Estriol combined with glatiramer acetate for women with relapsing-remitting multiple sclerosis: a randomised, placebo-controlled, phase 2 trial

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Summary

Background **Relapses of multiple sclerosis decrease during pregnancy, when the hormone estriol is increased. Estriol treatment is anti-inflammatory and neuroprotective in preclinical studies. In a small single-arm study of people with multiple sclerosis estriol reduced gadolinium-enhancing lesions and was favourably immunomodulatory. We assessed whether estriol treatment reduces multiple sclerosis relapses in women.**

Methods **We did a randomised, double-blind, placebo-controlled phase 2 trial at 16 academic neurology centres in the USA, between June 28, 2007, and Jan 9, 2014. Women aged 18–50 years with relapsing-remitting multiple sclerosis were randomly assigned (1:1) with a random permuted block design to either daily oral estriol (8 mg) or placebo, each in combination with injectable glatiramer acetate 20 mg daily. Patients and all study personnel, except for pharmacists and statisticians, were masked to treatment assignment. The primary endpoint was annualised relapse rate after 24 months, with a significance level of p=0·10. Relapses were confirmed by an increase in Expanded Disability Status Scale score assessed by an independent physician. Analysis was by intention to treat. The trial is registered with ClinicalTrials.gov, number NCT00451204.**

Findings **We enrolled 164 patients: 83 were allocated to the estriol group and 81 were allocated to the placebo group. The annualised confirmed relapse rate was 0·25 relapses per year (95% CI 0·17–0·37) in the estriol group versus 0·37 relapses per year (0·25–0·53) in the placebo group (adjusted rate ratio 0·63, 95% CI 0·37–1·05; p=0·077). The proportion of patients with serious adverse events did not differ substantially between the estriol group and the placebo group (eight [10%] of 82 patients *vs* ten [13%] of 76 patients). Irregular menses were more common in the estriol group than in the placebo group (19 [23%] *vs* three [4%], p=0·0005), but vaginal infections were less common (one [1%] *vs* eight [11%], p=0·0117). There were no differences in breast fibrocystic disease, uterine fibroids, or endometrial lining thickness as assessed by clinical examination, mammogram, uterine ultrasound, or endometrial lining biopsy.**

Interpretation **Estriol plus glatiramer acetate met our criteria for reducing relapse rates, and treatment was well tolerated over 24 months. These results warrant further investigation in a phase 3 trial.**

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Introduction

Multiple sclerosis is an autoimmune, neurodegenerative disease of the CNS.[1] Relapses are decreased by more than 70% during the last trimester of pregnancy,[2] when oestrogen and progesterone concentrations are highest.[3] Pregnancy is a state of temporary immune modulation enabling survival of the fetus as a half-foreign allograft.[4] Other cell-mediated autoimmune diseases, such as psoriasis and rheumatoid arthritis, also improve during pregnancy.[5]

Estriol is an oestrogen unique to pregnancy, made by the fetal–placental unit, and reaches highest concentrations in the last trimester. We postulated that increased concentrations of estriol might mediate a decrease in relapses. Preclinical studies of multiple sclerosis showed that estriol treatment has both anti-inflammatory and neuroprotective properties, mediated through binding to oestrogen receptors expressed in the immune system and the CNS.[6] In a small phase 2, single-arm, crossover clinical trial[7] of estriol treatment for ten women with multiple sclerosis, monthly brain MRI showed significant reductions in gadolinium-enhancing lesions during 6 months of treatment compared with 6 months before treatment. Peripheral blood mononuclear cells had significantly increased expression of interleukin 5 and interleukin 10 and decreased concentrations of tumour necrosis factor (TNF) α and matrix metalloproteinase 9.[8,9] When estriol treatment was discontinued for 6 months, both enhancing lesions and immune responses returned to pre-treatment levels. Furthermore, when estriol was

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Research in context

Evidence before this study

We searched PubMed for studies published between Jan 1, 1980, and June 25, 2015, with the terms "multiple sclerosis" and "estriol". We included clinical trials, clinical observations, and preclinical studies, both in vitro and in vivo in animals and human beings. We also searched abstracts from the American and the European Committees for Treatment and Research in Multiple Sclerosis from the past 5 years. Besides laboratory studies, we found one single-arm, crossover clinical trial of estriol for multiple sclerosis. ClinicalTrials.gov lists an ongoing double-blind, placebo-controlled trial of estriol treatment for relapsing-remitting and progressive multiple sclerosis with cognitive testing as the primary outcome (registration number NCT01466114).

Added value of this study

This study is the first randomised, placebo-controlled trial of oral estriol treatment for women with relapsing-remitting multiple sclerosis to be completed. We showed the safety and beneficial effects on relapse rates compared with placebo.

Implications of all the available evidence

These findings are consistent with the hypothesis that increased concentrations of estriol during pregnancy might mediate, at least in part, the protective effect of pregnancy on relapse rates. A phase 3 study of estriol in relapsing-remitting multiple sclerosis is needed to test these findings and to explore potential effects on disabilities.

administered again for 4 additional months, combined with a progestin for uterine protection,[10] the reduction in enhancing lesions and immunomodulation returned.[7–9]

Estriol has been used for several decades throughout Europe and Asia for treating menopausal symptoms.[11–13] A Women's Health Initiative study[14] done in 2002 to assess whether premarin (a complex mix of conjugated oestrogens) protects against coronary heart disease in menopausal women aged 50–79 years was stopped prematurely because of an increased risk for cardiovascular disease and breast cancer, whereas the number of colorectal cancers and hip fractures decreased. Premarin includes estradiol, an oestrogen that is present at low concentrations in women with normal menstrual cycles and in oral contraceptives. Oral contraceptives have also been associated with cardiovascular risks in non-menopausal women, particularly in those who smoke. Whether oral contraceptives are a cause of breast cancer or if they enable breast cancer to be detected earlier is unclear.[15] The distinction between estradiol and estriol is important, because oestrogens are not all alike.[16] Estriol binds to ERα and ERβ weaker than does estradiol, and it binds to ERβ stronger than it does to ERα.[17] Oestrogenic effects on breast cancer and cardiovascular disease are mediated by ERα. Indeed, ERβ binding can antagonise ERα binding in some tissues,[17] with estriol treatment being protective in preclinical models of breast cancer.[18] Finally, estradiol also induces uterine endometrial proliferation, which can lead to cancer.[19] Thus, women taking oral contraceptives or hormone replacement therapy are not treated with unopposed oestrogens,[10] but rather in combination with a progestin to protect the uterus.[15] Estriol is a weaker stimulator of endometrial proliferation than is estradiol, such that it can be taken unopposed for up to a year, but after that, it too should be taken in combination with a progestin for uterine protection.[15]

Here, we report results of a phase 2 clinical trial to test the safety and efficacy of oral estriol as an add-on treatment in women with relapsing-remitting multiple sclerosis.

See Online for appendix

For the **protocol** see http://neurology.ucla.edu/media/attachments/files/51/Estriol_Relapse_Trial_Protocol.pdf

Methods

Study design and participants

We did this double blind, placebo-controlled, randomised, parallel group trial at 16 academic neurology clinics in the USA, starting on June 28, 2007, with the last clinic visit on Jan 9, 2014, and last follow-up questionnaire on July 10, 2014. Eligible patients were women aged 18–50 years, with a diagnosis of relapsing-remitting multiple sclerosis according to the McDonald criteria,[20] a baseline score of 0–4·5 on the Expanded Disability Status Scale (EDSS), and relapsing disease activity in the previous 24 months. Key exclusion criteria were progressive multiple sclerosis, taking glatiramer acetate for more than 2 months before randomisation, currently smoking, and taking other concurrent disease-modifying or hormonal treatments (appendix pp 6–8). The study was approved by the ethics committee at each site and participants provided written informed consent at screening. The National Institutes of Health appointed a data and safety monitoring board, which monitored safety and efficacy (appendix p 4) with representatives from the National Institutes of Health and the National Multiple Sclerosis Society serving as observing members. The protocol is available online.

Randomisation and masking

A statistician who had no further role in the trial randomly assigned patients (1:1) to receive oral estriol (8 mg daily) or oral placebo (matched by appearance and taste) using a computer-generated code with random permuted block design (block size six). We stratified randomisation by glatiramer acetate treatment during screening, using Zelen's method[21] to ensure that treatment assignment was balanced within each clinic.

Patients, treating physicians, and all investigators assessing outcomes were masked to treatment assignment.

Study statisticians and pharmacy staff were not masked to treatment, but they had no interaction with patients.

Procedures

All patients started glatiramer acetate injections (20 mg/day) within 2 months of randomisation. Patients provided their own glatiramer acetate. To avoid patients taking unopposed oestrogens, patients in the estriol group also received a progestin (norethindrone 0·7 mg) daily for 2 weeks every 3 months, starting at 6 months; patients in the placebo group received a second placebo matched to progestin. After 24 months of treatment, a 4-week taper began for both estriol and placebo (appendix p 21).

Examining neurologists did the EDSS assessments, whereas treating neurologists managed patient care including treatment of relapses, and gynaecologists managed gynaecological issues. The appendix pp 9–14 shows details of the blood laboratory tests for safety, methods for measuring estriol blood concentration, MRI analyses of enhancing lesions[7] and brain volumes,[22,23] and voxel-based morphometry for regional loss of grey matter.[24]

Outcomes

The primary endpoint was annualised confirmed relapse rate at 24 months. A confirmed relapse was defined as new neurological symptoms or worsening of pre-existing symptoms, lasting at least 48 h in a participant who had been neurologically stable or improving in the previous 30 days, accompanied by an objective neurological change (worsening by 0·5 points on the EDSS or worsening by ≥1·0 points on the pyramidal, cerebellar, brainstem, or visual functional system scores),[25] not due to fatigue alone and not associated with fever or infection.

The secondary endpoints were time to first confirmed relapse, annualised relapse event rate, and time to first relapse event, all at 24 months. A relapse event was defined as meeting the criteria for a confirmed relapse without documentation of a change in EDSS score. Other prespecified outcomes included safety, blood estriol concentration (at 3 months, 6 months, 12 months, 18 months, and 24 months), proportion of patients with confirmed disability progression (defined as an increase of EDSS of ≥1·0 point in participants with baseline score of ≥1·0, or an increase of ≥1·5 points for those with a baseline score of 0, each sustained for at least 6 months), change from baseline in EDSS score, Modified Fatigue Impact Scale (MFIS) score, Beck Depression Inventory (BDI) score, Multiple Sclerosis Quality of Life (MS QoL) score, Multiple Sclerosis Functional Composite (MSFC) score, and Paced Auditory Serial Addition Test (PASAT) score, as well as enhancing lesions, T2 lesions, and brain volume by MRI at 24 months.

All analyses were planned at 24 months, the end of the study. We also did analyses at 12 months. The National Institutes of Health would not fund a trial with a placebo-only comparator arm for this duration because approved treatments are available. However, because glatiramer acetate takes 9 months to reach full potency,[26] the first 9 months of the study approximated a comparison of estriol only with placebo only, which could be captured by an analysis at 12 months.

We did post-hoc analyses of whole grey matter volume, cortical grey matter volume, white matter volume, PASAT scores stratified by median PASAT score at baseline, brain volumes by MRI stratified by presence or absence of enhancing lesions, proportion of patients with enhancing lesions, correlation of estriol concentration with relapses and with enhancing lesions, and correlation of PASAT scores with grey matter volumes (appendix pp 10,11).

Statistical analysis

We calculated that we would need a sample size of 150 patients to provide 80% power to detect a 33% reduction in relapse rates in the estriol group compared with the placebo group (0·75 in the estriol group *vs* 1·18 in the placebo group) at 24 months with a two-sided significance level of 0·10.

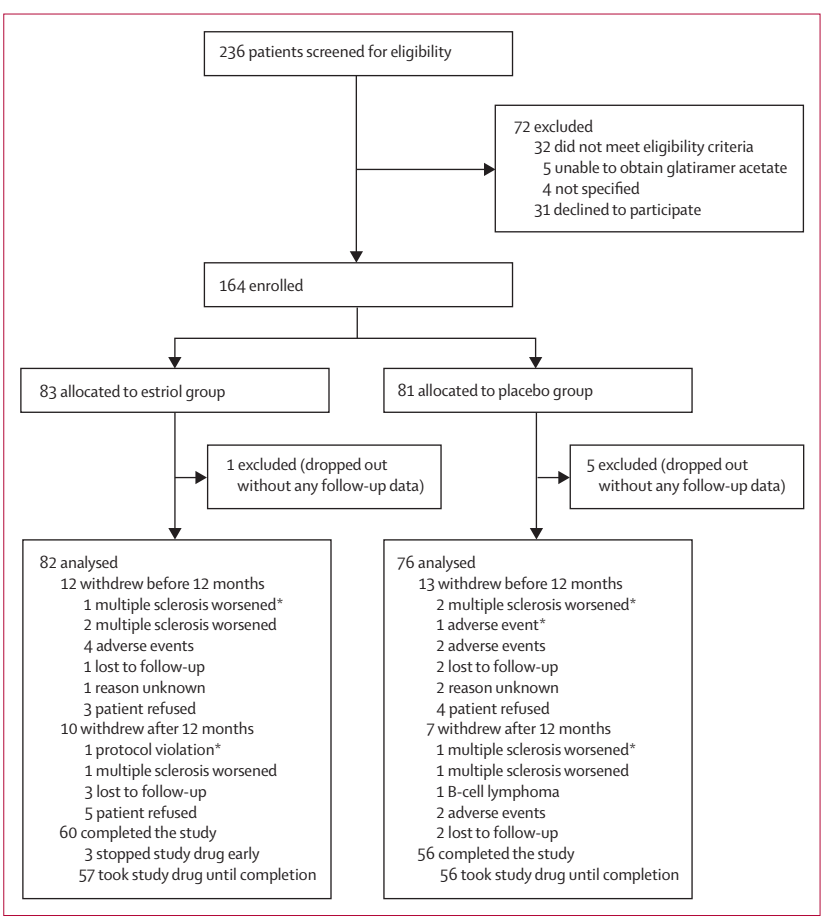

Figure 1: **Trial profile**
*Investigator's decision to withdraw.

We assessed the difference in annualised confirmed relapse rates between groups with a negative binomial regression model adjusted for age, baseline EDSS score (<2 *vs* ≥2), number of relapses in the 12 months before the study (≤1 *vs* >1), time since diagnosis (<1 *vs* ≥1 year), previous glatiramer acetate treatment (none *vs* previous or current), and previous interferon beta treatment (yes *vs* no). Annualised relapse rates included all events for the entire 24 months or those occurring until the last visit adjusted for the time for which the participant was in the study.

Phase 2 trials of relapsing-remitting multiple sclerosis often have a biomarker as their primary endpoint. By contrast, we used a clinical outcome for three reasons: the National Institutes of Health want to focus on relapses as a basis for powering a future phase 3 trial, pregnancy decreases relapses,[2] and a small phase 2 trial with monthly MRI scans has already been done.[7] Because this trial was phase 2, we used a significance level of $\alpha=0\cdot10$ for all analyses, as has been used in cancer trials[27] and in phase 2 trials of stroke,[28] amyotrophic lateral sclerosis,[29] and Parkinson's disease.[30] The use of $p<0\cdot10$ as significant in phase 2 trials was considered stringent enough to assess the potential for clinical efficacy of a new intervention, while controlling for false positives and avoiding the much higher costs of the larger sample sizes needed to achieve a p value of less than $0\cdot05$.[27,28]

	Estriol group (n=82)	Placebo group (n=76)
Age (years)	37·7 (7·6)	37·1 (7·3)
Ethnic origin*		
White	65 (79%)	62 (82%)
Black	9 (11%)	7 (9%)
Hispanic	7 (9%)	6 (8%)
Other	1 (1%)	1 (1%)
Time since diagnosis (years)	3·3 (4·6)	2·9 (4·5)
Number of previous relapses		
Within 1 year before screening	1·5 (0·7)	1·5 (0·7)
Within 2 years before screening	2·0 (0·7)	2·3 (0·9)
Previous glatiramer treatment		
Never	25 (30%)	27 (36%)
Before screening	17 (21%)	6 (8%)
During screening	40 (49%)	43 (57%)
Previous treatment with any interferon beta?†		
No	59 (72%)	50 (66%)
Yes	23 (28%)	26 (34%)
Mean EDSS score‡	2·2 (1·2)	2·1 (1·1)
EDSS score at baseline		
0	9 (11%)	6 (8%)
1·0 or 1·5	16 (20%)	21 (28%)
2·0 or 2·5	27 (33%)	24 (32%)
3·0 or 3·5	25 (30%)	22 (29%)
4·0	4 (5%)	2 (3%)
5·5	1 (1%)§	1 (1%)§
Number of gadolinium-enhancing lesions	1·0 (2·3)	0·9 (2·1)
Active lesions on brain MRI?		
No	55 (67%)	53 (70%)
Yes	26 (32%)	22 (29%)
Volume of lesions on T2-weighted images (cm^3)	6·8 (8·9)	7·7 (11·2)

Data are mean (SD) or n (%). EDDS=Expanded Disability Status Scale. *Self-reported. †Patients may have received more than one previous multiple sclerosis drug. ‡Scores on the EDSS ranged from 0 to 10, with higher scores indicating a greater degree of disability. §This patient had a score of 4·5 at the first screening visit but 5·5 at baseline.

Table 1: **Baseline characteristics of the intention-to-treat population**

For the analysis of time to first relapse, we used Kaplan-Meier analysis and log-rank tests to estimate and compare the proportion of patients with first relapse at each timepoint. We used a Cox proportional hazards model to compare the proportion of patients with relapse at 12 months and 24 months, adjusting for age, baseline EDSS score (<2 *vs* ≥2), number of relapses in the 12 months before the study (≤1 *vs* >1), duration of multiple sclerosis (<1 *vs* ≥1 year), previous glatiramer acetate treatment (never *vs* past or current), and previous interferon treatment (yes *vs* no). We used mixed effects models to analyse repeated measurement outcomes with the random effect of participant to account for within patient correlation. For the exploratory analyses of EDSS, PASAT, fatigue, depression, and quality of life,[31] we used a linear mixed effects model to compare treatment groups at 12 months and 24 months. We used a mixed effects negative binomial regression model and linear mixed effects model to compare enhancing lesion number and volume (log-transformed) between treatment groups at all follow-up visits, and a mixed effects logistic model to compare the number of participants with gadolinium-enhancing lesions, with a linear mixed effects model to compare the percentage change in brain volumes between treatment groups. We also used a mixed effects logistic regression model to assess the association between the number of enhancing lesions and the occurrence of relapses and estriol concentrations. Finally, we used a linear mixed effects model to assess the association between PASAT change and percentage brain volume change, between PASAT change and estriol concentration, and between compliance and estriol concentration. We did all the analyses for the intention-to-treat population, which included all patients who were enrolled and for whom data existed after taking at least one dose of study drug. The appendix shows details of the sensitivity analyses.

This study is registered with ClinicalTrials.gov, number NCT00451204.

Role of the funding source

None of the funding sources had any role in the collection, analysis, or interpretation of data, or writing of the article. The National Institutes of Health had a role

	Estriol group (n=82)	Placebo group (n=76)	Estriol group *vs* placebo group	p value
At 24 months				
Confirmed relapse				
Annualised relapse rate (95% CI)	0·25 (0·17–0·37)	0·37 (0·25–0·53)	0·63 (0·37–1·05)*	0·077
Probability of first relapse (95% CI)	33·3% (23·8–45·4)†	42·9% (32·1–55·5)†	0·63% (0·36–1·09)¶	0·096
Relapse event				
Annualised relapse rate (95% CI)	0·32 (0·22–0·46)	0·46 (0·32–0·65)	0·65 (0·39–1·08)*	0·098
Probability of first relapse event (95% CI)	40·5% (30·0–53·0)†	46·9% (35·9–59·3)†	0·70% (0·42–1·17)¶	0·179
At 12 months				
Confirmed relapse				
Annualised relapse rate (95% CI)	0·25 (0·16–0·40)	0·48 (0·33–0·69)	0·49 (0·28–0·88)*	0·016
Probability of first relapse (95% CI)	22·8% (15·0–33·7)†	33·1% (23·5–45·2)†	0·58% (0·31–1·10)¶	0·095
Relapse event				
Annualised relapse rate (95% CI)	0·33 (0·22–0·50)	0·61 (0·44–0·84)	0·52 (0·31–0·86)*	0·012
Probability of first relapse event (95% CI)	30·7% (21·7–42·3)†	40·3% (30·0–52·7)†	0·65% (0·37–1·14)¶	0·131

The primary outcome was confirmed relapse rate at 24 months; other analyses at 24 months were secondary and analyses at 12 months were exploratory. *Adjusted rate ratio. †Percentage of patients with relapse. ¶Adjusted hazard ratio.

Table 2: **Primary, secondary, and exploratory outcomes related to relapses**

in designing the study. All authors had full access to all of the data in the study. The corresponding author had final responsibility for the decision to submit for publication.

Results

We screened 236 patients, 164 of whom we enrolled (figure 1). 158 participants received study drug and had at least one visit thereafter. Of the 158 patients, 82 were assigned to the estriol group and 76 were assigned to the placebo group. Baseline characteristics were balanced across groups (table 1). The proportion of complications was similar in each group (60 [73%] of 82 patients in the estriol group *vs* 56 [74%] of 76 in the placebo group).

After 24 months, the confirmed relapse rate was 0·25 relapses per year (95% CI 0·17–0·37) in the estriol group versus 0·37 relapses per year in the placebo group (0·25–0·53), with an adjusted rate ratio of 0·63 (0·37–1·05; p=0·077; table 2, appendix p 22). The annualised relapse event rate was also reduced in the estriol group compared with the placebo group (table 2, appendix p 22). Time to confirmed relapse was also significantly lower in the estriol group than in the placebo group according to our threshold of p<0·1, but time to relapse event was not (figure 2, table 2). We had similar results for the exploratory analyses of data up to 12 months: the annualised confirmed relapse rate was reduced in the estriol group compared with the placebo group, as were the annualised relapse event rate and the time to first confirmed relapse, but the time to first relapse event did not differ significantly between groups (table 2, appendix p 22).

Estriol was well tolerated, with no substantial differences between groups in the number or proportion of patients with serious adverse events (table 3). Laboratory abnormalities were the same in each group

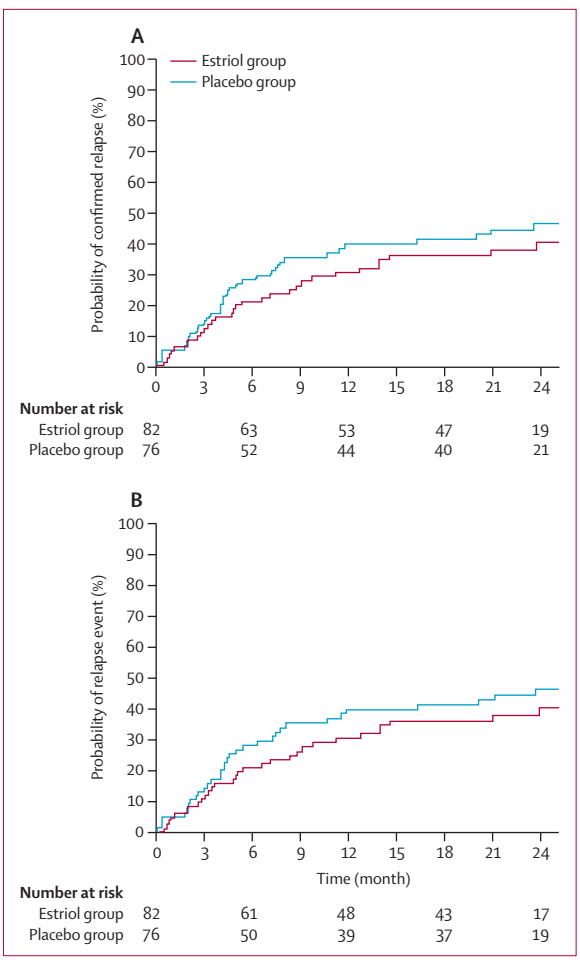

Figure 2: **Kaplan-Meier plots of probability of first relapse**
(A) Confirmed relapse over 24 months, and (B) relapse events over 24 months.

(data not shown). Irregular menses were more common in the estriol group than in the placebo group, whereas vaginal infections were less common (table 3). We recorded no substantial differences in breast fibrocystic disease and no mammograms were positive for malignancy (table 3). Uterine fibroids occurred in similar proportions in each group (table 3). There was no substantial difference between groups in the number of patients with uterine endometrial thickness greater than 8 mm (table 3). Finally, because oestrogen and progesterone can cause some irregularity in menstrual cycles, we used criteria for clinically relevant increased menstrual flow in an algorithm with uterine lining thickness to trigger a uterine endometrial lining biopsy (detailed in the protocol). There was little difference between treatment groups in the proportion of patients who had these biopsies (table 3), and no biopsy showed abnormal proliferation.

Total serum estriol concentration increased in the estriol group (appendix pp 15, 23) and remained high through to 12 months. Estriol concentration then decreased, with a significant decrease by 24 months (16·2 ng/mL [SD 25·3] at 3 months *vs* 10·1 ng/mL [SD 6·9] at 24 months; difference 5·7, 95% CI 1·8–9·6; p=0·003). When we assessed estriol concentrations in participants who completed the study, concentrations were again

	Estriol group (n=82)		Placebo group (n=76)		p value
	Events (n)	Patients (n; %)	Events (n)	Patients (n; %)	
Adverse events					
Any adverse event	480	76 (93%)	392	67 (88%)	0·21
Most common events					
Upper respiratory tract infection	33	22 (27%)	38	26 (34%)	0·31
Glatiramer acetate injection area abnormalities	25	21 (26%)	15	12 (16%)	0·13
Irregular menses or spotting	26	19 (23%)	4	3 (4%)	0·0005
Urinary tract infection	23	15 (18%)	16	10 (13%)	0·34
Fatigue	15	13 (16%)	10	8 (11%)	0·30
Depression or anxiety	14	12 (15%)	10	9 (12%)	0·56
Menstrual flow amount increased	12	11 (13%)	8	6 (8%)	0·24
Headache	11	9 (11%)	12	11 (14%)	0·55
Nausea or vomiting	9	7 (9%)	5	5 (7%)	0·61
Glatiramer acetate injection systemic reaction (dyspnoea, hot flushes)	7	7 (9%)	2	2 (3%)	0·17
Sinusitis	6	6 (7%)	14	10 (13%)	0·24
Arm or leg numbness, tingling	7	6 (7%)	10	7 (9%)	0·70
Gastroenteritis	7	5 (6%)	4	3 (4%)	0·72
Dizziness	5	4 (5%)	10	7 (9%)	0·30
Vision problems (blurry, double)	6	4 (5%)	7	7 (9%)	0·30
Back pain	5	4 (5%)	5	5 (7%)	0·74
Menstrual cramp	4	4 (5%)	5	4 (5%)	1·00
Insomnia	4	4 (5%)	4	4 (5%)	1·00
Heart palpitation	2	2 (2%)	4	4 (5%)	0·43
Shingles	2	2 (2%)	4	4 (5%)	0·43
Vaginal infection	1	1 (1%)	9	8 (11%)	0·012
Adverse events leading to discontinuation	5	5 (6%)	5	5 (6%)	1·00
Severe adverse events*	8	8 (10%)	12	10 (13%)	0·54
Relapse of multiple sclerosis†	2	2 (2%)	6	5 (7%)	0·27
Pregnancy termination	2	2 (2%)	0	0	0·50
Urinary tract infection	1	1 (1%)	1	1 (1%)	1·00
Migraine headache-related eye pain	1	1 (1%)	0	0	1·00
Heart failure or pacemaker implantation	1	1 (1%)	0	0	1·00
Pyelonephritis	1	1 (1%)	0	0	1·00
Sedation after taking non-prescribed non-study drug	0	0	1	1 (1%)	0·49
Acute appendicitis	0	0	1	1 (1%)	0·49
B-cell lymphoma	0	0	1	1 (1%)‡	0·49
Car accident caused by body numbness	0	0	1	1 (1%)	0·49
Right knee replacement	0	0	1	1 (1%)	0·49

(Table 3 continues on next page)

	Estriol group (n=82)		Placebo group (n=76)		p value
	Events (n)	Patients (n; %)	Events (n)	Patients (n; %)	
(Continued from previous page)					
Other safety events monitored					
Uterine					
Endometrium thickness >8 mm on ultrasound	32	24 (29%)	41	27 (36%)	0·46
Endometrial biopsy samples taken§	11	9 (11%)	10	6 (8%)	0·48
Fibroids (on ultrasound)	8	8 (10%)	8	8 (11%)	0·91
Abnormal proliferation on biopsy	0	0	0	0	NA
Breast					
Fibrocystic disease on clinical examination	5	5 (6%)	4	4 (5%)	1·00
Mammogram with malignancy	0	0	0	0	NA

Includes all patients who took at least one dose of study drug; however, the six patients who dropped out shortly after baseline visit did not have safety evaluation data and were excluded from the safety analysis. Laboratory abnormalities are only reported if they occurred in at least 5% of patients in either group. *All admitted to hospital, but none had severe or immediately life-threatening conditions. †Both patients in the estriol group discontinued the study, one before and one after 12 months; three patients in the placebo group discontinued the study, one before and two after 12 months. ‡This patient discontinued the study at the time of B-cell lymphoma diagnosis after being on the study for 12 months and died 17 months later. §Two patients had two biopsies each in the estriol group and two patients had three biopsies each in the placebo group; no abnormal proliferation was found.

Table 3: **Adverse events and serious adverse events**

significantly decreased between 3 months and 24 months (data not shown), suggesting poor compliance as the reason for the difference. In a post-hoc analysis, we assessed compliance using pill counts and calendars and found strong correlations between estriol concentrations and compliance in the estriol group (regression coefficient 1·12 [SE 0·34]; p=0·001). Overall, compliance at 24 months was much the same in the estriol group (n=58; mean 0·88, SD 0·19; median 0·96, IQR 0·87–0·99) and the placebo group (n=55; mean 0·89, SD 0·13; median 0·94, IQR 0·84–0·99). Estriol concentration was inversely correlated with relapses (regression coefficient –0·21 [SE 0·11]; p=0·057), and gadolinium-enhancing lesions (–0·77 [SE 0·34]; p=0·028).

We recorded significant improvements with estriol compared with placebo for fatigue after 24 months (p=0·009; table 4). Cognitive testing as measured by PASAT showed no differences at 24 months; however, at 12 months, PASAT scores were significantly greater in the estriol group than in the placebo group (table 4), and in a post-hoc analysis of patients with a score of less than the median of 55 at baseline, improvements at 12 months were 4·7 points in patients who received estriol and 1·6 in patients who received placebo (p=0·011), whereas there was no significant effect in patients with higher scores at baseline (p=0·694; table 4). We found no significant difference for EDSS, depression score, MS QoL, or MSFC (table 4). Because estriol concentrations were low at 24 months, which is when PASAT scores were no longer significantly improved in the estriol group compared with the placebo group, we assessed correlations between estriol concentration and PASAT score. Estriol concentration was directly correlated with PASAT score (regression coefficient 0·36 [SE 0·17]; p=0·03). We recorded similar benefits of estriol treatment on 7/24 Spatial Recall cognitive testing (appendix p 16).

Table 5 shows the results of prespecified exploratory and post-hoc MRI outcomes. We recorded no differences between groups for the prespecified endpoints related to enhancing or T2 lesions or whole brain volume. Post-hoc analyses showed a difference between groups at 12 months for cortical grey matter (p=0·056) and white matter (p=0·090). In patients without enhancing lesions at baseline, those in the estriol group had larger cortical grey matter volume than did those in the placebo group (p=0·043), whereas in patients who had enhancing lesions at baseline, those in the estriol group had smaller white matter volumes than did those in the placebo group (0·012). The appendix (p 24) shows localisation of the estriol treatment effect on grey matter by voxel-based morphometry.

Post-hoc analysis showed that cortical grey matter volume by MRI correlated directly with PASAT cognitive test improvement in estriol-treated participants (regression coefficient 0·82 [SE 0·38]; p=0·032), but not in placebo-treated participants (regression coefficient –0·17 [SE 0·38]; p=0·660).

Discussion

Estriol treatment reduced relapse rates compared with placebo in patients with relapsing-remitting multiple sclerosis. The relapse rate ratio between the two groups was nearly the same as in the original sample size calculation; however, the relapse rates for both groups were considerably lower than expected. As a result, the power of the study was reduced to 74%, but the primary endpoint of a reduction in annualised confirmed relapse rates at p<0·1 was reached. Our results suggest that estriol might have a role in decreased relapses during pregnancy.

Estriol is considered to be the safest oestrogen on the basis of data for its worldwide use for menopausal

	Estriol group (n=82)	Placebo group (n=76)	Estriol group *vs* placebo group*	p value
Percentage of patients with disability progression over 24 months (95% CI)†	11·4 (5·9 to 21·7)	15·8 (8·8 to 27·6)	0·81 (0·32 to 2·07)‡	0·664
EDSS score§				
Baseline (estriol group n=82, placebo group n=76)	2·22 (1·16), 2·3 (1·5 to 3·0)	2·13 (1·11), 2·0 (1·5 to 3·0)		
Change from baseline at 12 months (estriol group n=69, placebo group n=63)	−0·13 (1·06), 0 (−0·5 to 0·5)	−0·06 (1·11), 0 (−0·5 to 0·5)	−0·14 (−0·47 to 0·19)	0·404
Change from baseline at 24 months (estriol group n=56, placebo group n=56)	−0·29 (0·98), −0·5 (−1·0 to 0·5)	−0·05 (1·13), 0·0 (−0·5 to 0·5)	−0·22 (−0·57 to 0·12)	0·198
Fatigue score§				
Baseline (estriol group n=82, placebo group n=76)	33·7 (19·4), 32 (17·0 to 50·0)	31·1 (19·0), 28·5 (17·0 to 44·5)		
Change from baseline at 12 months (estriol group n=70, placebo group n=62)	−3·4 (13·5), −3 (−10 to 5)	−0·6 (15·1), −0·5 (−8 to 9)	−2·6 (−6·7 to 1·5)	0·218
Change from baseline at 24 months (estriol group n=58, placebo group n=56)	−5·0 (14·0), −3 (−10 to 4)	0·5 (12·8), 1 (−6 to 7)	−5·7 (−10·0 to −1·4)	0·009
Depression score§				
Baseline (estriol group n=82, placebo group n=76)	11·3 (9·2), 10 (4 to 17)	10·9 (10·0), 9 (5 to 14)		
Change from baseline at 12 months (estriol group n=70, placebo group n=62)	−1·6 (7·1), −2 (−5 to 1)	−0·6 (9·4), −0·5 (−5 to 3)	−0·6 (−2·9 to 1·6)	0·583
Change from baseline at 24 months (estriol group n=59, placebo group n=56)	−2·6 (6·0), −3 (−6 to 1)	−1·2 (6·8), −2 (−5 to 2)	−1·1 (−3·5 to 1·3)	0·368
MS QoL—physical score¶				
Baseline (estriol group n=82, placebo group n=76)	64·3 (19·3), 64·2 (50·7 to 78·7)	66·3 (19·0), 69·2 (49·4 to 83·0)		
Change from baseline at 12 months (estriol group n=69, placebo group n=62)	4·6 (12·2), 3·2 (−2·6 to 10·0)	1·5 (16·2), 2·4 (−8·0 to 10·2)	2·4 (−2·0 to 6·8)	0·279
Change from baseline at 24 months (estriol group n=58, placebo group n=56)	5·1 (16·7), 3·9 (−2·4 to 12·2)	3·1 (13·8), 4·6 (−5·0 to 11·4)	2·2 (−2·3 to 6·8)	0·338
MS QoL—mental score¶				
Baseline (estriol group n=82, placebo group n=76)	80·7 (24·1), 86·3 (68·2 to 98·7)	83·5 (24·5), 92·9 (69·0 to 103·0)		
Change from baseline at 12 months (estriol group n=69, placebo group n=62)	3·0 (20·3), −0·8 (−8·8 to 13·5)	−0·5 (23·6), 0·2 (−10·7 to 12·2)	2·1 (−4·3 to 8·5)	0·525
Change from baseline at 24 months (estriol group n=58, placebo group n=56)	4·6 (20·2), 4·6 (−3·9 to 15·9)	0·8 (20·7), 2·4 (−6·3 to 10·4)	2·8 (−3·9 to 9·5)	0·419
MSFC score¶				
Baseline (estriol group n=82, placebo group n=76)	−0·04 (0·69), 0·05 (−0·46 to 0·47)	0·06 (0·79), 0·24 (−0·44 to 0·75)		
Change from baseline at 12 months (estriol group n=70, placebo group n=58)	0·12 (0·37), 0·09 (−0·06 to 0·29)	0·06 (0·38), 0·06 (−0·17 to 0·32)	0·07 (−0·05 to 0·20)	0·262
Change from baseline at 24 months (estriol group n=60, placebo group n=54)	0·10 (0·35), 0·09 (−0·06 to 0·29)	0·08 (0·43), 0·10 (−0·21 to 0·35)	0·03 (−0·10 to 0·16)	0·629
PASAT score¶||				
Baseline (estriol group n=82, placebo group n=76)	51·0 (8·9), 55 (45 to 58)	52·3 (9·1), 56 (49 to 59)		
All patients				
Change from baseline at 12 months (estriol group n=70, placebo group n=61)	1·93 (5·59), 1·0 (0·0 to 4·0)	0·13 (4·46), 0·0 (−1·0 to 2·0)	1·62 (−0·03 to 3·27)	0·054
Change from baseline at 24 months (estriol group n=60, placebo group n=55)	1·07 (4·04), 1·0 (−1·5 to 3·0)	1·11 (4·29), 0·0 (−1·0 to 3·0)	−0·11 (−1·81 to 1·60)	0·902
Patients with baseline score <55				
Change from baseline at 12 months (estriol group n=33, placebo group n=25)	4·70 (6·56), 4·0 (2·0 to 6·0)	1·60 (5·99), 1·0 (0·0 to 5·0)	3·00 (0·68 to 5·32)	0·011
Change from baseline at 24 months (estriol group n=26, placebo group n=23)	2·31 (5·25), 3·5 (−3·0 to 6·0)	2·96 (5·88), 4·0 (−2·0 to 6·0)	−0·33 (−2·74 to 2·07)	0·785
Patients with baseline score ≥55				
Change from baseline at 12 months (estriol group n=37, placebo group n=36)	−0·54 (2·88), 0·0 (−1·0 to 1·0)	−0·89 (2·62), 0·0 (−1·5 to 0·5)	0·43 (−1·71 to 2·57)	0·694
Change from baseline at 24 months (estriol group n=34, placebo group n=32)	0·12 (2·47), 0·0 (−1·0 to 1·0)	−0·22 (1·79), 0·0 (−1·0 to 1·0)	0·04 (−2·14 to 2·22)	0·971

All outcomes were exploratory, except for analysis of PASAT score above and below 55, which was post hoc. MS QoL=Multiple Sclerosis Quality of Life. PASAT=Paced Auditory Serial Addition Test. MSFC=MS Functional Composite. EDSS=Expanded Disability Status Scale. Data are mean (SD), median (IQR), unless stated otherwise. *Data are mean difference (95% CI) unless stated otherwise. †Calculated with the Kaplan-Meier product-limit method; progression defined as EDSS increase of at least 1·0 point in participants with a baseline score of 1·0 or higher, or an increase of at least 1·5 points in participants with a baseline score of 0, each sustained for at least 6 months. ‡Adjusted hazard ratio (95% CI), estimated by Cox proportional hazard regression; adjusted for age and baseline EDSS (<2 *vs* ≥2). §A negative change indicates improvement. ¶A positive change indicates improvement. ||To estimate the difference of PASAT score change between the two study groups for patients with baseline PASAT scores above or below the median, we included dichotomised baseline PASAT score (<55 *vs* ≥55) and its interaction terms with treatment and month in the model and all patients' follow-up data were used; $p_{interaction}$=0·038 for 24 months, $p_{interaction}$=0·092 for 12 months.

Table 4: **Exploratory and post-hoc endpoints related to disability**

symptoms over 40 years.[11–13] The standard daily dose for menopause symptoms (2 mg) is generally lower than the dose we used, although some studies have used up to 16 mg. In this study, a dose of 8 mg induced an estriol concentration equivalent to that present early in the second trimester of pregnancy, consistent with previous findings.[7] Because estriol concentrations continue to increase during pregnancy, the blood concentration induced by estriol treatment was below that usually present in the third trimester, when the effect of pregnancy in protection against relapse is greatest.[2] Even so, we postulated that the concentration achieved might be sufficient to exert protective effects. We targeted second trimester levels to induce third trimester protection. This dose was safe and well tolerated, including in the uterus and breast. However, as is the case with all new drugs, the long-term effects will be unknown until larger and longer studies are done.

The greater occurrence of irregular menses in the estriol group than in the placebo group was not surprising because oral contraceptives are known to cause menstrual irregularity, and estriol decreases vaginal flora and promotes urogenital health.[32,33] If estriol was not safer than other oestrogens, the risk-to-benefit

	Estriol group (n=82)	Placebo group (n=76)	Estriol group *vs* placebo group*	p value
Enhancing lesion volume†				
At baseline (mean [SD], median [IQR])	79·7 (220), 0·0 (0·0 to 32)	54·2 (126), 0·0 (0·0 to 25·0)		
Change from baseline at 12 months	n=69	n=62		
Mean (SD), median (IQR)	−51·2 (202), 0·0 (−21·6 to 0·0)	−18·7 (184), 0·0 (0·0 to 0·0)	−12·5 (−69·0 to 44·1)	0·665
Change from baseline at 24 months	n=55	n=55		
Mean (SD), median (IQR)	−39·3 (196), 0·0 (−29·0 to 0·0)	−34·0 (120), 0·0 (−13·9 to 0·0)	−1·6 (−62·6 to 59·5)	0·960
Number of enhancing lesions†				
At baseline (mean [SD], median [IQR])	1·0 (2·3), 0·0 (0·0 to 1·0)	0·9 (2·1), 0·0 (0·0 to 1·0)		
Change from baseline at 12 months	n=68	n=62		
Mean (SD), median (IQR)	−0·9 (2·2), 0·0 (−1·0 to 0·0)	−0·5 (1·8), 0·0 (0·0 to 0·0)	0·89 (0·54 to 1·45)	0·631
Change from baseline at 24 months	n=55	n=55		
Mean (SD), median (IQR)	−0·9 (2·5), 0·0 (−1 to 0·0)	−0·5 (2·4), 0·0 (−1 to 0·0)	0·89 (0·54 to 1·48)	0·655
Proportion of patients with enhancing lesion on MRI				
At baseline (%; 95% CI)	32·1 (21·9 to 42·3)	29·3 (19·0 to 39·6)		
At 12 months (%; 95% CI)	14·5 (6·2 to 22·8)	21·0 (10·8 to 31·1)	0·30 (0·07 to 1·31)	0·110
At 24 months (%; 95% CI)	14·6 (5·2 to 23·9)	14·6 (5·2 to 23·9)	0·66 (0·13 to 3·40)	0·616
Total T2 volume†				
At baseline (mean [SD], median [IQR])	6·8 (8·9), 3·9 (1·3 to 8·5)	7·7 (11·1), 3·0 (1·4 to 8·3)		
Change from baseline at 12 months	n=69	n=63		
Mean (SD), median (IQR)	1·5 (3·4), 0·6 (0·0 to 2·8)	1·5 (3·1), 0·6 (0·1 to 2·3)	−0·0 (−1·0 to 1·0)	0·938
Change from baseline at 24 months	n=56	n=56		
Mean (SD), median (IQR)	2·3 (4·0), 0·8 (−0·1 to 3·0)	1·4 (2·7), 0·7 (0·0 to 2·1)	0·7 (−0·3 to 1·8)	0·174
Brain volume at baseline (all patients)§				
At baseline (mean [SD], median [IQR])				
Whole brain	1604 (62), 1607 (1571 to 1651)	1602 (51), 1602 (1569 to 1635)		
Whole grey matter	954 (51), 957 (917 to 985)	926 (52), 967 (931 to 1002)		
Cortical grey matter	754 (46), 755 (722 to 784)	761 (42), 762 (738 to 790)		
White matter	650 (35), 650 (630 to 670)	640 (37), 635 (612 to 664)		
Percentage change from baseline (mean [SD], median [IQR])‡				
Whole brain at 12 months	−0·50 (0·70), −0·49 (−0·93 to 0·00)	−0·50 (0·64), −0·43 (−1·04 to −0·12)	−0·00 (−0·23 to 0·23)	0·988
Whole brain at 24 months	−0·89 (0·82), −1·00 (−1·23 to −0·33)	−0·78 (0·73), −0·91 (−1·26 to −0·12)	−0·02 (−0·26 to 0·22)	0·877
Whole grey matter at 12 months	−0·48 (0·82), −0·50 (−0·99 to −0·06)	−0·69 (0·71), −0·68 (−1·19 to −0·16)	0·21 (−0·05 to 0·47)	0·108
Whole grey matter at 24 months	−0·96 (0·75), −0·94 (−1·35 to −0·52)	−0·95 (0·76), −0·93 (−1·47 to −0·34)	0·12 (−0·16 to 0·39)	0·411
Cortical grey matter at 12 months	−0·44 (0·92), −0·51 (−0·99 to −0·11)	−0·72 (0·80), −0·67 (−1·32 to −0·13)	0·29 (−0·01 to 0·58)	0·056
Cortical grey matter at 24 months	−0·96 (0·86), −0·94 (−1·35 to −0·42)	−1·04 (0·87), −0·93 (−1·57 to −0·35)	0·23 (−0·09 to 0·54)	0·156
White matter at 12 months	−0·51 (1·06), −0·16 (−1·16 to 0·22)	−0·20 (0·83), −0·11 (−0·67 to 0·41)	−0·29 (−0·63 to 0·04)	0·090
White matter at 24 months	−0·77 (1·28), −0·66 (−1·33 to −0·09)	−0·54 (1·12), −0·56 (−1·18 to 0·38)	−0·20 (−0·56 to 0·15)	0·261
Brain volume (no enhancing lesions at baseline)				
Percentage change from baseline (mean [SD], median [IQR])‡				
Whole brain at 12 months	−0·35 (0·58), −0·41 (−0·67 to 0·03)	−0·49 (0·63), −0·43 (−1·01 to −0·09)	0·10 (−0·16 to 0·36)	0·429
Whole brain at 24 months	−0·72 (0·80), −0·87 (−1·12 to −0·29)	−0·80 (0·76), −0·99 (−1·31 to −0·09)	0·08 (−0·19 to 0·36)	0·540
Whole grey matter at 12 months	−0·45 (0·77), −0·48 (−0·98 to −0·06)	−0·71 (0·74), −0·68 (−1·20 to −0·16)	0·24 (−0·05 to 0·53)	0·106
Whole grey matter at 24 months	−0·94 (0·81), −0·91 (−1·71 to −0·62)	−0·99 (0·78), −0·93 (−1·56 to −0·35)	0·14 (−0·16 to 0·45)	0·358
Cortical grey matter at 12 months	−0·39 (0·89), −0·43 (−0·97 to −0·01)	−0·74 (0·84), −0·64 (−1·41 to −0·09)	0·34 (0·01 to 0·67)	0·043
Cortical grey matter at 24 months	−0·96 (0·89), −0·98 (−1·91 to −0·54)	−1·11 (0·89), −0·93 (−1·64 to −0·35)	0·29 (−0·06 to 0·63)	0·106
White matter at 12 months	−0·19 (0·95), 0·06 (−0·69 to 0·41)	−0·14 (0·72), −0·11 (−0·58 to 0·43)	−0·10 (−0·48 to 0·28)	0·620
White matter at 24 months	−0·41 (1·13), −0·35 (−0·93 to 0·09)	−0·51 (1·18), −0·51 (−1·04 to 0·38)	−0·00 (−0·40 to 0·39)	0·988

(Table 5 continues on next page)

	Estriol group (n=82)	Placebo group (n=76)	Estriol group *vs* placebo group*	p value
(Continued from previous page)				
Brain volume (patients with enhancing lesions at baseline)				
Percentage change from baseline (mean [SD], median [IQR])‡				
Whole brain at 12 months	−0·81 (0·77), −0·65 (−1·31 to −0·29)	−0·53 (0·67), −0·49 (−1·14 to −0·19)	−0·23 (−0·58 to 0·12)	0·206
Whole brain at 24 months	−1·13 (0·82), −1·04 (−1·30 to −0·88)	−0·74 (0·67), −0·66 (−1·12 to −0·34)	−0·25 (−0·61 to 0·11)	0·182
Whole grey matter at 12 months	−0·61 (0·84), −0·54 (−1·05 to −0·26)	−0·65 (0·64), −0·64 (−1·08 to −0·15)	0·08 (−0·31 to 0·47)	0·698
Whole grey matter at 24 months	−1·00 (0·68), −0·98 (−1·22 to −0·49)	−0·83 (0·71), −0·92 (−1·25 to −0·28)	−0·02 (−0·42 to 0·38)	0·923
Cortical grey matter at 12 months	−0·60 (0·92), −0·57 (−1·10 to −0·26)	−0·67 (0·72), −0·69 (−0·98 to −0·19)	0·08 (−0·37 to 0·53)	0·722
Cortical grey matter at 24 months	−0·95 (0·85), −0·93 (−1·39 to −0·23)	−0·82 (0·80), −0·93 (−1·29 to −0·27)	0·02 (−0·44 to 0·48)	0·919
White matter at 12 months	−1·09 (1·03), −0·92 (−2·00 to −0·18)	−0·36 (1·06), −0·13 (−0·69 to 0·16)	−0·67 (−1·19 to −0·15)	0·012
White matter at 24 months	−1·32 (1·31), −1·20 (−2·11 to −0·57)	−0·62 (0·97), −0·84 (−1·47 to 0·24)	−0·58 (−1·11 to −0·04)	0·034

Enhancing lesion volume and number, total T2 volume, and whole brain volume were exploratory outcomes. Because of the distribution of the data (with few patients having enhancing lesions), the most appropriate approach to assess enhancing lesion volume and number was to assess the proportion of patients with enhancing lesion activity. Whole grey matter, cortical grey matter, and white matter volumes, stratified by patients with or without enhancing lesions at baseline, were post-hoc outcomes. *Data are mean difference (95% CI) except for number of enhancing lesions, which is mean of lesions number ratio (95% CI), and for proportion of patients with enhancing lesion on MRI, which is odds ratio (95% CI). †Negative change indicates improvement. ‡Negative change indicates worsening. §To estimate the difference of brain volume change between the two study groups for patients with and without enhancing lesions at baseline, we included baseline enhancing lesion number (present *vs* absent) in the model and used follow-up data for all patients; $p_{interaction}$=0·058 for white matter at 12 months.

Table 5: **Exploratory and post-hoc MRI endpoints**

ratio of oral contraceptives and hormone replacement would be controversial in healthy individuals for whom no toxic effects are acceptable. However, for patients with a disabling disease such as multiple sclerosis, the risk-to-benefit ratio is different.

Some patients who experienced menstrual irregularity might have then deduced their treatment allocation, but follow-up questionnaires did not suggest substantial unmasking. Furthermore, unmasking would not have affected the primary outcome because each relapse was confirmed by an increase in the EDSS score as determined by an independent examiner who was not aware of adverse events.

Post-hoc MRI studies using volumetry at 12 months showed less cortical grey matter atrophy in the estriol group than in the placebo group. This effect was independently confirmed by voxel-based morphometry to show which grey matter regions were preserved by treatment. Furthermore, patients in the estriol group without enhancing lesions had less cortical grey matter atrophy than did those in the placebo group, suggesting a direct neuroprotective effect independent from anti-inflammatory effects, which is consistent with preclinical studies.[6]

Cortical grey matter atrophy on brain MRI has been associated with cognitive dysfunction in patients with multiple sclerosis.[34,35] In addition, oestrogen treatment improves cognitive dysfunction in women without multiple sclerosis who have had ovariectomy,[36] and oestrogen treatment of ovariectomised animals increases dendritic spines and synapses in cerebral grey matter.[37–39] We showed that higher serum estriol concentrations might be needed for beneficial effects on cognition. Cortical grey matter sparing in the estriol group compared with the placebo group was lost at 24 months, when both estriol concentrations and PASAT scores had decreased. Indeed, we detected correlations between PASAT improvement and cortical grey matter sparing in all participants and in the estriol group, but not in the placebo group.

By contrast, patients in the estriol group with enhancing lesions had more white matter atrophy compared with those in the placebo group at both 12 months and 24 months, consistent with pseudoatrophy.[40] A pseudoatrophy or anti-inflammatory effect in white matter in the estriol group would be consistent with the reduction in relapse rates. Whether maintenance of higher estriol concentrations at 24 months in all participants in the estriol group could have resulted in more robust effects on relapses or enhancing lesions is unknown. Achieving large reductions in these outcomes at 24 months compared with 12 months was challenging given: (1) the low level of relapse activity in this population, as shown by the few enhancing lesions at baseline, (2) the small sample size, and (3) the fact that all patients were treated with glatiramer acetate, which reduces relapse rates within 24 months of starting treatment.[26] Future studies with participants with more actively relapsing disease, a larger sample size, or placebo alone as a comparator would be necessary to test the effect of sustained concentrations of estriol.

Limitations of our study included the small sample size, requiring our findings to be tested in a larger phase 3 study. Although treatment with estriol was safe and well tolerated for 24 months, assessing the long-term risk of treatment with estriol will require larger, longer studies, as well as post-marketing experience. Our findings might encourage pilot trials for other

cell-mediated autoimmune diseases that go into remission during pregnancy—ie, rheumatoid arthritis and psoriasis. However, estriol treatment should not be considered in primarily antibody-mediated autoimmune diseases such as lupus, since lupus tends to worsen during pregnancy.[5] Whether estriol treatment could be used in men with multiple sclerosis is unknown. Estriol treatment was protective in male mice in preclinical studies,[6] so it could also be efficacious in men. However, because estriol treatment in men does not have the history of widespread use that treatment in women has, early phase safety studies are needed before its efficacy in men with multiple sclerosis can be addressed. Although menstrual effects would not be an issue in men, breast enlargement or possibly other unexpected adverse events could occur.

In view of the current practice of aggressive treatment of relapsing-remitting multiple sclerosis to reduce disease activity as soon as possible after diagnosis,[41] a phase 3 trial of estriol in combination with glatiramer acetate is warranted. In addition, further studies are needed to investigate estriol for progressive multiple sclerosis given the neuroprotective effects of oestrogens,[6] and our promising exploratory findings of sparing grey matter atrophy and improving cognition. Finally, because estriol is a simple biological molecule, it would be less expensive than many treatments[42] and more accessible to economically disadvantaged patients throughout the world.

Contributors

RRV, HW, and RE designed the study. TCJW, JB, JTB, JRC, AHC, SD-J, CCF, EMF, BG, DJ, LHK, SL, GP, MKR, ATR, JR, and DMW collected data. RRV, HW, TCJW, NLS, KN, FK, NI, JB, AJM-G, DLA, CHT, and RE analysed the data. RRV, HW, TCJW, NLS, KN, FK, AJM-G, DLA, CHT, and RE wrote the report. RRV, HW, TCJW, NLS, KN, FK, NI, JTB, JRC, AHC, SD-J, CCF, EMF, BG, DJ, LHK, SL, GP, MKR, ATR, JR, DMW, AJM-G, DLA, CHT, and RE interpreted data and revised the report.

Data safety and monitoring board

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Declaration of interests

RRV has received research grants from the National Institutes of Health and National Multiple Sclerosis Society, has received personal payment as a consultant from Synthetic Biologics, and is an inventor on a patent for estriol owned by UCLA. DLA has received payment from NeuroRx, has received research grant support from Biogen, and has received personal payment from Biogen, EMD Serono, Genentech, Genzyme, Hoffman-La Roche, Innate Immuntherapy, Medlmmune, Mitsubishi, Novartis, Receptos, Acorda, Sanofi-Aventis, Teva, and Xenoport. JTB has received payment for consultation from Novartis, and research grant support from Biogen Idec. JRC has received research grants from National Multiple Sclerosis Society, Novartis, Sun Pharma, and Diogenix, and has received personal payment from Novartis, Teva, and Biogen Idec. AHC has received personal payment for consulting, acting on a scientific advisory board, or speaking from Biogen Idec, Genzyme/Sanofi-Aventis, Hoffman-La Roche, Teva Neuroscience, Novartis, and Mallinckrodt, and has received research support from Hoffman-La Roche, EMD Serono, and Teva Neuroscience. SD-J has received personal payment for consulting, acting on a scientific advisory board, or speaking from Serono, Novartis, Bayer, Teva, Genetech, Genzyme, Mallinckrodt, and TG Pharmaceuticals, has received research support from Teva, Biogen Idec, and Serono, and has received research grants from Teva and Biogen Idec. EMF has received personal payment for speaking and consultation from Teva, Genzyme, Novartis, and Acorda. BG's family holds stock in Biogen and Pfizer. DJ has received personal payment for consulting and acting on a scientific advisory board or speaking from Questcor Pharmaceuticals, Teva Neuroscience, and Genzyme, and has received clinical trial support from Biogen Idec and Novartis. SL has received research support from Novartis, Biogen, Teva, Genzyme, Genentech, Berlex, Cognition Pharmaceuticals, UCB Pharmceuticals, Serono, Acorda, Sun Pharma, Opexa, and Actelion. MKR has received personal payment for consulting, acting on a scientific advisory board, or speaking from Biogen Idec, Revalesio, Novartis, and Roche, and has received research support from Diogenix. ATR has received payment for editorial activities from Medlink/Neurobase. JR has received research support from Teva and Biogen. DMW has received research support from Genentech, Genzyme, Alexion, and TerumoBCT. JB, RE, CCF, NI, LHK, FK, AJM-G, KN, GP, NLS, CHT, HW, and TCJW declare no competing interests.

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