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Cumulative estrogen exposure, number of menstrual cycles, and Alzheimer's risk in a cohort of British women

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Summary The effect of estrogen on Alzheimer's Disease (AD) risk has received substantial research and media attention, especially in terms of hormone replacement therapy. But reproductive history is also an important modifier of estrogenic exposure, and deserves further investigation. Importantly, there is wide variation in reproductive patterns that modifies estrogen exposure during the reproductive span, which previous AD studies have not incorporated into their calculations. We measured degree of Alzheimer's-type dementia in a cohort of elderly British women, and collected detailed reproductive and medical history information, which we used to estimate number of months with estrogen exposure and number of months with menstrual cycles. Using Cox proportional-hazards models, we find that longer duration of estrogen exposure may have a protective effect against AD risk, such that for every additional month with estrogen, women experienced on average a 0.5% decrease in AD risk ($N = 89$, $p = 0.02$). More menstrual cycles may also have a protective effect against AD risk, although this result was of borderline statistical significance ($p < 0.10$). These results build upon previous methodologies by taking into account a variety of parameters including oral contraceptive use, breastfeeding, post-partum anovulation, abortions, and miscarriages. Additionally, Cox models revealed that longer reproductive span, age > 21 at first birth, and more months in lifetime spent pregnant had protective effects against AD risk.

1. Introduction

Estrogen has been implicated in Alzheimer's Disease (AD) risk and etiology. Various aspects of reproductive history determine the cumulative duration of estrogen exposure a woman

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experiences in her lifetime. Because there is substantial variation between women's reproductive histories and use of hormone-containing therapies, there is substantial variation in lifetime exposure to estrogen. Here, we investigate whether differences in cumulative exposure to estrogen and differences in specific aspects of reproductive history influence risk of AD in a cohort of elderly British women.

A range of studies have demonstrated estrogen's role in inhibiting and reversing AD-specific brain insults. In in vitro and animal model studies, estrogen has been shown to inhibit amyloid-β formation, promote amyloid-β clearance, inhibit neuronal apoptosis pathways, inhibit tau hyperphosphorylation, and reduce brain oxidative stress and inflammation, among other neuroprotective functions (see Supplementary Material section 2 for list of references). Nonetheless, a range of medical and epidemiological studies indicate possible heterogeneous effects of estrogen or confounders, so we present a literature review on this topic in Section 4.

1.1. Combined reproductive history features

Two studies have attempted to combine estrogen-altering life-history traits to calculate their cumulative effects in comparison to AD risk. Rasgon et al. (2005) added the duration of reproductive span (years between menarche and menopause) with the duration of time spent using hormone replacement therapy (HRT). They found that those with a higher composite number of years of estrogen exposure exhibited less cognitive decline.

Smith et al. (1999) created a more complex measurement for determining lifetime estrogen exposure. The effects of age at menarche, age at menopause, parity, duration of estrogen replacement therapy (ERT) use, postmenopausal weight, and years since menopause were given standardized (z) scores, which were then accumulated to create an estrogen exposure index. They found a correlation between their estrogen exposure index and cognitive function, which was stronger after they corrected for age and depression.

2. Methods

We propose an original method for a more comprehensive determination of lifetime exposure to estrogen based on number of months spent with exposure to estrogen. Women ages 70—100 along with family member(s) and/or carer(s) were recruited for participation through nursing homes, churches, community centers, the Alzheimer's Society, and a retired employee community. Participants received a modest gift voucher. The protocol was approved by the University of Cambridge Human Biology Research Ethics Committee.

Each session consisted of an interview collecting information about reproductive history, medical history, and factors that would potentially confound the relationship between AD and hormone exposure, or could obscure determination of dementia status. Exclusionary criteria included non-Alzheimer's-type dementia (e.g. vascular) or possible brain injury (e.g. head impact injury, brain tumor). Ten cases were excluded from analysis post-interview because of these criteria. Dementia status was measured by the Clinical Dementia Rating (CDR) scale, consisting of a 60—90 min interview conducted in two parts, one with the proband and the other with an informant (her relative or carer). Interviews were conducted by a researcher certified in CDR rating by the Washington University School of Medicine, a credential that requires high inter-rater reliability between the trainee and "gold standard" (Morris, 1997). In the CDR, probands are evaluated in six categories: memory; orientation; judgment and problem solving; home and hobbies; community affairs; personal care. The "sum of boxes" was used as a continuous variable, as has become standard in clinical trials (Coley et al., 2011; O'Bryant et al., 2008), computed from the sum of each category score, creating a scale from 0 to 18.

2.1. Variable calculations

2.1.1. Total lifetime duration of estrogen exposure

To estimate the number of months women spent in their lifetimes exposed to estrogen, we measured reproductive span as menopausal minus menarcheal age, subtracted the number of months spent breastfeeding, and for those pregnancies after which there was no breastfeeding, 1.5 months were subtracted to approximate the typical delay before ovulatory cycling resumes in such cases (Tulchinsky, 1980). Duration of post-menopause ERT use was added.

Rasgon et al. (2005) previously investigated the effect of total duration of estrogen exposure on AD risk, which they estimated by adding reproductive span to duration of ERT use after menopause. Given their significant results, we tested the same parameter ("Rasgon variable").

2.1.2. Number of menstrual cycles

No previous study known to the authors has investigated the relationship between number of menstrual cycles and AD risk. Cancer studies have estimated number of menstrual cycles by considering different combinations of the following variables: reproductive span, full-term pregnancies, abortions, miscarriages, breastfeeding, oral contraceptive (OC) use, infertility, period regularity (see Supplementary Material section 2 for list of references). Our methods are similar to these techniques, employing more variables at once than previous studies.

The number of months with menstrual cycles was computed as reproductive span, in months, minus months spent pregnant (including miscarriages, abortions, stillbirths, and child-bearing pregnancies), months spent breastfeeding, and months spent using OC. For pregnancies followed by no breastfeeding, cycling was assumed to resume 1.5 months post parturition (Tulchinsky, 1980).

2.1.3. Age at first birth

Age at first birth was taken as a woman's age at her first childbearing pregnancy, including stillbirths. This dataset did not contain nulliparas. While it would have been possible to instead utilize information for any pregnancy including incomplete ones, there is not biological evidence that incomplete pregnancy produces the equivalent long-term decrease in estrogen levels as full-term pregnancies. Additionally, recall of age at miscarriage or abortion was often vague and difficult to verify through family interview.

Ryan et al. (2009) found a relevant trend based on whether women had their first child in their twenties versus earlier. Our cohort contained only three individuals who had

Table 1 Cohort size.

	Total participants	Age at AD onset	Reproductive history info	Family history of dementia info
Cases included	133	123	99	89
Missing info or excluded cases	0	10	24	10

This table reports the number of cases for which we were able to collect essential information for this study. Cox models were fitted for a sample size of 89 individuals.

their first child before age twenty, and so to investigate this possible effect, we modified Ryan's variable so that each category contained a sufficient number of cases to perform statistics. To this end, we created a variable of whether women first gave birth after age 21.

2.1.4. Age at Alzheimer onset

For those women with CDR-SOB scores of 0.5 or above, age at onset was estimated. We chose 0.5 as the point of origin, despite the fact that it is not yet considered AD, in order to capture the full progression of disease progression from the earliest stages of cognitive decline for maximum accuracy. Exclusion of the ''Questionable Dementia'' CDR 0.5 category would have excluded CDR-SOB scores of 0.5—4 and two GDS categories (Table S1). Based on published norms of disease progression (Reisberg et al., 2010), a scale of CDR-SOB scores and standard amount of time spent in each dementia phase was created, interpolating CDR-SOB scores between the endpoints of other scales' categories (see Supplementary Material section 1, and Fox et al., 2013). In this way, women's age at AD onset was estimated, i.e. when CDR-SOB would have gone from 0 to 0.5, back extrapolated from degree of dementia at time of interview. This estimate of onset is a monotonic transformation from CDR-SOB score, and therefore qualifies as a surrogate measure of AD status. Any detected statistical association with outcome variables can be safely interpreted as an association with AD risk according to principles of surrogate variables.

2.2. Statistical methods

Cox proportional hazards models were fitted to investigate the relationship between estrogen exposure and AD risk. Prior to analysis, variables were checked for normal distribution. Variables that did not meet this criterion were transformed to maximize data symmetry. The Cox models utilized a measurement of the time between age 50 and AD onset as time-scale. Those women who were determined to have CDR-SOB = 0 at interview were included in the model as right-censored cases, as is standard in survival analysis. All analyses were performed using the R language and environment for statistical computing.

Cox models were initially designed to control for all potentially confounding factors (Table S3). Models in which the predictive variable did not contribute a significant effect were not further explored. For models in which the predictive variable contributed a significant effect, control variables that contributed at least borderline significance to the model fitting ($p < 0.1$) were retained, and extraneous variables were omitted (Table S4).

All Cox models included the following control variables: the woman's age at interview, exponentiated age at interview, education history, family history of dementia. Models with predictive variables related to pregnancy controlled for OC use, those with predictive variables related to ERT use controlled for a list of six common menopause symptoms, reproductive span models controlled for surgical menopause and ERT use, and models with menstrual cycles as predictive variable controlled for regularity of periods. Those women aged 90 and older at time of interviews would have been too old to have access to OC and HRT at ages during which they might have used them, so in the models with estrogen pills as predictive variables, we controlled for a variable of whether or not a woman was age 90+ at interview. A list of all statistical models is detailed in Table S3.

3. Results

In order to test the role of cumulative estrogen exposure on AD risk in a cohort of British women, reproductive and medical history information were collected, and degree of Alzheimer's-type dementia was measured. All women in this study were White British currently living in England. In total 133 interviews were performed, and ten cases were omitted from analyses due to factors that could confound or mask estrogen's effects on AD risk, including hormone disorders, brain injury, and diagnoses of other types of dementia. 24 cases were excluded from analyses due to missing reproductive history data, and 10 more cases were excluded due to missing data on family history of dementia. As a result, the Cox models were fitted for a sample size of 89 probands (Table 1). Descriptive statistics for this cohort are reported in Tables 2 and 3. There were 51 individuals with CDR-SOB = 0 (indicating no sign of dementia) with a mean age of 79, and 38 individuals with CDR-SOB $>$ 0 (indicating any sign of dementia) with a mean age of 85.

To investigate the role of estrogen exposure in AD risk, we considered how number of months with estrogen exposure and number of months with menstrual cycles affected AD risk in our cohort. We also considered age at first birth, as well as the individual aspects of reproductive history used to calculate the aforementioned composite variables. Cox models revealed that longer estrogen exposure, more menstrual cycles, longer reproductive span, age $>$ 21 at first birth, and more months in lifetime spent pregnant had protective effects against AD risk (Table 4). For each Cox model that yielded significant results, scatterplots of the Martingale residuals revealed that the model fits were not unduly influenced by particular cases.

3.1. Total months with estrogen exposure

More months with exposure to estrogen during the lifetime was negatively associated with AD risk (Fig. 1). For every additional month with estrogen, women experienced a 0.5%

Table 2 Participant recruitment and cohort descriptive statistics.

Year of interview:	2010: 8 (4/4)	2011: 65 (40/25)	2012: 16 (7/9)			
Interview location:	Participant's home: 45 (30/15)	Nursing home: 20 (3/17)	Cambridge University office: 21 (15/6)	Medical office: 3 (3/0)		
Participant's place of birth:	Cambridge: 19 (12/7)	London: 18 (13/5)	Other Southern England: 23 (13/10)	Northern England: 13 (4/9)	Scotland, Wales, Ireland: 13 (7/6)	Outside UK: 3 (2/1)
CDR-SOB >0:	False: 51 (''controls'')	True: 38 (''patients'')				
Family history of dementia	No: 55 (32/23)	Parent or sibling with dementia: 17 (8/9)	NA: 17 (11/6)			
Parity	Nulliparous: 0	Parous: 89 (51/38)				
Age first birth >21	No: 11 (6/5)	Yes: 78 (45/33)				
COC use:	No: 66 (35/31)	Yes: 23 (16/7)				
Any hormone replacement therapy:	No: 67 (33/34)	Yes: 22 (18/4)				
Hysterectomy	No: 47 (22/25)	Yes: 26 (15/11)	NA: 16 (14/2)			
Regular periods	No: 6 (2/4)	Yes: 38 (16/22)	NA: 45 (33/12)			
Bilateral oophorectomy	No: 60 (30/30)	Yes: 6 (3/3)	NA: 23 (18/5)			
Religion:	Church of England: 74 (45/29)	Church of Scotland/ Wales: 3 (1/2)	Other Christian: 11 (5/6)	Jewish: 1 (0/1)		
Education	Schooling to age 16 or less: 78 (43/35)	Schooling beyond age 16: 11 (8/3)				
Smoking history	Never or <1 year: 45 (29/16)	1—10 years: 6 (4/2)	10—20 years: 5 (2/3)	>20 years: 19 (6/13)		
Alcohol consumption:	≤ 2 servings per day: 69 (38/31)	>2 servings per day: 6 (3/3)				

Each row represents a different variable. Numbers in parentheses present the breakdown for probands with CDR-SOB = 0/CDR-SOB >0. Any effect of age at participation is corrected by back-extrapolating age-at-onset. Controls are included in Cox models as right-censored cases. NA = not available.

Table 3 Cohort age and reproductive features.

	Controls				Patients			
	Min	Median	Max	SD	Min	Median	Max	SD
Age at interview	70	77	97	6.53	72	86	98	5.65
Age at menarche (years)	10	13	16	1.47	7	13.14	18	1.93
Age at menopause (years)	36	50	60	5.53	33	50	60	6.06
Reproductive span	288	449.9	576	63.68	228	440.1	576	79.85
Rasgon variable	288	480	684	84.70	228	440.1	612	85.01
Cumulative estrogen exposure (months)	274	465	682.5	87.61	222	423.1	608.8	87.33
Number of full-term pregnancies	1	3	6	1.37	1	2.5	11	1.95
Months spent pregnant in lifetime	9	27	51	10.33	9	19.5	93	16.22
Age at first birth	17	26	39	4.14	20	25	34	3.37
Number of ovulatory cycles in lifetime	82	390	565.5	99.00	56	382.5	554	107.45
Duration COC use (months)	0	0	360	84.35	0	0	312	65.62
Duration ERT use (months)	0	0	336	75.51	0	0	120	20.19

For "Controls" (CDR-SOB = 0) column, $N = 51$. For "Patients" (CDR-SOB > 0) column, $N = 38$. COC: combined oral contraceptives; ERT: estrogen replacement therapy; Rasgon variable: reproductive span + ERT duration.

decrease in AD risk ($p = 0.02$). When comparing women above and below the cohort median number of months with estrogen exposure in their lifetimes, the ratio of hazards was 0.36 ($p < 0.01$), the lower hazard for women with above-median estrogen exposure. In other words, women who were above the cohort median number of months with estrogen exposure had only 64% of the AD risk of women who were below the cohort median ($p < 0.01$). We also looked at a simpler variable used to estimate estrogen exposure, as reproductive span + HRT duration (Rasgon et al., 2005). There was a significant negative relationship between this estimate of estrogen exposure and AD risk, such that each additional month for "Rasgon variable" corresponded to 0.56% reduction in AD risk ($p = 0.01$).

3.2. Reproductive span and number of menstrual cycles

We tested the relationship between reproductive span and AD risk, and found a marginally statistically significant result ($p < 0.10$) such that each additional month between menarche and menopause corresponded to a 0.45% decrease in AD risk.

Number of menstrual cycles was estimated as number of months between menarche and menopause free from OC use, pregnancy, breastfeeding, and post-partum anovulation. A larger number of menstrual cycles was associated with lower AD risk. Each additional cycling month corresponded to a 0.3% reduction in AD risk, but this result was of only marginal statistical significance ($p < 0.10$). Due to limitations in study design (see Section 5.1) this may not be a sufficiently accurate estimation method to detect the biological relationship between lifetime number of cycling months and AD.

3.3. Pregnancy

Women who spent above the cohort median number of months pregnant in lifetime exhibited lower AD risk. Women above the cohort median had only 63% of the AD risk of women below the cohort median number of months spent pregnant ($p < 0.01$).

Table 4 Results of Cox regressions.

Parameter	coef	exp(coef)	se(coef)	p-Value	96% CI
Estrogen exposure (months)	−0.004991	0.995021	0.002203	0.0235*	(0.9907, 0.9993)
Estrogen exposure (median)	−1.0269	0.3581	0.3843	0.00754**	(0.004118, 0.005289)
Number of ovulatory cycles	−0.003144	0.996861	0.001708	0.0656†	(0.9935, 1)
Rasgon variable	−0.005569	0.994447	0.002279	0.0145*	(0.01356, 0.01499)
Reproductive span	−0.004526	0.995484	0.002532	0.0739†	(0.9906, 1)
Age first birth > 21	−1.060676	0.346222	0.517636	0.0405*	(0.1255, 0.9549)
Months pregnant lifetime (above median)	−0.992827	0.370528	0.349751	0.00453**	(0.18669, 0.7354)

Rasgon variable: reproductive span + ERT duration. Each row represents the results of one Cox model summarized by the parameter listed in the first column, and further described in Tables S3 and S4. This table reports the partial likelihood point estimate for the effect of the parameters listed, the corresponding exponentiated value, the standard error, the p-value for the relative sharp null hypothesis and the 95% confidence interval. The model fitting statistics, likelihood ratio test results, and score (logrank) test results are reported in Table S4. Tests included 89 individuals, and there were 38 total observed failure events.

* $p < 0.05$.

** $p < 0.01$.

† $p < 0.10$.

Women who first gave birth after they were 21 years of age had lower AD risk compared to women who gave birth earlier. Comparing two hypothetical women who are identical except one of them gave birth for the first time after age 21 and the other before, the AD hazards ratio is 0.35, the lower hazard for the woman who gave birth after age 21 ($p < 0.05$). Giving birth after age 21 contributed a 0.35-fold reduction in AD risk.

3.4. Non significant statistical outcomes

There were no statistically significant effects on AD risk from the following estrogen-altering aspects of reproductive history when each was considered as the primary predictive variable with appropriate controls (Table S3): age at menarche, age at menopause, parity, age at first birth as an interval variable, regularity of menstrual cycles, use and duration of COC or HRT. Given our small sample size, we encourage further research into these topics.

4. Discussion

Duration of exposure to estrogen may modify women's risk of AD. Women differ in their duration of estrogen exposure due to differences in reproductive history and use of estrogen-containing therapies. We present a new, more comprehensive way to measure individual women's lifetime duration of exposure to estrogen by taking into account various aspects of reproductive history. In our cohort, more months with exposure to estrogen cumulatively in the lifetime was associated with lower AD risk (Fig. 1). We also present tentative evidence that more menstrual cycling months could be associated with reduced AD risk. Women who first gave birth after age 21 exhibited lower AD risk compared to women who first gave birth earlier. Women who spent more total months pregnant had lower AD risk than women who spent fewer months pregnant.

Reproductive history is an important modifier of women's lifetime exposure to estrogen. Different reproductive states are characterized by substantially different natural doses of estrogen, and the lengths and patterns of these phases vary widely between individuals. Here we review previous studies of reproductive history features mediating exposure to estrogen and any association with later-life cognitive health, and discuss our own findings in this context.

4.1. Endogenous estrogen

4.1.1. Reproductive span

Our results indicated a marginally significant protective effect of longer reproductive span against AD risk, and this effect was not detected when age at menarche or age at menopause were considered individually. Previous authors have explored these questions with varied results. Earlier age at menarche has been associated with less cognitive impairment in later life (Rasgon et al., 2005; Ryan et al., 2009), and decreased risk of dementia (Kim et al., 2003) and of AD (Hong et al., 2001; Paganini-Hill and Henderson, 1994), though others found no significant association (Henderson et al., 2003). Among elderly women, later age at menopause correlated with less cognitive decline (Lebrun et al., 2005; McLay et al., 2003; Rasgon et al., 2005), higher IQ

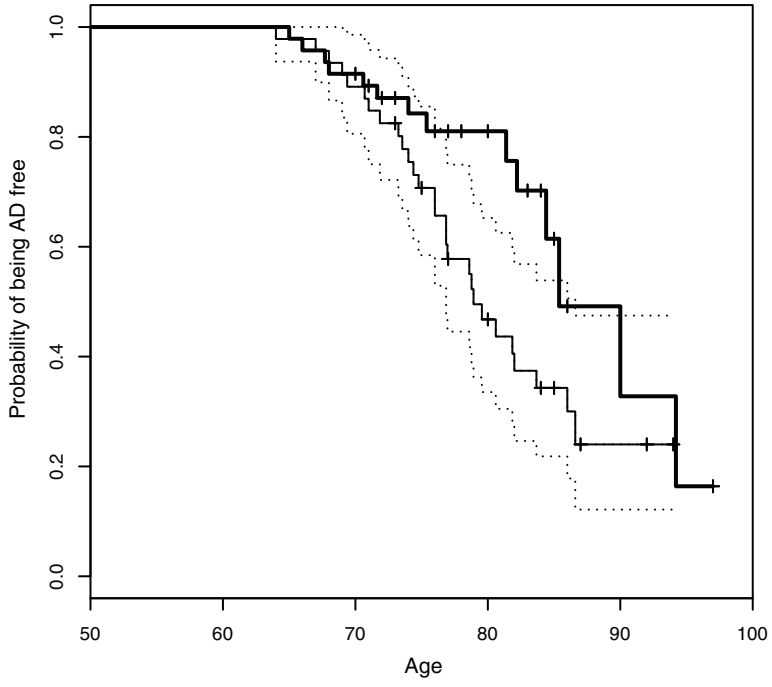

Figure 1 Women with more months of estrogen exposure in lifetime had lower AD risk. For each value of age, the Kaplan–Meier plot reports the probability of being AD-free for women with months of estrogen exposure lower than the sample median (lower curve) and above the sample median (bold, upper curve). Pointwise 95% confidence bands for the lower curve are shown (dotted lines). This plot gives a visual sense of the effect's magnitude, while the results in Table 4 represent a more meaningful analysis utilizing the detailed information available for months of estrogen exposure.

(Whalley et al., 2004), reduced dementia risk (Kim et al., 2003), reduced AD risk (Hong et al., 2001), later AD onset (Sobow and Kloszewska, 2003), and later AD onset in women with Down's Syndrome (Cosgrave et al., 1999; Schupf et al., 2003). Other studies found no significant association between age at menopause and cognitive decline (Colucci et al., 2006; Paganini-Hill and Henderson, 1994). Elderly women who had longer reproductive spans showed better cognitive function (Heys et al., 2011; Rasgon et al., 2005; Ryan et al., 2009), but other studies found no significant trend (Colucci et al., 2006; Henderson et al., 2003; Low et al., 2005), and one study revealed that for women who carried at least one ApoE-ε4 allele, AD risk was higher for the group that had the longer reproductive spans (Geerlings et al., 2001).

4.1.2. Age at first birth

Although length of reproductive period influences lifetime estrogen exposure, reproductive activity during this phase alters the frequency and level of endogenous estrogen production. Evidence suggests that parity alters estrogen levels, with parous women having lower endogenous estrogen than nulliparas (Bernstein et al., 1985). Previous authors have generally hypothesized that despite the high levels of estrogen during pregnancy, parity should increase AD risk given the longer-term reduction in estrogen levels (e.g. Beeri et al., 2009; McLay et al., 2003). Age at first birth may influence lifetime exposure to estrogen by decreasing the age at which estrogen levels drop with first gravid event. As such, it would be predicted that later age at first birth would result in higher lifetime estrogen levels and decreased risk of AD. Indeed, Ryan et al. (2009) found that elderly women who had their first child in their twenties had better cognitive function than women who had had their first child earlier. Conversely, Heys et al. (2011) reported that the later a woman's first birth, the worse her cognitive function in later life.

In our cohort, whether a woman gave birth for the first time after age 21 was a statistically powerful modifier of AD risk (Table 4), similar to the observation of Ryan et al. (2009). This finding is consistent with the fact that maternal baseline estrogen levels are lower after the first gravid event compared to before (Bernstein et al., 1985), so later age at first birth would indicate more years spent in a nulliparous state of higher estrogen levels.

4.1.3. Parity

4.1.3.1. Nulliparous versus parous women. Given parity's longterm decrease in estrogen levels, nulliparas would be expected to experience higher lifetime levels of estrogen. Some authors have found that in comparison to parous women, nulliparas had less cognitive decline (McLay et al., 2003) and lower risk of AD (Ptok et al., 2002). One study (Beeri et al., 2009) looked at the brains of their cohort pre and post mortem, and found that compared to nulliparas, parous women had more signs of AD neuropathy but the difference was only statistically significant for neuritic plaques in the amygdala. Corbo et al. (2007) found that among non-ApoE-ε4 carriers only, nulliparas had later onset of AD. Conflictingly, Colucci et al. (2006) found that nulliparas had a greater risk of AD than parous women, consistent with evidence that parity makes the brain more responsive to estrogen in later life in rats (Barha and Galea, 2011).

4.1.3.2. Number of pregnancies. Given the fact that pregnancy represents a state of increased estrogen, one could argue that with increasing parity there should be increasing protection against AD. This is especially true because the estrogen characteristic of pregnancy is estriol (E3), which may have stronger anti-AD properties than the other estrogens (Morinaga et al., 2007, 2011). Higher parity has been associated with better memory ability in elderly women (Henderson et al., 2003), and ERT has been shown to have a beneficial effect on cognitive function that improved with increasing parity (Dunkin et al., 2005), although this effect was not statistically significant. Despite the results of those two studies, there has been more robust evidence for the opposite trend. Increasing parity has been associated with worse cognitive function (Heys et al., 2011) and earlier onset of AD (Colucci et al., 2006; Sobow et al., 1999). In one cohort, women who had had three or more pregnancies had earlier onset of AD and triple the risk of having AD (Colucci et al., 2006), and in another study, women who had five or more pregnancies had worse cognitive impairment compared with those who had fewer pregnancies (Rasgon et al., 2005).

We found that women who spent more cumulative months pregnant exhibited reduced AD risk (Table 4), similar to effects in some previous studies (Dunkin et al., 2005; Henderson et al., 2003) although this trend was weak and non-significant in Dunkin et al. (2005). Also, not all relevant studies have reported a beneficial effect (Beeri et al., 2009; Colucci et al., 2006; Heys et al., 2011; Rasgon et al., 2005). Nonetheless, our finding is consistent with the fact that during pregnancy, women experience increasing levels of estrogen with plasma estrogen concentration typically rising from 2 ng/ml in the first trimester to 14.5 ng/ml in the third trimester (Tulchinsky and Little, 1994). Such phases of estrogen exposure may have long-term neuroprotective effects.

4.1.4. Breastfeeding

Two studies have considered the relationship between breastfeeding duration and AD risk. Shorter mean breastfeeding duration corresponded to reduced dementia incidence among elderly women in a Chinese cohort (Heys, 2010; Heys et al., 2011). We previously demonstrated a protective effect of breastfeeding against AD in our British cohort (Fox et al., 2013). While the latter finding is not consistent with estrogen's protective effects, other aspects of lactational endocrinology may override estrogen's neuroprotection in the case of breastfeeding.

4.1.5. Menstrual cycles

Ovulatory menstrual cycles expose women to a unique hormonal environment characterized by estrogen and progesterone surges. While there have not been previous studies exploring menstrual cycling in connection with AD, there have been studies looking at the effect of menstrual cycling on risk of reproductive cancers, due to the ovarian hormones' well-established involvement in reproductive cancer risk. These studies have generally found that cycling regularity and more cycles in lifetime increase reproductive cancer risk (see Supplementary Material section 2 for references).

Here, we find no effect from cycle regularity, and a negative relationship between number of menstrual cycles

and AD risk. This effect was of marginal statistical significance, and we encourage further research into this question.

4.1.6. Body mass index

We were not able to measure BMI in this study, but it should be mentioned as an important indicator of estrogen levels. Our assessment of the number of months a woman was exposed to estrogen is limited by our lack of BMI data. Elderly women with higher BMI have higher plasma concentrations of estrogens (Szymczak et al., 1998) because adipose tissue continues to aromatize androgens produced by the adrenal glands (Deslypere et al., 1985). Adipose tissue represents the main influence on postmenopausal estrogen levels in women. The estrogenic benefit of high BMI is balanced by the fact that higher adiposity is associated with decreased cardiovascular health and decreased insulin sensitivity, both of which increase AD risk (Newman et al., 2005; Rasgon and Jarvik, 2004). The effect BMI has on AD risk has been described as bimodal, with midlife high BMI increasing risk and late life high BMI decreasing risk (see Supplementary Material section 2 for list of references) (Fitzpatrick et al., 2009). However, one study found that elderly women who develop AD may be more likely to be overweight (Gustafson et al., 2003). Nonetheless, BMI is an important modifier of post-menopausal estrogen exposure and should be included in future studies.

4.1.7. Bone mineral density

We were not able to measure bone mineral density (BMD) in this study, but it should be mentioned as an important alternative tactic for estimating a woman's lifetime exposure to estrogen. Bone tissue contains estrogen receptors, and estrogen inhibits bone resorption (see Supplementary Material section 2 for list of references). Higher BMD may be a risk factor for breast cancer (see Supplementary Material section 2 for list of references), particularly ER-positive. High postmenopausal BMD has been associated with long reproductive span (Ito et al., 1995), and high parity (Murphy et al., 1994). Low postmenopausal BMD has been associated with the inverses of these measures and longer amenorrhea (Supplementary Material section 2).

Among elderly women, lower BMD has been associated with higher risk of AD. Women in the lowest quartile of hip BMD had more than double the incidence of AD (Tan et al., 2005). Low BMD has also been associated with increased AD risk, earlier age at AD onset and worse cognitive performance on a variety of measurements including memory (see Supplementary Material section 2 for list of references).

4.2. Exogenous estrogen

Some studies have found that women who use ERT are at reduced risk for developing AD, but other studies have found neutral or even opposite effects, with ERT use increasing AD risk or having no effect (see Supplementary Material section 2 for list of references). The first large long-term clinical trial of this kind, the Women's Health Initiative Memory Study (WHIMS), found no statistically significant difference between AD risk in the ERT group versus the placebo group, calling into question the protective effects of estrogen (Shumaker et al., 1998). While some researchers have interpreted these findings as evidence against estrogen's neuroprotection, others believe that the statistical significance is not reflective of the actual effect, or that ERT began too long after menopause, thus after a critical window of time during which the brain is still sensitive to estrogen's effects (Bagger et al., 2005; Whitmer et al., 2011; Zandi et al., 2002).

5. Conclusions

5.1. Study limitations

This study was limited to a small cohort of White British women currently residing in England. Further research should explore whether the relationship between cumulative estrogen exposure and AD risk is relevant across different ethnic and regional groups. The data utilized in this study rely heavily on recall, though the accuracy of information was maximized by conducting interviews with family and carers who could confirm, correct, and contribute to the data being collected.

A limiting factor in our estimation of number of menstrual cycles is the wide range in lactation amenorrhea duration (Gray et al., 1990). Also, each month with the possibility of ovulation does not necessarily result in ovulation, and anovulatory cycles are a common occurrence. It is important to note that hormone levels vary between fully competent cycles, follicular or luteal suppressed cycles, anovulatory cycles, and oligomenorrheic or amenorrheic phases (Ellison et al., 1993), which we are not able to distinguish here. In sum, our assessment of the impact of estrogen levels during possible cycling months is limited by a number of factors and other study designs may better be able to assess this relationship. Our analyses were not designed to measure the potential for different impacts of estrogen exposure at different life phases. Other research methodologies are better able to gauge this information.

The method of AD diagnosis is inherently flawed by the limitations of the CDR as a diagnostic instrument. Even the most comprehensive interview-based diagnostic techniques are merely estimations in the absence of post-mortem brain analysis. Because one researcher (MF) performed all assessments, this study did not suffer from inter-rater variability. A limitation of this cross-sectional study is that age at AD onset is estimated rather than observed. While there is variation in rate of disease progression, this estimation method was selected because the alternatives—relying on proband recall, family/carer recall, or clinical diagnosis—would have been more biased. Another potential bias is that healthy individuals were more likely to volunteer themselves, while individuals with more severe dementia were more likely to be volunteered by a family member or carer. However, there is no reason to believe that such bias would be related to estrogen exposure.

5.2. Estradiol, estriol, estrone

It should be noted that throughout the life course, different estrogens are dominant. During the reproductive span with the exception of pregnancy, estradiol (E2) is the most abundant and potent of the estrogens. During pregnancy, estriol (E3) is most abundant and most potent. Post-menopause, endogenous estrogen is mostly estrone (E1) and ERT contains mostly estrone. The AD-relevant literature has suggested

that all three estrogens are neuroprotective, positively associated with cognitive function, and protective against AD in rodent models (Morinaga et al., 2007, 2011). While E2 and E1 appear to have equivalent anti-AD potencies, E3 may have a more powerful beneficial effect (Morinaga et al., 2011), possibly indicating pregnancy-related benefits. The clinical literature has focused on E1 in post-menopausal women, possibly accounting for some of the inconsistencies in the clinical literature (Asthana, 2003). Our study highlights E2 and E3's effects, and future research should distinguish between cumulative exposures to different estrogens.

5.3. Concluding remarks

Evidence from a range of sources suggests that estrogen has neuroprotective effects that may reduce risk of AD. Here, we demonstrate that reproductive history modifies estrogen exposure in a way that is relevant to AD etiology. Total months exposed to estrogen was calculated taking into account a number of features of reproductive history, and the resulting variable was significantly negatively associated with AD risk. More menstrual cycles, longer reproductive span, more cumulative months pregnant, and later age at first birth were associated with reduced AD risk. Use of OC and HRT did not affect AD risk in this cohort. Future research should include BMI and BMD in investigating reproductive history, estrogens, and AD. The differences in reproductive history between individuals should be considered when assessing individual women's particular AD risk.

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Conflict of interest

The authors have no conflicts of interest. The protocol was approved by the University of Cambridge Human Biology Research Ethics Committee.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.psyneuen.2013.08.005.

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