

# Effect of Estrogen Therapy on Gallbladder Disease

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**C**HOLELITHIASIS, OR GALLSTONE disease, is estimated to affect between 10% and 15% of the US population, with 1 million new diagnoses yearly.<sup>1</sup> Symptoms arise in about one third of individuals with gallstones, with about 80% of symptomatic patients experiencing biliary colic.<sup>2</sup> Furthermore, cholecystitis usually arises from obstruction in the cystic duct due to gallstones.<sup>3</sup> Cholecystectomy procedures can be a good proxy indicator for symptomatic gallstone disease in clinical research.<sup>4</sup> In 2002, there were 432 000 inpatient cholecystectomies performed,<sup>5</sup> and the estimated total number of cholecystectomies, including laparoscopic procedures, numbered as high as 770 000 in 1996,<sup>6</sup> which has been associated with an expense of more than \$2 billion annually.<sup>7</sup>

Gallstone formation is thought to rely on 3 factors: supersaturation of biliary cholesterol due to hepatic hypersecretion, nucleation of cholesterol monohydrate crystals, and gallbladder hypomotility.<sup>7,8</sup> The liver has estrogen receptors, and the presence of endogenous estrogens causes cholesterol saturation in the bile, inhibition of chenodeoxycholic acid secretion, and increased cholic acid content.<sup>9</sup> Progestins inhibit gallbladder contraction, encourage bile stasis, and have been

**Context** Estrogen therapy is thought to promote gallstone formation and cholecystitis but most data derive from observational studies rather than randomized trials.

**Objective** To determine the effect of estrogen therapy in healthy postmenopausal women on gallbladder disease outcomes.

**Design, Setting, and Participants** Two randomized, double-blind, placebo-controlled trials conducted at 40 US clinical centers. The volunteer sample was 22 579 community-dwelling women aged 50 to 79 years without prior cholecystectomy.

**Intervention** Women with hysterectomy were randomized to 0.625 mg/d of conjugated equine estrogens (CEE) or placebo (n=8376). Women without hysterectomy were randomized to estrogen plus progestin (E+P), given as CEE plus 2.5 mg/d of medroxyprogesterone acetate (n=14 203).

**Main Outcome Measures** Participants reported hospitalizations for gallbladder diseases and gallbladder-related procedures, with events ascertained through medical record review. Cox proportional hazards regression was used to assess hazard ratios (HRs) and 95% confidence intervals (CIs) using intention-to-treat and time-to-event methods.

**Results** The CEE and the E+P groups were similar to their respective placebo groups at baseline. The mean follow-up times were 7.1 years and 5.6 years for the CEE and the E+P trials, respectively. The annual incidence rate for any gallbladder event was 78 events per 10 000 person-years for the CEE group (vs 47/10 000 person-years for placebo) and 55 per 10 000 person-years for E+P (vs 35/10 000 person-years for placebo). Both trials showed greater risk of any gallbladder disease or surgery with estrogen (CEE: HR, 1.67; 95% CI, 1.35-2.06; E+P: HR, 1.59; 95% CI, 1.28-1.97). Both trials indicated a higher risk for cholecystitis (CEE: HR, 1.80; 95% CI, 1.42-2.28; E+P: HR, 1.54; 95% CI 1.22-1.94); and for cholelithiasis (CEE: HR, 1.86; 95% CI, 1.48-2.35; E+P: HR, 1.68; 95% CI, 1.34-2.11) for estrogen users. Also, women undergoing estrogen therapy were more likely to receive cholecystectomy (CEE: HR, 1.93; 95% CI, 1.52-2.44; E+P: HR, 1.67; 95% CI, 1.32-2.11), but not other biliary tract surgery (CEE: HR, 1.18; 95% CI, 0.68-2.04; E+P: HR, 1.49; 95% CI, 0.78-2.84).

**Conclusions** These data suggest an increase in risk of biliary tract disease among postmenopausal women using estrogen therapy. The morbidity and cost associated with these outcomes may need to be considered in decisions regarding the use of estrogen therapy.

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shown to decrease the gallbladder's response to cholecystokinin.<sup>9</sup> One study found that exogenous estrogens, given either transdermally or orally, affected physiologic markers in a pattern that favored gallstone formation.<sup>10</sup>

Observational evidence suggests that estrogen therapy, including the use of oral contraception and postmenopausal estrogen therapy, is an impor-

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tant risk factor for gallbladder disease.<sup>8</sup> Observational studies indicate up to a 2.5-fold increased risk of biliary tract conditions related to estrogen therapy.<sup>11-16</sup> To our knowledge, the only previous randomized trial examining biliary tract outcomes after estrogen therapy in postmenopausal women was the Heart and Estrogen/progestin Replacement Study (HERS), which included women with known cardiovascular disease. HERS data revealed a 38% increased risk for biliary tract surgery in the estrogen therapy group.<sup>4</sup> Thus, limited clinical trial evidence is available to provide unbiased estimates of the true effects and risks of estrogen therapy in postmenopausal women.

The Women's Health Initiative (WHI) postmenopausal hormone trial consisted of 2 randomized components, in which women with hysterectomy were randomized to receive estrogen alone or placebo, and those without hysterectomy received either a combination of estrogen and progestin (E+P) or placebo.<sup>17</sup> The major findings of these trials have been previously reported.<sup>18,19</sup> Together, these trials represent the largest randomized, double-blind study of hormone use in postmenopausal women, and offer the most reliable and current documentation of the full risk/benefit profile of the use of hormone therapy in postmenopausal women. This report provides new evidence on the effect of estrogen therapy, with or without progestin, on gallbladder disease incidence and gallbladder surgical outcomes.

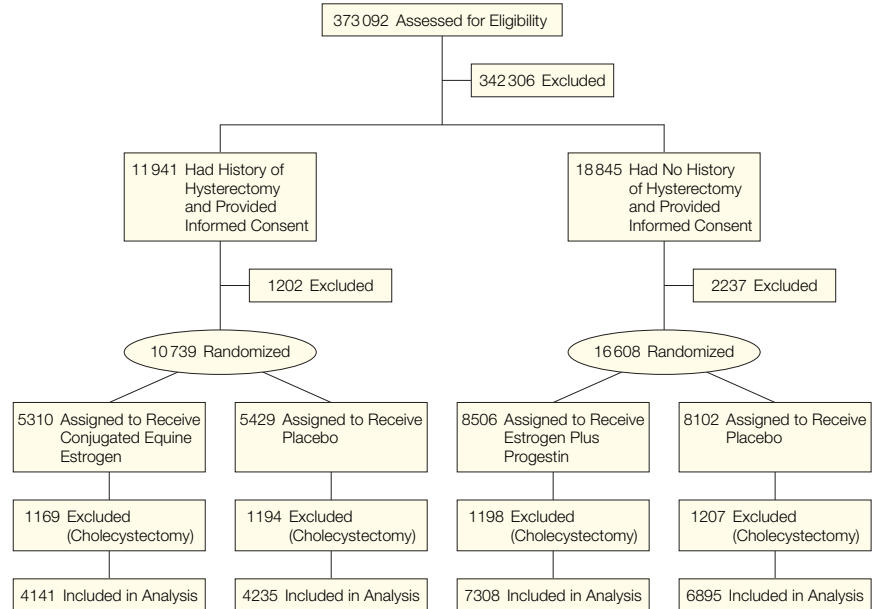
## METHODS

### Participants

This presentation is based on information from the estrogen therapy portions of the WHI clinical trials, although 29% were simultaneously enrolled in the dietary modification arm and 59% in the calcium/vitamin D arm of this complex trial. A detailed discussion of the methods of the WHI trials and the principal findings of this trial are available elsewhere.<sup>19,20</sup>

Women were recruited into the trial at 40 clinical centers, largely via direct

**Figure 1.** Profile of the Estrogen Therapy Arms of the Women's Health Initiative



Individuals were excluded prior to randomization for safety reasons such as history of breast cancer (4.5%), or clinic staff impression that a woman was not a good candidate for the study (4.8%). Some women were not included in the trial if the stratum was closed (4.6%). The majority of exclusions were due to lack of interest or no informed consent for the estrogen therapy component of the Women's Health Initiative (81.2%).

mail. Participants ( $n = 22\,579$  community-dwelling women) were aged 50 to 79 years at the initial screening visit and they provided written informed consent. The National Institutes of Health and institutional review boards for all participating institutions approved the WHI protocols and consent forms. The screening and enrollment algorithm is shown in FIGURE 1.

Women were excluded if they had any illness that suggested less than a 3-year survival, had a prior cholecystectomy or gallbladder disease, had a contraindication to using the study medications, or were deemed to be at risk of poor medication adherence. Women with hysterectomy were eligible for the estrogen-alone trial, but women without hysterectomy were eligible for the E+P trial due to the concerns about risk of endometrial cancer. Eligible participants were randomized in equal proportions to receive estrogen therapy or placebo using computers and a stratified permuted block

algorithm.<sup>20,21</sup> Women in the estrogen alone arm received either placebo or 0.625 mg/d of conjugated equine estrogens (CEE) (Premarin; Wyeth, Philadelphia, Pa) using blinded dispensing. Women in the E+P trial received either placebo or 0.625 mg/d of CEE plus 2.5 mg/d of medroxyprogesterone acetate, administered as a single tablet (Prempro; Wyeth).

Reductions in dosage or frequency were permitted in order to alleviate unacceptable adverse effects, although dosage alterations were generally conducted without unblinding the patients or study gynecologists. In some cases, an unblinding officer provided the clinic gynecologist with the treatment assignment if it was needed due to safety or symptom management. Although the amount of unblinding that may have occurred with the participants is unmeasured, the clinic gynecologists were unblinded at rates of 1.9% and 1.5% for the assigned treatment and placebo groups for the CEE

trial, and 40.5% and 6.8% for the E+P trial. Clinic gynecologists were not involved in the assessment of outcomes.

Study participants were contacted by telephone 6 weeks after randomization to assess symptoms and to reinforce adherence. Follow-up for clinical events was undertaken every 6 months with a mandatory clinic visit annually. At each semiannual contact, standardized information on specific symptoms and safety concerns, as well as major health events, was collected. As described in detail, the E+P trial was discontinued after a recommendation from the data and safety monitoring board concluded that the overall harms outweighed the benefits of receiving E+P.<sup>19</sup>

### Study Population, Data Collection, and Outcomes

The original publication of global findings from the estrogen-alone trial described the results for 5310 women in the CEE group and 5429 in the placebo group.<sup>18</sup> The original publication of the E+P trial reported the findings of 8506 women receiving E+P and 8102 receiving placebo.<sup>19</sup> This total population from both hormone studies comprise the analytic groups for this report, except that those reporting a history of gallbladder disease or cholecystectomy at entry were eliminated from the analyses, resulting in the exclusion of 1169 women in the CEE group and 1194 in the placebo group for the estrogen-alone trial, as well as 1198 women in the active hormone arm and 1207 in the placebo arm of the E+P trial.

At baseline, an extensive history was collected in a standardized fashion using self-administered forms, interviews, and clinical examinations. Weight and height were measured using a calibrated balance beam scale and a wall-mounted stadiometer. Body mass index (BMI) was calculated as weight in kilograms divided by the square of height in meters. Race and ethnicity were self-reported using predefined categories and were used to explore for treatment effect differences in subgroup analyses. Other baseline variables, including clinical, demographic, social,

and behavioral characteristics, were obtained from self-administered, structured questionnaires.<sup>20</sup>

### Main Outcome Measures

Gallbladder and gallstone disease outcomes were directly reported by the participants in the semiannual safety monitoring questionnaire. When participants noted any of these outcomes, medical and hospital records were obtained with participant consent. Completion rates for clinical record acquisition and adjudication were estimated overall to be about 96%. Outcomes used in this analysis were taken from *International Classification of Diseases-Ninth Revision* and *Current Procedural Terminology* codes. A global outcome for surgical procedures was constructed to include all gallbladder and biliary tract procedures except diagnostic procedures and those that were solely related to the pancreas; this category included *Current Procedural Terminology* codes (51.0-51.04, 51.2-51.81 and 51.84-51.99). The vast majority of the procedures were cholecystectomies. Similarly, a global disease index was constructed using *International Classification of Diseases-Ninth Revision* codes from medical and hospital records that included all designations of acute or chronic gallbladder inflammation (574.0-574.2, 574.3-574.41, 574.6-574.81, and 575.0-575.12) and all gallbladder or biliary tract stone disease (codes 574-574.91). In most instances, participants with a disease code also had a procedural code. In all of the analyses presented here, an individual in an outcome category is only counted once, regardless of the number of related codes present.

### Statistical Analyses

This study was a secondary analysis of a large trial designed and sufficiently powered to detect clinically relevant changes in the primary outcomes of coronary heart disease, hip fracture, and breast cancer, but the study was not powered for gallbladder outcomes, as these were not the primary end points for the study. Associations between

baseline variables and randomization assignment were assessed using *P* values from  $\chi^2$  tests for categorical variables and 2-sample *t* tests for continuous variables. All statistical analyses were conducted using SAS statistical software version 9.0 (SAS Institute Inc, Cary, NC). All outcomes analyses used time-to-event methods and were based on the intention-to-treat principle; all tests used a .05 level of significance. Outcome comparisons are presented as hazard ratios (HRs) and nominal 95% confidence intervals (CIs) from Cox proportional hazards models, stratified by age, and by the dietary modification and/or calcium/vitamin D trial randomization arm. Since calcium/vitamin D participants were randomized to the calcium/vitamin D trial 1-2 years after randomization in the hormone program, adjustment for calcium/vitamin D trial participants used the calcium/vitamin D randomization date as the only time-dependent covariate in the main analyses. The proportionality assumption was tested by including a time  $\times$  treatment interaction term in the Cox model, in addition to the treatment indicator. No adjustments were made for multiple tests over time, as this adjustment would be too conservative; however, we included a Bonferroni-adjusted 95% CI for multiple end points. We calculated the number needed to harm as the reciprocal of the absolute risk difference between taking the active drug and control.

Kaplan-Meier plots describe gallbladder disease and procedure event rates over time. Sensitivity analyses examining the effect of nonadherence were conducted by repeating these analyses after censoring events occurring 6 months after a participant became nonadherent (prospectively defined for adherence monitoring purposes as consuming <80% of study pills as determined by pill count or starting non-study prescribed hormone therapy during the most recent 6-month study interval). Therefore, a treatment indicator was used in the sensitivity analysis as a time-dependent variable.

Potential effect modification with participant baseline characteristics including recognized gallbladder disease risk factors was assessed in expanded proportional hazards models that included the designated risk factor and randomization assignment as main effects and the interaction between them. *P* values for possible interactions were computed from likelihood ratio tests, comparing models with and without the interaction term. Women with missing values for the risk factors in a given analy-

sis were excluded from the analysis. Subgroup comparisons were tested for potential effect modification including age, BMI, physical activity, prior hormone or aspirin use, non-aspirin analgesics use (including nonsteroidal anti-inflammatory medicines), or cholesterol-lowering statins at baseline. Additionally, race/ethnicity subgroups were examined without statistical testing due to small numbers in some categories. As a result, less than 1 of the 7 tests for both the CEE trial and the E + P trial would be

expected to be significant at the .05 level by chance alone.

## RESULTS

Baseline characteristics for the entire study cohort were presented elsewhere.<sup>17,19</sup> A total of 22 579 women were analyzed, 8376 in the CEE arm and 14 203 in the E + P arm. The proportion of women who were excluded from the analysis based on history of a cholecystectomy was higher for the CEE trial compared with the E + P trial (22.0% vs

**Table 1.** Baseline Descriptive Characteristics of the Women's Health Initiative

Characteristics	No. of Participants (%)*			
	CEE Trial		E + P Trial	
	CEE (n = 4141)	Placebo (n = 4235)	E + P (n = 7308)	Placebo (n = 6895)
Age at screening, mean (SD), y	63.4 (7.2)	63.5 (7.4)	63.1 (7.1)	63.2 (7.1)
50-59	1309 (31.6)	1346 (31.8)	2502 (34.2)	2354 (34.1)
60-69	1854 (44.8)	1913 (45.2)	3282 (44.9)	3086 (44.8)
70-79	978 (23.6)	976 (23.0)	1524 (20.9)	1455 (21.1)
Race/ethnicity				
White	3083 (74.5)	3130 (73.9)	6133 (83.9)	5796 (84.1)
Black	675 (16.3)	726 (17.1)	491 (6.7)	519 (7.5)
Hispanic	217 (5.2)	230 (5.4)	377 (5.2)	319 (4.6)
American Indian	28 (0.7)	26 (0.6)	19 (0.3)	26 (0.4)
Asian/Pacific Islander	77 (1.9)	67 (1.6)	179 (2.4)	156 (2.3)
Unknown	61 (1.5)	56 (1.3)	109 (1.5)	79 (1.1)
Family income, US \$				
<10 000	321 (8.2)	339 (8.5)	359 (5.2)	329 (5.0)
10 000-19 000	750 (19.2)	750 (18.8)	1026 (14.9)	919 (14.1)
20 000-34 999	1168 (29.8)	1133 (28.4)	1902 (27.5)	1745 (26.8)
35 000-49 999	735 (18.8)	794 (19.9)	1462 (21.2)	1382 (21.2)
50 000-74 999	588 (15.0)	592 (14.9)	1222 (17.7)	1213 (18.6)
≥75 000	353 (9.0)	376 (9.4)	935 (13.5)	929 (14.3)
Education				
0-8 y	126 (3.1)	99 (2.4)	162 (2.2)	138 (2.0)
Some high school	260 (6.3)	276 (6.6)	290 (4.0)	293 (4.3)
High school diploma	948 (23.1)	898 (21.4)	1356 (18.7)	1312 (19.2)
School after high school	1722 (42.0)	1832 (43.6)	2898 (39.9)	2585 (37.8)
College degree or higher	1040 (25.4)	1097 (26.1)	2563 (35.3)	2519 (36.8)
BMI, mean (SD)†	29.6 (6.0)	29.7 (6.2)	28.1 (5.7)	28.1 (5.7)
Normal (<25.0)	959 (23.3)	933 (22.2)	2375 (32.6)	2273 (33.2)
Overweight (25-<30)	1461 (35.5)	1546 (36.7)	2629 (36.1)	2455 (35.8)
Obese (>30)	1700 (41.3)	1731 (41.1)	2271 (31.2)	2126 (31.0)
Waist/hip ratio, mean (SD)	0.8 (0.1)	0.8 (0.1)	0.8 (0.1)	0.8 (0.1)
Glasses of alcohol/wk				
None	535 (13.1)	550 (13.1)	809 (11.1)	783 (11.5)
Past consumption	955 (23.3)	945 (22.5)	1177 (16.2)	1118 (16.3)
<1/mo	591 (14.4)	580 (13.8)	1003 (13.8)	931 (13.6)
<1/wk	780 (19.0)	830 (19.7)	1466 (20.2)	1277 (18.7)
1-<7 wk	836 (20.4)	905 (21.5)	1856 (25.6)	1780 (26.0)
≥7/wk	402 (9.8)	395 (9.4)	945 (13.0)	949 (13.9)

(continued)

14.5%,  $P < .001$ ). The treatment and control groups were balanced on key baseline demographic and disease risk factors (TABLE 1). The only variable that was significantly different ( $P < .05$ ) between the placebo and the E+P treatment group was the frequency of moderate to strenuous exercise (Table 1), although the differences were small and the statistical significance was driven by the large number of women enrolled in the trial. Past or current use of hormone therapy was reported by 26% of

the women at the start of the E+P study, whereas 47% of women with hysterectomy had used hormone therapy. A 3 month wash-out period was required by current users before being eligible for randomized treatment assignment. Among prior hormone users, most use was of short duration ( $< 5$  y). More than 30% of the participants were obese ( $BMI \geq 30$ ) and another 36% were overweight ( $BMI 25 - < 30$ ) among women without hysterectomy, but the women with hysterectomy had an obesity rate

of 41% with an additional 36% overweight.

The estrogen-alone trial was stopped February 29, 2004, with a mean follow-up period of 7.1 years and a maximum of 10.2 years,<sup>18</sup> with comparable adherence between the CEE and the placebo group (57% each). As the E+P trial was halted early on July 7, 2002, the mean length of follow-up in this analysis was 5.6 years for participants who received placebo and 5.7 years for participants who received the as-

**Table 1.** Baseline Descriptive Characteristics of the Women's Health Initiative (cont)

Characteristics	No. of Participants (%)*			
	CEE Trial		E + P Trial	
	CEE (n = 4141)	Placebo (n = 4235)	E + P (n = 7308)	Placebo (n = 6895)
Cups of caffeinated coffee/d				
None	1500 (36.5)	1533 (36.6)	2400 (33.1)	2299 (33.6)
1	640 (15.6)	658 (15.7)	980 (13.5)	882 (12.9)
2-3	1404 (34.2)	1378 (32.9)	2622 (36.2)	2484 (36.3)
4-5	416 (10.1)	445 (10.6)	893 (12.3)	884 (12.9)
$\geq 6$	144 (3.5)	172 (4.1)	347 (4.8)	295 (4.3)
Smoking				
Never	2117 (51.6)	2099 (50.1)	3584 (49.5)	3409 (50.1)
Past	1557 (38.0)	1635 (39.0)	2887 (39.9)	2672 (39.3)
Current	426 (10.4)	456 (10.9)	770 (10.6)	720 (10.6)
Episodes/wk of moderate or strenuous activity $> 20$ min				
None	817 (21.5)	769 (20.1)	1162 (17.6)	1097 (17.0)
Some	1696 (44.5)	1786 (46.7)	2824 (42.9)	2766 (42.8)
2- $< 4$	579 (15.2)	543 (14.2)	1084 (16.5)	983 (15.2)
$\geq 4$	716 (18.8)	727 (19.0)	1518 (23.0)	1618 (25.0)
Hormone use				
Never	2189 (52.9)	2210 (52.2)	5397 (73.9)	5132 (74.5)
Past	1445 (34.9)	1466 (34.6)	1417 (19.4)	1336 (19.4)
Current†	507 (12.2)	557 (13.2)	491 (6.7)	424 (6.2)
Duration of prior hormone use, y				
$< 5$	1058 (25.5)	1089 (25.7)	1308 (17.9)	1239 (18.0)
5- $< 10$	352 (8.5)	385 (9.1)	372 (5.1)	312 (4.5)
$\geq 10$	542 (13.1)	551 (13.0)	231 (3.2)	211 (3.1)
Duration of oral contraceptive use, y				
Nonuser	2524 (61.0)	2624 (62.0)	4124 (56.4)	3939 (57.2)
$< 5$	947 (22.9)	909 (21.5)	1699 (23.3)	1489 (21.6)
5- $< 10$	375 (9.1)	366 (8.6)	714 (9.8)	700 (10.2)
$\geq 10$	290 (7.0)	334 (7.9)	769 (10.5)	764 (11.1)
Medication use				
Nonaspirin analgesics§	1228 (29.7)	1287 (30.4)	1734 (23.7)	1701 (24.7)
Aspirin	886 (21.4)	911 (21.5)	1565 (21.4)	1521 (22.1)
Statin	305 (7.4)	330 (7.8)	487 (6.7)	428 (6.2)

Abbreviations: BMI indicates body mass index, calculated as weight in kilograms divided by the square of height in meters; CEE, conjugated equine estrogens; E + P, estrogen plus progestin.

\*Subgroup totals may not sum to number randomized because of missing data.

†Means tested on the log scale.

‡A 3-month washout period was required prior to randomization.

§Includes use of acetaminophen, ibuprofen, and other anti-inflammatory medications, but does not include aspirin.

signed treatment, with a maximum of 8.6 years at the time of trial closure (TABLE 2). Forty-two percent of those randomized to E+P and 38% of those randomized to placebo were noncompliant at some time during the follow-up period.

For the CEE trial, the annual incidence rate for any gallbladder event (cholecystitis, cholelithiasis, or cholecystectomy) was 78 per 10000 person-years for the CEE group compared with 47 per 10,000 person-years for the placebo group (Table 2). The observed annual incidence rate of any gallbladder event was 55 per 10000 person-years for the E+P group, compared with 35 per 10000 person-years for the placebo group (Table 2). Receipt of both CEE and E+P significantly increased the risk for gallbladder procedures, which were predominantly cholecystectomies (192 in the CEE group vs 104 in the placebo group) (HR, 1.93; nominal 95% CI, 1.52-2.44;  $P < .001$ ), and 190 cholecystectomies in the E+P vs 107 in the placebo group (HR, 1.67; nominal 95% CI, 1.32-2.11;  $P < .001$ ). No association between the randomization status and other noncholecystectomy biliary tract procedures was seen in either trial (27 procedures in the CEE vs 24 in the placebo group) (HR, 1.18; nominal 95% CI, 0.68-2.04;  $P = .56$ ), and 24 procedures in the E+P

vs 15 in the placebo group (HR, 1.49; nominal 95% CI, 0.78-2.84;  $P = .23$ ). Both hormone treatment groups presented a significant excess risk of both cholecystitis (HR, 1.80; nominal 95% CI, 1.42-2.28;  $P < .001$  for the CEE trial and HR, 1.54; nominal 95% CI, 1.22-1.94;  $P < .001$  for the E+P trial), and cholelithiasis (HR, 1.86; nominal 95% CI, 1.48-2.35;  $P < .001$  and HR, 1.68; nominal 95% CI, 1.34-2.11;  $P < .001$ ) for CEE and for E+P, respectively. No significant interactions were found with BMI, physical activity, prior hormone use, or the use of aspirin, non-aspirin analgesic medicines, or cholesterol-lowering statins at baseline (all  $P$  values for interaction  $\geq .20$ ). There was a suggestion of an increased HR with increasing age in the CEE trial ( $P = .06$ ); however, this association was not seen in the E+P trial.

The Kaplan-Meier estimates of cumulative hazards of any gallbladder procedure, any gallbladder disease event, and the global gallbladder disease or procedure measure showed a divergence starting in the first year of randomization, with CEE separating earlier (FIGURE 2 and FIGURE 3). Bonferroni-adjusted CIs were calculated to adjust for having multiple end points in the study, resulting in a 95% Bonferroni-adjusted CI, 1.26-2.21 for the global gallbladder procedures and dis-

eases; a 95% Bonferroni-adjusted CI, 1.33-2.49 for global gallbladder procedures only; and a 95% Bonferroni-adjusted CI, 1.34-2.39 for global gallbladder diseases for the CEE trial. For the E+P trial, the results were a 95% Bonferroni-adjusted CI, 1.19-2.13 for the global gallbladder procedures and diseases, 95% Bonferroni-adjusted CI, 1.19-2.24 for global gallbladder procedures only, and 95% Bonferroni-adjusted CI, 1.20-2.17 for global gallbladder diseases for the E+P trial. When women were censored 6 months after they became nonadherent to randomization assignment in the sensitivity analysis, the resultant HR for the global gallbladder procedures and diseases outcome was 2.19 (nominal 95% CI, 1.65-2.90;  $P < .001$ ) for the CEE trial (Figure 2D), and (HR, 2.24; nominal 95% CI, 1.71-2.92;  $P < .001$ ) for the E+P trial (Figure 3D).

## COMMENT

These WHI findings demonstrate, for the first time in a randomized, double-blind trial, in otherwise healthy postmenopausal women, that the risk of adverse biliary tract outcomes was substantially increased by estrogen alone or E+P, and the HRs for gallbladder events were similar in the 2 arms. These findings suggest that oral estrogens are causally associated with gallbladder dis-

**Table 2.** Incidence (Annualized Percentage) of Gallbladder Disease or Procedures by Randomization Assignment in the Women's Health Initiative Estrogen Trials

	CEE Trial				E + P Trial			
	Estrogen, No. (%) (n = 4141)	Placebo, No. (%) (n = 4235)	HR (95% CI)	P Value*	Estrogen, No. (%) (n = 4141)	Placebo, No. (%) (n = 4235)	HR (95% CI)	P Value*
Follow-up time, mean (SD), y	7.1 (1.6)	7.1 (1.6)			5.7 (1.3)	5.6 (1.3)		
Global gallbladder procedure/disease†	228 (0.78)	143 (0.47)	1.67 (1.35-2.06)	<.001	228 (0.55)	135 (0.35)	1.59 (1.28-1.97)	<.001
Global gallbladder procedure	197 (0.67)	113 (0.37)	1.82 (1.45-2.30)	<.001	196 (0.47)	113 (0.29)	1.63 (1.29-2.06)	<.001
Cholecystectomy	192 (0.65)	104 (0.34)	1.93 (1.52-2.44)	<.001	190 (0.46)	107 (0.28)	1.67 (1.32-2.11)	<.001
Other biliary tract procedures	27 (0.09)	24 (0.08)	1.18 (0.68-2.04)	.56	24 (0.06)	15 (0.04)	1.49 (0.78-2.84)	.23
Global gallbladder disease	223 (0.76)	130 (0.43)	1.79 (1.44-2.22)	<.001	223 (0.54)	130 (0.34)	1.61 (1.30-2.00)	<.001
Cholecystitis	186 (0.63)	107 (0.35)	1.80 (1.42-2.28)	<.001	192 (0.46)	117 (0.30)	1.54 (1.22-1.94)	<.001
Cholelithiasis (gallbladder or biliary calculi)	197 (0.67)	110 (0.36)	1.86 (1.48-2.35)	<.001	208 (0.50)	116 (0.30)	1.68 (1.34-2.11)	<.001

Abbreviations: CEE indicates conjugated equine estrogens; E + P, estrogen plus progestin; CI, 95% confidence intervals; HR, hazard ratios.

\* $P$  values from Cox proportional hazards analyses stratified by age and randomization status in the Women's Health Initiative Dietary Modification and Calcium and Vitamin D trials.

†Events include the first of any diagnosis of gallbladder disease including cholecystitis or calculi, as well as biliary tract procedures, including cholecystectomy.

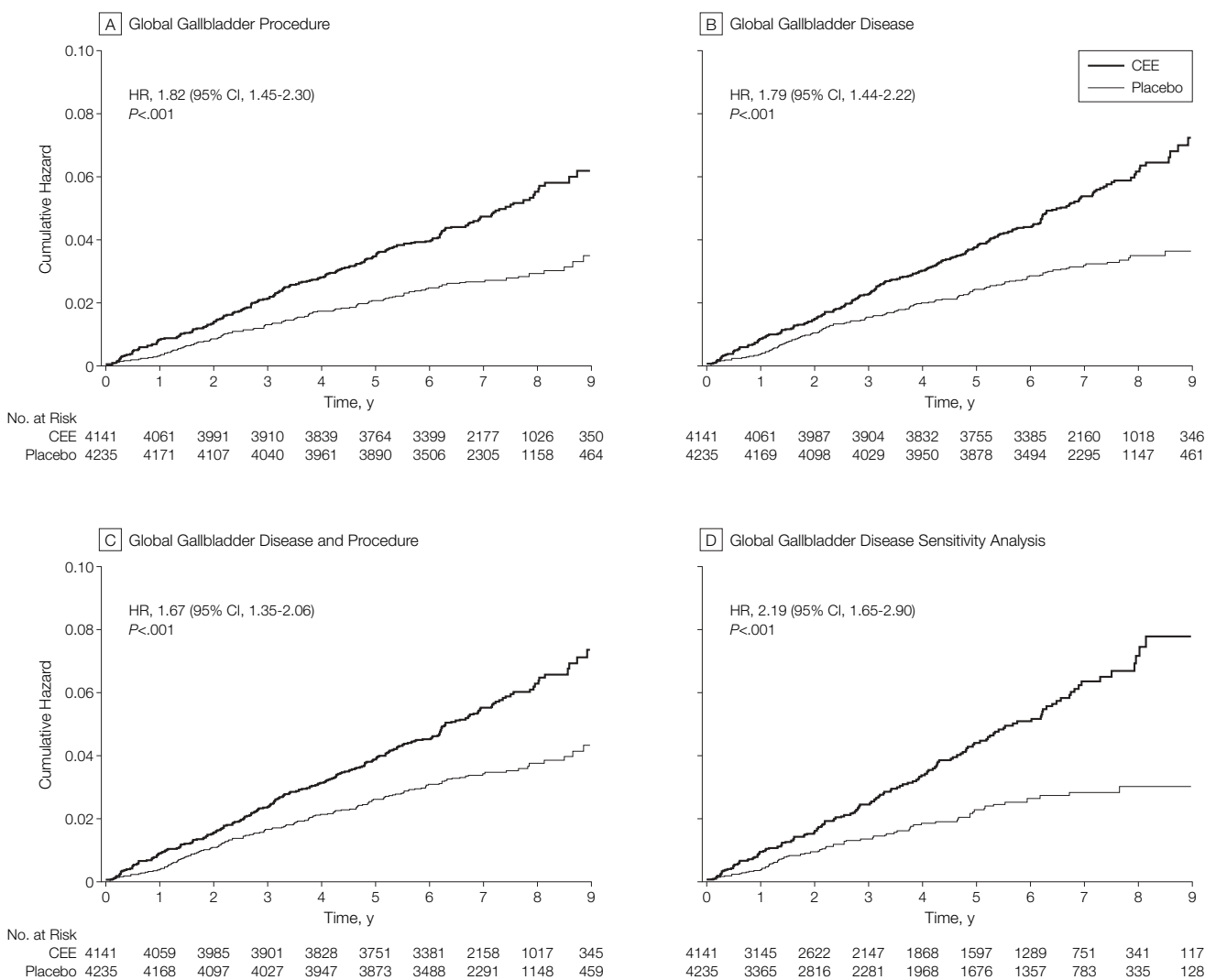
eases, and the magnitude of the effect is not influenced greatly by the presence or absence of progestins. These findings are in the same direction, but of a greater magnitude, than the findings of the HERS, the only other large randomized controlled trial of this therapy, which showed a 38% increase in hospitalizations for gallbladder disease over 4.2 years follow-up among women with known cardiovascular disease at entry.<sup>4</sup> Biliary tract surgery was more common in HERS participants, although,

with 7% of those randomized to the E+P arm having surgery vs 5% of the placebo group, and the effect of E+P appeared to diminish over time. The findings of the present study suggest that the risk continues to increase with longer exposure to E+P.

Estrogens appear to influence several key steps in gallstone formation. One study of postmenopausal women found that exogenous CEEs given orally, or estradiol given transdermally, decreased nucleation time in vitro,

increased cholesterol saturation index, and increased biliary arachidonate and prostaglandin E<sub>2</sub> levels.<sup>10</sup> The authors asserted that this pattern of findings resulted in both an increased propensity to form cholesterol crystals and an excess saturation of biliary cholesterol,<sup>10</sup> which along with hypomotility of the gallbladder are thought to be key requirements for gallstone formation.<sup>7,8</sup> The effect of estrogen therapy has also been studied in men who received exogenous estrogens. In an au-

**Figure 2.** Kaplan-Meier Estimates of Cumulative Hazards for Any Gallbladder Outcomes in the Estrogen-Alone Trial



CEE indicates conjugated equine estrogens; CI, confidence interval; HR, hazard ratio. Procedure (A) or disease (B) among women receiving CEE or placebo. Any gallbladder disease or procedure during follow-up was used to comprise a global index (C). A sensitivity analysis was conducted with censoring occurring at 6 months after nonadherence with randomization assignment (D).

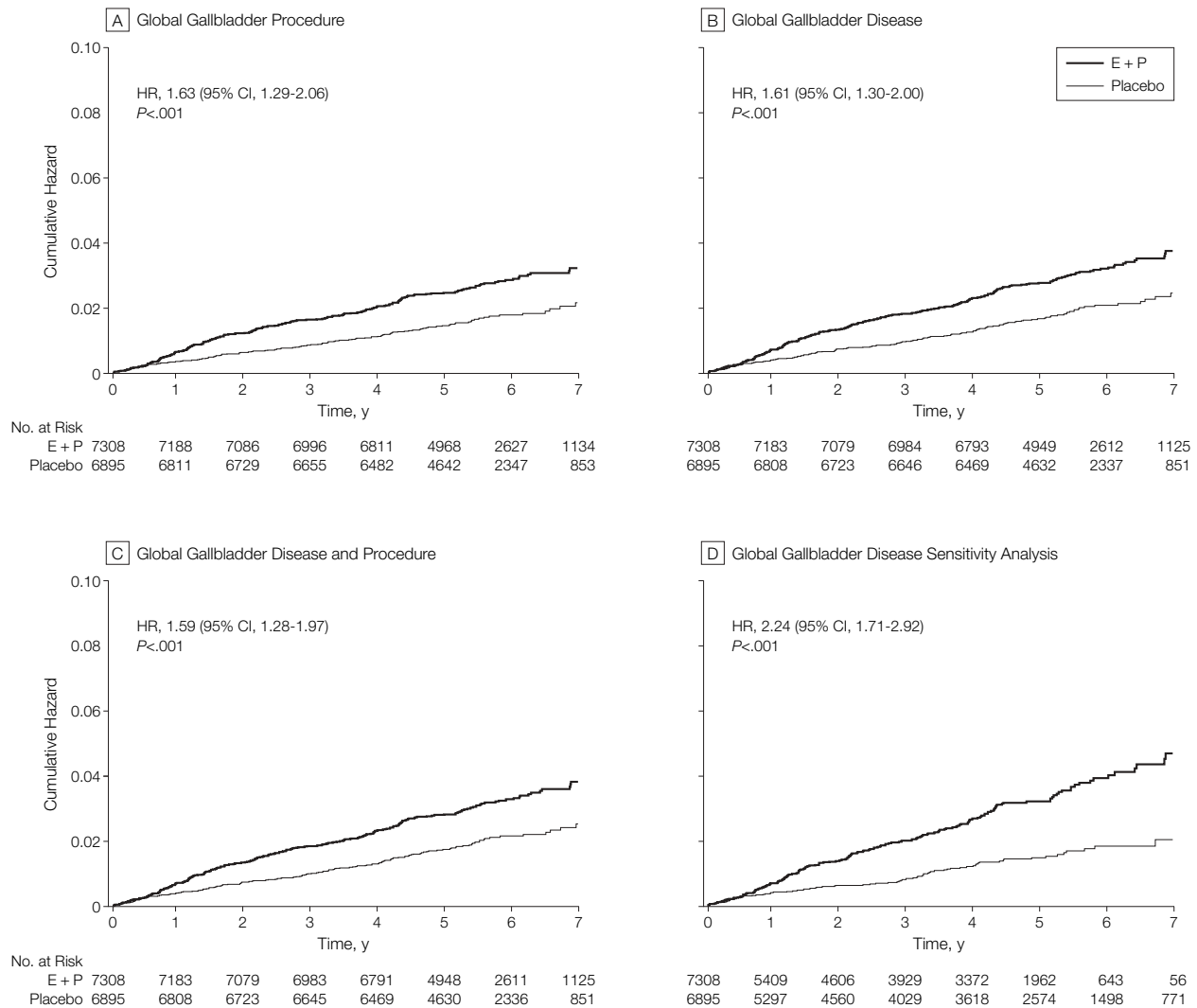
topsy study of men randomized to estrogen therapy for the treatment of prostate cancer, the rate of cholecystectomy was higher, but the prevalence of cholelithiasis among estrogen users was lower at time of death.<sup>22</sup>

Prior observational studies have explored the role of estrogen therapy in gallbladder disease, dating as far back as 1974.<sup>23</sup> The Nurses' Health Study found an increased risk of cholecystitis among participants taking exogenous estrogens, with a relative risk of 2.1 for cur-

rent hormone users, with higher risks associated with longer duration of use and greater doses.<sup>11</sup> The Atherosclerosis Risk in Communities study group found that former and current hormone users had a significantly increased risk of hospitalized gallbladder disease (relative risk, 1.76 for current users and 1.84 for former users), and this effect persisted in multivariate models including age, race, and BMI.<sup>13</sup> Mamdani et al found that Canadian women who recently started using estrogen

therapy had a 1.9 age-adjusted relative risk for cholecystectomy.<sup>14</sup> However, not all studies have found increased risk for gallstone disease. One small case-control study of 200 women found no significant excess odds for gallstone surgery among women using estrogen.<sup>15</sup> A cross-sectional study of 1765 Danish women reported an association between estrogen therapy and gallstones detected by ultrasound, but this association did not persist in multivariate analyses.<sup>16</sup>

**Figure 3.** Kaplan-Meier Estimates of Cumulative Hazards for Any Gallbladder Outcomes in the E + P Trial



CI indicates confidence interval; E + P, estrogen plus progestin; HR, hazard ratio. Procedure (A) or disease (B) among women receiving E + P or placebo. Any gallbladder disease or procedure during follow-up was used to comprise a global index (C). A sensitivity analysis was conducted with censoring occurring at 6 months after non-adherence with randomization assignment (D).

The WHI study results provide strong evidence of a causal association between estrogen therapy and gallstone disease because randomized trials are the best study design to provide unbiased estimates of effect. Nonetheless, some limitations of the study should be acknowledged. The generalizability of these findings is limited to mostly healthy postmenopausal women who took part in the WHI, and who were selected for willingness to comply with the treatment regimen. Thus, a "healthy volunteer" effect may explain the low overall rate of gallbladder disease in this study compared with other studies, although the randomized design verifies the internal validity of the effects of CEE and E+P in this study. The exclusion of women with reported disease at baseline was conducted post hoc, although the numbers were comparable between the randomized groups. Also, the baseline prevalence of disease was significantly higher among the women with hysterectomy (22% vs 14% of women without hysterectomy), and the incidence rates remained higher in the women with hysterectomy throughout the follow-up period. Changes in BMI may also have an effect on gallbladder outcomes, as both obesity and rapid weight loss are thought to contribute to gallstone formation, and estrogen therapy and a history of hysterectomy are associated with greater BMI. In this analysis, we conducted a subgroup analysis to test for differences in treatment effect depending on baseline BMI and found no significant interaction. However, we did not adjust for changes in BMI while using the study medication, because weight change may be part of the causal pathway between estrogen therapy and gallbladder disease.

Another potential source of bias could result from differential noncompliance to the study drug, if adverse effects in the treatment groups were more likely than among the placebo group. This problem is unlikely to strongly influence the results because the noncompliance rates were similar between the active hormone and the placebo users

for both trials and the statistical analysis was performed within an intention-to-treat framework. When participants were censored at 6 months after noncompliance in the sensitivity analysis, the estimated effects of the active hormones were increased on gallbladder disease outcomes due to the reduction in "crossover" or misclassification between the groups.

Ascertainment bias may be a threat to the findings, if estrogen users in both trials were more likely to have diagnostic suspicion of gallbladder disease when compatible symptoms emerged. We cannot fully rule this out, but think it is unlikely for several reasons. First, our trial findings are consistent with prior observational data. Also, the blinding appeared to work well across the trial until vaginal bleeding caused by the active drug supervened in some participants, who then may have been unblinded. The unblinding rates for the clinical gynecologists were higher for the active hormones in both trials, although the E+P trial had a substantially higher unblinding rate. However, the clinic gynecologists were not involved in the assessment of outcomes. It was not possible to determine the proportion of participants who were indirectly unblinded based on their perceptions of randomization status. We believe that vaginal bleeding would not lead to enhanced surveillance for gallbladder disease. In instances where symptoms may have caused study drug discontinuation, the results would have been biased toward the null hypothesis. The sensitivity analysis, which censored individuals after 6 months after study drug noncompliance, revealed an increased risk of the outcome among estrogen therapy users. Also, the primary trial findings for E+P showed that some conditions were less commonly found in the treatment group, including colon cancer. We feel that these findings argue against biases from differential unblinding and ascertainment.

Finally, the findings for this study cannot necessarily be extrapolated to other types of estrogen therapy and

other routes of administration. Further study is needed to help determine if transdermal administration of estrogen therapy has the same effect on biliary tract outcomes, since the first pass effect would be lessened.

In summary, this large randomized trial showed an important increase in gallbladder disease and surgical procedures among women who were randomized to using either oral CEE or E+P. Among women with hysterectomy, CEE contributed to 78 events per 10000 person-years, which is 31 excess events per 10000 women annually. In the E+P trial, there were 55 events per 10000 person-years, which is an excess of 20 events per 10000 women annually. The number needed to harm, which is the number of women who would undergo the interventions in 1 year in order to cause 1 excess adverse gallbladder outcome, were calculated to be 323 for CEE and 500 for the E+P arms. The women in this study were not selected to have preexisting cardiovascular disease as was the case in the HERS, and were geographically dispersed across 40 clinical sites. Therefore, these results should be more generalizable to healthy postmenopausal women in the United States without previous gallbladder disease and less likely to be biased than observational study cohort data. The increased risk for gallbladder events should be addressed in the decision-making process for women considering menopausal hormone therapy.

**Author Contributions:** Ms Rodabough had full access to all of the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis.

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**Analysis and interpretation of data:** Cirillo, Wallace, Rodabough, Greenland, LaCroix, Limacher, Larson.

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**Statistical analysis:** Cirillo, Rodabough, Larson.

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**REFERENCES**

1. Agrawal S, Jonnalagadda S. Gallstones, from gallbladder to gut: management options for diverse complications. *Postgrad Med.* 2000;108:143-153.
2. Ahmed A, Cheung RC, Keeffe EB. Management of gallstones and their complications. *Am Fam Physician.* 2000;61:1673-1680, 1687-1688.
3. Santen S. Cholecystitis and biliary colic [eMedicine Journal Web site] [serial online]. 2001;2. Available at: <http://www.emedicine.com/emerg/topic98.htm>. Accessed December 23, 2004.
4. Simon JA, Hunninghake DB, Agarwal SK, et al. Effect of estrogen plus progestin on risk for biliary tract surgery in postmenopausal women with coronary artery disease: the Heart and Estrogen/Progestin Replacement Study. *Ann Intern Med.* 2001;135:493-501.
5. DeFrances CJ, Hall MJ. *National Hospital Discharge Survey: Advance Data From Vital and Health Statistics.* Hyattsville, Md: National Center for Health Statistics; 2004. No. 342.
6. Owings MF, Kozak LJ. Ambulatory and inpatient procedures in the United States, 1996. *National Center for Health Statistics.* Available at: [http://www.cdc.gov/nchs/data/series/sr\\_13/sr13\\_139.pdf](http://www.cdc.gov/nchs/data/series/sr_13/sr13_139.pdf). Accessibility verified December 2, 2004.
7. Sterling RK, Shiffman ML. Nonsteroidal antiinflammatory drugs and gallstone disease: will an aspirin a day keep the gallstones away? *Am J Gastroenterol.* 1998;93:1405-1407.
8. Donovan JM. Physical and metabolic factors in gallstone pathogenesis. *Gastroenterol Clin North Am.* 1999;28:75-97.
9. George ED, Schluger LK. Special women's health issues in hepatobiliary diseases. *Clinics in Family Practice.* 2000;2:155-169.
10. Uhler ML, Marks JW, Voigt BJ, Judd HL. Comparison of the impact of transdermal versus oral estrogens on biliary markers of gallstone formation in postmenopausal women. *J Clin Endocrinol Metab.* 1998;83:410-414.
11. Grodstein F, Colditz GA, Stampfer MJ. Postmenopausal hormone use and cholecystectomy in a large prospective study. *Obstet Gynecol.* 1994;83:5-11.
12. Nelson HD, Humphrey LL, Nygren P, Teutsch SM, Allan JD. Postmenopausal hormone replacement therapy: scientific review. *JAMA.* 2002;288:872-881.
13. Boland LL, Folsom AR, Rosamond WD. Atherosclerosis Risk in Communities Study I: hyperinsulinemia, dyslipidemia, and obesity as risk factors for hospitalized gallbladder disease: a prospective study. *Ann Epidemiol.* 2002;12:131-140.
14. Mamdani MM, Tu K, van Walraven C, Austin PC, Naylor CD. Postmenopausal estrogen replacement therapy and increased rates of cholecystectomy and appendectomy. *CMAJ.* 2000;162:1421-1424.
15. Kakar F, Weiss NS, Strite SA. Non-contraceptive estrogen use and the risk of gallstone disease in women. *Am J Public Health.* 1988;78:564-566.
16. Jorgensen T. Gall stones in a Danish population: fertility period, pregnancies, and exogenous female sex hormones. *Gut.* 1988;29:433-439.
17. Stefanick ML, Cochrane BB, Hsia J, Barad DH, Liu JH, Johnson SR. The Women's Health Initiative postmenopausal hormone trials: overview and baseline characteristics of participants. *Ann Epidemiol.* 2003;13(9 suppl):S78-S86.
18. Women's Health Initiative Steering Committee. Effects of conjugated equine estrogen in postmenopausal women with hysterectomy: the Women's Health Initiative randomized controlled trial. *JAMA.* 2004;291:1701-1712.
19. Writing Group for the Women's Health Initiative. Risks and benefits of estrogen plus progestin in healthy postmenopausal women: principal results from the Women's Health Initiative randomized controlled trial. *JAMA.* 2002;288:321-333.
20. The Women's Health Initiative Study Group. Design of the Women's Health Initiative clinical trial and observational study. *Control Clin Trials.* 1998;19:61-109.
21. Anderson GL, Manson J, Wallace R, et al. Implementation of the Women's Health Initiative study design. *Ann Epidemiol.* 2003;13(9 suppl):S5-17.
22. Everson RB, Byar DP, Bischoff AJ. Estrogen predisposes to cholecystectomy but not to stones. *Gastroenterology.* 1982;82:4-8.
23. Surgically confirmed gallbladder disease, venous thromboembolism, and breast tumors in relation to postmenopausal estrogen therapy. *N Engl J Med.* 1974;290:15-19.