis

All hormone concentrations were logarithmically transformed for analysis to reduce skew. A very few outliers (one to two per hormone) were removed to prevent undue influence of extreme values. The resulting distributions were virtually normal in many cases as judged by the Shapiro-Wilk statistic, and in no case was the distribution of log concentration severely asymmetrical. Simple correlations between baseline and follow-up hormone level were calculated by the Spearman formula (26), which is based on ranks and therefore unaffected by log transformation. After analysis, all transformed variables were retransformed into natural units for presentation.

To describe the trends for each hormone, we used a repeated-measures regression model, illustrated by Fig. 1, simultaneously estimating the cross-sectional trend between subjects at baseline (*left*), the longitudinal trend within individuals between baseline and follow-up (*center*), and the cross-sectional trend between subjects at follow-up (*right*). The model allowed both the level and the trend in hormone concentration to differ between men who were in apparent good health and those who were not. Figure 1 depicts data from a random sample of 50 men in whom the hormone level differed by health status but the trend did not, as shown by separate but parallel lines.

To specify the regression model mathematically, we denote time of measurement by T, log hormone concentration by Y_T, and age by a_T, with $T = 1$ for baseline, $T = 2$ for follow-up. The interval between measurements is thus $\Delta a = a_2 - a_1$. Indicator variables H_1 and H_2 are defined as

TABLE 2. Hormone levels at baseline and follow-up, MMAS

	Baseline (1987–89)	Follow-up (1995–97)
Age	55.2 ± 8.7 yr[a]	62.7 ± 8.3 yr
Total T	5.2 ± 1.8 ng/ml (18.0 ± 6.1 nmol/liter)	4.5 ± 1.6 ng/ml (15.7 ± 5.6 nmol/liter)
Free T	0.097 ± 0.039 ng/ml (0.34 ± 0.14 nmol/liter)	0.075 ± 0.032 ng/ml (0.26 ± 0.11 nmol/liter)
Albumin-bound T	1.9 ± 0.9 ng/ml (6.5 ± 3.0 nmol/liter)	1.5 ± 0.6 ng/ml (5.1 ± 2.1 nmol/liter)
SHBG	32 ± 16 nmol/liter	36 ± 17 nmol/liter
DHT	0.26 ± 0.17 ng/ml (0.91 ± 0.58 nmol/liter)	0.35 ± 0.20 ng/ml (1.19 ± 0.70 nmol/liter)
AAG	7.7 ± 4.0 ng/ml (26.5 ± 13.7 nmol/liter)	7.7 ± 5.0 ng/ml (26.6 ± 17.2 nmol/liter)
DHEA	2.3 ± 1.6 ng/ml (8.1 ± 5.6 nmol/liter)	1.9 ± 1.0 ng/ml (6.5 ± 3.6 nmol/liter)
DHEAS	2.6 ± 1.5 μg/ml (7.0 ± 4.1 nmol/liter)	1.6 ± 1.0 μg/ml (4.4 ± 2.6 nmol/liter)
Cortisol	17.4 ± 5.7 μg/dl (479 ± 156 nmol/liter)	15.9 ± 5.1 μg/dl (439 ± 141 nmol/liter)
FSH	6.6 ± 7.1 IU/liter	8.5 ± 8.4 IU/liter
LH	5.2 ± 3.5 IU/liter	5.8 ± 4.3 IU/liter
Estrone	42 ± 20 pg/ml (156 ± 75 pmol/liter)	33 ± 21 pg/ml (124 ± 78 pmol/liter)
PRL	7.1 ± 3.9 ng/ml	11.5 ± 10.1 ng/ml

[a] Mean (SD).

TABLE 3. Assay methods and variance parameters for hormones and metabolites measured in MMAS at baseline (1987–89) and follow-up (1995–97)

	Period	Assay method	Coefficient of variation (%)	
			Intra-assay	Inter-assay
Total T	Baseline	Diagnostic Products kit	5.4	8.0
	Follow-up	Diagnostic Products kit	5.8	9.0
Free T	Baseline	Centrifugal ultrafiltration (22)	6.0	7.1
	Follow-up	Centrifugal ultrafiltration (22)	5.1	8.9
Albumin-bound T	Baseline	Centrifugal ultrafiltration (23)	5.0	6.0
	Follow-up	Centrifugal ultrafiltration (23)	3.1	10.3
SHBG	Baseline	Filtration assay (22)	8.0	10.9
	Follow-up	Orion diagnostics kit	4.5	7.9
DHT	Baseline	RIA (24)	10.9	12.2
	Follow-up	RIA (24)	3.2	9.3
AAG	Baseline	Diagnostic Systems Laboratories kit	4.9	7.3
	Follow-up	Diagnostic Systems Laboratories kit	4.4	6.6
DHEA	Baseline	RIA (24)	2.6	5.2
	Follow-up	Wein Labs kit	2.9	6.5
DHEAS	Baseline	RIA (24, 25)	4.1	8.9
	Follow-up	ICN Biomedicals kit	3.0	7.9
Cortisol	Baseline	Ciba Corning kit	4.3	5.4
	Follow-up	Diagnostic Products kit	5.0	8.2
FSH	Baseline	Ciba Corning kit	5.1	6.4
	Follow-up	IMX Abbott Diagnostics kit	—	5.0
LH	Baseline	Ciba Corning kit	5.5	9.5
	Follow-up	IMX Abbott Diagnostics kit	—	6.9
Estrone	Baseline	RIA (24)	4.6	9.9
	Follow-up	RIA (24)	5.0	10.1
PRL	Baseline	Ciba Corning kit	3.6	8.7
	Follow-up	IMX Abbott Diagnostics kit	—	5.0

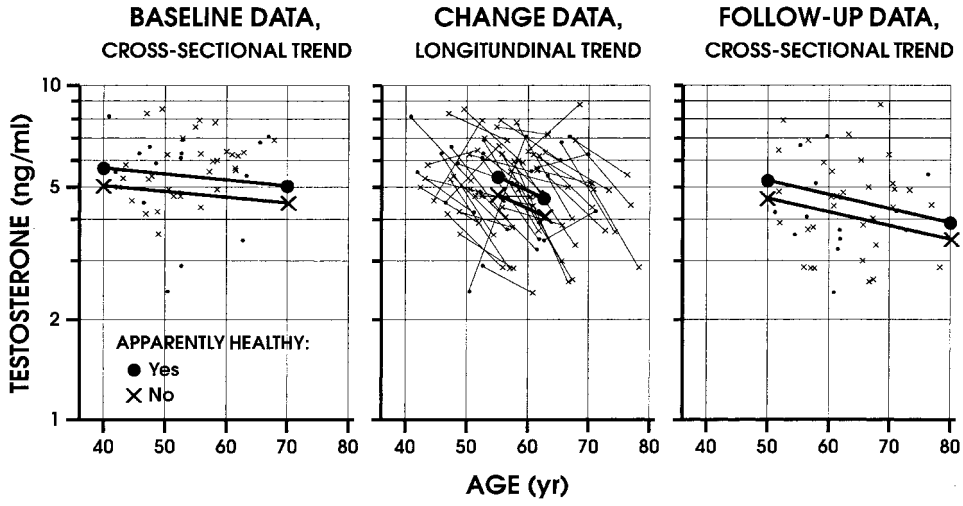

FIG. 1. Regression model for simultaneous estimation of cross-sectional age trends (within time, between subjects) and longitudinal age trends (within subject, between times) for androgens, metabolites, and other hormones in MMAS, illustrated by T values from 50 randomly selected men. Fitted model shows health status affecting T level but not affecting cross-sectional or longitudinal age trends, as indicated by separate but parallel lines.

TABLE 4. Cross-sectional and longitudinal age trend in hormones and metabolites measured in MMAS at baseline (1987–89) and follow-up (1995–97)[a]

	Cross-sectional trend (%/yr)			Longitudinal trend (%/yr)	
	Baseline	Follow-up	P^b	Estimate	95% CI[c]
Total T	−0.3	−0.8	0.005	−1.6	−2.0, −1.3
Free T	−1.0	−1.7	0.004	−2.8	−3.2, −2.3
Albumin-bound T	−0.9	−2.0	0.001	−2.5	−3.0, −2.1
SHBG	1.2	1.6	0.08, NS	1.3	0.8, 1.6
DHT	(0.0)	(0.0)	0.94, NS	3.5	3.0, 4.1
AAG	−1.1	−1.6	0.06, NS	(−0.4)	−0.9, 0.1
DHEA	−3.1	−2.3	0.02	−1.4	−2.0, −0.9
DHEAS	−2.2	−2.4	0.37, NS	−5.2	−5.6, −4.7
Cortisol	(0.0)	(−0.1)	0.51, NS	−0.9	−1.2, −0.7
FSH	2.0	1.9	0.82, NS	3.1	2.5, 3.6
LH	1.3	1.4	0.64, NS	0.9	0.5, 1.4
Estrone	(0.2)	−0.8	0.001	−3.6	−4.0, −3.1
PRL	−0.4	(−0.1)	0.28, NS	5.3	4.9, 5.8

[a] Serum concentrations log-transformed for analysis. Trend estimates in parentheses are not significantly different from zero; $P > 0.05$.
[b] Testing hypothesis that cross-sectional trends were equal within baseline data and follow-up data. NS, Not significantly different; $P > 0.05$.
[c] CI, Confidence interval.

1 if the subject was in apparent good health at baseline and follow-up, respectively, and 0 otherwise. The regression model is expressed as follows:

$$Y_T = \mu + \theta H_T + \beta_1 a_T + (\beta_2 - \beta_1)(T-1)a_T + \gamma H_T a_T + \lambda(a_T - a_1) + \delta + \epsilon_T,$$

or equivalently,

$$Y_1 = \mu + \theta H_1 + (\beta_1 + \gamma H_1)a_1 + \delta + \epsilon_1 \text{ (baseline)}$$

$$Y_2 = \mu + \theta H_2 + (\beta_2 + \gamma H_2)a_2 + \lambda\Delta a + \delta + \epsilon_2 \text{ (follow-up)}.$$

The term μ is a constant representing the general level of hormone. The coefficient θ is the effect of apparent good health on the general level. The coefficients β_1 and β_2 are cross-sectional age trends at baseline and follow-up, respectively. The parameter γ is the effect of apparent good health on the cross-sectional age trend. The coefficient λ is the longitudinal age trend. The constant δ represents a participant's unique characteristics affecting his hormone level at both time points. The values of δ are assumed to be drawn from a Gaussian distribution with mean 0 and variance V_δ. The residual error terms ϵ_1 and ϵ_2 represent measurement error and all other sources of random variance applying separately to the two measurements. The residual errors, assumed Gaussian with mean 0 and variance V_ϵ, are independent of each other and of the subject term δ. The correlation between a participant's baseline and follow-up values, adjusted for age and all other variables in the regression model, is given by $\rho = V_\delta/(V_\delta + V_\epsilon)$.

The Statistical Analysis System MIXED procedure (27) was used to fit the regression model, producing estimates and confidence intervals for all coefficients and the intraclass correlation ρ. The coefficients representing differences or linear trends in log hormone (θ, β_1, β_2, λ) were converted for presentation to percentage differences or trends per unit time; for example, the longitudinal trend is reported as $100\% \times (10^\lambda - 1)$. Statistically significant refers to inference based on either a 95% confidence interval or an asymptotic partial F test with 5% Type I error rate per predictor variable.

Additional terms were considered for the repeated-measures model, including terms for period (T) and acceleration of the longitudinal trend. Period was strongly confounded with the longitudinal trend, with parameter correlations exceeding 0.99, because of the concentration of data collection at two discrete time points. Period effects, although plausible in theory, could therefore not be pursued meaningfully. A second-order term for longitudinal trend [$(\Delta a)^2$] was likewise strongly confounded with the linear trend, and the coefficient could not be estimated. A term expressing modulation of the longitudinal trend by general health ($H \times \Delta a$ interaction) proved to be statistically insignificant in all analyses and was discarded.

To corroborate the estimates of longitudinal trend, we restricted the analysis to men with observations at both times and conducted conventional regression analysis with the individual's change in log hormone concentration as dependent variable, controlling for baseline age and health status. The resulting estimates of longitudinal trend were very close to those obtained from the repeated-measures model.

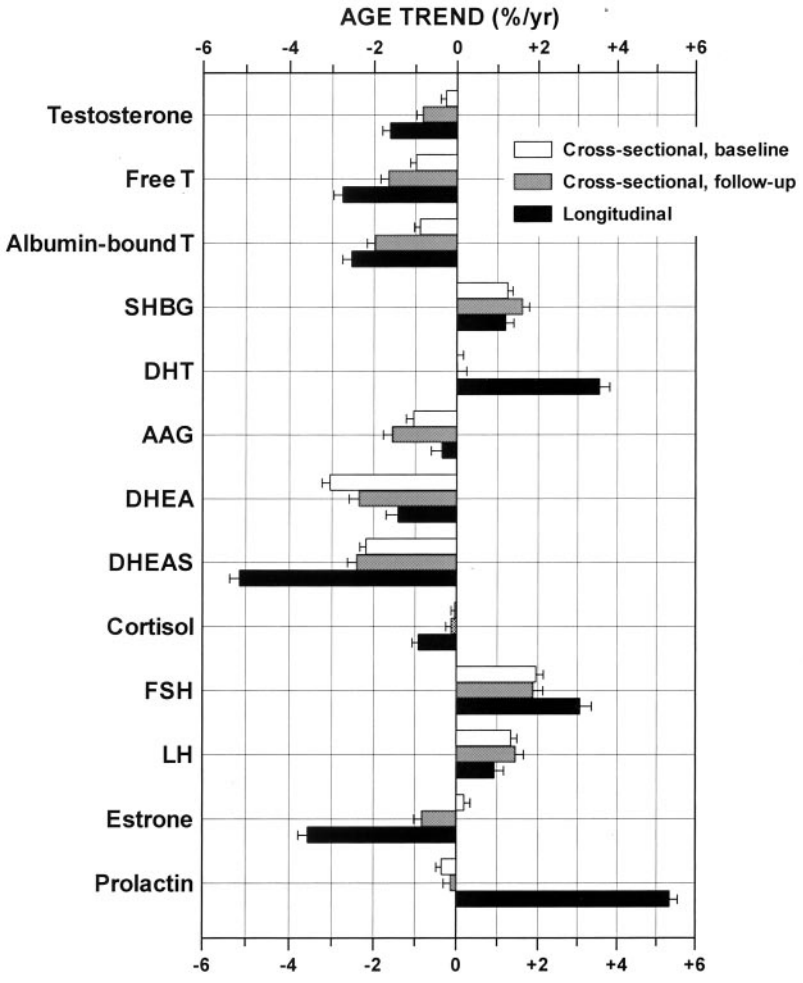

FIG. 2. Cross-sectional and longitudinal trends of T, other androgens and metabolites, and related hormones in middle-aged men, participants in MMAS, 1987–97.

Results

Cross-sectional and longitudinal trend estimates are presented in Table 4 and illustrated in Fig. 2. Within the follow-up data, total T declined cross-sectionally with age by 0.8%/yr, whereas both free and albumin-bound T declined at about 2%/yr. Each of those cross-sectional trends was significantly steeper within the follow-up data than within the baseline data ($P < 0.005$). The cross-sectional trend for SHBG in the follow-up data was an increase of 1.6%/yr, similar to that seen at baseline. The longitudinal decline within subjects between baseline and follow-up was 1.6%/yr for total T and 2–3%/yr for bioavailable T, both considerably steeper than the cross-sectional declines among subjects within the two measurement times. SHBG rose longitudinally at 1.3%/yr, a rate similar to the cross-sectional rise.

DHT showed a flat cross-sectional age trend within the follow-up data, just as it had at baseline, but the longitudinal trend within subjects between baseline and follow-up was a sharp increase of 3.5%/yr. The cross-sectional trend for androstanediol glucuronide steepened slightly between baseline and follow-up, from a decline of 1.1%/yr to a decline of 1.6%/yr, but no significant longitudinal trend was evident.

DHEA and DHEA sulfate (DHEAS) showed steep cross-sectional declines of 2–3%/yr in the follow-up data, as they had in the baseline data. Both DHEA and DHEAS showed steep longitudinal declines between baseline and follow-up as well. The cross-sectional age trend for cortisol was virtually flat within both measurement times, with a slight longitudinal decline.

The pituitary gonadotropins FSH and LH showed increases with age, both cross-sectionally and longitudinally. The cross-sectional trend in estrone was flat within the baseline data but declined significantly at 0.8%/yr within the follow-up data, with a marked longitudinal decrease of 3.6%/yr between baseline and follow-up. PRL varied only slightly cross-sectionally within both measurement periods but underwent a sharp longitudinal increase of 5.3%/yr.

The indicator of apparent good health, defined as absence of obesity, excessive drinking, and chronic illness (diabetes, heart disease, hypertension, ulcer, cancer), added 10–15% to the level of several hormones, especially the androgens (Table 5). The indicator did not affect the longitudinal trends (data not shown), but in those men with apparent good health, the reciprocal cross-sectional trends in T (downward) and LH (upward) within each measurement time were significantly attenuated. Cross-sectional trends in the other hormones were not affected.

Intrasubject correlations, shown in Table 6, were on the order of 0.50 before adjustment for age, ranging from 0.16 for estrone to 0.77 for FSH. Age accounted for a good deal of the

TABLE 5. Effects of apparent good health on level and cross-sectional age trend in hormones and metabolites measured in MMAS, 1987–97[a]

	Difference in level, AH − Not AH (%)	Cross-sectional age trend (%/yr)[b]		
		AH	Not AH	*P*
Total T	12.6	(−0.1)	−0.6	0.04
Free T	8.7	−1.0	−1.3	0.24, NS
Albumin-bound T	4.7	−1.1	−1.4	0.24, NS
SHBG	11.3	1.4	1.4	0.82, NS
DHT	19.3	(0.3)	(−0.1)	0.29, NS
AAG	(−1.7)	−1.4	−1.2	0.51, NS
DHEA	15.7	−2.3	−2.9	0.06, NS
DHEAS	15.8	−2.0	−2.4	0.17, NS
Cortisol	(2.3)	(−0.2)	(−0.1)	0.59, NS
FSH	(−4.9)	1.9	1.9	0.81, NS
LH	(−2.0)	0.9	1.5	0.03
Estrone	(−2.8)	(−0.1)	(−0.2)	0.60, NS
PRL	(−2.3)	−0.5	−0.2	0.36, NS

[a] Apparently healthy (AH) defined as absence of self-reported chronic disease, prescription medication, body mass index over 29 kg/m^2, and alcohol consumption over 600 ml/wk ethanol. AH had no significant effect on longitudinal trends. Estimates in parentheses are not significantly different from zero; $P > 0.05$.

[b] Serum concentrations log-transformed for analysis. Trend estimate is weighted average of baseline and cross-sectional trends. *P* tests hypothesis that AH had no common effect on the baseline and cross-sectional trends. NS, Not significantly different; $P > 0.05$.

correlation, as shown by the 2- to 3-fold decrease in correlation resulting from regression modeling. Similarly, the fraction of variance in hormone level attributable to individual variability, which ranged above 20% in several cases, was severely reduced when an appropriate amount was attributed to age and apparent health, leaving under 1% in every case attributable to any other individual characteristics (Table 6).

TABLE 6. Within-subject correlation between baseline (1987–89) and follow-up (1995–97) for hormones and metabolites measured in MMAS

	Correlation		Fraction of variance[a]	
	Simple[b]	Adjusted[c]	Simple	Adjusted
Total T	0.46	0.17	0.209	0.028
Free T	0.25	0.15	0.061	0.024
Albumin-bound T	0.23	0.10	0.053	0.009
SHBG	0.60	0.20	0.356	0.039
DHT	0.28	0.16	0.078	0.026
AAG	0.62	0.21	0.385	0.044
DHEA	0.47	0.19	0.222	0.036
DHEAS	0.57	0.24	0.322	0.056
Cortisol	0.23	0.11	0.051	0.013
FSH	0.77	0.26	0.590	0.067
LH	0.50	0.14	0.251	0.021
Estrone	0.16	0.00	0.025	0.000
PRL	0.48	0.21	0.227	0.046

[a] Fraction attributable to individual variability; equal to correlation squared.

[b] Spearman (rank-based) correlation.

[c] From regression analysis of log-transformed hormone concentrations, removing effects of cross-sectional and longitudinal age trends and apparent good health.

Discussion

Methodological issues

Until recently, most studies of the age course of T were compromised by methodological difficulties. Samples tended to be small and subject to the selection biases inherent in clinic-based research or volunteer enrollment (28). Diurnal variation in T levels, which is more pronounced in younger than older men (29), was not always accounted for. Some studies addressed total serum T concentration, whereas others concentrated solely on free T (1–2% of the total) or bioavailable T (free plus albumin-bound, 30–40% of total). Some disparities may have resulted from differing methods for assay of free T (30).

The MMAS overcame several of these methodological problems. The cohort was randomly selected and therefore representative of middle-aged Massachusetts men in the baseline year, although not of the entire United States, racial minorities being minimal. A complete set of T measurements was made, including total, free, and albumin-bound fractions and SHBG level. Blood was drawn consistently in the morning and assayed by a single laboratory.

Many of our assays remained the same between baseline and follow-up (Table 3). For these assays, there was no drift over time in the laboratory's measurements. For other assays, the methodology changed because a more efficient method became available. In these instances, a careful evaluation of both methods was performed to ensure that results by the two methods were similar before a change was instituted. Because of these procedures, methodological alterations can be ruled out as a major source of variance in the analysis of longitudinal trends.

Because total T levels as originally reported for MMAS baseline appeared systematically lower than in other studies (9), the samples were subsequently reassayed by newer methods. The new methodology produced higher T levels with no change in the percentage cross-sectional age trend. In the present analysis, we used the newer values for baseline T.

The regression model used for this report is an example of repeated-measures statistical analysis, in that it treats each measurement as a separate outcome but also accounts for correlation among the observations on a given individual (31). Compared with analysis of individual changes, analysis of covariance, or two-stage methods using summary statistics, repeated-measures analysis has the advantage that it

can accommodate different patterns of missing and nonmissing observations for each subject. No bias results from missing data, so long as one can assume that the likelihood of missing data is predicted by variables included in the regression model. For MMAS, the implication is that even if a participant was not reinterviewed and had no follow-up hormone measurement, his baseline value could be included in the analysis, contributing partial information. We confirmed that straightforward analysis of changes in men with complete data produced estimates of longitudinal trend very close to those obtained by repeated-measures analysis.

Hormone trends

The import of a decline in T is wide-ranging because of its ubiquitous role in male physiology, regulating gonadal function and affecting libido, aggressive behavior, mood, muscle mass, liver function, lipid regulation, bone formation, erythropoiesis, and immune function (32). Several large cross-sectional studies have come to consensus on a 1–2% annual decline in free or bioavailable T, offset by a rise in SHBG, resulting in a net decline in total T. The baseline MMAS estimated the rate of decline in total T at 0.4%/yr, the net result of free T declining at a rate of 1.2% and SHBG rising at 1.2%. Importantly, the rate of decline was the same in apparently healthy men as in those reporting chronic illness, obesity, alcoholism, prescription medication, or prostate problems. Other investigators have reported total T declining at 0.7%/yr (12, 33) or 0.5%/yr (34, 35). Longitudinal studies have confirmed the intrasubject rate of decline of total T as follows: 0.2%/yr in one report (36), 0.11 ng/ml·yr (0.38 nmol/liter·yr) in another (37), and 0.036 ng/ml·yr (0.124 nmol/liter/yr) in a third report (4).

The decline in total T after age 40 may represent a combination of factors: reduction in number, function, or responsiveness of testicular secretory cells; failure of receptor-modulated T production (hypothalamic-pituitary insensitivity); compensatory adjustment of serum-binding fractions, leading to sequestering of T by SHBG; and response to other physiological changes such as depression or insulin resistance (38). Lower T could be considered a beneficial adaptation to the risk of prostate cancer in older men, in that malignant growth is promoted through binding of T at a cell-surface receptor, although a relation between circulating T levels and prostate cancer risk has not been seen consistently (39, 40).

Both DHT and its further metabolite androstanediol glucuronide (AAG) have been implicated in prostate cancer promotion. As with T, a prospective relation with incident prostate cancer has been difficult to demonstrate, possibly because tissue and serum androgen levels are not entirely concordant (40, 41). DHT and AAG also have value as markers of pancreatic cancer (42). AAG declined slightly with age in the baseline MMAS sample (9) and in the data of Belanger *et al.* (11). DHT showed no cross-sectional age trend in the baseline MMAS (9) or in other studies (10).

The observed longitudinal rise in DHT of 3.5%/yr, not previously reported, stands in contrast to cross-sectional studies describing stable circulating and intraprostatic DHT (10, 43). This unexpected finding, which we confirmed by alternative analytic methods, might be an artifactual bias, or it might be evidence of a genuine physiological phenomenon. The two most common sources of bias in a longitudinal study are laboratory drift and selective attrition of the cohort. Laboratory drift is unlikely because of the quality-control procedures detailed above. Selective attrition of the older, less healthy men may have left more of those with higher DHT in the follow-up sample, giving an artifactual increase. Apparent good health was indeed associated cross-sectionally with higher DHT (Table 4). Arguing against such a mechanism, however, is the fact that mean DHT at baseline was not significantly higher in the men who were ultimately remeasured (0.27 ng/ml; 0.93 nmol/liter) than in those ultimately lost to follow-up (0.26 ng/ml; 0.90 nmol/liter). Moreover, controlling the analysis as we did for health status at both time points should have eliminated any artifactual change in DHT attributable to better health of the retained sample.

Among the biological mechanisms that might explain a rise in DHT are an alteration in metabolism and an adjustment in protein binding. Because 98% of DHT is produced by peripheral conversion from T, a rise in DHT in the presence of declining T might be due to an increase in 5α-reductase activity in liver, skin, or prostate tissue (44) or a decrease in catabolism of DHT (45). The binding capacity for DHT in serum might increase as a result of the rise in SHBG concentration. Of the relatively little DHT in circulation (about 10% of T levels), approximately 99% is bound to protein, compared with 98% of T (46). Free DHT has been reported to decrease with age (43). Given the small absolute amount but high percentage of tightly bound DHT in circulation, a relatively small perturbation in the DHT production pathway could lead to a small but real increase in serum levels.

Clinically, higher concentration of DHT might compensate in part for the decrease in T in terms of preserving certain facets of androgenic activity, considering that DHT is the more potent androgen with higher affinity for the androgen receptor. As an adaptation to aging, preserving tissue-specific androgenic activity may be particularly relevant to progressive male-pattern balding and prostate physiology, including development of benign prostatic hypertrophy.

The adrenal steroid DHEA is viewed by many as a general marker of good health and is sold over the counter as anti-aging therapy with the support of a small body of experimental evidence in humans (47). DHEA and DHEAS, the most plentiful steroid in serum, decline in concentration with age more markedly than other hormones (48), corroborating the experimental evidence of their involvement in age-related health problems (49). MMAS showed DHEA and DHEAS declining cross-sectionally at 2–3%/yr (9). Most prominently in the epidemiological literature, low DHEA has been alternately implicated and exonerated as a predictor of ischemic heart disease (16, 50).

General agreement is that in men, the pituitary gonadotropins FSH and LH, which serve to stimulate T secretion and sperm production, respectively, increase in serum concentration with age (37). In the baseline MMAS, LH was higher by 1.3%/yr of age and FSH at 1.9%/yr (9). The rise in FSH and LH is consistent with the decline in T, considering that a low T level signals the hypothalamic-pituitary axis to re-

lease FSH and LH (32). That feedback mechanism is altered with old age, which may explain why T can decline despite elevated LH (51).

PRL, another anterior pituitary hormone, has been reported to increase with age in both animal and human studies (52–56). Our observed longitudinal rise of 5.3%/yr is consistent with the loss of hypothalamic-pituitary regulatory function that occurs with aging. The increase in PRL has been ascribed to an age-related decline in dopamine, the neurotransmitter responsible for inhibiting PRL secretion (57, 58). Recent reports showing the presence of PRL receptors in prostate epithelium suggest local, glandular production of PRL by the prostate and a possible indirect role for PRL in prostate carcinogenesis (59, 60).

The age trend of serum estrogen in men has been reported variously as declining (8, 35) or steady (61). Estrogens were invariant with age in the baseline MMAS (9). Estrone showed a significant cross-sectional decline at follow-up in MMAS and a longitudinal decline comparable to that of bioavailable T, suggesting that the ratio of T to estrone is held steady or possibly increases.

Mechanisms of trend

The steepening of a cross-sectional age trend is easily explained by positing a curvilinear age course, as illustrated in Fig. 3a. That simple model would imply, however, that the longitudinal age trend within individual participants, represented by the chord slope in Fig. 3a, must be intermediate between the cross-sectional age trends at the two measurement times, represented by tangent slopes in the figure. To the contrary, we consistently found that the longitudinal trend was steeper than the cross-sectional trend within either measurement time.

An alternative explanation is that men with differing covariate status follow parallel age trends, with one group on a lower track as illustrated in Fig. 3b. An aging man might jump from one curve to another as his covariate status changes, adding a component of longitudinal change to the cross-sectional age course. In Fig. 3, the covariate is apparent good health, which accounted for a 10–15% parallel displacement of the cross-sectional age trend for several hormones (Table 5). An aging man who incurred a significant change in health status would cross from the upper track to the lower track, losing hormone level at a greater rate than if he had maintained his good health. The fraction of men in apparent good health did indeed decline from 26% at baseline to 18% at follow-up (Table 1), despite the greater rate of follow-up among men who were healthier at baseline, because more men lost their apparent good health between baseline and follow-up (47%) than regained it (7%).

Limitations

The interpretability of these results may be limited by several factors inherent in the community-based design. Data were collected in the men's homes, restricting the physical measures to an essential few and the medical history to self-report of a number of chronic illnesses and behavioral variables that can be determined reliably in that setting (13, 14). Blood work was limited by cost and logistics to the battery of lipid and hormone assays central to the main themes of the study. Our indicator of apparent good health was therefore necessarily a crude composite based on fundamental data rather than on detailed clinical measures. More specific hypotheses concerning particular conditions and hormones, such as the relation of DHEAS to cardiovascular disease, have been tested with these data (16), and more such studies are readily conceived. The present report was deliberately focused on obtaining a precise and comprehensive set of estimates for cross-sectional and longitudinal aging trends, derived from a common data set using a sophisticated statistical model, and, as a first step toward studying the determinants of those trends, examining their dependence on a general indicator of health status.

An important consideration for interpreting any longitudinal study is the influence of selective attrition. The men lost to follow-up were older and less healthy at baseline than the cohort average. These and other demographic differences noted above in the men lost to follow-up (fewer Caucasian, married, employed, or highly educated) may have biased the estimates of longitudinal trend, although such effects were

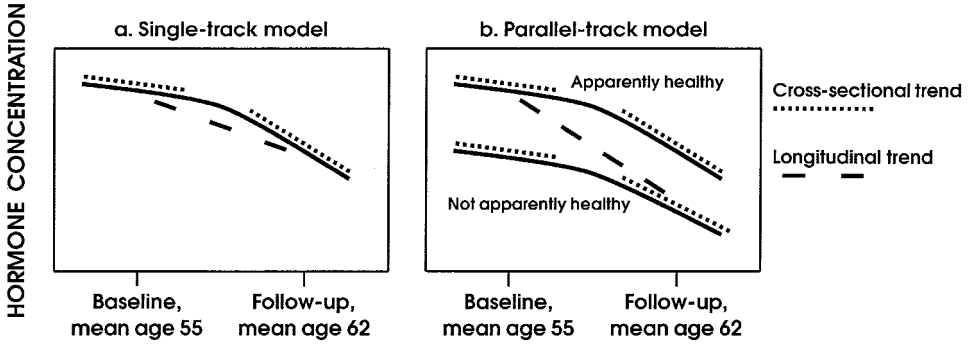

FIG. 3. Model used in analysis of hormone trends in MMAS, 1987–97. Schematic diagrams illustrate two possibilities for joint operation of cross-sectional and longitudinal age trends. A, Population mean hormone level follows a single track, declining with age at an accelerating rate (*curved line*). Estimate of longitudinal trend (*dashed line*) lies in between cross-sectional estimates at baseline and follow-up (*dotted lines*). B, An independent factor such as apparent good health influences hormone level, resulting in parallel tracks. With aging, a fraction of the population moves from better health (*top track*) to poorer health (*bottom track*), causing the estimated longitudinal decline to be steeper than the cross-sectional trend at either baseline or follow-up. All parameters of this family of models were simultaneously estimated from combined baseline and follow-up data by repeated-measures regression analysis.

necessarily indirect because the lost men showed no significant difference from the cohort average T, DHEA, or DHT level at baseline. Bias might also arise if the remeasured men, being initially more healthy, were more likely to jump the track in the sense of Fig. 3 when their health worsened, which would be manifested as a steepening of the longitudinal trend. The fact that apparent health as defined in this analysis did not account completely for the discrepancies between cross-sectional and longitudinal trend emphasizes that more refined indicators are needed to delineate the influences of health status on hormone level and trend.

A limitation imposed for practical reasons on the MMAS design was the confinement of data collection to two relatively narrow periods, 1987–89 and 1995–97. In such a two-point design one must allow that the observed longitudinal trends may simply represent period effects, *i.e.* differences affecting all men in a particular period of measurement, regardless of age. The modest variation in measurement interval, ranging from 7.1–10.4 yr, was not enough to prevent period and longitudinal change from being almost completely confounded when we tried to separate them analytically. Another possibility, more remote, is that birth cohort (period minus age) is the driving factor behind both the cross-sectional and the longitudinal trends. Given the thorough confounding of age, period, longitudinal trend, and cohort in the design of MMAS, we have given primary weight to interpretations in terms of individual (age) effects, particularly because with physiological variables such as hormone level, age is the most plausible determinant of the three.

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Address all correspondence and requests for reprints to: John B. McKinlay, Ph.D., New England Research Institutes, 9 Galen Street, Watertown, Massachusetts 02472. E-mail: johnm@neri.org.

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