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The effect of tibolone and continuous combined conjugated equine oestrogens plus medroxyprogesterone acetate on progression of carotid intima–media thickness: the Osteoporosis Prevention and Arterial effects of tiboLone (OPAL) study

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KEYWORDS
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Selective tissue oestrogenic activity regulator

Aims At the time of the design of the Osteoporosis Prevention and Arterial effects of tiboLone (OPAL) study in 1996, oral hormone therapy (HT) was assumed to reduce cardiovascular risk. The evidence mainly came from the effects of combined conjugated equine oestrogens plus medroxyprogesterone acetate (CEE/MPA) therapy. Other HT regimes had not been studied widely. Tibolone, a selective tissue oestrogenic activity regulator, has several effects on cardiovascular risk factors, one of which is HDL lowering. Because the overall effect of tibolone on cardiovascular risk was unknown, the OPAL study was designed.

Methods and results The OPAL study was a three-arm, randomized, placebo-controlled, double-blind study to determine the effect of tibolone (2.5 mg daily) and of CEE/MPA (0.625/2.5 mg daily) over 3 years on progression of carotid intima–media thickness (CIMT) in 866 healthy post-menopausal women. The women were recruited from six US and five European centres. The primary outcome was change in mean common CIMT. Annual common CIMT progression rates in the tibolone and CEE/MPA groups were higher than in the placebo group: 0.0077 mm [95% confidence interval (CI) 0.0051–0.0103] in the tibolone group, 0.0074 mm (0.0048–0.0099) in the CEE/MPA group, and 0.0035 mm (0.009–0.0061) in the placebo group. The differences with placebo (0.0042 mm/year for tibolone and 0.0039 mm/year for CEE/MPA) were statistically significant. HDL cholesterol increased in CEE/MPA group and was lowered in the tibolone group.

Conclusion Both tibolone and CEE/MPA showed increased progression of common CIMT. Translation of the increased common CIMT progression of the CEE/MPA group into cardiovascular disease risk could not fully explain the observed increased cardiovascular risk as observed in the Women's Health Initiative study. This suggests that the net effect of tibolone and CEE/MPA on cardiovascular events may depend on the combined effects on the arterial wall, clotting factors, and possibly inflammation.

Introduction

At the time of the design of the Osteoporosis Prevention and Arterial effects of tiboLone (OPAL) study in 1996, oral hormone therapy (HT) was assumed to reduce cardiovascular disease risk. This was based on a wealth of data from observational studies.[1–4] Analyses from the Nurses Health Study had indicated that the increased use of HT might explain ~9% of the observed decrease in coronary heart disease risk among women from 1980 to 1994.[5] Most of the evidence on cardiovascular risk was based on effects of combined conjugated equine oestrogens plus medroxyprogesterone acetate (CEE/MPA) therapy, and other HT regimes had not been studied widely. Also, it had been recognized that HT was related to an increased risk of breast cancer.[6,7] Tibolone, a selective tissue oestrogenic activity regulator, appeared to be neutral on the breast[8] and generally had favourable effects on lipids [reduction of triglycerides, very LDL (VLDL), and lipoprotein (a)] with the exception of

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its effects on HDL cholesterol, which is typically lowered.[9] This reduction has been shown to be, in part, a result of increasing hepatic lipase activity, whereas the function of HDL in reverse cholesterol transport was not impaired.[10] The net effect of these changes on the development of atherosclerosis and cardiovascular disease risk was unknown. We set out to evaluate the effect of tibolone (2.5 mg daily) and of CEE/MPA (0.625 + 2.5 mg daily) on progression of carotid intima–media thickness (CIMT) compared with placebo in healthy post-menopausal women.

Methods

General

The rationale and design of the OPAL study has been described in detail elsewhere.[11] In short, the OPAL study is a three-arm, randomized, placebo-controlled, double-blind trial to determine the effect of tibolone (2.5 mg tablet daily) and continuous combined CEE plus MPA (0.625 + 2.5 mg tablets daily) on the progression of intima–media thickness of the carotid arteries and bone mineral density (BMD) of the lumbar vertebrae and proximal femur in post-menopausal women. A total of 866 healthy women, aged 45–79 were recruited in six US and five European centres (Appendix). Duplicate carotid ultrasound examinations of the common carotid artery, the carotid bifurcation, and the internal carotid artery were performed at baseline. After randomization, ultrasound examinations were repeated every 6 months for 36 months following baseline, with another duplicate examination at the end of the study. The primary outcome was the change in mean common CIMT defined as the average of the intima–media thickness measurements performed circumferentially at pre-defined angles for the near and far walls of the distal 10 mm of the right and left common carotid arteries.[11] The aim was to determine the extent to which 3 years of treatment with tibolone or CEE/MPA affects progression of common CIMT when compared with placebo in healthy post-menopausal women. In addition, the effects of the two treatment regimens, relative to placebo on (i) progression of the mean of the maximum CIMT of the 12 walls of the carotid artery, (ii) BMD of the lumbar vertebrae and proximal femur, and (3) quality of life were examined.

Approval for the conduction of the OPAL study was obtained from the institutional review boards of the participating clinics, and written informed consent was obtained from all study participants.

Subjects were randomized to receive tibolone, CEE/MPA, or placebo at a 1:1:1 ratio from 11 sites. Randomization was stratified within each site. The sample size was based on the estimate that 142 subjects per treatment arm would be required for a statistically meaningful comparison of active treatment groups to placebo with a two-sided alpha of 0.05 and 90% power to detect a difference between the tibolone group and placebo of −0.0185 mm/year change in mean common CIMT with a standard deviation of 0.048 mm/year. The progression rates and effect estimates were based on findings in the 'oestrogen users' subgroup of the Asymptomatic Carotid Atherosclerosis Progression Study.[12] On the basis of experience gained from other studies using these compounds, conservative post-randomization discontinuation of blinded treatment rates of 30% for the first year and 10% for each of the following years were assumed. It was estimated that enrolment of 756 subjects would result in 142 subjects who would complete the 3-year treatment period per group.

The study was designed so that the study population would reflect a large proportion of women who might potentially use tibolone or HT. Therefore, the exclusion criteria were mainly limited to contraindications for CEE/MPA or tibolone, a high probability of experiencing serious side effects, or a low likelihood of completing the study, as detailed elsewhere.[11] In short, the participants were healthy post-menopausal women, aged 45–79. Menopausal status was defined as being without menses for ≥1 year. When the last menstruation date was unclear, women needed to fulfill the Food and Drug Administration criteria for menopause [oestradiol ≤20 pg/mL (or 73 pmol/L)] and a follicle-stimulating hormone level ≥40 mIU/mL). For the US sites, women needed to have an intact uterus. Users of oral HT, androgens, or selective oestrogen receptor modulators were 'washed out' for 8 weeks. Users of transdermal or local sex steroids were 'washed out' for 4 weeks. Participants were required to have a body mass index (BMI) > 19 kg/m^2 but ≤32 kg/m^2. Finally, the near and far walls of the common carotid arteries had to be visualized in such a way that reliable CIMT measurements could be taken.

Carotid ultrasound and CIMT measurements

The OPAL carotid ultrasound protocol has been described in detail elsewhere.[11] In short, standardized longitudinal B-mode images were obtained of the near and far walls of the arterial segment extending from 10 to 20 mm proximal to the tip of the flow divider into the common carotid artery, the near and far walls of the carotid bifurcation beginning at the tip of the flow divider and extending 10 mm proximal to the tip of the flow divider, and the near and far walls of the proximal 10 mm of the internal carotid artery. In addition, the Meijer's Carotid Arc® was used which enabled the sonographer to indicate at which angle of interrogation the transducer was located during image selections. In all 11 centres, the same high resolution B-mode ultrasound system was used (Acuson Aspen, Mountain View, CA, USA), with identical pre-sets and a 7.0 (10–5.0) MHz linear array transducer. The entire ultrasound examination was recorded on super VHS videotape, including the oral comments given by the sonographer, for off-line central QC and reading. All sonographers completed a uniform certification program.

All ultrasound scans were read using Image Pro® software on which a dedicated software program was added to ensure standardized settings across reading stations and continents. On each image, the visualized blood–intima and media–adventitia boundaries were marked with a computer mouse-controlled caliper within the defined segment. For the CIMT measurements, the trailing edges were traced on the near wall boundaries and the leading edges on the far wall boundaries. In a study with complete data acquisition, the right common carotid artery contributes data on mean, minimum, and maximum near wall and far wall CIMTs and lumen diameter for each of the selected angles, i.e. 60°, 90°, 120°, 150°, 180° and the optimal angle of interrogation. The same applies for the left common carotid artery and corresponding angles. For the carotid bifurcation and the internal carotid artery, emphasis was on the maximum CIMT only, which was measured at all selected angles. All readers completed a uniform training program.

CIMT reproducibility was assessed by estimation of the intraclass correlation coefficient (ICC). The ICC for mean common CIMT was 0.88 for the two duplicate scans at baseline and 0.87 for the duplicate scans at the end of study. The ICC for repeated scans during the course of the study (after last randomization and before first completing subjects) was 0.87. The results for the mean maximum (meanMax) CIMT (secondary outcome) were 0.85, 0.91, and 0.87, respectively. The between core laboratory reproducibility data from 25 study scans read by both laboratories twice during the study showed a systematic difference between the labs, Europeans readers read common CIMT 0.07 mm thicker than the US readers with an ICC of 0.70.

Primary and secondary outcome

The primary outcome was the change in mean common CIMT. Mean common CIMT was defined as the average of the intima–media thickness measurements performed within a 10 mm segment at the right near wall, right far wall, left near wall, and left far wall of the carotid arteries. The secondary outcome was the change in meanMax CIMT. MeanMax was defined as the average of the 12 segment-specific maximal CIMT measurements.

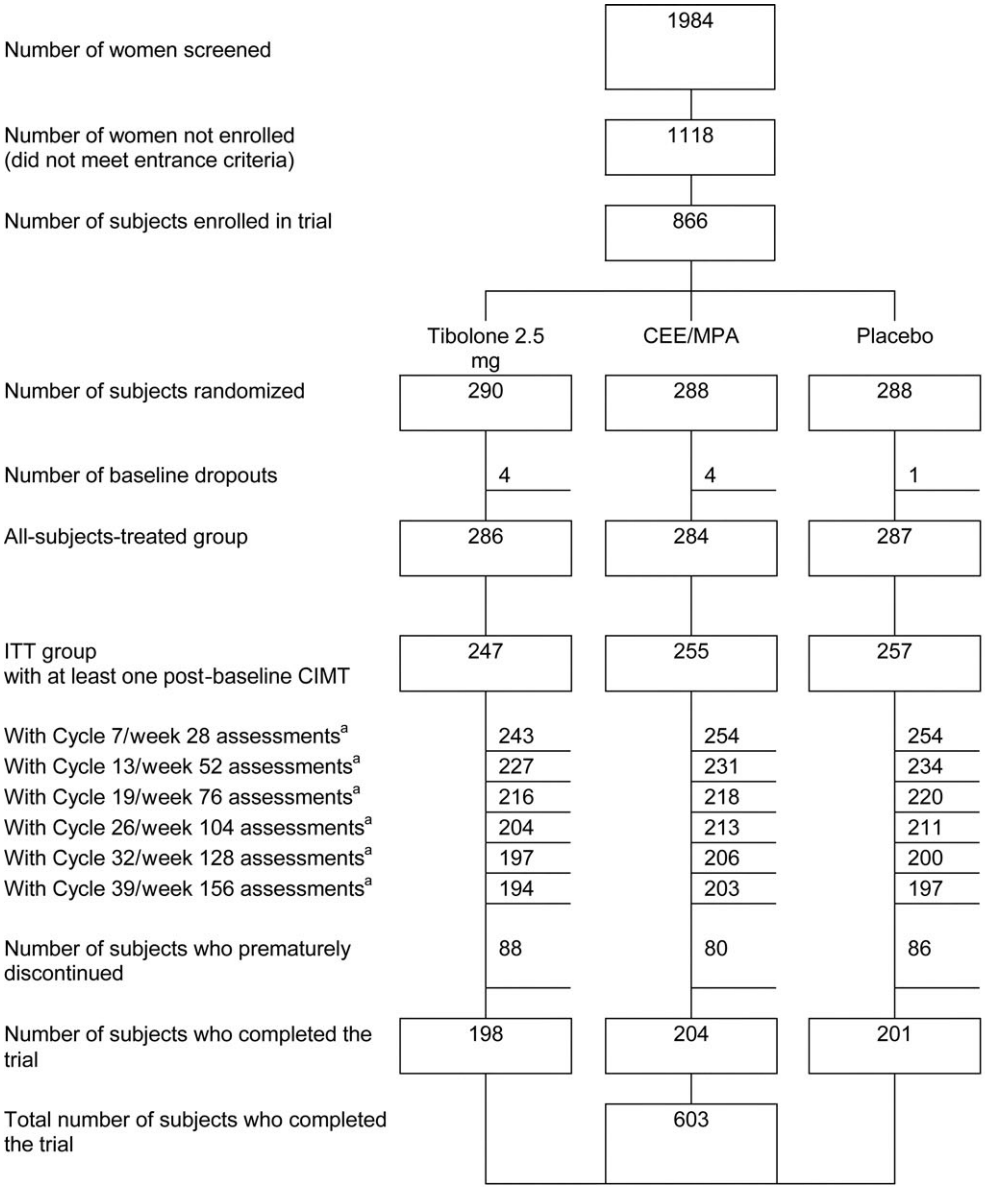
Data analysis

The primary analysis of CIMT progression was based on a linear random coefficient (Laird–Ware) model using real visit days, treatment, and clinical centre as independent variables.[13] For each participant, the intercept and slope of CIMT change over time was assumed to be a normally distributed random variable with different means for the three treatment groups. The mean slope for each active treatment group (tibolone and CEE/MPA) was compared with that for the placebo group using linear contrasts and a 5% significance level. No adjustment for type I error was made as the primary comparison was between placebo and tibolone and the other comparisons were secondary. All analyses were based on an intent-to-treat approach (ITT), i.e. the ITT group consists of all subjects, including those who withdrew from blinded medication, who received at least one dose of study drug, and who had at least one post-baseline assessment of CIMT. Complete subjects had nine CIMT measurement points, and the minimum number of CIMT measurements was three (two duplicate baseline and one follow-up measurement).

Exploratory analyses were performed in strata of continent (Europe/United States), statin use (yes/no), baseline LDL level [below and above 3.37 mmol/L (130 mg/dL)], baseline HDL level [below and above 1.55 mmol/L (60 mg/dl)], hysterectomy (yes/no), baseline BMI (below and above 25 kg/m^2) and age (below and above 60 years) and time since menopause (below and above the median).

Results

The disposition of the study subjects is given in *Figure 1*. Out of 866 subjects randomized, 759 (89%) subjects received at least one dose of study drug and had at least one post-baseline assessment of CIMT. This group comprised the ITT group. The baseline characteristics of the ITT study population



CEE/MPA, conjugated equine estrogens (0.625 mg)/medroxyprogesterone acetate (2.5 mg).
[a]Numbers of subjects of whom a mean CCIMT assessment was made at the indicated time point. Cycle 7/week 28 = since seven cycles of the HT treatment have been passed since basline. As a cycle has a duration of 4 weeks, the time point is 28 weeks after baseline.

Figure 1 Schematic presentation of the number of patients participating in the OPAL study.

(overall and across groups) and that of those randomized were similar. Baseline characteristics of the study population are given in *Table 1*. The main characteristics were balanced across treatment groups. As expected, there was a gradual decline in percentage of subjects on treatment over the study period, which was similar across groups and well within the pre-defined limits (*Table 2*).

The ITT analysis showed that the annual common CIMT progression rates in the tibolone and CEE/MPA groups were statistically, significantly higher than in the placebo group: 0.0077 mm (95% CI 0.0051–0.0103) in the tibolone group, 0.0074 mm (0.0048–0.0099) in the CEE/MPA group, and 0.0035 mm (0.009–0.0061) in the placebo group (*Table 3*). For the meanMax CIMT, no statistically significant differences in progression rates across groups were found (*Table 3*). In addition to the ITT analyses, analyses were performed in which adjustments were made for reader, reading centre, clinical centre, lumen diameter changes (for common CIMT only), and presence/absence of a uterus. These additional analyses did not change the direction, the magnitude, or the significance of the results of the initial ITT analysis and therefore only the latter is reported. The crude results of the CIMT measurements over time are presented in *Figure 2*.

Common CIMT progression rates appeared to differ across continents. This is presented in *Table 4*. The comparisons between mean common CIMT of tibolone and placebo and CEE/MPA and placebo were statistically significant for the European data, but not for the US data. Apart from the differences in common CIMT progression rates, the European subjects had higher lipids, higher blood pressure levels, and more were smokers at baseline. Furthermore, hysterectomized women were allowed to be enrolled into the study in Europe but not in the United States and comprised 29.7% of the European study population.[11] The effects of tibolone and CEE/MPA on meanMAX CIMT progression were not statistically significant in either the European data or the US data (*Table 4*).

The results of the stratified analyses for the common CIMT progression are given in *Table 5*. A relatively high baseline HDL level, a BMI <25 kg/m^2, and younger age was generally related to a reduced progression of common CIMT. Overall, the common CIMT progression rates in the treatment groups were higher than that in the placebo group. The

Table 1 Baseline characteristics of the study population with at least one post-baseline CIMT assessment, by assigned treatment group

	Tibolone	CEE/MPA	Placebo	Total
Number of subjects	247	255	257	759
Age (years)	58.9 (6.8)	58.6 (6.6)	59.0 (6.6)	58.8 (6.7)
Height (cm)	163.4 (6.2)	163.5 (6.4)	163.9 (5.8)	163.6 (6.1)
Weight (kg)	67.6 (8.7)	67.9 (9.0)	66.9 (9.0)	67.5 (8.9)
Body mass index (kg/m^2)	25.3 (3.0)	25.4 (3.0)	24.9 (2.8)	25.2 (2.9)
Caucasian race (%)	237 (95.9%)	247 (96.8%)	246 (95.7%)	730 (96%)
Time since menopause (years)	10.6 (7.6)	10.3 (7.6)	10.9 (7.9)	10.6 (7.7)
Intact uterus (%)	198 (80.1%)	211 (82.7%)	221 (85.9%)	630 (83.1%)
Previous HT use (%)	118 (47.7%)	124 (48.6%)	112 (43.5%)	354 (46.6)
Alcohol consumption (drinks/day)	0.5 (0.9)	0.6 (1.0)	0.5 (0.8)	0.5 (0.9)
Current alcohol drinkers (%)	78 (31.5%)	86 (33.7%)	81 (31.5%)	245 (32.2%)
Current cigarette smoking (%)	47 (19.0%)	47 (18.4%)	43 (16.7%)	137 (18.0%)
Heart rate (b.p.m.)	70.4 (9.6)	69.5 (8.5)	70.2 (9.2)	70.1 (9.1)
Systolic blood pressure (mmHg)	129 (15.8)	129 (15.2)	129 (16.0)	129 (15.7)
Diastolic blood pressure (mmHg)	77.0 (9.4)	76.1 (10.3)	76.3 (10.3)	76.5 (10.0)
Glucose (mmol/L)	5.5 (0.9)	5.4 (0.6)	5.4 (1.1)	5.4 (0.9)
Total cholesterol (mmol/L)	6.2 (1.2)	6.3 (1.1)	6.2 (1.1)	6.3 (1.1)
LDL cholesterol (mmol/L)	4.1 (1.1)	4.1 (1.0)	4.0 (1.0)	4.0 (1.0)
HDL cholesterol (mmol/L)	1.6 (0.4)	1.6 (0.4)	1.6 (0.4)	1.6 (0.4)
Triglycerides (mmol/L)	1.3 (0.8)	1.4 (0.7)	1.3 (0.7)	1.3 (0.7)
Mean common CIMT (mm)	0.72 (0.11)	0.72 (0.10)	0.73 (0.12)	0.72 (0.11)
Mean Max CIMT (mm)	1.097 (0.22)	1.11 (0.22)	1.09 (0.22)	1.1 (0.22)

Table 2 Compliance to treatment, by assigned treatment

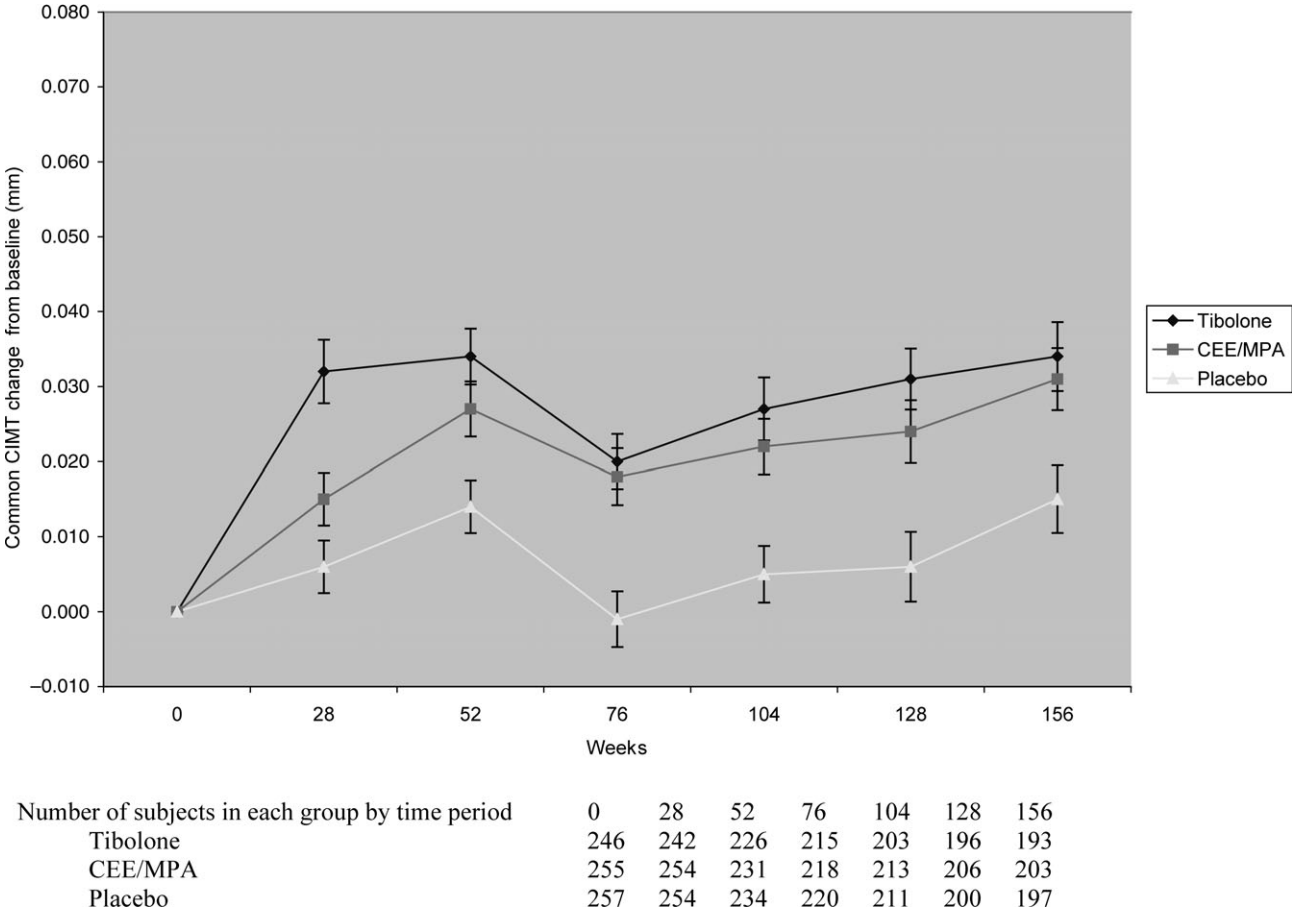
	Tibolone	CEE/MPA	Placebo	Overall
Randomized (%)	290 (100%)	288 (100%)	288 (100%)	866 (100%)
ITT for CIMT[a]	247 (85%)	255 (89%)	257 (89%)	759 (89%)
Completing 1 year with treatment	227 (78%)	228 (79%)	230 (80%)	685 (80%)
Completing 2 years with treatment	207 (72%)	217 (75%)	209 (73%)	633 (73%)
Completing 3 years with treatment	198 (68%)	204 (71%)	201 (70%)	603 (70%)

Values are number of subjects with percentages in parentheses.
[a]ITT, with at least one CIMT measurement at follow-up.

Table 3 Mean (95% CI) progression rate of the mean common CIMT and the progression rate of the MeanMax CIMT, by treatment group (ITT)

Parameter		Estimate (mm/year)	95% CI (mm/year)	*P*-value[a]
Common CIMT progression rate	Tibolone 2.5 mg	0.0077	(0.0051–0.0103)	
	CEE/MPA	0.0074	(0.0048–0.0099)	
	Placebo	0.0035	(0.0009–0.0061)	
Difference in progression rate compared to placebo	Tibolone 2.5 mg	0.0042	(0.0005–0.0079)	0.03
	CEE/MPA	0.0039	(0.0003–0.0075)	0.04
MeanMax CIMT progression rate	Tibolone 2.5 mg	0.0033	(−0.0021–0.0086)	
	CEE/MPA	−0.0017	(−0.0070–0.0037)	
	Placebo	0.0016	(−0.0038–0.0070)	
Difference in progression rate compared to placebo	Tibolone 2.5 mg	0.0017	(−0.0059–0.0093)	0.67
	CEE/MPA	−0.0033	(−0.0109–0.0043)	0.40

CEE/MPA = 0.625/2.5 mg.
[a]Pairwise comparison of the regression rate with respect to placebo. *P*-value is obtained from Laird–Ware model with slope and intercept as random effects.



Number of subjects in each group by time period	0	28	52	76	104	128	156
Tibolone	246	242	226	215	203	196	193
CEE/MPA	255	254	231	218	213	206	203
Placebo	257	254	234	220	211	200	197

Figure 2 The effects of 3 years treatment with tibolone, CEE/MPA, and placebo on common CIMT in post-menopausal women (mean ± SE). The data are crude (no adjustments were made).

ratios of progression rate in the treatment group and in the placebo group seemed identical across strata of age (below or above 60). The ratios, however, were higher among women with higher baseline HDL levels compared to women with lower HDL levels, in women with a higher baseline LDL levels compared to women with lower LDL baseline levels, in women with a lower BMI compared to women with higher BMI levels and in women with a longer time since menopause compared to women with a short time since menopause (*Table 5*).

The effect of tibolone and CEE/MPA on cardiovascular risk factors is presented in *Table 6*. The most notable differences between both treatment arms were the effects on weight, LDL cholesterol, HDL cholesterol, and triglycerides.

Table 4 Unadjusted progression rate of the mean common CIMT and of mean maximum CIMT, by treatment group (ITT) and continent

	Europe					United States				
	Mean (mm/year)	95% CI	Difference in progression with placebo (mm/year)		*P*-value	Mean (mm/year)	95% CI	Difference in progression with placebo (mm/year)		*P*-value[a]
			Mean	95% CI				Mean	95% CI	
Common Carotid IMT										
Placebo	−0.0024	−0.0057–0.0010				0.0122	0.0085–0.0158			
CEE/MPA	0.0033	−0.0001–0.0067	0.0057	0.0009–0.0105	0.021	0.0131	0.0095–0.0166	0.0009	−0.0042–0.0059	0.730
Tibolone	0.0043	0.0009–0.0078	0.0067	0.0019–0.0115	0.006	0.0124	0.0087–0.0160	0.0002	−0.0050–0.0053	0.948
MeanMax Carotid IMT										
Placebo	−0.0039	−0.0111–0.0033				0.0122	0.0048–0.0196			
CEE/MPA	0.0040	−0.0113–0.0032	0.0001	−0.0103–0.0101	0.978	0.0034	−0.0037–0.0105	0.0089	−0.0019–0.0014	0.090
Tibolone	0.0031	−0.0042–0.0104	0.0069	−0.0032–0.0173	0.181	0.0035	−0.0037–0.0107	0.0087	−0.0019–0.0016	0.098

CEE/MPA = 0.625/2.5 mg.
[a]Pairwise comparison of the regression rate with respect to placebo. *P*-value is obtained from Laird–Ware model with slope and intercept as random effects.

A total of 114 women had a serious adverse event during the treatment period (44 in the tibolone group, 31 in the CEE/MPA group, and 39 in the placebo group). Most were musculoskeletal disorders, reflecting a variety of symptoms ranging from meniscus lesions, sprained ankles, fractures, and removal of osteosynthesis material. Nine subjects were diagnosed with a malignant breast neoplasm (four in the tibolone group, one in the CEE/MPA group, and four in the placebo group) and two were diagnosed with uterine cancer (one in the tibolone group and one in the placebo group). Cardiovascular events (rhythm disturbances, angina pectoris, coronary heart disease, transient ischemic attacks, stroke, pulmonary embolism, and venous thrombosis) occurred in five subjects in the tibolone group, nine in the CEE/MPA group, and four in the placebo group. The frequency of adverse events leading to discontinuation of the study was 22.7% in the tibolone group, 22.9 % in the CEE/MPA, and 16.4% in the placebo group. Most of these discontinuations were because of headache, breast pain, and vaginal bleeding. Two women died during the study, both had been assigned to the placebo group.

Discussion

The OPAL study is a randomized controlled trial directly assessing the effect of tibolone and CEE/MPA, regimes currently available to alleviate post-menopausal symptoms, on progression of atherosclerosis, as assessed with common CIMT. From our findings, it is apparent that both tibolone and CEE/MPA lead to an increased progression of common CIMT compared with placebo, which reached statistical significance.

Several issues regarding the design and findings in the study need to be addressed. First, in the OPAL study, progression of common CIMT was chosen as the primary endpoint as a measure of atherosclerosis and a proxy for cardiovascular risk. This choice was based on the need to examine the relevance of HDL changes induced by tibolone with respect to atherosclerosis progression and on the view that a change in common CIMT predicts cardiovascular disease. Several lines of evidence favour this latter notion. Observational studies have uniformly shown that increased common CIMT is related to an increased risk of vascular disease.[14] Also, progression of common CIMT has been shown to predict future cardiovascular events.[15] In addition, the guidelines of the AHA working group on use of non-invasive techniques to study effects of interventions recommend CIMT measurements as a proxy for atherosclerosis and an alternative outcome for vascular events.[16] Although progression of the common CIMT has been the most frequently used measure, some preference has been given to the progression of the meanMax CIMT as there is a trend towards greater predictability when data from all segments are used.[17] Considered together, our results for the common CIMT and meanMAX CIMT outcomes provide strong evidence that neither CEE/MPA nor tibolone favourably affect atherosclerosis and cardiovascular risk in healthy post-menopausal women. Secondly, the CIMT ITT analysis was based on a smaller number of subjects than was initially randomized. This was due to discontinuing subjects who mostly withdrew within 3 months after randomization. Although the OPAL study was designed to record as many CIMT measurements as possible from those who withdrew

Table 5 Mean (95% CI) progression rate of the mean common CIMT by treatment group and in strata of several risk factors (ITT)

Parameter	Tibolone (2.5 mg)				CEE/MPA				Placebo			
	n	Estimate	CI		*n*	Estimate	CI		*n*	Estimate	CI	
			Lower	Upper			Lower	Upper			Lower	Upper
Statin (yes)	20	0.0215	0.0102	0.0327	13	−0.0040	−0.0183	0.0103	19	−0.0009	−0.0125	0.0108
Statin (no)	227	0.0062	0.0036	0.0088	242	0.0081	0.0056	0.0106	238	0.0039	0.0013	0.0065
Baseline LDL ≤ 3.37 mmol/L*	68	0.0063	0.0018	0.0108	63	0.0113	0.0067	0.0159	68	0.0082	0.0035	0.0129
Baseline LDL > 3.37 mmol/L	177	0.0082	0.0050	0.0113	189	0.0061	0.0030	0.0091	189	0.0019	−0.0012	0.0049
Baseline HDL ≤ 1.55 mmol/L*	131	0.0098	0.0058	0.0139	119	0.0104	0.0061	0.0147	118	0.0068	0.0025	0.0110
Baseline HDL > 1.55 mmol/L	114	0.0052	0.0020	0.0085	134	0.0052	0.0022	0.0081	139	0.0005	−0.0025	0.0036
Hysterectomy (yes)	49	0.0087	0.0015	0.0159	44	0.0043	−0.0032	0.0118	36	−0.0028	−0.0117	0.0061
Hysterectomy (no)	198	0.0074	0.0046	0.0101	211	0.0081	0.0054	0.0107	221	0.0045	0.0018	0.0071
Baseline BMI ≤ 25 kg/m^2	123	0.0058	0.0021	0.0096	121	0.0051	0.0014	0.0088	136	0.0014	−0.0022	0.0050
Baseline BMI > 25 kg/m^2	124	0.0095	0.0059	0.0131	134	0.0096	0.0061	0.0131	121	0.0059	0.0022	0.0096
Age ≤60 years	155	0.0060	0.0031	0.0088	163	0.0067	0.0039	0.0095	153	0.0027	0.0002	0.0057
Age >60 years	92	0.0109	0.0058	0.0161	92	0.0085	0.0035	0.0136	104	0.0046	0.0001	0.0092
Time since menopause ≤10 years	129	0.0073	0.0041	0.0105	134	0.0062	0.0030	0.0093	135	0.0039	0.0007	0.0071
Time since menopause >10 years	117	0.0084	0.0042	0.0126	120	0.0088	0.0046	0.0129	120	0.0033	0.0009	0.0076

*LDL level of 3.367 mmol/L = 130 mg/dL; HDL level of 1.554 mmol/L = 60 mg/dL. Because of the relatively small number of subjects in some strata, the results can be influenced by 'outlying' data, and thus care should be exercised when interpreting these results.

Table 6 Percentage change in cardiovascular risk factor from baseline, by treatment group

Risk factor	Tibolone Mean (95% CI)	CEE/MPA Mean (95% CI)	Placebo Mean (95% CI)
Weight (kg)	2.6 (1.76 to 3.40)	1.4 (0.52 to 2.24)	1.5 (0.53 to 2.45)
Systolic blood pressure (mmHg)	−0.5 (−2.31 to 1.31)	−0.7 (−2.47 to 1.07)	−0.7 (−2.48 to 1.08)
Diastolic blood pressure (mmHg)	−2.0 (−3.96 to −0.04)	−1.7 (−3.75 to 0.35)	−1.8 (−3.84 to 0.24)
Heart rate (b.p.m.)	−1.1 (−3.53 to 1.33)	0.9 (−1.09 to 2.89)	−0.8 (−2.97 to 1.37)
Total cholesterol (mmol/L)	−9.3[a] (−11.4 to −7.26)	−8.1[a] (−10.3 to −6.04)	−2.5 (−4.37 to −0.73)
LDL cholesterol (mmol/L)	−1.2 (−4.31 to 1.91)	−19.6[a] (−22.9 to −16.3)	−3.5 (−6.37 to −0.59)
HDL cholesterol (mmol/L)	−21.7[a] (−24.3 to −19.2)	9.2[a] (6.59 to 11.79)	1.2 (−1.46 to 3.80)
Triglycerides (mmol/L)	−10.1[a] (−16.2 to −4.06)	38.5[a] (30.51 to 46.51)	13.6 (7.64 to 19.66)
Glucose (mmol/L)	−5.7 (−7.69 to −3.73)	−3.1 (−4.50 to −1.68)	−1.7 (−3.43 to 0.05)

[a]Significantly different from placebo.

from the study, we decided *a priori* that a subject should have been in the study for at least 6 months in order to have a close out CIMT scan performed. In order to examine whether withdrawal (i.e. absence of a post-baseline CIMT measurements) might have affected our results, we studied whether baseline characteristics, risk factors for CIMT progression, and assignment of treatment were related to the presence or absence of a post-baseline CIMT measurement. In these analyses (data not shown), neither the baseline characteristics and risk factors nor the treatment assignment was related to the absence of a post-baseline CIMT measurement. These results suggest that any bias in the final study findings that might have come from excluding subjects with no post-baseline measurement is regarded as minimal, and thus we do not believe that this has affected the validity of the findings. Finally, the OPAL study is the first study on CIMT progression using two core laboratories (one in the US and one in Europe). Although we used an identical ultrasound protocol and identical reading stations for off-line assessment of CIMT, some differences may have remained. Europeans readers read 0.07 mm thicker than the US readers with an ICC of 0.70. Data from repeat readings within each core laboratory were inconsistent, but suggest that there may have been some temporal drift in the measurement process, either due to changes in the behaviour of individual readers over time or due to turnover in the pool of certified readers from the beginning to the end of the study. Although such a temporal change may affect the absolute magnitude of progression rates, it is unlikely to bias the treatment comparisons, as the phenomenon is randomly distributed across treatment arms.

Previous randomized controlled trials on the effect of HT on progression of CIMT have shown neutral effects[18–20] or beneficial effects of the treatment.[21] The trials differ in populations (low risk/high risk), in time since menopause (short/long), in treatment regimen (opposed/unopposed/17 beta

oestradial), in sample size (from 35 to 181 per arm), in duration of follow-up (1–4 years), in design (single/multicentre), in used methodology to measure CIMT both in acquisition of the images (single/multiple angles) as well as off-line reading (manual/edge detection), and in primary outcome (common CIMT/meanMax CIMT). These factors limit direct comparison between studies of progression rates and treatment effect, but do not generally affect the validity of the results of each study separately. Several uncontrolled small observational studies have been performed on the effect of tibolone on CIMT. These studies showed no differences between tibolone and control groups[22,23] or beneficial effects of tibolone.[24] Thus far, the OPAL study is the only randomized controlled trial showing an increased progression of common CIMT in the treated groups compared with placebo. The OPAL study result is in agreement with results from trials that showed no effect of CEE/MPA[20] or 17 beta oestradiol[19] on meanMAx CIMT progression.

From *Figure 2*, where raw unadjusted common CIMT measurements are presented over time, it seems that most of the progression in common CIMT occurs in the first year of the study, after which progression appears to level off. One of the most intuitive reasons may be either adaptation by an effect of treatment on lumen diameter or differences in reading behaviour over time. We have, at length, tried to explain this phenomenon by looking into additional adjustments for lumen diameter, reader, and time, but were unable to fully account for the phenomenon, and it thus remains unexplained.

In clinical studies, tibolone had shown favourable effects of lipids [reduction of triglycerides, VLDL, and lipoprotein (a)], with the exception of reduction in HDL cholesterol.[9] The OPAL study was performed to evaluate whether this HDL-lowering effect of tibolone may adversely affect cardiovascular risk, as estimated by progression of common CIMT. The OPAL findings showed that common CIMT progression was similar for both tibolone and CEE/MPA treatment, even though CEE/MPA increased HDL cholesterol. Also, the progression of the meanMax CIMT did not differ between CEE/MPA or tibolone. Hence, it may be that the net effects of tibolone on risk factors are similar to the net effects of CEE/MPA with respect to atherosclerosis progression.[25] Alternatively, it may be that the effects of tibolone on HDL do not have an additional adverse atherogenic effect. Recently published HDL efflux studies suggested that the lowering of HDL cholesterol in post-menopausal women by tibolone was not associated with changes in cholesterol efflux capacity or paraoxonase activity and therefore is possibly of minor significance to cardiovascular risk.[10,26] Preclinical studies showed that there was no increase in atherosclerosis in rabbits and monkeys treated with tibolone.[27,28]

Given that increased progression of common CIMT relates to increased risk of cardiovascular events, our findings for CEE/MPA are in line with recent trials showing an increased risk of cardiovascular events in post-menopausal women treated with CEE/MPA.[29–33] In addition, our findings for meanMax CIMT agree with trials using alternative markers of cardiovascular disease risk, which have generally reported no beneficial effect of HT on coronary atherosclerosis progression,[34–36], brachial reactivity,[37–39], or CIMT progression.[18–20] Yet in the Estrogen in the Prevention of Atherosclerosis trial, a randomized, double-blind, placebo-controlled trial that evaluated the effect of unopposed micronized 17 beta oestradiol (1 mg daily) on 2-year progression of common CIMT in 222 healthy post-menopausal women without pre-existing cardiovascular disease but with LDL cholesterol levels $\geq$3.37 mmol/L, a reduced progression was found in the actively treated group.[21]

The OPAL study was not designed to examine the effect of the interventions on the occurrence of cardiovascular events. Yet, it may be of importance to relate the observed increased progression in common CIMT to the risk of cardiovascular events. The Atherosclerosis Risk in Communities (ARIC) study among women aged 45–64 showed by relating baseline common CIMT to the occurrence of coronary heart disease events that an increase in common CIMT of 0.19 mm increases risk of coronary heart disease by 92% (95% CI 66–122).[40] The observed difference in annual common CIMT progression of tibolone and CEE/MPA with placebo was 0.0042 and 0.0039 mm, respectively. On the basis of the point estimates from the ARIC study, the increase in common CIMT progression rates seen with tibolone and CEE/MPA, assuming a linear relationship between common CIMT and time, translates into a relative risk of coronary heart disease of 1.47 and 1.36% per year, respectively. The recent Women's Health Initiative (WHI) study results showed that women on CEE/MPA have a 24% increased risk of cardiovascular events over a period of 5.2 years, an average annual increased risk of 4.6%.[29] In interpreting these estimates, one should acknowledge possible differences between ARIC and OPAL that may affect estimates of CIMT and its relation to risk of events, as well as differences between WHI and OPAL in baseline characteristics that may affect the risk of cardiovascular events (WHI women were older and 18% was a minority population). For example, the risk estimate from ARIC was based on a CIMT estimate determined at one occasion with a reported ICC of around 0.60.[40] This may have lead to an underestimation of the magnitude of the risk relation. Alternatively, the relative risk estimate of CEE/MPA in WHI may have been biased upwards due to the premature end of the trial. Perhaps more important, however, is the notion that CIMT reflects atherosclerosis only and not the combination of thrombosis, inflammation, and atherosclerosis, which may eventually lead to cardiovascular events. In this respect, it may be important to note that tibolone has been shown to shift the haemostasis parameters to a more fibrinolytic profile,[41] where CEE/MPA have been shown to increase the risk for venous thrombo-embolic events.[29]

It has been suggested that the effect of HT may be modified by the duration of menopause (the shorter the menopause, the more beneficial the effect of HT).[42] Evidence to support this comes not only from observational studies,[42] but also from studies on post-menopausal cynomolgus monkeys. In the latter studies, treatment was started immediately after surgical ovariectomy.[27,43] Subgroup analyses of the WHI study showed, although not statistically significant, that among those with a menopause duration of $>$20 years CEE/MPA was related to a higher increased risk for cardiovascular disease (hazard ratio 1.71) than among those with a menopause duration $<$10 years (hazard ratio 0.89). Results from stratified analyses from the OPAL study may support that view. Moreover, this issue will be further addressed in a recently started trial

to investigate the effect of oestrogen and oestrogen–progestin on CIMT progression in women <55 years.

In conclusion, both tibolone and CEE/MPA showed increased progression of common CIMT. Translating the increased common CIMT progression of the CEE/MPA group into cardiovascular disease risk could not fully explain the observed increased cardiovascular risk in the WHI study. This suggests that the net effect of tibolone and CEE/MPA on cardiovascular events may depend on the combined effects on the arterial wall, clotting factors, and possibly inflammation.

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