Effect of oestrogen during menopause on risk and age at onset of Alzheimer's disease

Ming-Xin Tang, Diane Jacobs, Yaakov Stern, Karen Marder, Peter Schofield, Barry Gurland, Howard Andrews, Richard Mayeux

Summary

Background Oestrogen use by postmenopausal women has many health benefits, but findings on the effect of oestrogen in Alzheimer's disease are conflicting. Oestrogen promotes the growth and survival of cholinergic neurons and could decrease cerebral amyloid deposition, both of which may delay the onset or prevent Alzheimer's disease. To investigate whether use of oestrogen during the postmenopausal period affects the risk of Alzheimer's disease, we studied 1124 elderly women who were initially free of Alzheimer's disease, Parkinson's disease, and stroke, and who were taking part in a longitudinal study of ageing and health in a New York City community.

Methods Relative risks and age-at-onset distributions were calculated from simple and adjusted Cox proportional hazards models. Standard annual clinical assessments and criterion-based diagnoses were used in follow-up (range 1–5 years).

Findings Overall, 156 (12·5%) women reported taking oestrogen after onset of menopause. The age at onset of Alzheimer's disease was significantly later in women who had taken oestrogen than in those who did not and the relative risk of the disease was significantly reduced (9/156 [5·8%] oestrogen users *vs* 158/968 [16·3%] non-users; 0·40 [95% CI 0·22–0·85], p<0·01), even after adjustment for differences in education, ethnic origin, and apolipoprotein-E genotype. Women who had used oestrogen for longer than 1 year had a greater reduction in risk; none of 23 women who were taking oestrogen at study enrolment has developed Alzheimer's disease.

Interpretation Oestrogen use in postmenopausal women may delay the onset and decrease the risk of Alzheimer's disease. Prospective studies are needed to establish the dose and duration of oestrogen required to provide this benefit and to assess its safety in elderly postmenopausal women.

Lancet 1996; **348:** 429–32
See Commentary page 420

Gertrude H Sergievsky Center (M-X Tang PhD, D Jacobs PhD, Y Stern PhD, K Marder MPH, P Schofield MD, R Mayeux MD)**; Taub Center for Alzheimer's Disease Research** (D Jacobs, Y Stern, K Marder, H Andrews PhD, R Mayeux)**; Departments of Neurology** (M-X Tang, D Jacobs, Y Stern, K Marder, P Schofield, R Mayeux) **and Psychiatry** (Y Stern, B Gurland MD, R Mayeux) **and Division of Epidemiology** (R Mayeux) **(School of Public Health); Stroud Center for the Study of Quality of Life, Columbia University, College of Physicians and Surgeons** (B Gurland)**; and Division of Statistics, New York State Psychiatric Institute** (H Andrews)**, New York, USA**

Correspondence to: Dr Richard Mayeux, Gertrude H Sergievsky Center, 630 West 168th Street, Columbia University, New York, NY 10032, USA

Introduction

Use of oestrogen by postmenopausal women has many health benefits.[1,2] Oestrogen has been used as a treatment[3,4] for Alzheimer's disease, which affects a substantial proportion of elderly women.[5] One study reported that oestrogen use was associated with a lower risk of mortality related to Alzheimer's disease,[6] although a subsequent case-control study did not confirm this association.[7] Oestrogen promotes the growth of cholinergic neurons,[8,9] stimulates the secretase metabolism of the amyloid precursor protein,[10] and may interact with apolipoprotein E.[11] All these factors could affect the risk of Alzheimer's disease.

We examined the effect of a history of oestrogen use on the development of Alzheimer's disease among elderly women living in New York. Our hypothesis was that oestrogen use might lower the risk of incident Alzheimer's disease.

Methods

1282 non-demented elderly women were identified in a community-based study of ageing in northern Manhattan, New York City. The Health Care Financing Administration provided access to a random sample of Medicare recipients in the community. Additional potential participants were consecutively identified from records at senior centres and elderly housing sites in the same community. We wrote to potential participants identified by either method explaining that they had been selected to take part in a study of ageing. Participation rates were 77% at the senior centres and housing sites and 72% in the Medicare sample; these rates did not differ by ethnic origin. Each participant underwent a 90 min face-to-face interview then a standard assessment, which included a medical history, physical and neurological examination, and a brief (about 1 h) battery of neuropsychological tests.[12,13] The criteria for eligibility were: no evidence of cognitive impairment on detailed psychometric assessment at the initial interview; no history of stroke or Parkinson's disease; and at least one subsequent annual follow-up assessment.

Information about oestrogen use was available for 1227 (95·7%) women. From this group we excluded 52 (4·2%) women with Parkinson's disease, 45 (3·7%) with stroke, and six (0·5%) with both disorders. Thus, we restricted the analysis to 1124 women—352 (31%) women identified at the senior centres and housing sites and 772 (69%) from the Medicare sample. The Columbia University Institutional Review Board reviewed and approved this project.

A standard history of oral oestrogen use was obtained from all women at study entry by a trained interviewer as part of the risk-factor questionnaire. The test-retest reliability of the overall questionnaire had been previously established.[14] Questions about oestrogen use had good test-retest reliability (κ=0·65). If a woman had ever taken oestrogen, she was asked the ages at which she began and stopped. She was asked how old she was at the onset of the menopause and whether it occurred naturally or as a result of surgery. We asked the women to name the type of oestrogen preparation they used but not the dosages.

For diagnosis of dementia, medical records and imaging studies were used, as well as data from the initial and follow-up

	At risk	Alzheimer's disease*	Healthy	Relative risk (95% CI)
No oestrogen use	968	158 (16·3%)	810	1·0
Oestrogen use	156	9 (5·8%)	147	0·40 (0·22–0·85)
Total	1124	167 (14·9%)	957	

*Total number of incident cases over whole study period (cumulative incidence).

Table 1: **Relative risk of incident Alzheimer's disease associated with use of oestrogen during postmenopausal period**

study examinations. The diagnosis was established by consensus among an independent group of physicians and neuropsychologists from this information. This group were not aware of information on oestrogen use at any point during the diagnostic process. The diagnosis of dementia[15] required evidence of cognitive decline on the neuropsychological test battery and impairment in social or occupational function. All data were examined to identify the aetiology of dementia with standard criteria[16] for the diagnosis of Alzheimer's disease.

Ethnic origin was classified by self-report on the format of the 1990 United States Census Bureau (Census of Population and Housing, 1990).

For apolipoprotein E (*APOE*) genotyping, genomic DNA was amplified by PCR, and subjected to *Cfo*I restriction analysis with *APOE* primers and conditions modified from those described by Hixson and Vernier.[17,18]

Demographic characteristics and a history of oestrogen use in women who did and did not develop Alzheimer's disease were compared by means of χ^2 tests for categorical variables and ANOVA for continuous variables. Age, ethnic origin, and education were compared among women who did and women who did not use oestrogen, and then among those who did and did not develop Alzheimer's disease.

The Cox proportional hazard model was used to calculate the relative risk of developing Alzheimer's disease associated with use of oestrogen, and survival analysis was used to plot the age-at-onset distributions for women who did and did not use oestrogen. Subsequent models were stratified to assess the effects of education, *APOE* genotype, and ethnic origin on the relative risk; these covariates were then included in multivariate models. Because older women entering the study had a higher probability of developing Alzheimer's disease than younger women, we stratified the analysis by the median age at baseline to reduce a possible age cohort effect. We used martingale methods to check the proportional hazards assumption.[19] The annual incidence rate was estimated and the incidence rate ratio was calculated by standard methods.

Results

The mean age of the participating women was 74·2 years (SD 7·0) and the mean duration of education 9·2 years (SD 4·6). 400 (36%) of the women were African-American, 431 (38%) were Hispanic, and 293 (26%) were caucasian. During follow-up, which ranged from 1 to 5 years after the initial interview, 167 (14·9%) women developed Alzheimer's disease. The women who developed Alzheimer's disease were older than those who did not (78·5 [7·7] *vs* 73·7 [6·6] years, p=0·001) and had had fewer years of education (6·7 [4·2] *vs* 9·6 [4·5] years, p=0·001). Age at menopause was similar for women who did and did not develop Alzheimer's disease.

Oestrogen use	At risk	Alzheimer's disease*	Healthy	Relative risk (95% CI)
None	968	158 (16·3%)	810	1·0
Unknown	31	3 (9·7%)	28	1·3 (0·40–4·20)
≤1 year	67	5 (7·5%)	62	0·47 (0·20–1·10)
>1 year	58	1 (1·7%)	57	0·13 (0·02–0·92)

*Total number of incident cases over whole study period (cumulative incidence).

Table 2: **Duration of oestrogen use**

156 women reported that they had used oestrogen after the onset of the menopause. The average duration of oestrogen use was 6·8 years (range 2 months to 49 years). Women who had used oestrogen were younger than those who had not (73·0 [6·3] *vs* 74·4 [7·1] years, p=0·01) and had had more years of education (10·2 [4·8] *vs* 9·0 [4·5] years, p=0·005). Fewer African-American women than caucasian or Hispanic women had used oestrogen (41 [10·3%] *vs* 115 [15·9%], p=0·007). Women who took oestrogen were more likely than those who did not to have had a hysterectomy (78 [50·0%] *vs* 256 [26·4%], p=0·0001), and to have had onset of the menopause at an earlier age (45·4 [8·1] *vs* 47·0 [7·7], p=0·06). A greater proportion of women who had undergone hysterectomy than of those who had natural menopauses had used oestrogen for longer than 1 year (23/227 [10·1%] *vs* 35/897 [4·0%], p=0·0001). The majority of women used tablets containing conjugated oestrogens (Premarin). The most frequent reasons for discontinuation of oestrogen use were uterine bleeding, fear of cancer, or physician's advice.

A history of oestrogen use was significantly less common among women who developed Alzheimer's disease than in women who remained free of the disease (table 1, p=0·0006). There was no difference in the use of oestrogen between the women excluded at baseline for Parkinson's disease or stroke and the disease-free women (Parkinson's disease 17%, stroke 10%, disease-free 16%, p=0·51).

The age at onset for Alzheimer's disease, stratified by the median age at entry into the study, was significantly later among women who used oestrogen than among women who never used oestrogen (log-rank test p<0·01). The relative risk of Alzheimer's disease associated with a history of oestrogen use was 0·40 (95% CI 0·22–0·85; p=0·01, table 1). Adjustment for ethnic group, years of education, and participation group (senior centre or housing *vs* Medicare sample) did not significantly change the relative risk (0·5 [0·25–0·9], p=0·02).

The Cox proportional hazards model was also used to examine the effect of duration of oestrogen use on the risk of Alzheimer's disease (table 2). 31 (20%) oestrogen users did not recall their duration of oestrogen use. The association with Alzheimer's disease among these women (unknown group in table 2) was not significantly different from that in non-users, which suggests that they may not have used oestrogen at all or for only a brief period. Women who took oestrogen for 1 year or less (average 4 months) had a lower risk of Alzheimer's disease than never-users, of borderline statistical significance (p=0·06). The relative risk of Alzheimer's disease for women who took oestrogen for longer than a year (average 13·6 years) was significantly reduced (0·13 [0·02–0·92], p<0·01). There was a significant linear trend in the effect of duration of oestrogen use on disease risk (log-rank test, p=0·0034, figure). 23 women were still taking oestrogen at enrolment in the study, and none has developed Alzheimer's disease during the study period.

To check the assumptions of the Cox regression model, we plotted the martingale residuals against age at onset.[19] Data for all but three subjects fell within the predicted horizontal band around 0, satisfying the assumptions of proportional hazards.

Among women whose data satisfied criteria for

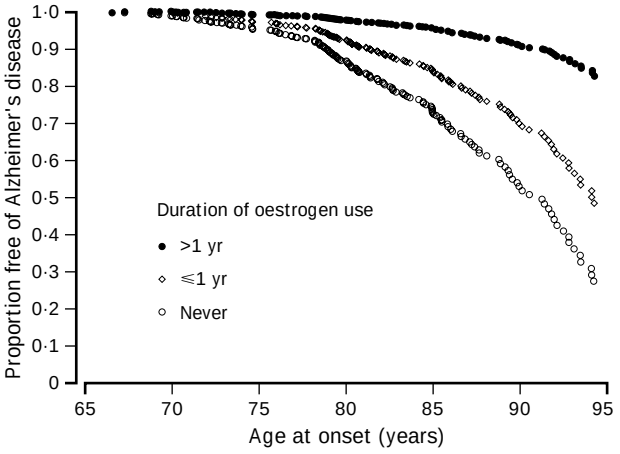

Figure: **Survival analysis plot of distribution by age of proportion of individuals remaining unaffected according to duration of oestrogen use**

Duration of use >1 year, average=13·6 years; ⩽1 year, average=4 months. Women classified as unknown duration of use (table 2) included in reference group.

inclusion in the analysis, 181 (16·1%) had no additional follow-up information. These women were similar to the 943 with additional follow-up in age (mean 75·4 [SD 7·8] *vs* 74·2 [6·8] years), education (9·3 [4·7] *vs* 9·2 [4·6] years), ethnic origin (African-American 62 [34·3%] *vs* other 338 [35·8%]), and oestrogen use (22 [12·5%] *vs* 134 [14·2%]). For women with additional follow-up, the duration did not differ significantly by history of oestrogen use or by the development of Alzheimer's disease (p=0·7).

APOE genotypes were available for 604 (53·7%) of the 1124 women. The risk of incident Alzheimer's disease was higher for women with *APOE*ε4 than for those with other genotypes (ε4/ε4 3·9 [1·5–9·8]; one ε4 allele 1·2 [0·7–1·6]). None of the nine *APOE*ε4 homozygous women had taken oestrogen (three cases, six controls). The relative risk for Alzheimer's disease associated with oestrogen use among *APOE*ε4 heterozygous women was reduced (0·13 [0·02–0·95]) as was that among women with other *APOE* genotypes (0·4 [0·2–0·9]).

The estimated annual incidence rate of Alzheimer's disease was 2·7% among women who took oestrogen compared with 8·4% among those who did not take oestrogen. The overall incidence rate ratio was 0·4 (0·2–0·7), similar to that estimated in the Cox proportional hazards model. 68 patients (41% of the 167 patients) showed mild impairment of cognitive function but met criteria for inclusion. We repeated the analysis after excluding these mildly impaired women to estimate the relative risk of more advanced disease (n=99), but the results of the Cox model were similar (relative risk [0·25–0·9], p=0·04) as was the incidence rate ratio (0·24 [0·09–0·69]; incidence rate oestrogen 1·2% *vs* no oestrogen 5·4%). The annual incidence rates are within the ranges reported previously in East Boston.[20]

Discussion

These results suggest that a history of oestrogen use during the postmenopausal period significantly delays the onset of Alzheimer's disease and lowers the risk of disease. The duration of oestrogen use seems to be important in risk reduction because women with a history of long-term use had the lowest risk. Our study differs from previous investigations of Alzheimer's disease[6,7] in that all women were interviewed about the use of oestrogen and examined before the onset of disease.

Paganini-Hill and Henderson[6] reported a reduction with oestrogen use in mortality related to Alzheimer's disease in a nested case-control study; 13 979 (61%) participants completed a health survey by mail, but follow-up was limited to health records and death certificates. Longer periods of oestrogen use and younger age at menarche were also associated with lower mortality.

Graves et al[21] found no difference between patients with Alzheimer's disease and controls in the frequency of oestrogen replacement, though sex distribution was not described. Brenner and colleagues[7] found no evidence that oestrogen replacement therapy affected the risk of Alzheimer's disease; though no significant association was observed, the odds ratio for oral oestrogen use associated with Alzheimer's disease was less than one. That study[7] had sufficient power to detect a reduction in risk similar to that in our study but the study design[3] may explain why the outcomes differ. Brenner and colleagues' main comparison was of the odds that patients with Alzheimer's disease used oestrogen and the odds that controls used oestrogen. By contrast, we classified women on the basis of a history of oestrogen use and compared the cumulative risk that Alzheimer's disease would develop among oestrogen-users and never-users. Our results (figure) show that oestrogen use does not prevent Alzheimer's disease, but that it seems to delay the onset of the disease. The case-control design used by Brenner et al[7] would not allow examination of differences in the age-at-onset distribution.

Toran-Allerand and colleagues[8] identified colocalisation of oestrogen-receptor binding sites with the mRNA for nerve growth factors and their receptors in developing neurons of rodent basal forebrain.[22] The degeneration of these regions in Alzheimer's disease may cause the loss of memory and other cognitive functions. Their findings imply that oestrogen and nerve growth factors influence synthesis and release of nerve growth factors or alternatively promote survival, differentiation, regeneration, and plasticity. In a study of rats that had undergone oophorectomy, those treated with 17β-oestradiol did better on a memory task than those deprived of oestrogen; these animals also showed preservation of neurons in the basal forebrain as well as a return to near-normal concentrations of mRNA for nerve growth factors.[9,23,24]

Oestrogen protects hippocampal neurons in culture exposed to excitotoxins, oxidative stress, or amyloid β.[25] Cells cultured with 17β-oestradiol accumulate a soluble form of the amyloid precursor protein by enhancing the amount or activity of α-secretase.[10] An exogenous source of oestrogen in postmenopausal women, even for a limited time, might foster the survival of neurons and limit the amount of amyloid β deposition, thus delaying the onset of overt manifestations of Alzheimer's disease.

The association between *APOE*ε4 and Alzheimer's disease has been extensively investigated,[26,27] and our results are similar to other population estimates.[28] Oestrogen use was associated with a reduction in the risk of Alzheimer's disease among women heterozygous for ε4 and among women with other *APOE* genotypes, but we were unable to assess the effect of oestrogen among women homozygous for ε4.

This study has limitations because the design was observational. Oestrogen use was assessed by history. Oestrogen use was less common among African-American

women and more likely among better-educated women. Although we adjusted for some of these factors in our analyses, we cannot exclude the possibility that oestrogen use reflects a lifestyle characteristic or an as yet unidentified exposure or bias that accounts for the effect observed. We believe that a prospective trial of oestrogen in perimenopausal women to delay the onset of Alzheimer's disease is justified, once more data on the safety, dosage, and duration of oestrogen treatment required become available.

We thank David Wilder and Rafael Lantigua (Stroud Center for the Study of Quality of Life and Center for Geriatrics and Gerontology at Columbia University) for their help in the design and conduct of the registry and Medicare survey; John Shinin, Maria Gonzales, Harold Brown, and Roseann Costa for technical assistance; interview team from the Sergievsky Center and the Center for Geriatrics and Gerontology at Columbia University; and Nicole Schupf, Wei-Yen Tsai, Allen Hauser, and Dominique Toran-Allerand for review of this work.

This study was supported by federal grants AG07232, AG10963, AG08702, and RR00645 and the Charles S Robertson Memorial Gift for Alzheimer's disease research from the Banbury Fund. M-XT is supported by a Faculty Scholar Award (95–045) from the Alzheimer's Disease and Related Disorders Association.

References

1 Bush TL, Cowan LD, Barrett-Connor E, et al. Estrogen use and all-cause mortality: preliminary results from the Lipid Research Clinics Program Follow-Up Study. *JAMA* 1983; **249:** 903–06.

2 Ettinger B, Friedman GD, Bush T, Quesenberry CP. Reduced mortality associated with long-term postmenopausal estrogen therapy. *Obstet Gynecol* 1996; **87:** 6–12.

3 Fillit H, Weinreb H, Cholst I, et al. Observations in a preliminary open trial of estradiol therapy for senile dementia—Alzheimer's type. *Psychoneuroendocrinology* 1986; **11:** 337–45.

4 Henderson VW, Paganini-Hill A, Emanuel CK, Dunn ME, Buckwalter JG. Estrogen replacement therapy in older women: comparisons between Alzheimer's disease cases and nondemented control subjects. *Arch Neurol* 1994; **51:** 896–900.

5 Rocca WA, Amaducci LA, Schoenberg BS. Epidemiology of clinically diagnosed Alzheimer's disease. *Ann Neurol* 1986; **19:** 415–24.

6 Paganini-Hill A, Henderson VW. Estrogen deficiency and risk of Alzheimer's disease in women. *Am J Epidemiol* 1994; **140:** 256–61.

7 Brenner DE, Kukull WA, Stergachis A, et al. Postmenopausal estrogen replacement therapy and the risk of Alzheimer's disease: a population-based case-control study. *Am J Epidemiol* 1994; **140:** 262–67.

8 Toran-Allerand CD, Miranda RC, Bentham WD, et al. Estrogen receptors colocalize with low affinity nerve growth factor receptors in cholinergic neurons of the basal forebrain. *Proc Natl Acad Sci USA* 1992; **89:** 4668–72.

9 Simpkins JW, Singh M, Bishop J. The potential role for estrogen replacement therapy in the treatment of the cognitive decline and neurodegeneration associated with Alzheimer's disease. *Neurobiol Aging* 1994; **15** (suppl 2): S195–97.

10 Jaffe AB, Toran-Allerand CD, Greengard P, Gandy SE. Estrogen regulates metabolism of Alzheimer's amyloid beta precursor protein. *J Biol Chem* 1994; **269:** 13065–68.

11 Honjo H, Tanaka K, Kashiwagi T, et al. Senile dementia-Alzheimer's type and estrogen. *Hormone Metab Res* 1995; **27:** 204–07.

12 Stern Y, Andrews H, Pittman J, et al. Diagnosis of dementia in a heterogeneous population: development of a neuropsychological paradigm and quantified correction for educated. *Arch Neurol* 1992; **49:** 453–60.

13 Pittman J, Andrews H, Tatemichi T, et al Diagnosis of dementia in a heterogeneous population: a comparison of paradigm-based diagnosis and physician's diagnosis. *Arch Neurol* 1992; **49:** 461–67.

14 Mayeux R, Ottman R, Tang M-X, et al. Genetic susceptibility and head injury as risk factors for Alzheimer's disease among community-dwelling elderly persons and the first-degree relatives. *Ann Neurol* 1993; **33:** 494–501.

15 American Psychiatric Association Diagnostic and statistical manual of mental disorders, 3rd edn, revised. Washington, DC: American Psychiatric Association, 1987: 205–24.

16 McKhann G, Drachman D, Folstein M, Katzman R, Price D, Stadland E. Clinical diagnosis of Alzheimer's disease: report of the NINCDS-ADRDA Work Group under the auspices of the Department of Health and Human Services Task Force on Alzheimer's disease. *Neurology* 1984; **34:** 939–44.

17 Hixson J, Vernier D. Restriction isotyping of human apolipoprotein E by gene amplification and cleavage with HhAl. *J Lipid Res* 1991; **31:** 545–48.

18 Maestre G, Ottman R, Stern Y, et al. Apolipoprotein-E and Alzheimer's disease: ethnic variation in genotypic risks. *Ann Neurol* 1995; **37:** 254–59.

19 Collet D. Modelling survival data in medical research. London: Chapman and Hall, 1994: 153–54.

20 Herbert LE, Scherr PA, Beckett LA, et al. Age-specific incidence of Alzheimer's disease in a community population. *JAMA* 1995; **273:** 1354–59.

21 Graves AB, White E, Koepsell TD, et al. A case-control study of Alzheimer's disease. *Ann Neurol* 1990; **28:** 766–74.

22 Miranda RC, Sohrabji F, Toran-Allerand CD. Estrogen target neurons co-localize the mRNAs for the neutrophins and their receptors during development: a basis for the interactions of estrogen and the neurotrophins. *Mol Cell Neurosci* 1993; **4:** 510–25.

23 Singh M, Meyer EM, Simpkins JW. The effect of ovariectomy and estradiol replacement on brain-derived neurotrophic factor messenger ribonucleic acid expression in cortical and hippocampal brain regions of female Sprague-Dawley rats. *Endocrinology* 1995; **136:** 2320–24.

24 Singh M, Meyer EM, Huang FS, Millard WJ, Simpkins JW. Ovariectomy reduces ChAT activity and NGF mRNA levels in the frontal cortex and hippocampus of the female Sprague Dawley rat. *Abstr Soc Neurosci* 1993; **19:** 1254.

25 Goodman Y, Bruce AJ, Cheng B, Mattson MP. Estrogens attenuate and corticosterone exacerbates excitotoxicity, oxidative injury and amyloid β-peptide toxicity of hippocampal neurons. *J Neurochem* 1996; **66:** 1836–44.

26 Saunders AM, Strittmatter WJ, Schmechel D, et al. Association of apolipoprotein E allele e4 with late-onset familial and sporadic Alzheimer's disease. *Neurology* 1993; **43:** 1467–72.

27 Roses AD, Strittmatter WJ, Pericak-Vance MA, Corder EH, Saunders AM, Schmechel DE. Clinical application of apolipoprotein E genotyping to Alzheimer's disease. *Lancet* 1994; **343:** 1564–65.

28 Henderson AS, Easteal S, Jorm AF, et al. Apolipoprotein E allele ε4, dementia, and cognitive decline in a population sample. *Lancet* 1995; **346:** 1387–90.