Equine Estrogens Impair Nitric Oxide Production and Endothelial Nitric Oxide Synthase Transcription in Human Cells Compared with the Natural 17β- Estradiol

Laura Novensà Casas
Cardiología experimental
IDIBAPS
Background
Gender differences in CVD incidence

Why are the women protected?

Next at Harvard...

Why does aspirin prevent heart attacks in men but not women?

Because women don't like science?
The hormonal theory

Estrogen protects females mammals at a cellular level, so that the incidence and severity of cardiovascular disease in females (rats, dogs or humans) will be lower than in males.
Estradiol

**Relaxing Factors ↑**
- Constricting Factors ↓
- Anti-Oxidant ↑
- Cholesterol ↓
- RASS ↓
- Antimitogenic Effects ↑

**KIDNEY**
- Glomerular Remodeling
- Glomerulosclerosis
- Renal Arteriolar Remodeling

**BLOOD VESSEL**
- Vascular Remodeling
- Vasoconstriction
- SMC Growth
- ECM Deposition
- Endothelial Damage

**HEART**
- Cardiac Remodeling
- CF/MC Growth
- ECM Deposition

**Cardiovascular disease**
Risks and Benefits of Estrogen Plus Progestin in Healthy Postmenopausal Women

Principal Results From the Women’s Health Initiative Randomized Controlled Trial

Writing Group for the Women’s Health Initiative Investigators
Women’s Health Initiative (WHI)

Coronary Heart Disease

- HR, 1.29
- 95% nCI, 1.02-1.63
- 95% aCI, 0.85-1.97

Stroke

- HR, 1.41
- 95% nCI, 1.07-1.85
- 95% aCI, 0.86-2.31

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Estrogen Replacement Therapy: before and after the Women’s Health Initiative (WHI)

Before WHI

- Estradiol
  - Relaxing Factors $\uparrow$
  - Constricting Factors $\downarrow$
  - Anti-Oxidant $\uparrow$
  - Cholesterol $\downarrow$
  - RASS $\downarrow$
  - Antimitogenic Effects $\uparrow$

$\downarrow$ Cardiovascular Disease

After WHI

- Estradiol
  - Venous thromboembolism $\uparrow$
  - Stroke $\uparrow$
  - Coronary heart disease $\uparrow$
  - Cholesterol $\varnothing$

$\uparrow$ Cardiovascular Disease
WHI?

- Dose regimen
- Association of estrogens with progestins
- Administration route
- Type of estrogen
- Average age of women beginning the trial
WHI?

- Dose regimen
- Association of estrogens with progestins
- Administration route
- Type of estrogen
- Average age of women beginning the trial
Equine estrogens vs natural estrogens

“I’m in an experimental program that treats menopause with ostrich hormones. Now I only get hot flashes when I’m laying an egg.”
• To compare the action of CEE and human estrogens in the NO production

• To determine the mechanisms of NO modulation by the different estrogens
RESULTS
NO production in HAEC

<table>
<thead>
<tr>
<th>BASAL</th>
<th>NT</th>
<th>E2</th>
</tr>
</thead>
<tbody>
<tr>
<td>E1</td>
<td>Eq</td>
<td>Eqn</td>
</tr>
</tbody>
</table>

The images show green fluorescence intensity, indicating NO production in HAEC cells under different conditions.
NO production in HAEC

* P<0.05 vs Estradiol

**Graph:**
- DAF-2 Fluorescence (Fold Increase) vs Hormone (nM)
- Y-axis: 0 to 4
- X-axis: 0 to 1000
- Curves for different hormones:
  - Estradiol (E2)
  - Estrona
  - Equilenina
  - Equilina

**Legend:**
- **Estradiol** (Red)
- **Equilina** (Blue)
- **Estrona** (Yellow)
- **Equilenina** (Green)
Metabolites concentration

NO$_2$/NO$_3$

![Graph showing metabolites concentration (NO$_2$/NO$_3$) with annotations and asterisks indicating significance at P<0.05 vs Estradiol.](image)
eNOS expression

Normalized copies of eNOS mRNA/µg RNA

* p<0.05 vs Estradiol
#p<0.05 vs NT
Transcriptional activity

Luciferase Activity (Fold Increase)

- E2
- E1
- 17α-E2
- Eq
- 17α-Eq
- 17β-Eq
- Eqn
- 17α-Eqn
- 17β-Eqn

* P<0.05 vs Estradiol
# P<0.05 vs ERα
Molecular structure

- Estrone (E1)
- 17-β-estradiol (E2)
- 17-α-estradiol (17α-E2)
- Equilin (Eq)
- 17-β-equilin (17β-Eq)
- 17-α-equilin (17α-Eq)
- Equilenin (Eqn)
- 17-β-equilenin (17β-Eqn)
- 17-α-equilenin (17α-Eqn)
17 position

17-β-estradiol (E2)  +  NO production  -  17-α-estradiol (17α-E2)
17 position

17β-E2

E1

17α-E2

A

B

C

17β-estradiol (E2)

17α-estradiol (17α-E2)

estrone (E1)
B ring saturation

17-β-estradiol (E2) 17-β-equilenin (17β-Eqn) 17-β-equilenin (17β-Eqn)

NO production

+  NO production  -
B ring saturation

17-β-estradiol (E2) > 17-β-equilenin (17β-Eq) > 17-β-equilenin (17β-Eqn)

Flexibilidad

pKa 10.54
pKa 10.41
pKa 9.91
Equine estrogens increase NO production less effectively than naturally occurring estrogens, as a result of their lesser ability to activate the ERα-mediated increase of eNOS promoter activity and eNOS transcription.

Chemical moiety and stereochemistry at position 17 and the degree of ring B saturation in estrogenic compounds play a significant role in ERα-transcriptional activity.
MOLTES  GRÀCIES!!!