Endogenous Sex Hormones, Cognitive Decline, and Future Dementia in Old Men

Mirjam I. Geerlings, PhD,[1,2] Dorothea Strozyk, MD,[2] Kamal Masaki, MD,[3] Alan T. Remaley, MD, PhD,[4] Helen Petrovitch, MD,[3] G. Webster Ross, MD,[3] Lon R. White, MD,[3] and Lenore J. Launer, PhD [2]

Objective: To estimate the association of endogenous levels of bioavailable testosterone and estradiol with risk for cognitive decline and dementia in old men.

Methods: Within the population-based, prospective Honolulu-Asia Aging Study, 2,974 men, aged 71 to 93 years, without dementia were reexamined 3 times over an average of 6 years for development of dementia and cognitive decline. Cognitive decline was measured with the Cognitive Abilities Screening Instrument. Incident dementia was diagnosed according to standard criteria. A total of 134 men experienced development of Alzheimer's disease (AD; including 40 cases with contributing cerebrovascular disease) and 44 experienced development of vascular dementia.

Results: Adjusting for age and other covariates, testosterone was not associated with risk for dementia (using Cox regression analyses) or cognitive decline (using random coefficient analyses). However, higher levels of estradiol were associated with risk for AD (hazard ratio per standard deviation increase, 1.25; 95% confidence interval, 1.05–1.47) and AD with cerebrovascular disease (hazard ratio, 1.19; 95% confidence interval, 1.02–1.38). Also, compared with the lowest tertile of estradiol, men in the middle and highest tertile of estradiol had 0.24 and 0.28 points lower Cognitive Abilities Screening Instrument scores, respectively, for each year increase in age.

Interpretation: In old men, endogenous testosterone levels are not associated with risk for cognitive decline and AD, whereas higher estrogen levels increase risk for cognitive decline and AD.

Ann Neurol 2006;60:346–355

The impact of dementia on society and health care is a growing concern, given the increase of the elderly population. It is expected that with the increase of the elderly population, the prevalence of Alzheimer's disease (AD), the most common cause of dementia, will triple to 13 million people in the United States by 2050.[1]

Sex hormones have been identified as factors that modulate the risk for dementia. Recent studies have examined the association of dementia with exogenous and endogenous levels of estrogens in postmenopausal women. Earlier observational studies found reduced risk for cognitive decline and dementia with higher levels of estrogen,[2,3] findings that are biologically plausible and supported by findings in animal studies.[4–6] More recently, however, we observed that higher endogenous levels of estrogens in postmenopausal women, as measured by length of reproductive period and level of plasma estradiol, were associated with an increased risk for dementia, poorer memory performance, and smaller hippocampal volumes.[7–9] Our findings from these observational studies were confirmed by findings from the Women's Health Initiative Memory Study, a large, randomized, double-blind, placebo-controlled trial where older women free of dementia at baseline who were receiving estrogen therapy (estrogen alone or estrogen plus progestin) had an increased risk for cognitive impairment and dementia compared with women receiving placebo.[10,11]

These unexpected results in women increased interest in the effect of sex hormones on cognition and the risk for dementia in older men. In older men, testosterone levels gradually decline with age, and low testosterone levels have been associated with an increased risk for cardiovascular disease.[12] Risk for cognitive decline and dementia may be increased in older men with low testosterone levels through this increased cardiovascular risk, but other more direct biological mechanisms are also plausible.[13] The existing studies examining associations of sex hormones with cognition and dementia in older men, however, are conflicting.[8,9,14–17]

From the [1]University Medical Center Utrecht, Julius Center for Health Sciences and Primary Care, Utrecht, The Netherlands; [2]Laboratory of Epidemiology, Demography, and Biometry, National Institute on Aging, National Institutes of Health, Bethesda, MD; [3]Pacific Health Research Institute, Honolulu, HI; and [4]Department of Laboratory Medicine, National Institutes of Health, Bethesda, MD.

Received Jan 30, 2006, and in revised form May 19. Accepted for publication May 20, 2006.

Published online Jul 24, 2006, in Wiley InterScience (www.interscience.wiley.com). DOI: 10.1002/ana.20918

Address correspondence to Dr Geerlings, University Medical Center Utrecht, Julius Center for Health Sciences and Primary Care, Stratenum 6.131, PO Box 85500, 3508 GA Utrecht, The Netherlands. E-mail: m.geerlings@umcutrecht.nl

In this study, we examined whether endogenous levels of bioavailable testosterone and estradiol were associated with the risk for cognitive decline and development of dementia, including AD and vascular dementia. Data are from the prospectively studied, population-based cohort of Japanese American men who participated in the Honolulu-Asia Aging Study (HAAS).

Subjects and Methods

Honolulu-Asia Aging Study Population

The HAAS was established in 1991 to study neurodegenerative diseases.[18] The HAAS is a continuation of the Honolulu Heart Program, a prospective population-based study[19] of coronary heart disease and stroke in Japanese American men born between 1900 and 1919 living on the island of Oahu, Hawaii. The baseline examination of the Honolulu Heart Program was conducted from 1965 through 1968, after which participants were further evaluated in 1968 through 1970 (examination 2) and in 1971 through 1974 (examination 3). Of the original Honolulu Heart Program cohort, 3,734 subjects (80% of those eligible), ranging in age from 71 to 93 years, agreed to participate in the HAAS (1991–1993, examination 4). Participants were reexamined twice for dementia after the HAAS baseline assessment in 1994 through 1996 (examination 5) and 1997 through 1999 (examination 6). At each examination, physical measurements, demographic information, and medical information were collected. The study was approved by the Institutional Review Board of the Kuakini Medical Center and the Honolulu Department of Veterans Affairs. All participants, or their caretakers if subjects were demented, provided written informed consent.

Dementia and Cognitive Assessment

Assessment of dementia was performed in 1991 to 1993, 1994 to 1996, and 1997 to 1999 (examinations 4–6). Assessment of cognitive functioning was performed at examinations 4 through 7 (1999–2000). At each examination, participant cognitive status was evaluated with the 100-point Cognitive Abilities Screening Instrument (CASI), a combination of the Hasegawa Dementia Screening scale, the Folstein Mini-Mental State Examination, and the Modified Mini-Mental State Test.[20] The CASI is a well-recognized instrument for the assessment of cognitive function validated among Japanese and Western sample populations.[21] To identify the subgroup for further dementia evaluation, we used CASI score and age as described in detail elsewhere.[18,22] Evaluation of clinical dementia included a proxy interview, detailed neuropsychological assessment, neurological examination, and neuroimaging.[18] A consensus committee that included a neurologist and at least two other physicians expert in geriatric medicine and dementia determined the final diagnosis of clinical dementia. Dementia was diagnosed according to Diagnostic and Statistical Manual of Mental Disorders, Third Edition, Revised and Fourth Edition criteria.[23,24] Probable and possible AD were diagnosed following the criteria of the National Institute of Neurological and Communicative Disorders and Stroke and the Alzheimer's Disease and Related Disorders Association (NINCDS-ADRDA).[25] Vascular dementia was diagnosed using the criteria of the California Alzheimer's Disease Diagnostic and Treatment Centers guidelines.[26] Other dementias included those due to chronic alcohol abuse, brain tumor, subdural hematoma, Parkinson's disease, Lewy body disease, Pick's disease, trauma, vitamin B12 deficiency, hypothyroidism, progressive supranuclear palsy, and unknown causes. Diagnostic categories used for analyses were AD (without contributing cerebrovascular disease [CVD]), AD with or without CVD, and vascular dementia.

Sex Hormone, Sex Hormone–Binding Globulin, and Albumin Measurements

Measurement of sex hormones have been described in detail elsewhere.[27] In brief, fasting blood samples were drawn in 1991 to 1993 as part of the baseline measurements and stored at −70°C. A quantitative competitive immunoassay (IMMULITE 2000, Diagnostic Product Co., Los Angeles, CA) was used to measure 17-β estradiol (total-E2) with an intraassay variation coefficient of 4.3%, interassay variation coefficient of 5.2%, and calibration range of 20 to 2,000pg/ml. Levels of 17-β estradiol less than 20pg/ml (<73.4pmol/L), which is less than the typical calibration range, were estimated using the same curve-fitting program that the IMMULITE analyzer uses automatically to calculate higher levels. This involves converting the relative light unit from the immunoassay into an absolute unit by fitting the standard master calibration curve. For 33% of the samples, E2 was quantitated using the extended calibration range; although these levels are less precisely measured, the individuals should be correctly ranked relative to the others.

Total testosterone (total-T) was measured by a chemiluminescent enzyme immunoassay (IMMULITE 2000), with an intraassay variation coefficient of 7.4%, an interassay variation coefficient of 7.7%, and a sensitivity of 0.1ng/ml, ranging from 0.1 to 16ng/ml. Sex hormone–binding globulin (SHBG) was measured by IMMULITE 2000 immunometric assay, and albumin was measured by the bromocresol green method using a colometric immunoassay (intraassay variation coefficient of 2%, interassay variation coefficient of 3%).

The non–SHBG-bound fractions (ie, the bioavailable fractions) of testosterone and estradiol consist of the free and albumin-bound hormones and are thought to better reflect the active component of the hormone.[28,29] Bioavailable and free fractions (ie, not SHBG- and not albumin-bound) of testosterone and estradiol were calculated using testosterone, estradiol, SHBG, and albumin as Södergård and colleagues[30] and van den Beld and colleagues[28] described.

Other Variables

Other measurements at examination 4 included age, years of formal education, smoking habits, alcohol intake, physical activity, body mass index (BMI), the five components of the metabolic syndrome (fasting plasma glucose, serum triglycerides, serum high-density lipoprotein [HDL] cholesterol, waist circumference, and hypertension), diabetes mellitus, coronary heart disease, stroke, apolipoprotein E (APOE) genotype, and depressive symptoms. We categorized smoking habits as current, former, and never smoker. In the data

analysis, never smoker was used as the reference group. Alcohol intake was categorized as no alcohol, one to two drinks per day, and three or more drinks per day. An index of physical activity was calculated by summing the products of five levels of habitual activity in a day by corresponding intensity factor.[31] The physical activity index was analyzed as a continuous variable, with higher scores indicating higher activity. Height and weight were measured, and BMI was calculated as weight in kilograms divided by squared height in meters. The metabolic syndrome was defined according to the following criteria: fasting glucose of at least 110mg/dl, triglycerides of at least 150mg/dl, HDL cholesterol less than 40mg/dl, hypertension, or waist circumference of more than 102cm.[32] Serum cholesterol was measured in previously frozen blood specimens collected from subjects in a nonfasting state using Auto-Analyzer methods (Technicon, Terrytown, NY).[33] Systolic and diastolic blood pressure values correspond to the mean of three measurements made on the left arm while the subject was seated. Hypertension was defined as blood pressure of at least 140/90mm Hg or antihypertensive medication use. In the analyses, the number of risk factors (zero to five) of the metabolic syndrome was entered into the model. Diabetes mellitus was assessed according to World Health Organization criteria based on self-report of a doctor's diagnosis, use of oral hypoglycemic medications or insulin, or fasting and postchallenge glucose levels measured at examination 4.[34] History of cardiovascular disease (coronary heart disease and stroke) was derived by continuous surveillance of hospital discharge and death records on Oahu from 1965 through 1997. Study physicians reviewed hospital and physician records to confirm the diagnoses. Events were categorized relative to examination 4 into no cardiovascular disease at baseline (examination 4) versus prevalent cardiovascular disease at examination 4. APOE genotyping was obtained by standard DNA amplification and restriction isotyping[35] at the Bryan Alzheimer's Disease Research Center at Duke University. Subjects were categorized as APOE ε4-positive if they carried at least one copy of the ε4 allele, and ε4-negative otherwise. Depressive symptomatology was measured with the 11-question version of the Center for Epidemiologic Studies Depression Scale; a cutoff score of 9 or greater indicated depression.[36]

Study Samples

Of the 3,734 participants of the HAAS, 3,168 participants had complete measures of albumin, SHBG, total-T, and total E2. Compared with the 3,168 subjects with hormone measures, the 566 without hormone measures were somewhat older, had lower CASI scores, and higher total and HDL cholesterol (using t test or χ^2 test, all $p < 0.05$). Of the 3,168 participants with hormone measurements, 194 were diagnosed with dementia at baseline, leaving a cohort free of dementia at baseline of 2,974 men.

Of the 2,974 men free of dementia at baseline, 2,300 had complete follow-up information and 223 men were diagnosed with incident dementia during a follow-up of, on average, 6.1 years (range, 4.4–7.8 years). Of those 674 subjects who did not participate in follow-up examinations, 68% died and 32% refused (n = 218). Compared with those subjects with complete follow-up, those 218 who did not participate in subsequent dementia evaluations had fewer years of education, lower CASI score, and higher albumin, but did not differ in age or E2 or T levels. The analytical sample for the dementia analyses consisted of the 2,300 men free of dementia at baseline with complete follow-up information, and the analytical sample for the cognitive decline analyses consisted of the 2,974 men free of dementia at baseline, regardless of whether they had complete follow-up information and whether they experienced dementia or remained free of dementia during follow-up.

Data Analysis

INCIDENT DEMENTIA. The associations of levels of testosterone and estradiol with incident dementia were estimated with Cox proportional hazards models with delayed entry and age as the timescale.[37] The age of onset of dementia was set at the midpoint of the interval between the last examination without dementia and the first follow-up examination with dementia. Subjects who died or did not participate in subsequent follow-up examinations were censored at the time of their last evaluation. The proportional hazards assumption was checked using a log versus log minus log plot. In the Cox proportional hazards models, hormone levels were examined in two ways: as a continuous variable expressed as increase per standard deviation (SD) and as tertiles. In the first model, we adjusted for age (timescale); in the second model, we added years of education (continuous), smoking habits (former vs never smoker and current vs never smoker), alcohol intake (no alcohol intake vs one to two drinks per day, and three drinks or more vs one to two drinks per day), physical activity index (continuous), BMI (continuous), number of risk factors of the metabolic syndrome (zero to five), diabetes (yes vs no), APOE allele (one or two ε4 alleles vs no ε4 allele), and depression (depressed as indicated by a score of 9 or higher vs not depressed) as covariates. Because dementia risk associated with testosterone or estradiol may be different for different APOE ε4 alleles,[9,38,39] we repeated the Cox proportional hazards analyses in strata of APOE ε4 allele (one or two ε4 alleles vs no ε4 allele).

COGNITIVE DECLINE. The longitudinal associations of hormone levels with risk for cognitive decline were estimated using random coefficient analyses with robust standard errors, in which the rates of change in cognitive functioning over time (ie, intercepts and slopes) were fitted as random effects (proc mixed statement in SAS; SAS Institute, Cary, NC). This statistical method takes into account the multiple observations per subject that are likely to be correlated. The fixed effect of the main determinant (testosterone and estradiol, respectively) was entered in two ways: as tertiles and as a continuous variable expressed as increase per SD. In the same model, the fixed effect of age (the time variable) was entered, as well as the interaction between the hormone level and age. The estimate of interest of these analyses is the coefficient of the interaction between hormone level and age (time). This coefficient represents the rate of change in cognitive functioning over time (in this analysis: age) as a function of hormone level. To graphically represent the model, we calculated mean CASI scores across tertiles of hormone level from the fully adjusted random coefficient models for

Table 1. Baseline Characteristics (Means and Percentages) of the Cohort Free of Dementia according to Tertiles of Bioavailable Testosterone

Characteristics	Bio-T[a]			
	Low Tertile (n = 991)	Middle Tertile (n = 992)	High Tertile (n = 991)	All (N = 2974)
Age, yr	78.5 ± 4.9	77.1 ± 4.3	76.6 ± 3.5	77.4 ± 4.3
Education, yr	10.6 ± 3.3	10.5 ± 3.2	10.7 ± 3.0	10.6 ± 3.2
CASI score	83.4 ± 14.0	85.5 ± 10.0	86.3 ± 9.2	85.1 ± 11.3
Never smoker, %	35.4	32.7	33.9	34.0
No alcohol intake, %	45.3	41.9	39.9	42.3
Physical activity index score	30.8 ± 4.6	31.3 ± 4.7	31.2 ± 4.8	31.1 ± 4.7
BMI, kg/m^2	23.7 ± 3.5	23.5 ± 3.1	23.4 ± 2.8	23.5 ± 3.1
Waist circumference, cm	86.7 ± 9.5	86.2 ± 8.5	85.5 ± 7.9	86.1 ± 8.7
Systolic blood pressure, mm Hg	150.8 ± 23.0	150.1 ± 23.5	148.3 ± 23.1	149.7 ± 23.2
Diastolic blood pressure, mm Hg	79.4 ± 12.0	80.9 ± 11.0	80.5 ± 10.6	80.2 ± 11.2
Metabolic syndrome, %	25.5	24.4	19.5	23.1
Diabetes mellitus, %	38.8	34.2	34.2	35.7
Coronary heart disease, %	11.0	11.7	10.9	11.2
CVA, %	3.6	3.5	4.4	3.9
Depressive symptomatology, %	8.9	9.1	11.2	9.8
APOE ε4 homozygote or heterozygote, %	16.8	20.0	18.4	18.4
Albumin, gm/dl	4.38 ± 0.4	4.50 ± 0.4	4.64 ± 0.4	4.51 ± 0.41
SHBG, nmol/L	56.6 ± 26.2	55.4 ± 21.7	56.4 ± 20.6	56.1 ± 23.0
Total-T, nmol/L	11.8 ± 3.8	16.6 ± 3.6	22.1 ± 4.8	16.8 ± 5.9
Bio-T, nmol/L	5.6 ± 1.4	8.2 ± 0.6	11.3 ± 1.8	8.3 ± 2.7
Free-T, nmol/L	0.21 ± 0.05	0.30 ± 0.03	0.40 ± 0.07	0.30 ± 0.09
Total-E2, pmol/L	83.4 ± 52.5	95.0 ± 55.8	106.2 ± 52.8	94.9 ± 54.5
Bio-E2, pmol/L	53.4 ± 32.2	62.8 ± 36.1	71.9 ± 36.5	62.7 ± 35.8
Free-E2, pmol/L	2.0 ± 1.2	2.2 ± 1.3	2.5 ± 1.3	2.2 ± 1.3

Means and percentages are given for participants with valid observations.
Data were missing for smoking (n = 138), alcohol intake (n = 177), physical activity score (n = 146), body mass index (BMI; n = 55), depression (n = 280), and apolipoprotein E (APOE) genotype (n = 3).

[a]Low tertile bioavailable testosterone (bio-T) < 7.2nmol/L; middle tertile bio-T = 7.2–9.2nmol/L; high tertile bio-T ≥ 9.2nmol/L.

CASI = Cognitive Abilities Screening Instrument; CVA = cardiovascular accident; SHBG = sex hormone–binding globulin; total-T = total testosterone; total-E2 = total 17-β estradiol; bio-E2 = bioavailable 17-β estradiol.

four age groups according to the mean age of the sample at each examination (baseline and the three follow-up examinations). Adjustments were made as described earlier for dementia, with the exception that coronary heart disease and stroke were also entered as covariates.

In all analyses, missing values on covariates were replaced with dummy variables when variables were categorical or were given the mean value of the distribution of the study population when continuous. Missing values were present in smoking, alcohol intake, physical activity, BMI, glucose level, triglycerides, HDL, waist circumference, APOE, and depression. All variables had missing values of less than 6% with the exception of depression, where 9% of values were missing. Analyses were performed using SPSS, version 12 (SPSS, Chicago, IL) and SAS, version 8.2 (SAS Institute).

Results

In the cohort free of dementia (n = 2,974), the mean total-T level was 16.8nmol/L; the mean bioavailable testosterone (bio-T) and free-T levels were 8.3 and 0.3nmol/L, respectively. The mean total-E2 level was 94.9pmol/L, and the mean bio-E2 and free-E2 levels were 62.7 and 2.2pmol/L, respectively. With increasing testosterone levels, estradiol levels also increased. Men in the highest tertile of bio-T were, on average, 1.5 years younger than men in the lowest tertile of bio-T (Table 1). They also had a more favorable cardiovascular risk profile. Across tertiles of bio-E2, the average age, as well as other characteristics, was similar, but coronary heart disease and stroke were more common in men in the highest tertile of bio-E2 (Table 2).

Incident Dementia

Bio-T was not associated with risk for development of dementia, whether adjusted for age only or also for additional potential confounding or intermediate factors (Table 3). Bio-E2, however, was associated with an increased risk for AD. Per SD increase, the risk for AD increased by 25% (95% confidence interval [CI], 1.05–1.47). This relation was not explained by potential confounding factors. Entering tertiles of hormones instead of continuous measures did not indicate a threshold effect or nonlinearity and yielded comparable results for both bio-T (no association) and bio-E2 (increased risk with higher tertiles) (data not shown).

Table 2. Baseline Characteristics (Means and Percentages) of the Cohort Free of Dementia according to Tertiles of Bioavailable Estradiol

Characteristics	Bio-E2[a]		
	Low Tertile (n = 991)	Middle Tertile (n = 992)	High Tertile (n = 991)
Age, yr	77.4 ± 4.5	77.4 ± 4.3	77.3 ± 4.2
Education, yr	10.7 ± 3.2	10.6 ± 3.1	10.5 ± 3.3
CASI score	85.4 ± 11.4	85.4 ± 9.5	84.3 ± 12.7
Never smoker, %	32.4	34.2	35.3
No alcohol intake, %	43.1	42.5	41.4
Physical activity index score	31.1 ± 4.7	31.1 ± 4.9	31.0 ± 4.5
BMI, kg/m^2	23.2 ± 3.1	23.4 ± 3.0	24.0 ± 3.2
Waist circumference, cm	85.3 ± 8.9	86.2 ± 8.5	86.9 ± 8.6
Systolic blood pressure, mm Hg	150.3 ± 22.8	150.9 ± 23.9	147.9 ± 22.8
Diastolic blood pressure, mm Hg	80.2 ± 11.4	80.3 ± 11.5	80.3 ± 10.8
Metabolic syndrome, %	24.6	21.4	23.4
Diabetes mellitus, %	33.8	36.8	36.6
Coronary heart disease, %	9.7	10.4	13.5
CVA, %	3.0	3.0	5.5
Depressive symptomatology, %	8.5	10.4	10.3
APOE ε4 homozygote or heterozygote, %	20.0	18.0	17.1
Albumin, gr/dl	4.51 ± 0.4	4.49 ± 0.4	4.52 ± 0.5
SHBG, nmol/L	59.5 ± 25.5	56.1 ± 22.2	52.7 ± 20.4
Total-T, nmol/L	15.5 ± 5.7	17.1 ± 5.9	17.9 ± 5.8
Bio-T, nmol/L	7.4 ± 2.4	8.4 ± 2.5	9.2 ± 2.7
Free-T, nmol/L	0.27 ± 0.09	0.31 ± 0.09	0.33 ± 0.09
Total-E2, pmol/L	48.2 ± 16.6	87.3 ± 16.1	149.1 ± 56.6
Bio-E2, pmol/L	30.8 ± 9.5	57.1 ± 7.1	100.2 ± 35.3
Free-E2, pmol/L	1.1 ± 0.4	2.0 ± 0.3	3.5 ± 1.3

Means and percentages are given for those with valid observations.
Data were missing for smoking (n = 138), alcohol intake (n = 177), physical activity score (n = 146), body mass index (BMI; n = 55), depression (n = 280), and apolipoprotein E (APOE) genotype (n = 3).

[a]Low tertile bioavailable estradiol (bio-E2) < 45.2pmol/L; middle tertile bio-E2 = 45.2–69.6pmol/L; high tertile bio-E2 ≥ 69.6pmol/L.

CASI = Cognitive Abilities Screening Instrument; CVA = cardiovascular accident; SHBG = sex hormone–binding globulin; bio-T = bioavailable testosterone; total-T = total testosterone; total-E2 = total 17-β estradiol.

When we entered bio-T and bio-E2 together in the model, the risk estimates of both factors slightly decreased, but there remained a significant increased risk for bio-E2 on AD. The hazard ratios (HRs) in the fully adjusted model for bio-E2 and bio-T with AD were 1.23 (95% CI, 1.04–1.47) and 1.05 (95% CI, 0.85–

Table 3. Hazard Ratios per Standard Deviation Increase with Corresponding 95% Confidence Intervals of the Association of Bioavailable Testosterone and Estradiol with Risk for Dementia and Its Subtypes in the 2,300 Men Free of Dementia at Baseline and with Complete Follow-up Information

	AD (n = 94), HR (95% CI)[a]	AD ± CVD[b] (n = 134), HR (95% CI)	Vascular Dementia (n = 44), HR (95% CI)
Bio-T			
Model 1[c]	1.13 (0.92–1.38)	1.14 (0.96–1.34)	1.04 (0.77–1.40)
Model 2[d]	1.12 (0.91–1.38)	1.12 (0.95–1.33)	1.07 (0.79–1.46)
Bio-E2			
Model 1[c]	1.26 (1.06–1.49)	1.18 (1.02–1.37)	1.20 (0.94–1.54)
Model 2[d]	1.25 (1.05–1.47)	1.19 (1.02–1.38)	1.23 (0.95–1.59)

[a]Hazard ratios are per standard deviation (SD) increase; for bioavailable estradiol (Bio-E2), the SD is 36.2pmol/L, and for bioavailable testosterone (bio-T), the SD is 2.65nmol/L.
[b]Includes 94 cases of Alzheimer's disease (AD) with no cerebrovascular disease (CVD).
[c]Model 1: hazard ratio (HR) adjusted for age (timescale).
[d]Model 2: HR adjusted for age (timescale), years of education, Cognitive Abilities Screening Instrument (CASI) score at baseline, smoking habits, alcohol intake, body mass index, physical activity, metabolic syndrome, apolipoprotein ε4 allele, diabetes, and depression.

CI = confidence interval; bio-T = bioavailable testosterone.

Table 4. Hazard Ratios per Standard Deviation Increase with Corresponding 95% Confidence Intervals of the Association of Bioavailable Testosterone and Estradiol with Risk for Dementia and Its Subtypes across Apolipoprotein E Genotype in the 2,300 Men Free of Dementia at Baseline and with Complete Follow-up Information

	AD (no CVD), HR (95% CI)[a]	AD ± CVD, HR (95% CI)[a]	Vascular Dementia, HR (95% CI)[a]
No APOE ε4 allele	71 cases	102 cases	37 cases
Bio-T: model 1[b]	1.17 (0.93–1.46)	1.14 (0.94–1.38)	0.95 (0.68–1.32)
Bio-T: model 2[c]	1.18 (0.93–1.48)	1.15 (0.95–1.40)	1.00 (0.71–1.40)
APOE ε4 allele(s)	23 cases	32 cases	7 cases
Bio-T: model 1[b]	0.98 (0.62–1.53)	1.11 (0.76–1.63)	1.78 (0.80–3.97)
Bio-T: model 2[c]	0.91 (0.56–1.48)	0.96 (0.63–1.46)	1.53 (0.52–4.51)
No APOE ε4 allele	71 cases	102 cases	37 cases
Bio-E2: model 1[b]	1.21 (0.98–1.50)	1.16 (0.96–1.39)	1.20 (0.90–1.58)
Bio-E2: model 2[c]	1.17 (0.94–1.45)	1.12 (0.92–1.35)	1.19 (0.89–1.60)
APOE ε4 allele(s)	23 cases	32 cases	7 cases
Bio-E2: model 1[b]	1.36 (1.06–1.74)	1.25 (0.98–1.60)	1.20 (0.65–2.24)
Bio-E2: model 2[c]	1.44 (1.11–1.88)	1.37 (1.06–1.78)	1.06 (0.44–2.57)

[a]Hazard ratios (HRs) are per standard deviation (SD) increase; for bioavailable estradiol (bio-E2), the SD is 36.2pmol/L, and for bioavailable testosterone (bio-T), the SD is 2.65nmol/L.
[b]Model 1: HR adjusted for age (timescale).
[c]Model 2: HR adjusted for age (timescale), years of education, Cognitive Abilities Screening Instrument (CASI) score at baseline, smoking habits, alcohol intake, body mass index, physical activity, metabolic syndrome, diabetes, and depression.

AD = Alzheimer's disease; CVD = cerebrovascular disease; CI = confidence interval; APOE = apolipoprotein E.

1.30), respectively. For AD with or without contributing CVD, the HR of bio-E2 was 1.17 (95% CI, 0.99–1.37) and of bio-T was 1.08 (95% CI, 0.90–1.28); for vascular dementia, the risks were 1.22 (95% CI, 0.94–1.60) for bio-E2 and 1.02 (95% CI, 0.74–1.39) for bio-T.

Post hoc analyses excluding men with a history of coronary heart disease and stroke at baseline did not materially change the results. Post hoc analyses of the relation between the total-T/SHBG ratio, as a proxy for bioavailable testosterone, with dementia subtypes showed no significant associations. In the fully adjusted model, the HR of the total-T/SHBG ratio per SD (0.185nmol/L) increase for AD was 0.98 (95% CI, 0.77–1.24); for AD with or without contributing CVD, the HR was 0.99 (95% CI, 0.81–1.20); and for vascular dementia, the HR was 1.07 (95% CI, 0.91–1.25).

Stratifying by APOE genotype showed that the higher risk for AD with higher levels of bio-E2 was particularly present in men carrying one or two ε4 alleles (Table 4). However, the confidence intervals around the estimates in the two APOE genotype strata overlapped, indicating that the association of bio-E2 with risk for dementia was not significantly different for subjects with or without the APOE ε4 allele. Adding a product term of bio-E2 with APOE genotype also did not indicate significant interaction between bio-E2 and APOE ($p = 0.27$ for pure AD; $p = 0.29$ for AD including CVD; and $p = 0.68$ for VD). Stratifying bio-T by APOE allele did not indicate difference in risk according to APOE genotype (see Table 4). Adding a product term of bio-T with APOE genotype also did not show significant interaction between bio-T and APOE ($p = 0.86$ for pure AD; $p = 0.55$ for AD including CVD; and $p = 0.21$ for VD).

Cognitive Decline

At baseline (examination 4), the unadjusted mean CASI score was 85.1 (SD 11.3); at examinations 5 through 7, the unadjusted mean CASI scores were 82.2 (SD 12.0), 78.5 (SD 16.4), and 78.0 (SD 16.2), respectively. The mean ages at examination 4 through 7 were 77.4 (SD 4.3), 79.8 (SD 4.1), 82.6 (SD 3.9), and 83.9 years (SD 3.6), respectively.

Table 5 and Figure 1 show the results from the fully adjusted random coefficient model that estimated the rate of cognitive decline on the CASI as a function of time (age) and bio-T levels (tertiles). CASI score declined over time in the total population, but CASI scores did not significantly differ across tertiles of bio-T at each time point, nor did CASI score significantly change over time. When entered as a continuous variable, bio-T was also not significantly associated with CASI score or change in CASI score over time (data not shown).

Table 6 and Figure 2 show the results from the fully adjusted random coefficient model that estimated the rate of cognitive decline on the CASI as a function of time (age) and bio-E2 levels (tertiles). Again, CASI scores declined over time, but higher levels of bio-E2 were significantly associated with more rapid decline than lower levels of bio-E2. Compared with the lowest tertile of bio-E2, men in the middle and highest tertiles of bio-E2 had 0.24 and 0.28 points lower cognitive

Table 5. Longitudinal Associations of Bioavailable Testosterone with Change in Cognitive Functioning with Increasing Age, as Calculated from the Random-Effects Model in 2,974 Men Free of Dementia at Baseline

	Estimate	Standard Error	*p*
Intercept	170.97	5.66	<0.0001
Age	−1.20	0.06	<0.0001
Bio-T tertile low	0 (reference)		
Bio-T tertile middle	3.90	6.9	0.57
Bio-T tertile high	5.80	7.2	0.42
Age*bio-T tertile low	0 (reference)		
Age*bio-T tertile middle	−0.05	0.09	0.57
Age*bio-T tertile high	−0.07	0.09	0.42

Estimates are adjusted for years of education, smoking habits, alcohol intake, body mass index, physical activity, metabolic syndrome, diabetes, coronary heart disease, stroke, apolipoprotein E genotype, and depression.
bio-T = bioavailable testosterone.

Table 6. Longitudinal Associations of Bioavailable Estradiol with Change in Cognitive Functioning with Increasing Age, as Calculated from the Random-Effects Model in 2,974 Men Free of Dementia at Baseline

	Estimate	Standard Error	*p*
Intercept	160.87	4.84	<0.0001
Age	−1.07	0.05	−0.0001
Bio-E2 tertile low	0 (reference)		
Bio-E2 tertile middle	18.8	6.5	0.004
Bio-E2 tertile high	21.4	7.0	0.002
Age*bio-E2 tertile low	0 (reference)		
Age*bio-E2 tertile middle	−0.24	0.08	0.005
Age*bio-E2 tertile high	−0.28	0.09	0.002

Estimates are adjusted for years of education, smoking habits, alcohol intake, body mass index, physical activity, metabolic syndrome, diabetes, coronary heart disease, stroke, apolipoprotein E genotype, and depression.
bio-E2 = bioavailable estradiol.

scores, respectively, for each year increase in age (see Table 6). As can be seen from Figure 2, at age 77 years, the adjusted mean CASI score was 85.4 for men with bio-E2 levels in the lowest tertile, and this score declined to an average score of 77.9 at age 84 years. However, men with bio-E2 levels in the middle tertile declined more rapidly, from an average score of 85.6 points at age 77 years to an average score of 76.4 points on the CASI at age 84 years, which is 1.5 points lower than men with bio-E2 levels in the lowest tertile. Men in the highest tertile of bio-E2 also declined more rapidly, from an average score of 85.4 at age 77 years to an average score of 75.9 at age 84 years, which is 2 points lower than men in the lowest tertile of bio-E2. When entered as a continuous variable, bio-E2 was also significantly associated with increased risk for cognitive decline (data not shown).

Discussion

This study examined risk for cognitive decline and dementia associated with endogenous levels of bioavailable testosterone and estradiol in a large cohort of old men free of dementia at baseline. We observed that levels of bioavailable testosterone were not associated with risk for cognitive decline and incident dementia. In contrast, higher levels of bioavailable estradiol were

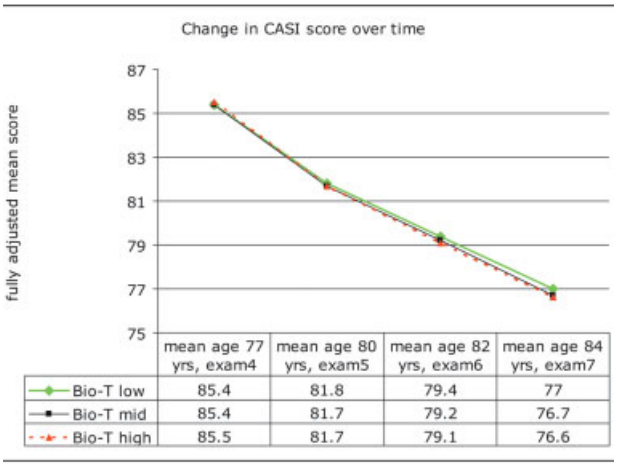

Fig 1. Mean Cognitive Abilities Screening Instrument (CASI) score across tertiles of bioavailable testosterone (bio-T) at 77, 80, 82, and 84 years, as calculated from the random-effects model in 2,974 men free of dementia at baseline. Means are adjusted for years of education, smoking habits, alcohol intake, body mass index, physical activity, metabolic syndrome, diabetes, coronary heart disease, stroke, apolipoprotein E genotype, and depression.

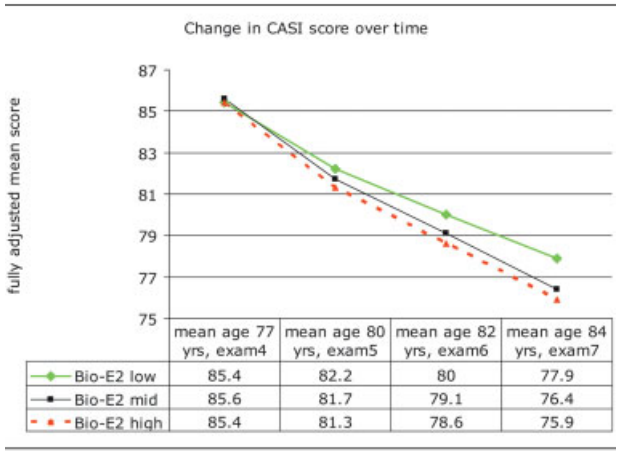

Fig 2. Mean Cognitive Abilities Screening Instrument (CASI) score across tertiles of bioavailable estradiol (bio-E2) at 77, 80, 82, and 84 years, as calculated from the random-effects model in 2,974 men free of dementia at baseline. Means are adjusted for years of education, smoking habits, alcohol intake, body mass index, physical activity, metabolic syndrome, diabetes, coronary heart disease, stroke, apolipoprotein E genotype, and depression.

associated with an increased risk for cognitive decline and AD. Furthermore, the increased risk for AD was somewhat stronger in APOE ε4 carriers. Thus, these results do not support the hypothesis that higher levels of testosterone or estradiol in older men are associated with a reduced risk for cognitive decline and dementia.

This study has several strengths. First, this is the largest cohort of older men in which risk for cognitive decline and dementia associated with sex hormones was examined. Other strengths are its study design: a population-based prospective cohort study. Furthermore, in the same cohort, we examined associations to cognitive decline and incident dementia of bioavailable testosterone and estradiol, and possible modification of APOE genotype; we were also able to correct for a large number of potential confounding factors.

This study also has limitations. First, 23% of the subjects was lost to follow-up, and this may have resulted in general biases toward finding no association. Second, due to small amounts of blood specimens available, we were not able to run the hormone assays in duplicate. Also, values of E2 less than 20pg/mL were less precisely measured, although the ranking of individuals should be maintained. This may also have led to risk estimates toward no association. Third, although the increased risk for cognitive decline and dementia associated with higher levels of estradiol were statistically significant, the comparisons were based on group differences and are difficult to interpret on an individual level.

To our knowledge, only one study has examined levels of testosterone with incident dementia as outcome, finding a protective effect of a higher total T/SHBG ratio, as an indicator of higher bioavailable T, on the risk for AD.[15] No association of total-T with AD was observed, nor was there an association of the total-T/SHBG ratio with all-cause dementia. When we analyzed the total-T/SHBG ratio with dementia outcomes, we did not find an association with any of the subtypes of dementia, however.

The finding of an increased risk for cognitive decline and dementia associated with increased levels of estradiol but not testosterone is of interest. It has been hypothesized that testosterone in men may maintain cognition via direct effects on the androgen receptors on the hippocampus or indirectly through aromatization to estradiol.[40] Our findings are not consistent with this hypothesis. One hypothesis to explain our findings is that testosterone and estradiol are both associated with increased risk for dementia, but that our study lacked power to find a significant association in testosterone, which may perhaps be less potent than estradiol on its affect on dementia.

An alternative hypothesis to explain our findings is that testosterone is not associated with future risk for dementia, and that the increased risk associated with higher estradiol is explained by increased aromatase activity in the brain, which may be associated with a neurodegenerative process. The enzyme aromatase is expressed in numerous sites of the brain, including the hippocampus. Estradiol synthesized within these sites is probably only biologically active at a local tissue level, and circulating levels may reflect this local metabolism.[41,42] Several experimental studies have found increased expression of aromatase and increase in local production of estradiol in injured brain areas[43]; this has been interpreted as an endogenous response of neural tissue to cope with neurodegeneration.[43] An alternative hypothesis to explain our findings is that neurodegeneration as a result of early AD leads to increased aromatase expression and consequent increase in estradiol production in the brain, which is then reflected in elevated serum levels of estradiol. This explanation is speculative, because we did not have measures of aromatase to examine our hypothesis. Also, from our data, we do not know to what extent the peripheral levels that we measured reflect the levels in the brain. If our hypothesis is correct, however, the results of our study would imply that the relatively high levels of estradiol that we observed in the men who experienced development of AD were a consequence or an early marker, rather than a cause of an incipient dementia process. It is conceivable, then, that the disease itself disrupts hormone regulation enough to cause the hormone imbalances we identified in the serum of the demented participants.

To date, little consensus exists as to what constitutes a normal sex hormone profile for an aging man or what specific effects the male andropause has on cognitive function. Future research should focus on defining a normal balance between testosterone and 17-β estradiol not only in the periphery, but also in the brain.

In conclusion, this large population-based study in men aged 71 to 93 years does not support the hypothesis that higher levels of testosterone or estradiol reduce the risk for cognitive decline and future dementia. Our findings of an increased risk for cognitive decline and AD associated with higher estradiol levels are similar to recent findings in postmenopausal women. Further studies are needed to examine whether there are mechanisms by which estradiol may increase risk for cognitive decline and dementia. From this study, however, it is not recommended to increase testosterone levels through androgen replacement therapy in healthy men to reduce the risk for dementia.

This study was supported by the NIH (National Institute on Aging [NIA], "Epidemiology of Brain Aging in the Very Old" 5UO1AG09349, "Epidemiology of Aging and Dementia–Autopsy Research" 2 RO1AG07155, L.R.W.; Intramural Research Program of the NIA; National Heart, Lung, and Blood Institute, contract NO1-HC-05102) and the American Heart Association (Grant-in-Aid 95-014560).

References

1. Hebert LE, Scherr PA, Bienias JL, et al. Alzheimer disease in the US population: prevalence estimates using the 2000 census. Arch Neurol 2003;60:1119–1122.
2. Yaffe K, Sawaya G, Lieberburg I, Grady D. Estrogen therapy in postmenopausal women: effects on cognitive function and dementia. JAMA 1998;279:688–695.
3. Yaffe K, Lui L-Y, Grady D, et al. Cognitive decline in women in relation to non-protein-bound oestradiol concentrations. Lancet 2000;356:708–712.
4. Gibbs RB, Aggarwal P. Estrogen and basal forebrain cholinergic neurons: implications for brain aging and Alzheimer's disease-related cognitive decline. Horm Behav 1998;34:98–111.
5. McEwen BS, Alves SE. Estrogen actions in the central nervous system. Endocr Rev 1999;20:279–307.
6. Solerte SB, Fioravanti M, Racchi M, et al. Menopause and estrogen deficiency as a risk factor in dementing illness: hypothesis on the biological basis. Maturitas 1999;31:95–101.
7. Geerlings MI, Ruitenberg A, Witteman JC, et al. Reproductive period and risk of dementia in postmenopausal women. JAMA 2001;285:1475–1481.
8. Geerlings MI, Launer LJ, de Jong FH, et al. Endogenous estradiol and risk of dementia in women and men: the Rotterdam Study. Ann Neurol 2003;53:607–615.
9. den Heijer T, Geerlings MI, Hofman A, et al. Higher estrogen levels are not associated with larger hippocampi and better memory performance. Arch Neurol 2003;60:213–220.
10. Shumaker SA, Legault C, Kuller L, et al. Conjugated equine estrogens and incidence of probable dementia and mild cognitive impairment in postmenopausal women: Women's Health Initiative Memory Study. JAMA 2004;291:2947–2958.
11. Espeland MA, Rapp SR, Shumaker SA, et al. Conjugated equine estrogens and global cognitive function in postmenopausal women: Women's Health Initiative Memory Study. JAMA 2004;291:2959–2968.
12. Hak AE, Witteman JC, de Jong FH, et al. Low levels of endogenous androgens increase the risk of atherosclerosis in elderly men: the Rotterdam study. J Clin Endocrinol Metab 2002;87:3632–3639.
13. Gouras GK, Xu H, Gross RS, et al. Testosterone reduces neuronal secretion of Alzheimer's beta-amyloid peptides. Proc Natl Acad Sci U S A 2000;97:1202–1205.
14. Barrett-Connor E, Goodman-Gruen D, Patay B. Endogenous sex hormones and cognitive function in older men. J Clin Endocrinol Metab 1999;84:3681–3685.
15. Moffat SD, Zonderman AB, Metter EJ, et al. Free testosterone and risk for Alzheimer disease in older men. Neurology 2004;62:188–193.
16. Yaffe K, Lui LY, Zmuda J, Cauley J. Sex hormones and cognitive function in older men. J Am Geriatr Soc 2002;50:707–712.
17. Muller M, Aleman A, Grobbee DE, et al. Endogenous sex hormone levels and cognitive function in aging men: is there an optimal level? Neurology 2005;64:866–871.
18. White L, Petrovitch H, Ross GW, et al. Prevalence of dementia in older Japanese-American men in Hawaii: the Honolulu-Asia Aging Study. JAMA 1996;276:955–960.
19. Syme SL, Marmot MG, Kagan A, et al. Epidemiologic studies of coronary heart disease and stroke in Japanese men living in Japan, Hawaii and California: introduction. Am J Epidemiol 1975;102:477–480.
20. Teng EL, Hasegawa K, Homma A, et al. The Cognitive Abilities Screening Instrument (CASI): a practical test for cross-cultural epidemiological studies of dementia. Int Psychogeriatr 1994;6:45–58.
21. Graves AB, Larson EB, Kukull W, et al. Screening for dementia in the community in cross-national studies: comparison between the Cognitive Abilities Screening Instrument and the Mini-Mental State Examination. In: Corain B, Iqbal K, Nicolini M, et al, eds. Alzheimer's disease: advances in clinical and basic research. New York: John Wiley & Sons, 1993:113–119.
22. Havlik RJ, Izmirlian G, Petrovitch H, et al. APOE-epsilon4 predicts incident AD in Japanese-American men: the Honolulu-Asia Aging Study. Neurology 2000;54:1526–1529.
23. American Psychiatric Association. Diagnostic and statistical manual of mental disorders. 3rd revised ed. Washington, DC: American Psychiatric Association, 1987.
24. American Psychiatric Association. Diagnostic and statistical manual of mental disorders. 4th ed. Washington, DC: American Psychiatric Association, 1994.
25. McKhann G, Drachman D, Folstein M, et al. Clinical diagnosis of Alzheimer's disease: report of the NINCDS-ADRDA Work Group under the auspices of Department of Health and Human Services Task Force on Alzheimer's Disease. Neurology 1984;34:939–944.
26. Chui HC, Victoroff JI, Margolin D, et al. Criteria for the diagnosis of ischemic vascular dementia proposed by the State of California Alzheimer's Disease Diagnostic and Treatment Centers. Neurology 1992;42(3 pt 1):473–480.
27. Irie F, Strozyk D, Peila R, et al. Brain lesions on MRI and endogenous sex hormones in elderly men. Neurobiol Aging (in press).
28. van den Beld AW, de Jong FH, Grobbee DE, et al. Measures of bioavailable serum testosterone and estradiol and their relationships with muscle strength, bone density, and body composition in elderly men. J Clin Endocrinol Metab 2000;85:3276–3282.
29. Vermeulen A, Verdonck L, Kaufman JM. A critical evaluation of simple methods for the estimation of free testosterone in serum. J Clin Endocrinol Metab 1999;84:3666–3672.
30. Södergård R, Bäckström T, Shanbhag V, Carstensen H. Calculation of free and bound fractions of testosterone and estradiol-17 beta to human plasma proteins at body temperature. J Steroid Biochem 1982;16:801–810.
31. Rodriguez BL, Curb JD, Burchfiel CM, et al. Physical activity and 23-year incidence of coronary heart disease morbidity and mortality among middle-aged men. The Honolulu Heart Program. Circulation 1994;89:2540–2544.
32. Executive Summary of the Third Report of the National Cholesterol Education Program (NCEP) Expert Panel on Detection, Evaluation, and Treatment of High Blood Cholesterol in Adults (Adult Treatment Panel III). JAMA 2001;285:2486–2497.
33. Nichaman MZ, Hamilton HB, Kagan A, et al. Epidemiologic studies of coronary heart disease and stroke in Japanese men living in Japan, Hawaii and California: distribution of biochemical risk factors. Am J Epidemiol 1975;102:491–501.
34. Peila R, Rodriguez BL, Launer LJ. Type 2 diabetes, APOE gene, and the risk for dementia and related pathologies: the Honolulu-Asia Aging Study. Diabetes 2002;51:1256–1262.

35. Hixson JE, Vernier DT. Restriction isotyping of human apolipoprotein E by gene amplification and cleavage with HhaI. J Lipid Res 1990;31:545–548.
36. Takeshita J, Masaki K, Ahmed I, et al. Are depressive symptoms a risk factor for mortality in elderly Japanese American men? the Honolulu-Asia Aging Study. Am J Psychiatry 2002; 159:1127–1132.
37. Commenges D, Letenneur L, Joly P, et al. Modelling age-specific risk: application to dementia. Stat Med 1998;17: 1973–1988.
38. Hogervorst E, Lehmann DJ, Warden DR, et al. Apolipoprotein E epsilon4 and testosterone interact in the risk of Alzheimer's disease in men. Int J Geriatr Psychiatry 2002;17:938–940.
39. Yaffe K, Haan M, Byers A, et al. Estrogen use, APOE, and cognitive decline: evidence of gene-environment interaction. Neurology 2000;54:1949–1954.
40. Cherrier MM, Matsumoto AM, Amory JK, et al. The role of aromatization in testosterone supplementation: effects on cognition in older men. Neurology 2005;64:290–296.
41. Simpson ER. Aromatase: biologic relevance of tissue-specific expression. Semin Reprod Med 2004;22:11–23.
42. Simpson ER, Davis SR. Minireview: aromatase and the regulation of estrogen biosynthesis—some new perspectives. Endocrinology 2001;142:4589–4594.
43. Garcia-Segura LM, Veiga S, Sierra A, et al. Aromatase: a neuroprotective enzyme. Prog Neurobiol 2003;71:31–41.