Categories

Fracture reduction E alone

**Incidence data:** women ages 50-54 USA. Annual incidence of major osteoporotic fractures 4.05 /1000/year equals 20.25 /1000/5 years. Reference source Ettinger B et al., Osteoporosis International 21:25-33, 2010 Revised statistics for fracture for FRAX calculations in the United States.

**Hazard ratio** 0.71 (0.64-0.80). Reference source: WHI Jackson RD Journal of Bone and Mineral research 21:817, 2006

**Calculations:** 20.25 x 0.71 = 14.38 ; 20.25-14.38= 5.87 /1000/5 years reduction in fractures

Fracture Reduction E+P

**Incidence data:** women ages 50-54 USA. Annual incidence of major osteoporotic fractures 4.05 /1000/year equals 20.25 /1000/5 years. Ettinger B et al., Osteoporosis International 21:25-33, 2010 Revised statistics for fracture for FRAX calculations in the United States.

**Hazard ratio:** 0.76 (0.69-0.83). Reference source: WHI Cauley JA JAMA 290:1729, 2003

**Calculations:** 0.76x 20.25=15.39; 20.25-15.39= 4.86/1000/5 years reduction in fractures

Diabetes reduction E alone and E+P combined

**Incidence data:** women ages 45-64 Reference source. Centers for Disease Control and Prevention Data and Trends 2007, page last modified February 27, 2009 10.5 /1000/year = 52.5/1000/5 years

**Hazard ratio:** 0.79 (0.67-0.93). Reference source: WHI Margolis KL et al Diabetologia 47:1175-1187, 2004

**Calculations:** 0.79x52.5=41.45; 52.5-41.45=11.05/1000/5 years reduction in diabetes

Breast Cancer E alone
Incidence data: women ages 50-54 from years 2002-2006, Reference Source: Surveillance and Epidemiology and End Results, Cancer of the Breast Invasive and Cancer of the Breast Non-invasive combined. Invasive breast cancer equals 229 and non-invasive 76.4 for a total of 296.4/100,000 per year equals 2.96 /1000/year equals 14.8/1000/5 years

Hazard ratio: 0.90 (0.73-1.12). Reference source Nurses Health Study; for use of estrogen alone for 5-9 years. Chen WY et al. Arch Int Med 166:1027-1032, 2006

Calculations: 0.90x14.8=13.3; 14.8-13.3= \textbf{1.48/1000/5 years reduction in breast cancer}

Breast Cancer E+ P

Incidence data: women ages 50-54 from years 2002-2006, Reference Source: Surveillance and Epidemiology and End Results, Cancer of the Breast Invasive and Cancer of the Breast Non-invasive combined. Invasive breast cancer equals 229 and non-invasive 76.4 for a total of 296.4/100,000 per year equals 2.96 /1000/year equals 14.8/1000/5 years

Hazard ratio: 1.46 (1.22-1.74). Reference Source: Nurses health study Colditz GA NEJM 332:1589-1593, 1995

Calculations: 1.46x14.8=21.6; 21.6-14.8= \textbf{6.8/1000/5 years increase in risk of breast cancer}

Colorectal Cancer E alone

Incidence data: women current age 50; Colorectal cancer risk by age; Centers for Disease Control and Prevention, last updated September 15, 2009; 0.53% (0.53/100) of women developed colorectal cancer over 10 years equals 5.3 /1000 women over 10 years equals 2.65 /1000/5 years

Hazard Ratio: 1.12 (0.77-1.63. Reference Source WHI Ritenbaugh C et al Cancer Epidemiology Biomarkers Prevention 17:2609-2618, 2008

Calculations: 1.12x 2.65=2.968; 2.969-2.65 = \textbf{0.32/1000/5 years increase}

Colorectal Cancer E+P

Incidence data: women current age 50; Colorectal cancer risk by age; Centers for Disease Control and Prevention, last updated September 15, 2009; 0.53% (0.53/100) of
women will develop colorectal cancer over 10 years equals 5.3 /1000 women over 10 years equals 2.65 /1000/5 years

**Hazard ratio:** 0.61 (0.42-0.87). Reference source WHI Chlebowski RT NEJM 350:991-1004, 2004

**Calculations:** $0.61 \times 2.65 = 1.61$; $2.65 - 1.61 = 1.24 /1000/5$ years decrease

**Overall Mortality E alone**

**Incidence data:** women ages 50-59, placebo arm of WHI (Rossouw J JAMA 297: 1465, 2007; 95 deaths per 4356 patients over a period of 6.3 years equals 17.3/1000/5 years

**Hazard ratio:** 0.71 (0.46-1.11) Reference Source Rossouw J JAMA 297: 1465, 2007

**Calculations:** $0.71 \times 17.3 = 12.8$; $17.3 - 12.8 = 5.02 /1000/5$ years reduction in deaths

**Overall Mortality E+P**

**Incidence data:** women ages 50-59, placebo arm of WHI (Rossouw J JAMA 297: 1465, 2007; 95 deaths per 4356 patients over a period of 6.3 years equals 17.3/1000/5 years

**Hazard Ratio:** 0.69 (0.45-0.63) for women ages 50-59 Reference Source Rossouw JAMA 297: 1465, 2007

**Calculations:** $0.69 \times 17.3 = 11.937$; $17.3 - 11.937 = 5.36 /1000/5$ year reduction

**Confirmatory data**

**Incidence data:** 3637 deaths in 121,700 women equals 30/1000 divided by 16 years of follow-up equals 1.8 deaths /1000 per years equals 9 deaths per 1000 per 5 years Reference source Nurses health Study Grodstein F et al NEJM 336:1769, 1997

**Hazard ratio** 0.54 (0.45-0.63) for use for 5-9 years; Grodstein et al NEJM 336:1769, 1997

**Calculations:** $0.54 \times 9 = 4.86$; $9 - 4.86 = 4.14 /1000/5$ years reduction
Coronary heart Disease E alone

**Incidence data:** ARIC Cohort Incidence Tables Table 4-1, 1987-2001
http://www.nhlbi.NIH.gov/resources/doc_IP_chtbk.pdf women ages 45-54 7.25/1000/5 years

**Hazard Ratio:** 0.63 (0.36-1.09) for ages 50-59 and 0.48 (0.20-1.17) for < 10 years postmenopausal; Reference source Rossouw J JAMA 297:1465, 2007

**Calculations:** 0.63x7.25=4.5675; 7.25-4.5675= 2.88/1000/5 years reduction for those 50-59 and 3.77/1000/5 years for those < 10 years postmenopausal

**Confirmatory data:** Nurses health Study NEJM 343:530, 2000; HR 0.56 (0.40-0.80); 3.19/1000/5 years reduction

Coronary heart Disease E+P

**Incidence data:** ARIC Cohort Incidence Tables Table 4-1, 1987-2001
http://www.nhlbi.NIH.gov/resources/doc_IP_chtbk.pdf women ages 45-54 7.25/1000/5 years

**Hazard ratio:** 1.29 (0.79-2.12 ) for those 50-59 and 0.88 (0.54-1.43) for those <10 years postmenopausal Reference source Rossouw J et al JAMA 297:1465, 2007

**Calculations:** 1.29x7.25=9.35; 9.35-7.25= 2.10/1000/5 year increase for women ages 50-59 and 0.88x7.25=6.38; 7.25-6.38= 0.87/1000/5 years decrease for those < 10 years postmenopausal

**Confirmatory study:** Nurses health Study. Reference source Epidemiology 19:771, 2008. Hazard ration 0.84 (0.61-1.14) , 0.42/1000/5 years reduction

Endometrial cancer E+P

**Incidence:** Women ages 50+, Reference source: Surveillance and Epidemiology and End Results, women of all races, from year 2006; 74.03 per 100,000 per year equals 3.7/1000/5 years

**Hazard ratio:** 0.81 (0.48-1.36) WHI JAMA 290:1739, 2003

**Calculations:** 0.81x3.3=2.992; 3.7-2.997= 0.70/1000/5 years reduction

Lung Cancer E+P

**Incidence:** women ages 50-54, all races. Reference source: Surveillance Epidemiology and End Results from SEER Cancer Statistics Review 1975-2006, Cancer of the Lung and Bronchi invasive . 44.8 per 100,000 and subtract small cell of 6.97 (all ages 2006) equals 1.89/1000/5 years
**Hazard ratio:** 1.23 (0.92-1.63) Reference source Chlebowski RT et al Lancet 374:1243-51, 2009

**Calculations:** 1.23 x 1.89 = 2.32; 2.32 - 1.23 = 0.43/1000/5 years increase

**Ovarian Cancer**

**Incidence:** women ages 50-54, all races, Surveillance Epidemiology and End Results data from SEER 1975-2006, Table 21.6. 21.7 per 100,000 per years equals 1.08/1000/5 years

**Hazard Ratio:** 1.58 (0.77-3.24) Reference source WHI Anderson GL et al, JAMA 290:1739-1746, 2003

**Calculations:** 1.58 x 1.08 = 1.71; 1.71 - 1.08 = 0.63/1000/5 years increase

**Veno-thrombotic episodes (VTE) E alone**

**Incidence:** Placebo group from Cushman et al for women ages 50-59 (Table 3) was 0.8 /1000/year or 4.0 /1000/year Reference Source Cushman M et al JAMA 292:1573-1580, 2004

**Hazard ratio:** estimated from annualized % of 0.12 for placebo and 0.16 for CEE or a hazard ratio of 1.33 Reference Source Curb JD et al Arch Int Med 186:772-780, 2006

**Calculations:** 1.33 x 4.0 = 5.32; 5.32 - 4.0 = 1.32/1000/5 years increase

**Veno-thrombotic episodes (VTE) E+P**

**Incidence:** Placebo group from Cushman et al for women ages 50-59 (Table 3) was 0.8 /1000/year or 4.0 /1000/year Reference Source Cushman M et al JAMA 292:1573-1580, 2004

**Hazard ratio:** 2.17 was estimated from annualized rate of 0.8/1000/year fro placebo and 1.9/1000/year for E+P

**Calculations:** 2.17 x 4.0 = 8.68; 8.68 - 4.0 = 4.68/1000/5 years increase

**Stroke E alone**

**Incidence:** Women from 45-54 years of age; Reference Source Incidence in Rochester, Minnesota from 1985 to 1989; A journal of Cerebral circulation 27: 373, 1996;; 64 /100,000/year equals 3.2/1000/year

**Hazard ratio:** 1.37 (1.09-1.73) Reference Source WHI Hendrix SL et al , Circulation 113:2425-34, 2006, patients of all ages in WHI (age did not influence the hazard ratios)

**Calculations:** 1.37 x 3.2 = 4.384; 4.384 - 3.2 = 1.18/1000/5 years increase

**Confirmatory data:** hazard Ratio from Nurses Health Study 1.39 (1.09-1.73); calculations: 1.39 x 3.2 = 4.48; 4.48 - 3.2 = 1.24/1000/5 years increase. Reference source Grodstein F et al Arch Int Med 168:861-866, 2008

**Stroke E+P**
**Incidence:** Women from 45-54 years of age; Reference Source Incidence in Rochester, Minnesota from 1985 to 1989; A journal of Cerebral circulation 27: 373, 1996;; 64 /100,000/year equals 3.2/1000/year

**Hazard ratio:** 1.31(1.02-1.68) Reference source Wassertheil-Smoller S JAMA 289:2673-84, 2003 for women of all ages ( age did not influence the hazard ratios)

**Calculations:** 1.31 x3.2=4.192; 4.192-3.2=0.99/1000/5 years increase

**Confirmatory data:** hazard ratio from Nurses health Study 1.27(1.04-1.56) ; calculations: 1.27x3.2=4.06; 4.06-3.2=0.86/1000/5 years increase Reference Source Grodstein F et al Arch Int med 168:861-866, 2008

**Cholecystitis E alone**

**Incidence:** 697 cases per 195,493 person years equals 17.8/1000/5 years Reference source Grodstein F et al Ob+Gyn 83: 5-11, 1994 ( data from Nurses health study utilized since the subjects were generally younger that those in the WHI who had an average age of 63).

**Hazard ratio:** 1.80 ( 1.42-2.28) Reference source WHI Cirillo DJ et al JAMA 293:330-339, 2005

**Calculations:** 1.80x17.8=32.04; 32.04-17.8=14.2/1000/5 years increase

**Cholecystitis E+P**

**Incidence:** 697 cases per 195,493 person years equals 17.8/1000/5 years Reference source Grodstein F et al Ob+Gyn 83: 5-11, 1994 ( data from Nurses health study utilized since the subjects were generally younger that those in the WHI who had an average age of 63).

**Hazard Ratio:** 1.54 (1.22-1.94) Reference source WHI Cirillo DJ et al JAMA 293:330-339, 2005

**Calculations:** 1.54x17.8=27.4; 27.4-17.8=9.61/1000/5 years increase
Supplemental Figure 1

Confluent MCF-7 cells grown in 6-well plated were fed with medium containing 5% DCC-FBS for 24 hours. The cells then were pretreated with 10 μM cell cycle inhibitor for one hour before a wound was made. Twenty-four hours after estradiol incubation, cells migrated across the start line were counted.
Boyden chamber assay: T47D cells (50,000 cells per well) were plated into the inserts with 8 nm pores of 24-well plates. The cells were stepped down to phenol red free RPMI with 5% DCC-FBS for 48 h and then incubated with 1 nM E2 or vehicle for another 48 h. The cells were fixed in 10% formaline and then stained with crystal violet after the cells on the upper surface of the chamber were removed by a cotton swab. The membrane was mounted on a microscope slide and the cells migrated through the pores were counted.
Supplemental Figure 3

(A) Western analysis of moesin phosphorylation in Thr558 after E₂ treatment. (B) Effect of ROCK inhibitor Y27632 on E₂-induced migration of T47D cells.