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The effect of conjugated equine oestrogen on diabetes incidence: The Women's Health Initiative randomised trial

Abstract *Aims/hypothesis:* Recent clinical trials have found that the combination of conjugated equine oestrogen (CEO) and medroxyprogesterone has a protective effect on the incidence of type 2 diabetes. To determine the effect of CEO alone on the incidence of diabetes mellitus in postmenopausal women, we analysed the results of the Women's Health Initiative oestrogen-alone trial. *Methods:* The Women's Health Initiative is a randomised, double-masked trial comparing the effect of daily 0.625 mg CEO with placebo during 7.1 years of follow-up of 10,739 postmenopausal women who were aged 50–79 years and had previously had a hysterectomy. Diabetes incidence was ascertained by self-report of treatment with insulin or oral hypoglycaemic medication. Fasting glucose, insulin and lipoproteins were measured in an 8.6% random sample of study participants, at baseline and at 1, 3 and 6 years. *Results:* The cumulative incidence of treated diabetes was 8.3% in the oestrogen-alone group and 9.3% in the placebo group (hazard ratio 0.88, 95% CI 0.77–1.01, *p*=0.072). During the first year of follow-

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up, a significant fall in insulin resistance (homeostasis model assessment of insulin resistance) in actively treated women compared with the control subjects (Year 1 baseline between-group difference −0.53) was seen. However, there was no difference in insulin resistance at the 3- or 6-year follow-up. *Conclusions/interpretation:* Post-menopausal therapy with oestrogen alone may reduce the incidence of treated diabetes. The effect is smaller than that seen with oestrogen plus progestin. CEO should not, however, be used with the intention of preventing diabetes, as its well-described adverse effects preclude long-term use for primary prevention.

Keywords Conjugated equine oestrogen · Diabetes mellitus · Postmenopausal · Randomised double-masked trial

Abbreviations CEO: conjugated equine oestrogen · HERS: Heart and Oestrogen/Progestin Replacement Study · HOMA-IR: Homeostasis model assessment of insulin resistance · HR: hazard ratio · O+P: oestrogen plus progesterone · SERM: selective oestrogen receptor modifier · WHI: Women's Health Initiative

Introduction

Recent studies have suggested that postmenopausal hormone use is associated with a lower incidence of diabetes. Over the 4 years of the Heart and Oestrogen/Progestin Replacement Study (HERS), the incidence of diabetes was 6.2% in the oestrogen plus progestin group and 9.5% in the placebo group (hazard ratio [HR]=0.65, 95% CI 0.48–0.89) [1]. The Women's Health Initiative (WHI) Oestrogen plus Progesterone (O+P) trial showed a similar protective effect [2]. After an average of 5.6 years of follow-up, the cumulative incidence of diabetes was 3.5% in the O+P group and 4.2% in the placebo group, which represented a 21% relative reduction in the risk of incident treated diabetes (HR=0.79, nominal 95% CI 0.67–0.93).

The effect of oestrogen and progestin individually has not been fully elucidated. A few studies have shown that medroxyprogesterone may have deleterious effects on glucose metabolism and diabetes incidence [3, 4]. For example, a study of Navajo women who used depot medroxyprogesterone intramuscularly found that users were more likely to develop diabetes than patients who had used any type of oral combination oestrogen–progestin contraception (odds ratio=3.8, 95% CI 1.8–7.9) [3]. Unopposed oestrogen, recommended only for women without a uterus, has been shown in observational studies to reduce the risk of developing type 2 diabetes. Over a decade ago, Manson and colleagues reported a reduced risk after following postmenopausal women enrolled in the Nurses' Health Study for 12 years [5]. More recently, Rossi and colleagues followed 673 postmenopausal women for an average of 3.7 years and found reduced incidence of diabetes among those who wore an oestrogen patch compared with women who took no hormones [6]. These findings suggest that use of a progestogen might increase the risk of diabetes, mitigating the beneficial effect of oestrogen on diabetes incidence. To determine if postmenopausal hormone therapy with oestrogen alone confers protection against diabetes and whether the effect is greater or less than that conferred by oestrogen plus progestin in combination, data from the recently concluded WHI oestrogen-alone trial were analysed.

Methods

Study design and population

The eligibility criteria, recruitment methods, study population characteristics, hormone regimens, randomisation, blinding, follow-up and main outcomes of the WHI oestrogen-alone trial have been previously published [7–9]. Briefly, 10,739 postmenopausal women who were aged 50–79 years and had undergone hysterectomy were recruited at 40 clinical centres throughout the USA. The primary outcome of the hormone trials was CHD, with other cardiovascular disease, breast and gynaecological cancers and hip fracture as pre-specified secondary outcomes [10]. Diabetes was a predetermined outcome measure. Major exclusion criteria were a history of previous breast cancer, any cancer within the previous 10 years except non-melanoma skin cancer, current use of corticosteroids, anticoagulants, tamoxifen or other selective oestrogen receptor modifiers (SERMs), and triglycerides >4.56 mmol/l. A history of venous thromboembolism was added as an exclusion criterion in 1997. Women who were unwilling to discontinue the use of menopausal hormone therapy were excluded, and a 3-month washout period was required for women who were current hormone users at the initial screening visit.

In addition to the oestrogen-alone trial, WHI also included three other randomised trials: a companion hormone trial of O+P in women who had an intact uterus (stopped in 2002) [11], a dietary modification trial examining the health outcomes of a low-fat eating pattern, and a trial of calcium and vitamin D supplementation [7]. Approximately 29% of women in the conjugated equine oestrogen (CEO) trial also participated in the dietary modification trial, where they were randomly assigned to participate in a low-fat dietary change group or continue their usual diet. Some 58% of participants in the CEO trial also participated in a trial of calcium plus vitamin D supplementation [7]. The study protocol and consent forms were approved by the institutional review board for each participating institution. Informed consent was obtained from all participants in the trial. The intervention portion of the oestrogen-alone trial was stopped early by the National Institutes of Health.

At baseline, participants were asked if a physician had ever told them they had 'sugar diabetes' when they were

not pregnant. Women who responded positively were considered to have self-reported diabetes at baseline and were excluded from further analysis. No medical record confirmation of self-reported diabetes was available.

Randomisation, intervention and blinding

A randomised permuted block algorithm, stratified by clinical centre site and age, was developed at the WHI Clinical Coordinating Center and implemented locally through a distributed study database. The study participants were provided CEO, 0.625 mg daily (Premarin; Wyeth Ayerst, Philadelphia, PA, USA), or a matching placebo. Participants, clinic staff, investigators and outcomes adjudicators were blinded to treatment assignment. Neither the clinic gynaecologist nor any of the staff or investigators involved with the clinical care of the participants was involved with study outcomes assessments.

If participants developed symptoms such as breast tenderness, flexibility in the dosage of oestrogen was allowed by reducing the number of days that study medication was taken. Permanent discontinuation of study medication was required for women who developed breast cancer, venous thromboembolism, meningioma, malignant melanoma or triglyceride levels >11.3 mmol/l, or who were prescribed oestrogen, testosterone or SERMs by their personal physicians. These participants were included in the primary intention-to-treat analysis presented here.

Data collection, follow-up and outcomes ascertainment

Volunteers completed three pre-randomisation screening visits during which baseline information was obtained. Height, weight, waist and hip circumferences, and blood pressure were measured by certified staff using standardised procedures [12]. Follow-up for clinical events occurred every 6 months. During the required annual clinic visits, anthropometric and blood pressure measurements were repeated, and adherence to study medication was assessed by pill counts. A standardised written protocol, centralised training of local clinic staff, local quality assurance activities, and periodic quality assurance oversight visits by the Clinical Coordinating Centre were used to maintain uniform data collection procedures at all study sites.

All women had blood drawn after an overnight 8-h fast at baseline and at the first annual visit. Serum and plasma samples were frozen at −70°C and shipped to the WHI central storage facility. A randomly selected paired (CEO and placebo) 8.6% sample of blood specimens was analysed at both time points for a set of 'core analytes' including glucose (serum), insulin (serum) and lipoproteins (EDTA-plasma). The random sampling procedure was stratified by age, clinical centre, hysterectomy status and ethnicity to oversample minority women. The 8.6% sample cohort also had fasting blood drawn using the same protocol at the third, sixth and ninth annual visits (ninth

annual visit blood not yet analysed). Insulin resistance was calculated from fasting glucose and insulin according to the homeostasis model assessment of insulin resistance (HOMA-IR) model: insulin/(22.5 exp[−ln(glucose/18)]), where the units for insulin are μU/ml and units for glucose are mmol/l [13]. A subject met the criteria for metabolic syndrome if at least three of the following conditions were met: a waist circumference >88 cm; triglycerides ≥1.69 mmol/l; HDL levels <1.28 mmol/l; glucose levels >6.1 mmol/l; systolic blood pressure ≥130 mmHg; or diastolic blood pressure ≥85 mmHg.

At each semi-annual contact, participants were asked, 'Since the date given on the front of this form, has a doctor prescribed any of the following pills or treatments?' Choices included 'pills for diabetes' and 'insulin shots for diabetes'. Thus, incident diabetes was defined as a self-report of a new physician diagnosis of diabetes treated with oral hypoglycaemic agents or insulin.

Statistical analysis

Glucose, insulin and lipoproteins were analysed on stored blood samples from 1,046 women at baseline, 909 women at Year 1 and 813 women at Year 3. Baseline variables were compared with either χ^2 or Fisher's exact tests for categorical variables or two-sample t tests for continuous variables. $p \leq 0.05$ was considered significant. Mean differences (at baseline and over time on study) in blood analytes, as well as blood pressure, BMI and waist circumference measures by treatment arm were assessed with t tests. Statistical analyses of blood analyte variables were weighted by race and/or ethnicity. The incidence of diabetes was assessed with a Cox proportional hazards model, stratified by age and dietary modification trial randomisation assignment (not randomised, randomised to intervention group, randomised to control group), in which time to a first self-report of treated diabetes was the outcome variable. A sensitivity analysis was performed to account for treatment non-adherence by comparing Kaplan–Meier cumulative hazard curves for incidence of diabetes for both an intention-to-treat analysis and with participants censored 6 months after becoming non-adherent (defined as taking <80% of study medication or starting open-label hormone therapy).

Interactions between nine baseline characteristics (age, race/ethnicity, BMI, waist circumference, hypertension, smoking, alcohol use, physical activity and metabolic syndrome) and treatment assignment were assessed in Cox proportional hazards models that included both the risk factor (where applicable, as a continuous variable for computing the p value) and randomisation assignment as main effects. Characteristics known to influence or be associated with diabetes were selected. p values for assessing possible interactions were computed from likelihood ratio tests, comparing models with and without the interaction term. Results from all nine comparisons are presented.

Table 1 Baseline characteristics of non-diabetic women randomised to oestrogen-alone or placebo in the WHI (*n*=9,712)[a]

	CEO (*n*=4,806)		Placebo (*n*=4,906)		*p* value
	n	% or mean (SD)	*n*	% or mean (SD)	
Age group at screening (years)	–	–	–	–	0.81
50–59	1,504	31.3	1,542	31.4	–
60–69	2,138	44.5	2,203	44.9	–
70–79	1,164	24.2	1,161	23.7	–
Race/ethnicity	–	–	–	–	0.40
White	3,687	76.7	3,743	76.3	–
Black	660	13.7	713	14.5	–
Hispanic	280	5.8	295	6.0	–
Asian/Pacific Islander	37	0.8	24	0.5	–
American Indian	75	1.6	67	1.4	–
Unknown	67	1.4	64	1.3	–
Hormone use	–	–	–	–	0.72
Never	2,459	51.2	2,477	50.5	–
Past user	1,716	35.7	1,759	35.9	–
Current user	630	13.1	667	13.6	–
Duration of prior hormone use (years)	–	–	–	–	0.83
<5	1,241	52.9	1,278	52.7	–
5–10	435	18.5	466	19.2	–
>10	670	28.6	682	28.1	–
BMI (kg/m^2)	–	–	–	–	0.47
<25	1,073	22.4	1,046	21.5	–
25–30	1,677	35.1	1,749	35.9	–
>30	2,032	42.5	2,079	42.7	–
Waist circumference (cm)	–	–	–	–	0.49
<88	2,222	46.3	2,233	45.6	–
>88	2,573	53.7	2,660	54.4	–
History of hypertension	–	–	–	–	0.70
Not hypertensive	2,424	54.0	2,477	54.5	–
BP ≥140/90 mmHg	849	18.9	876	19.3	–
Treated hypertension	1,217	27.1	1,190	26.2	–
Hyperlipidaemia	599	13.9	642	14.8	0.21
Smoking	–	–	–	–	0.14
Never	2,480	52.1	2,430	50.1	–
Past	1,776	37.3	1,891	39.0	–
Current	500	10.5	528	10.9	–
Alcohol use >1 drink/week	1,427	30.0	1,514	31.1	0.27
Lipid-lowering medication use	393	8.2	403	8.2	0.95
Aspirin use	914	19.0	943	19.2	0.80
History of myocardial infarction	132	2.7	132	2.7	0.87
History of angina	241	5.0	234	4.8	0.58
History of CABG/PTCA	96	2.0	75	1.6	0.08
History of stroke	61	1.3	71	1.4	0.45
History of DVT or PE	79	1.6	77	1.6	0.77
Metabolic syndrome, baseline[b]	178	35.2	181	34.2	0.75
Total expenditure from physical activity (MET)	4,406	9.8 (12.8)	4,420	9.8 (12.6)	0.98

CABG coronary artery bypass graft, *PTCA* percutaneous transluminal coronary angioplasty, *DVT* deep venous thrombosis, *PE* pulmonary embolism, *MET* metabolic equivalent of the task
[a] 1,027 subjects were deleted for self-reported previous diabetes
[b] Calculated only in those with baseline blood analysis available

Table 2 Baseline and follow-up measurements in the non-diabetic women randomised to oestrogen-alone or placebo in the WHI (measured in an 8.6% subsample)

	CEO			Placebo			Difference		p value
	n	Mean	SD	n	Mean	SD	Mean	SE	
Glucose (mmol/l)									
Baseline	509	5.42	0.97	532	5.42	0.82	0.00	0.07	1.00
Year 1 minus baseline	440	−0.22	0.69	450	0.02	0.59	−0.24	0.04	<0.01
Year 3 minus baseline	396	−0.19	1.03	403	0.00	0.78	−0.19	0.08	0.02
Year 6 minus baseline	332	0.13	1.10	340	0.23	0.89	−0.10	0.09	0.28
Insulin (mU/l)									
Baseline	501	11.98	7.75	524	12.46	7.05	−0.47	0.54	0.38
Year 1 minus baseline	425	−1.54	4.81	438	0.00	5.88	−1.55	0.46	<0.01
Year 3 minus baseline	370	0.33	8.12	376	0.94	6.95	−0.61	0.61	0.32
Year 6 minus baseline	327	−0.34	52.58	336	−1.56	7.82	1.22	1.83	0.51
Triglyceride (mmol/l)									
Baseline	511	1.78	1.26	532	1.81	0.92	−0.04	0.09	0.67
Year 1 minus baseline	439	0.31	0.94	450	−0.00	0.62	0.31	0.07	<0.01
Year 3 minus baseline	395	0.16	1.11	402	0.01	0.63	0.15	0.08	0.07
Year 6 minus baseline	332	0.10	1.37	341	0.01	0.88	0.09	0.12	0.43
Total cholesterol (mmol/l)									
Baseline	511	5.99	1.03	532	5.92	1.09	0.07	0.08	0.42
Year 1 minus baseline	439	−0.20	0.79	450	−0.11	0.67	−0.09	0.06	0.17
Year 3 minus baseline	395	−0.33	0.87	402	−0.20	0.84	−0.13	0.07	0.08
Year 6 minus baseline	332	−0.48	1.02	341	−0.38	1.11	−0.10	0.10	0.35
LDL (mmol/l)									
Baseline	499	3.71	0.94	525	3.68	0.96	0.04	0.08	0.63
Year 1 minus baseline	424	−0.56	0.68	441	−0.12	0.61	−0.44	0.06	<0.01
Year 3 minus baseline	383	−0.59	0.78	389	−0.19	0.77	−0.40	0.07	<0.01
Year 6 minus baseline	319	−0.67	0.97	332	−0.39	1.01	−0.28	0.10	<0.01
HDL (mmol/l)									
Baseline	507	1.49	0.38	531	1.42	0.38	0.06	0.03	0.03
Year 1 minus baseline	436	0.22	0.25	450	0.00	0.18	0.22	0.02	<0.01
Year 3 minus baseline	391	0.17	0.28	396	−0.02	0.21	0.19	0.02	<0.01
Year 6 minus baseline	328	0.14	0.31	340	−0.01	0.24	0.15	0.03	<0.01
HOMA-IR									
Baseline	500	2.94	2.12	523	3.11	2.15	−0.17	0.15	0.26
Year 1 minus baseline	424	−0.48	1.43	437	0.04	1.64	−0.53	0.12	<0.01
Year 3 minus baseline	370	−0.01	2.23	374	0.23	2.02	−0.25	0.18	0.16
Year 6 minus baseline	327	0.58	26.10	335	−0.18	2.71	0.76	0.88	0.39
Systolic BP (mmHg)									
Baseline	4,806	129.8	17.40	4,906	129.7	17.50	−0.16	0.35	0.64
Year 1 minus baseline	4,462	−1.5	16.17	4,539	−2.3	15.75	−0.78	0.34	0.02
Year 3 minus baseline	4,100	−0.9	17.18	4,184	−1.8	17.28	−0.92	0.38	0.02
Year 6 minus baseline	3,684	−1.3	18.81	3,719	−3.0	18.29	−1.73	0.43	<0.0001
Diastolic BP (mmHg)									
Baseline	4,805	76.6	9.19	4,906	76.6	9.32	−0.02	0.19	0.91
Year 1 minus baseline	4,463	−1.6	9.09	4,535	−1.6	8.86	0.04	0.19	0.82
Year 3 minus baseline	4,096	−2.2	9.52	4,183	−2.4	9.84	−0.14	0.21	0.52
Year 6 minus baseline	3,681	−4.0	10.32	3,715	−4.2	10.37	−0.18	0.24	0.45
BMI (kg/m²)									
Baseline	4,782	29.7	5.99	4,874	29.8	6.16	0.11	0.12	0.39
Year 1 minus baseline	4,399	−0.1	2.68	4,472	−0.1	2.61	0.00	0.06	0.96
Year 3 minus baseline	4,039	0.3	2.94	4,120	0.3	3.15	−0.01	0.07	0.87
Year 6 minus baseline	3,642	0.5	5.25	3,667	0.5	4.09	−0.03	0.11	0.76
Waist (cm)									
Baseline	4,795	90.7	13.70	4,893	90.7	13.43	0.04	0.28	0.88

Table 2 (continued)

	CEO			Placebo			Difference		*p* value
	n	Mean	SD	*n*	Mean	SD	Mean	SE	
Year 1 minus baseline	4,363	−0.5	6.76	4,436	−0.0	6.68	0.51	0.14	0.00
Year 3 minus baseline	2,026	0.3	8.61	2,006	0.8	7.86	0.44	0.26	0.09
Year 6 minus baseline	1,463	1.4	8.77	1,493	1.9	8.76	0.54	0.32	0.09
Excluding non-adherent participants by Year 1, Year 3 and Year 6									
Glucose (mmol/l)									
Year 1 minus baseline	281	−0.22	0.74	271	0.01	0.56	−0.24	0.06	<0.01
Year 3 minus baseline	133	−0.21	0.80	116	0.07	0.98	−0.28	0.13	0.04
Year 6 minus baseline	29	−0.18	1.19	28	0.15	1.11	−0.32	0.30	0.28
Insulin (mU/l)									
Year 1 minus baseline	274	−1.64	4.91	267	0.36	4.04	−2.00	0.48	<0.01
Year 3 minus baseline	126	0.22	5.59	107	2.78	6.09	−2.56	0.87	<0.01
Year 6 minus baseline	29	−2.99	4.34	28	−0.79	7.21	−2.20	1.90	0.25
HOMA-IR									
Year 1 minus baseline	273	−0.52	1.50	267	0.12	1.29	−0.63	0.14	<0.01
Year 3 minus baseline	126	−0.06	1.62	107	0.83	2.01	−0.89	0.28	<0.01
Year 6 minus baseline	29	−0.76	1.35	28	0.11	2.61	−0.87	0.66	0.19

Results

Of 10,739 participants, 1,027 (504 randomised to oestrogen-alone and 523 randomised to placebo) with a reported diagnosis of diabetes at baseline were excluded from further analysis. Of the remaining 9,712 participants, 4,806 were randomised to CEO alone and 4,906 to placebo. The average age was 64 years; 76% were non-Hispanic white, 14% African American and 6% Hispanic. Forty-three per cent had a baseline BMI >30 kg/m^2 (i.e. obese) and 54% a waist circumference >88 cm. Although there were no differences in mean blood pressure (130/77 mmHg) at baseline, treated hypertension was reported by 27%; another 19% had a baseline blood pressure measurement above 140/90 mmHg, and 14% reported a history of hyperlipidaemia or were using cholesterol-lowering medications. The two groups were well matched at baseline (Table 1).

There were no differences in blood chemistries at baseline (Table 2). In the 8.6% subset that had baseline blood analysis, the mean plasma glucose was 5.42 mmol/l and mean insulin 12.2 mU/l. Thirty-four per cent of the subsample with baseline blood analysis met the criteria for metabolic syndrome at baseline. There were also no differences in triglyceride, total cholesterol or LDL levels, but there was a small difference between the two groups in baseline HDL levels. The mean level for HDL in the oestrogen-alone participants was 1.49 mmol/l, slightly higher than the mean level in the placebo participants, 1.42 mmol/l (*p*=0.03).

After 1 year of study medications, blood glucose and insulin levels differed between groups (Table 2). Mean glucose decreased in the oestrogen-alone participants (Year 1 minus baseline: −0.22 mmol/l) while glucose levels increased in the placebo group (Year 1 minus baseline: 0.02 mmol/l, *p*<0.01 for oestrogen-alone vs placebo comparison). Insulin levels also decreased in the oestrogen-alone group (Year 1 minus baseline: −1.54 mU/l) while there was no change in participants taking placebo (Year 1 minus baseline: 0.00 mU/l, *p*<0.01 for oestrogen-alone vs placebo). This difference resulted in a decrease in insulin resistance measured by the HOMA model in the oestrogen-alone group (Year 1 minus baseline: −0.48) whereas there was slight increase in the placebo group (Year 1 minus baseline: 0.04, *p*<0.01 for oestrogen-alone vs placebo). At Year 3 of treatment, the glucose reduction was still present in the oestrogen-alone group (Year 3 minus baseline: −0.19 mmol/l) while the placebo group had no change (*p*=0.02 for oestrogen-alone vs placebo). By Year 6, an increase in glucose was seen in both groups (oestrogen-alone Year 6 minus baseline: 0.13 mmol/l vs placebo: 0.23 mmol/l, *p*=0.28). The change in insulin was similar between the two groups at both Year 3 and Year 6 (see Table 2) resulting in no difference in insulin resistance as calculated by HOMA. While there was no difference in the change in BMI between the two treatment groups, waist circumference decreased slightly in the oestrogen-alone group at 1 year (Year 1 minus baseline: −0.5 cm) while the placebo group did not change (*p*<0.01 for oestrogen-alone vs placebo). This difference was not seen at Years 3 or 6.

Nearly 54% of participants had already discontinued their study medication when the trial was stopped. Dropout and drop-in rates did not differ by study arm. If only participants adherent to study medication were considered (adherence defined as taking ≥80% of their medication), there was a decrease in blood glucose for the adherent oestrogen-alone group at Year 3 (Year 3 minus baseline: –0.21 mmol/l over the 3 years of follow-up vs placebo Year 3 minus baseline: 0.07 mmol/l, *p*=0.04 for oestrogen-alone vs placebo). Both groups saw increases in insulin levels (oestrogen-alone Year 3 minus baseline: 0.22 mU/ml vs

placebo Year 3 minus baseline: 2.78 mU/ml, $p<0.01$ for oestrogen-alone vs placebo). As a result, insulin resistance was reduced in the oestrogen-alone group (oestrogen-alone Year 3 minus baseline: −0.06; placebo Year 3 minus baseline: 0.83, $p<0.01$). At Year 6 only 29 adherent active treatment and 28 adherent placebo blood samples are available, precluding meaningful comparisons.

The incidence of diabetes was lower in the participants randomised to oestrogen alone, although the difference did not attain statistical significance (Table 3 and Fig. 1). After 7.1 years of follow-up, 8.3% of women (n=397,

annualised incidence 1.16%) in the oestrogen-alone group reported a new diagnosis of diabetes compared with 9.3% (n=455, annualised incidence 1.3%) in the placebo group (HR=0.88, 95% CI 0.77–1.01). Although not significant, the separation in incidence as shown in the Kaplan–Meier cumulative hazard curves is seen shortly after randomisation (Fig. 1). We examined several subgroups of participants and found no significant variation in treatment effect by age at entry, ethnicity, baseline BMI, baseline waist circumference, presence of hypertension, alcohol use, physical activity or metabolic syn-

Table 3 Self-reported diabetes incidence by treatment assignment overall and in subgroups

Outcomes	No. of patients (annualised, %)				HR[a]	95% CI	p value for interaction[b]
	CEO (n=4,787)		Placebo (n=4,887)				
Follow-up time, mean (SD) months	85.8	(18.5)	85.9	(19.1)	–	–	–
Incident diabetes	397	(1.16%)	455	(1.30%)	0.88	(0.77–1.01)	–
Subgroup analyses							
Age at screening (years)[c]	–	–	–	–	–	–	0.93
50–59	131	(1.17%)	159	(1.39%)	0.83	(0.66–1.05)	–
60–69	181	(1.20%)	198	(1.28%)	0.94	(0.77–1.15)	–
70–79	85	(1.06%)	98	(1.22%)	0.85	(0.64–1.14)	–
Race/ethnicity	–	–	–	–	–	–	0.94
White	265	(1.01%)	305	(1.14%)	0.87	(0.74–1.03)	–
Black	86	(1.84%)	101	(1.99%)	0.93	(0.70–1.24)	–
Hispanic	26	(1.32%)	29	(1.42%)	0.86	(0.51–1.47)	–
American Indian	5	(1.97%)	4	(2.38%)	1.35	(0.28–6.51)	–
Asian/Pacific Islander	10	(1.96%)	8	(1.70%)	1.10	(0.40–3.03)	–
Unknown	5	(1.08%)	8	(1.83%)	0.44	(0.12–1.60)	–
BMI (kg/m^2)	–	–	–	–	–	–	0.48
<25	28	(0.37%)	41	(0.55%)	0.66	(0.41–1.06)	–
≥25<30	99	(0.83%)	109	(0.87%)	0.95	(0.72–1.24)	–
>30	269	(1.86%)	302	(2.05%)	0.89	(0.76–1.05)	–
Waist circumference (cm)	–	–	–	–	–	–	0.58
<88	77	(0.48%)	92	(0.57%)	0.84	(0.62–1.14)	–
>88	318	(1.74%)	361	(1.91%)	0.89	(0.77–1.04)	–
History of hypertension	–	–	–	–	–	–	0.34
Not hypertensive	151	(0.89%)	157	(0.91%)	0.97	(0.78–1.22)	–
Treated or BP >140/90	219	(1.52%)	256	(1.76%)	0.85	(0.71–1.02)	–
Current smoker	–	–	–	–	–	–	0.02
No	361	(1.19%)	394	(1.27%)	0.93	(0.80–1.07)	–
Yes	31	(0.90%)	59	(1.61%)	0.54	(0.35–0.84)	–
Alcohol use	–	–	–	–	–	–	0.21
<1 drink/week	327	(1.38%)	391	(1.63%)	0.84	(0.72–0.97)	–
>1 drink/week	63	(0.62%)	63	(0.59%)	1.07	(0.75–1.52)	–
Physical activity (METs/week)[d]	–	–	–	–	–	–	0.31
<6.75	212	(1.30%)	242	(1.47%)	0.87	(0.72–1.04)	–
≥6.75	149	(1.05%)	150	(1.06%)	0.99	(0.79–1.24)	–
Metabolic syndrome[e]	–	–	–	–	–	–	0.75
No	13	(0.57%)	13	(0.54%)	1.18	(0.54–2.58)	–
Yes	40	(3.26%)	41	(3.26%)	0.98	(0.63–1.54)	–

[a] HRs and CIs are from Cox proportional hazards analyses stratified by age and randomisation status in the low-fat-diet trial
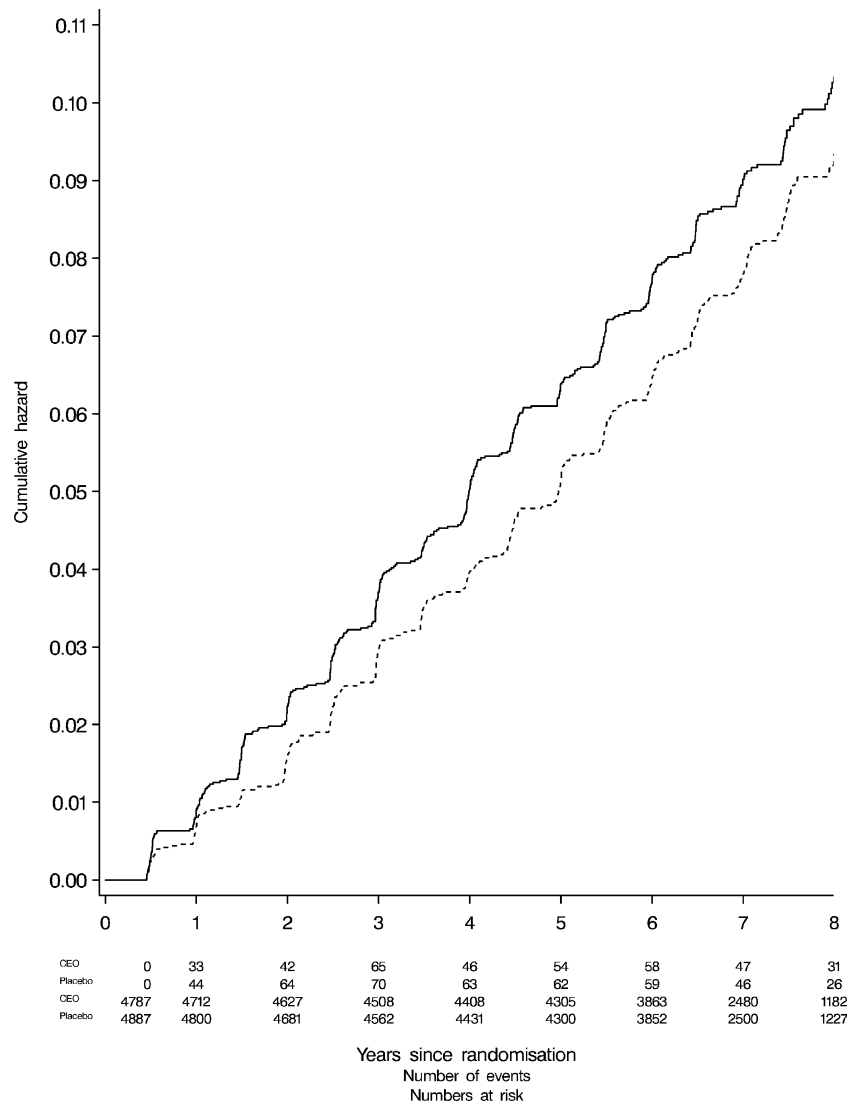
[b] p values from a likelihood ratio test comparing a model with the main effects for the variable of interest and intervention group vs a model with the main effects plus the interaction between the main effects

[c] Age subgroup models are only stratified by randomisation status in the low-fat-diet trial

[d] *MET* metabolic equivalent of the task

[e] The subsample with blood analysis was used

Fig. 1 Diabetes incidence by treatment arm; oestrogen-alone (*dashed line*) vs placebo (*solid line*). HR (95% CI) 0.88 (0.77–1.01)

	0	1	2	3	4	5	6	7	8
CEO	0	33	42	65	46	54	58	47	31
Placebo	0	44	64	70	63	62	59	46	26
CEO	4787	4712	4627	4508	4408	4305	3863	2480	1182
Placebo	4887	4800	4681	4562	4431	4300	3852	2500	1227

Years since randomisation
Number of events
Numbers at risk

drome at baseline (Table 3). However, the treatment effect was larger for current smokers than for non-smokers (non-smoker HR=0.93, 95% CI 0.8–1.07; current smokers HR=0.54, 95% CI 0.35–0.84, *p* value for smoking oestrogen-alone interaction=0.02).

When participants were censored who either took <80% of study pills or began open-label hormone therapy, the magnitude of the difference in incident diabetes between the groups was larger, with a 27% reduction in the risk of new, treated diabetes in the women who received active hormone treatment (HR=0.73, 95% CI 0.60–0.88).

Discussion

Our findings suggest that CEO alone given to postmenopausal women may protect against the development of diabetes. Although the difference in incidence did not quite reach nominal statistical significance, a clear trend toward a protective effect was seen with an HR of 0.88 (95% CI 0.77–1.01). This result further supports other studies of the effect of combination hormone therapy on

diabetes incidence. Both the O+P arm of the WHI study (HR=0.79, 95% CI 0.67–0.93) [2] and the HERS (HR=0.65, 95% CI 0.48–0.89) [1] found a significant reduction in the incidence of diabetes in women randomised to active treatment.

Blood glucose and insulin levels were significantly reduced after 1 year of CEO therapy in the WHI, but reduction did not persist at Years 3 or 6. CEO therapy also conferred a corresponding decrease in insulin resistance of 0.48 measured by the HOMA model at the end of Year 1 in the women randomised to active treatment. Similar effects on glucose and insulin have been seen in other studies. The Postmenopausal Oestrogen/Progestin Intervention Study, a placebo-controlled randomised trial of four hormone regimens included both a CEO-alone arm and CEO plus continuous medroxyprogesterone [14]. OGTT performed at baseline and 1 and 3 years showed modest reductions in both fasting glucose and insulin levels with any active therapy and a modest increase in the 2-h glucose. No significant difference by progesterone treatment group was seen. Smaller studies examining glucose and insulin levels after short-term use of CEO

[15], oestradiol [15, 16] or in cross-sectional analysis [17, 18] have shown similar effects.

The process underlying the change in glucose and insulin levels with oestrogen is not fully understood, but several mechanisms have been hypothesised. Oestrogen may have a direct effect on the secretion of insulin by the pancreas. Oestrogen receptors are present in pancreatic beta cells [19], and oestrogen increases the release of insulin in beta cell culture models [20]. Oestrogen may also decrease hepatic glucose production; hypoglycaemic clamp studies showed that hepatic glucose production was lower than expected in women on hormone therapy [21]. Similar reductions have been shown in animal experiments [22, 23]. Finally, oestrogen may reduce peripheral insulin resistance by reducing vascular reactivity in postmenopausal women with the use of both oral [24, 25] and transdermal oestrogen [24].

Other studies have shown an increase in glucose abnormalities and diabetes in premenopausal women using progesterone-only forms of contraception [3, 4, 26]. Given this background, a greater protective effect against diabetes was expected in the oestrogen-alone portion of the WHI trial, which was not found. We observed that the reduction in diabetes incidence reported here is less than that found in the O+P arm of the WHI study (HR=0.79, 95% CI 0.67–0.93) [2] and in the HERS trial (HR=0.65, 95% CI 0.48–0.89) [1].

Several possibilities may account for this difference. Most studies examining the metabolic effect of medroxyprogesterone have studied it as a contraceptive agent. When used to prevent contraception the doses of this progestin, 150 mg depot injected every 3 months, are considerably larger than that used in the WHI, where lower circulating levels would have been anticipated [27, 28]. However, it seems unlikely that differences in medroxyprogesterone dosage could explain both risk at high dosage and benefit at lower dosage. Women in the oestrogen-alone trial were significantly different from those in the O+P trial [29], particularly in characteristics that are associated with the development of diabetes such as age, BMI and waist circumference. This difference is demonstrated in the overall higher incidence of diabetes in the placebo group of each study arm (oestrogen-alone 9.3%, O+P 4.2% [2]). It is possible that the higher degree of baseline risk for diabetes of the women in the oestrogen-alone trial blunted the potential protective effect of the oestrogen although no increased risk by elevated BMI was found, making this an unlikely explanation. Finally, the low dose of medroxyprogesterone used in the WHI O+P study may have potentiated the effects of CEO on insulin secretion, as seen in rat pancreatic perfusion studies [19]. Further study is needed to elucidate the separate actions and interactions of the two hormones on glucose metabolism.

There are limitations to this study. There was no confirmation of the self-reported diabetes diagnosis with medical records, nor was it possible to determine the incidence of undiagnosed diabetes. Other studies that tested the validity of self-reported diabetes have found accuracies in the range of 64–96%, but none of these studies used a definition similar to that used in WHI [30–34]. Since 81.2% of participants in the WHI oestrogen-alone trial who self-reported treatment with diabetes at baseline medication were using an oral hypoglycaemic medication or insulin (unpublished data), it is unlikely that overreporting of diabetes treatment substantially affected our results. However, assessment of self-reported treated diabetes would have missed incident cases treated with lifestyle intervention and undiagnosed cases. It is unlikely that ascertainment of diabetes varied systematically by treatment arm given the masked status of the study design.

In conclusion, use of CEO alone appears to reduce the self-reported incidence of pharmacologically treated diabetes in postmenopausal women, but the overall diabetes protection from oestrogen-alone is less than that of O+P. The results of this study in conjunction with the other study results reviewed above support further investigation into the mechanism of the protective effect, examining oestrogen and progestin separately and in combination. At this time, the overall risk profile of CEO alone or O+P, including the increased risk for stroke, thrombotic disease and dementia, and the null effects on cardiovascular disease and breast cancer for oestrogen-alone for postmenopausal women, must be considered when weighing the possible beneficial effect on diabetes. Additionally, oestrogen-alone is licensed only for use in women who have had a hysterectomy, a small portion of the population. Given these risks, neither oestrogen-alone nor O+P should be recommended for the primary prevention of diabetes. However, these findings may provide useful information on the aetiology of diabetes that could be used to enhance prevention programmes. If the mechanism underlying the effects on diabetes incidence can be elucidated, it may be possible to exploit this through the development of other compounds thus further expanding the range of medications available to healthcare providers.

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