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# Bioavailable estradiol and age at onset of Alzheimer's disease in postmenopausal women with Down syndrome

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**Abstract**

Several lines of evidence suggest that loss of estrogen after menopause may play a role in the cognitive declines associated with Alzheimer's disease (AD). Women with Down syndrome (DS) experience early onset of both menopause and AD. This timing provides a model to examine the influence of endogenous estrogen deficiency on risk of AD. We hypothesized that low serum levels of bioavailable estradiol (E2) would be associated with increased risk of AD. One hundred and nineteen postmenopausal women with DS, 42–59 years of age, were ascertained through the New York State developmental disability service system and followed at 18-month intervals. Information from cognitive assessments, caregiver interviews, medical record review and neurological examination was used to establish the diagnosis of dementia. Women with DS who developed AD had lower levels of bioavailable E2, lower levels of total estradiol, higher levels of sex-hormone binding globulin, and lower levels of dehydroepiandrosterone sulfate at baseline than women who remained dementia free over the course of follow-up. Women who had low levels of bioavailable E2 at baseline were four times as likely to develop AD (HR = 4.1, 95% CI: 1.2–13.9) and developed AD, on average, 3 years earlier, than those with high levels of bioavailable E2, after adjustment for age, level of mental retardation, ethnicity, body mass index, history of hypothyroidism or depression and the presence of the apolipoprotein $\varepsilon$4 allele. Our findings support the hypothesis that reductions in estrogen following menopause can contribute to the cascade of pathological processes leading to AD.


*Keywords:* Endogenous estrogen; Bioavailable estradiol; Alzheimer's disease; Down syndrome

Estrogen has several neuroprotective effects and loss of estrogen after menopause may play a role in the cognitive declines associated with Alzheimer's disease AD [25]. In observational studies, postmenopausal women who used estrogen or hormonal replacement therapy (ERT/HRT) showed slower declines in cognitive function and decreased risk of AD [15,20,25,26] but not all studies have found positive effects [2,4,13]. The Women's Health Initiative Memory Study (WHIMS), a randomized trial of estrogen with and without progestin, reported more cognitive impairment and a twofold increase in risk of AD in treated women compared with women on placebo [18,19] casting doubt on the neuroprotective effects of ERT/HRT. Observational studies have also shown that ERT/HRT use may be associated with protective factors such as higher educational levels and better access to medical care, rather than with the neuroprotective effects of estrogen per se. Studies of endogenous estrogen are important to distinguish between these alternatives and to understand the role of estrogen in the pathogenesis of AD. Only a few studies have

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examined the relation of endogenous estrogen to risk of AD and the results have been inconsistent. Healthy nondemented women over 65 years of age with high serum concentrations of bioavailable or total estradiol were less likely to develop cognitive impairment than women with low serum estradiol concentrations [6,22,24], and serum estradiol levels were found to be lower in patients with late onset AD than in age-matched controls [12]. Several studies have found an association between elevated sex-hormone binding globulin (SHBG) or low bioavailable estradiol and cognitive decline or AD [9,17,24]. These findings suggest that non-protein bound (free) and loosely bound (bioavailable), rather than total serum estrogen, may be important in assessing the relationship between estrogen and AD.

In the current study we examined the relation of bioavailable estradiol to age at onset of AD in a cohort of postmenopausal women with Down syndrome (DS). Women with DS have high risk for AD, with onset 10–20 years earlier than women in the general population. The high risk for AD neuropathology and early onset of dementia may be due, at least in part, to triplication and overexpression of the gene for beta-amyloid precursor protein (APP), located on chromosome 21. The high risk for AD, together with an interval between onset of menopause and the onset of AD that is shorter than is typical in the general population, make postmenopausal women with DS an informative cohort for studying the relationship between loss of endogenous estrogen and cognitive decline related to AD.

The sample included 119 members of a community-based sample of 176 postmenopausal women with DS, 42–59 years of age, residing in New York State and participating in a longitudinal study of aging in adults with mental retardation (complete data were not available for the remaining 58 women, as discussed below). Participants were ascertained from the statewide service system and recruited with the help of state and voluntary service provider agencies. Subjects were eligible to participate in the study if a family member or correspondent provided informed consent, and participants also signed a form acknowledging their willingness to participate. The participation rate was 74.6%. Recruitment, informed consent and study procedures were approved by the Institutional Review Boards of the New York State Institute for Basic Research in Developmental Disabilities, Columbia University Medical Center and Columbia University Health Sciences.

Assessments included evaluations of cognition, functional and vocational abilities, behavioral/psychiatric conditions and health status. Assessments were repeated at 14–18 months over three cycles of data collection. Cognitive function was evaluated with a test battery used to assess cognitive functions that are typically affected in AD and designed for a wide range of intellectual function. A complete description of the battery is available in a previous study [27]. Participants showing declines in cognition or in adaptive behavior were evaluated by a study neurologist to confirm the presence of dementia and to determine the presence or absence of medical/psychiatric conditions other than AD that might result in or mimic dementia. Structured interviews were conducted with caregivers to collect information on changes in cognitive function, adaptive behavior and medical history. Past and current medical records were reviewed for all participants.

To determine the occurrence of dementia and classify dementia subtypes, information from all available sources was reviewed, including cognitive test scores, medical history, informant-based interviews on dementia and psychopathology, and neurological examination. Classification was made in a consensus conference regarding the presence or absence of dementia and its cause. We classified participants into two main groups: (1) as demented if there was a history of progressive memory loss, disorientation, and functional decline over a period of at least one year, and if no other medical or psychiatric conditions that might result in or mimic dementia were present (e.g., stroke) ($n = 38$) and (2) as nondemented if they were without cognitive or functional decline or if they showed some cognitive and/or functional decline but not of sufficient magnitude to meet criteria for dementia ($n = 81$). AD was the predominant form of dementia, accounting for 92% of the cases (35 of 38 cases). Participants suspected of having vascular or other non-AD forms of dementia were excluded from the analysis ($n = 3$). Age at meeting criteria for dementia was used to estimate age at onset of AD.

Non-fasting blood samples were collected between 10:00 a.m. and 3:00 p.m. Blood was separated in a refrigerated centrifuge and, after separation, sera were frozen at $-20\,^{\circ}$C until assay. Total estradiol and estrone (free + bound) were measured by a no-extraction solid-phase $^{125}$I-radioimmunoassay using commercial kits (Diagnostic Systems Laboratories, Inc. Webster, TX). Sensitivity or minimum detection level for estradiol was 4 pg/ml, and intra-assay and inter-assay coefficients of variation (CV) were 4.3% and 10.5%, respectively. Sensitivity or minimum detection level for estrone was 11 pg/ml and intra-assay and inter-assay CVs were 7.9% and 15.6%, respectively. Human follicle stimulating hormone (FSH), progesterone (P), Dehydroepiandrosterone sulfate (DHEAS) and sex-hormone binding globulin were measured by immunometric assays using Immulite systems (Diagnostic Products Corporation, Los Angeles, CA). We used two commercial controls for the SHBG assays, the first with a mean level of 4.8 nmol/l and the second with a mean level of 82 nmol/l. Sensitivity was 0.1 mIU/ml for FSH, 0.2 ng/ml for P, 30 μg/dl for DHEAS and 0.2 nmol/l for SHBG. Intra- and inter assay CVs were, respectively, 1.9% and 5.0% for FSH, 6.0% and 7.9% for P, 2.3% and 5.5% for DHEAS and 6.4% and 8.7% for SHBG. Bioavailable estradiol was measured by ammonium sulfate precipitation of SHBG-bound estradiol and calculated as the product of percentage non-SHBG bound and total estradiol. The inter-assay coefficient of variation is 13% [11].

Potential confounders, in addition to age, were level of mental retardation, body mass index (BMI), a history of past or current hypothyroidism or depression, ethnicity and the presence of the apolipoprotein E (APOE) ε4 allele. Level of mental retardation was classified into two groups: mild/moderate (IQ 35–70) and severe/profound (IQ ≤ 34), based on IQ scores obtained before onset of dementia. Ethnicity was classified as white or non-white. BMI was computed as weight in kilograms divided by squared height in meters (kg/m$^2$), and was measured at each assessment. History of past or current hypothyroidism and depression were ascertained by medical record review. All