Reproductive Steroid Hormones and Cardiovascular Function in Young Healthy Women

Paul Kaplan, M.D.
Clinical Professor
Division of Reproductive Endocrinology & Infertility
Department of Obstetrics & Gynecology
Oregon Health and Science University

Senior Research Associate
Department of Human Physiology
University of Oregon
Women’s Vascular Health & Hormones Research Team
Department of Human Physiology
University of Oregon

Christopher Minson, PhD
John Halliwill, PhD
Paul Kaplan, M.D.
• Jennifer Miner, M.S.
• Vienna Brunt, M.S.
• J. Cory Miner, B.S.

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Introduction

Hormone Replacement in Postmenopausal Women: Conflicting Data

Past Clinical Trials (HERS and WHI)

- No benefit or ↑ CV risk with E+P HRT in PM women
- Many Studies: Estrogen benefits vascular health
- MPA antagonizes E benefits in PM women & animals
Is Hormonal Information Being Translated to Younger Women?

- CVD is more prevalent in young women than in the past
- 72% of young women are on hormonal therapy
  - Gynecological or contraceptive purposes
- Progestin effects have been varied
  - Levonorgestrel (VLD) and MPA antagonize estrogen’s effects
  - Desogestrel (LD), etonorgestrel (ring), and drospirenone do not antagonize estrogen’s effects
- Unknown what progesterone does to the vasculature of young healthy women
Why Study Endothelial Function?


- Endothelial dysfunction has been found in young symptom-free subjects with risk factors for CVD, before atherosclerosis (Celermajer DS, et al. Lancet, 1992)

- Previous research demonstrated traditional risk factors fail to explain up to 50% of CVD morbidity and mortality
Endothelium-Dependent Vasodilation (FMD)

The rise in flow following a distal vascular occlusion creates a shear-stress across the brachial artery, causing the production and release of NO that is dependent on a healthy endothelium (Flow-Mediated Dilation or “FMD”).
Role of the Vascular Endothelium

Brachial Artery

VASCULAR SMOOTH MUSCLE

ENDOTHELIUM

Shear stress

NO

Adapted from Halliwill Lab
FMD Protocol

Baseline

FMD (% change) = ((FMD diameter – Baseline diameter) / Baseline diameter) * 100

Blood pressure cuff for 5 minute forearm occlusion (distal to ultrasound transducer)

Ultrasound transducer held in place with a clamp

Rest

Occlusion Cuff Inflated
(5 min)

Occlusion Cuff Deflated

1 min

3 min
Prognostic Role of FMD

- Prospective study of 2,264 PM Women (age 54 ± 6 years)
- Follow-up for 45 months
- Controlled for other risk factors
- 90 confirmed CV events
  - ↓ FMD = ↑ Cardiac Events: Cardiac-related death, myocardial infarction, revascularization procedure, TIA, Stroke
- “FMD was an independent risk determinant, and adds prognostic information above and beyond traditional risk factors”

Hormones change endothelial function across the menstrual cycle

FMD increases when estrogen is high during the follicular phase & decreases during the luteal phase.
Estrogen, medroxyprogesterone acetate, endothelial function, and biomarkers of cardiovascular risk in young women

Jessica R. Meendering,1 Britta N. Torgrimson,1 Nicole P. Miller,1 Paul F. Kaplan,1,2 and Christopher T. Minson1

1Department of Human Physiology, University of Oregon, Eugene, and 2Division of Reproductive Endocrinology and Infertility, Department of Obstetrics and Gynecology, Oregon Health and Sciences University, Portland, Oregon

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Meendering JR, Torgrimson BN, Miller NP, Kaplan PF, Minson CT. Estrogen, medroxyprogesterone acetate, endothelial function, and markers of cardiovascular risk in young women. Am J Physiol Heart Circ Physiol 294: H1630–H1637, 2008. First published February 15, 2008; doi:10.1152/ajpheart.01314.2007.—Medroxyprogesterone acetate (MPA) is widely known for its use in combination hormone therapy for postmenopausal women. However, MPA is also commonly used in young women for contraception and treatment of a number of gynecological conditions. Despite its widespread use, the cardiovascular effects of MPA in young women are unclear. Therefore, the purpose of this study was to determine the acute effects of MPA when used in combination with estradiol on raise questions about the use of progestins, and specifically MPA, in hormone treatments.

In addition to postmenopausal women, premenopausal women are also commonly prescribed MPA. MPA is used in the injectable progestin-only contraceptive Depo-Provera, which is a popular contraceptive choice, particularly for younger premenopausal women because of the ease of use and high compliance. Oral MPA hormone treatments are also used to treat a number of gynecological conditions in young women, such as endometriosis, polycystic ovarian syndrome, and irregular uterine bleeding (7). Despite numerous reports that MPA may impair lipid levels, several studies have shown no changes (18–19).
**Study Design**

1. **Study Days**
   - 1 2 3 4 5 6 7 8 9 10

2. **GnRH Antagonist in All Groups**
   - Estradiol
   - MPA + Estradiol
   - Estradiol
   - Estradiol
   - GnRH Suppression Only

3. **Groups**
   - **Group 1** (N = 10)
     - [age: 22±1, BMI: 23±1]
   - **Group 2** (N = 2)
     - [age: 22±1, BMI: 22±1]
   - **Group 3** (N = 2)
     - [age: 21±1, BMI: 22±3]

4. **Drugs and Dose**
   - GnRH antagonist = ganarelix 250µg/0.5 ml per day
   - Transdermal Estradiol = 0.1 mg/day
   - MPA = 5 mg per day
Endothelial-Dependent FMD in Group 3 (n=2)

FMD Response
(% Change in Brachial Artery Diameter from Baseline)

GnRHa Only

GnRHa Treatment

<table>
<thead>
<tr>
<th>Day</th>
<th>FMD Response</th>
</tr>
</thead>
<tbody>
<tr>
<td>Day 4</td>
<td>6</td>
</tr>
<tr>
<td>Day 7</td>
<td>6</td>
</tr>
<tr>
<td>Day 10</td>
<td>6</td>
</tr>
</tbody>
</table>
Endothelial-Dependent FMD in Group 2 (n=2)

FMD Response
(% Change in Brachial Artery Diameter from Baseline)

Day 4
GnRha
Day 7
GnRha+E2
Day 10
GnRha+E2+E2

P=0.026
Endothelial-Dependent FMD in Group 1 (n=10)

FMD Response
(% Change in Brachial Artery Diameter from Baseline)

Hormone Treatment

GnRh
GnRh + E2
GnRh + E2 + MPA

Day 4
Day 7
Day 10

P<0.001
Estradiol/MPA FMD Results

- Administration of E2 improved endothelium-dependent vasodilation (ED-FMD) in all groups.
- MPA antagonized the benefits of E2.
- There were no observed changes in endothelium-independent vasodilation in any group, consistent with a specific role for endothelial NO production.
Short-term oral progesterone administration antagonizes the effect of transdermal estradiol on endothelium-dependent vasodilation in young healthy women

Jennifer A. Miner, Emily R. Martini, Michael M. Smith, Vienna E. Brunt, Paul F. Kaplan, John R. Halliwill, and Christopher T. Minson

1Department of Human Physiology, University of Oregon, Eugene, and 2Division of Reproductive Endocrinology and Infertility, Department of Obstetrics and Gynecology, Oregon Health Sciences University, Portland, Oregon

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frequently prescribed progestogens, the need to understand the influence of progesterone on cardiovascular health is great.

One of the primary methods used to investigate the effect of sex hormones on vascular health is via flow-mediated dilation (FMD). FMD, measured as the percent change in brachial artery diameter in response to an increase in shear stress, has
Study Design

<table>
<thead>
<tr>
<th>Study Days</th>
<th>Progesterone</th>
<th>Progesterone + Estradiol</th>
<th>Estradiol</th>
<th>Progesterone + Estradiol</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 2 3 4 5 6 7 8 9 10</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Baroreflex | EDVD |
---|---|
N = 16 | N = 8 |
N = 15 | N = 9 |

GnRH antagonist = ganarelix 250µg/0.5 ml per day
Oral Progesterone = 200 mg per day
Transdermal Estradiol = 0.1 mg/day
Results
EDVD/FMD: Estradiol First

FMD Percentage vs. Hormonal Condition
Estradiol-First Group

A

FMD %

Day 4
GnRHa

Day 7
GnRHa+E2

Day 10
GnRHa+P4+E2

*
Results
EDVD/FMD: Progesterone First

FMD Percentage vs. Hormonal Condition
Progesterone-First Group

Day 4
Day 7
Day 10
Hormones as Predictor of FMD

• Multi-level prediction model
  – Comparing estrogen and progesterone levels in blood with percent FMD
  – Hormone levels across all subjects, regardless of condition
  – Nests observations within subjects

• Progesterone & estrogen both predict FMD:
  – Progesterone is associated with lower FMD (p=0.022)
  – Estrogen is associated with higher FMD (p=0.006)
Conclusions

• Acute administration of oral progesterone antagonizes the effect of estradiol on endothelial function at these study doses

• Progesterone is associated with decreased endothelial function, and estradiol with increased endothelial function

• Next Phase: Role of Testosterone ??
Assisted Reproductive Technologies: Present and Future
The Assisted Reproductive Technologies (ART)

- In Vitro Fertilization (IVF)
- Intracytoplasmic Sperm Injection (IVF/ICSI)
- Donor Oocyte IVF
- Frozen Embryo Thaw and Transfer
- In Vitro Maturation/Freezing of Oocytes
An hMG–hCG Cycle

- E₂ pg/mL
- P ng/mL

Follicular size (mm and [no.])

2 amp hMG

Intercourse + PCT

Cycle day

Adapted from Navot and Rosenwaks, 1987.
In Vitro Fertilization (IVF)

- Daily S/C or IM FSH/hMG injection
- Follicular monitoring with serum estradiol and transvaginal ultrasound
- HCG given to trigger ovulation (LH surge)
- Transvaginal oocyte retrieval and insemination
- Embryo culture and transcervical embryo transfer
- Embryo cryopreservation for future F.E.T.
- Pregnancy rate of 40-50 % per cycle
In IVF, eggs are harvested from the woman’s ovary and fertilized in the laboratory with sperm. The embryos are then transferred into the uterus.
Intracytoplasmic Sperm Injection (ICSI)

- Standard IVF Stimulation and oocyte retrieval
- Injection of a single sperm into each oocyte
- Embryo culture and transcervical embryo transfer
- Currently used in almost 50% of IVF cycles for treatment of male factor and unexplained causes
- Pregnancy rate of 40-50% per cycle
Intracytoplasmic Sperm Injection (ICSI)
Future Directions in Infertility Treatment

- The “-omics” Revolution
- Preimplantation genetic diagnosis (PGD)
  - with transgenic therapy?
  - Nuclear and/or cytoplasmic oocyte transfer
  - Oocyte Cryopreservation
  - Embryonic Stem Cell Line Development
The “-omics” Revolution in Infertility

- **Genomics**: The branch of molecular biology concerned with the structure, function, evolution, and mapping of genomes.

- **Proteomics**: The set of proteins expressed by the genetic material of an organism under a given set of environmental conditions.
The “-omics” Revolution in Infertility

- **Metabolomics**: The systematic study of the unique chemical fingerprints that specific cellular processes leave behind.

- **Embryomics**: The identification, characterization and study of the diverse cell types which arise during embryogenesis.
Future Directions in Infertility Treatment (con’t)

- Embryo Cloning - Reproductive/Therapeutic
- Embryonic Stem Cell Gamete Development
- Fertility Preservation Techniques (cancer)
- Adult Cell Gamete Cloning - sperm/oocyte
- Adult Somatic Cell Cloning
Preimplantation Genetic Diagnosis (PGD)

- **Goal:** Identify Genetically Abnormal Embryos
- **IVF/ICSI + Embryo Culture**
- **Blastomere Biopsy of 8-cell Embryo**
- **FISH/PCR Genetic Studies** (X, 21, single gene, etc.)
- **Transfer of Normal Blastocyst/Frozen Embryos**
PGD 8-cell Blastomere Biopsy
PGD FISH - Normal Embryo

White = Y
Yellow = X
Blue = 18
Red = 21
Green = 13
Oocyte Cryopreservation

- Preservation of Oocytes Prior to Fertilization
- TV Retrieval of Stimulated Oocytes
  - Future: Unstimulated Oocytes with IVM
- Desiccation and Cryopreservation
- Delayed Thaw and IVF/ICSI Embryo Culture
- Transfer of Healthy Embryos
Oocyte Desiccation for Cryopreservation
Is Oocyte Cryopreservation a Successful Option?

A. 69 women had all IVF oocytes frozen (12/04-12/06)
B. 254 oocytes thawed in 18 women for 24 transfers
C. 130 of 254 fertilized (52%)
D. 84 embryos transferred. Clinical pregnancy rate 11/24 (45.8%) per embryo transfer and 10/18 (55.6%) per patient.

Paulson R et al. PCOGS 10-12-07
Fertility Preservation

A Damage to follicular cells from radiation and chemotherapy
   Possible reduction of ovarian reserve
   Natural pregnancy
   Premature ovarian failure
   Donor egg or adoption

B In vivo
   Stimulation of follicle growth with exogenous hormones
   Aspiration of oocytes
   Mature oocyte
   Embryo
   Live birth

Ex vivo
   Emerging techniques
   Cryopreservation of ovarian cortical strips
   In vitro follicle maturation
   Transplantation of ovarian cortical strips in patient
   Embryo
# Fertility Preservation

<table>
<thead>
<tr>
<th>Group</th>
<th>Method</th>
<th>Cryopreservation</th>
<th>Treatment</th>
<th>Recipient</th>
<th>Concerns</th>
</tr>
</thead>
<tbody>
<tr>
<td>Women</td>
<td>Hormone stimulation</td>
<td>Zygote or embryo</td>
<td>Embryo transfer</td>
<td>Patient or gestational surrogate</td>
<td>Delay in cancer treatment</td>
</tr>
<tr>
<td>Postpubertal girls</td>
<td>Hormone stimulation</td>
<td>Mature oocyte</td>
<td></td>
<td></td>
<td>Hormone injections</td>
</tr>
<tr>
<td>Women</td>
<td>Hormone stimulation</td>
<td>Cumulus-oocyte complexes</td>
<td></td>
<td></td>
<td>Availability of appropriate sperm donor</td>
</tr>
<tr>
<td>Postpubertal girls</td>
<td>Laparoscopic oophorectomy</td>
<td>Ovarian transplantation</td>
<td>Patient</td>
<td></td>
<td>Potential reintroduction of cancer cells</td>
</tr>
<tr>
<td>Women</td>
<td>Laparoscopic oophorectomy</td>
<td>Ovarian cortical strips</td>
<td>In vitro follicle maturation and in vitro fertilization or ICSI with embryo transfer</td>
<td>Patient or gestational surrogate</td>
<td>Experimental</td>
</tr>
<tr>
<td>Men</td>
<td>Ejaculation</td>
<td>Sperm</td>
<td>ICSI with embryo transfer</td>
<td>Partner</td>
<td></td>
</tr>
<tr>
<td>Postpubertal boys</td>
<td>Testis biopsy</td>
<td>Testis</td>
<td>Stem-cell repopulation</td>
<td>Patient</td>
<td></td>
</tr>
</tbody>
</table>

Experimental
Stem Cell Gamete Production

A. Goal is cost-effective, ethically-acceptable source of sperm and oocytes
   - Reduction of risks with donor gametes
   - Alternative to somatic cell cloning

B. Potential treatment for cancer patients, age-related infertility, and severe male factor

C. Reduction of multiple gestation by SET
Mouse Embryonic Stem Cell Spermatid

NPR Dec. 2004
Mouse Blastocysts Fertilized by Embryonic Stem Cell Spermatids

NPR Dec. 2004
Milestones of infertility medicine

1969
Pergonal and human chorionic gonadotropin marketed

1967
Clomid comes on the market

1978
Louise Brown, first “test-tube baby,” born

1981
First IVF baby in America

1984
GIFT technique developed by Ricardo Asch of San Antonio
First “donor baby” (eggs and sperm) born to surrogate mother in Australia

1985
Maryland passes legislation requiring insurance coverage for IVF
First ultrasound-guided, nonsurgical IVF

1986
Richard Marrs delivers first U.S. baby developed from a frozen embryo

1987
ZIFT technique introduced
Lupron comes on the market

1990
Mark Sauer reports pregnancies in postmenopausal women

1991
First preimplantation genetic screening (for cystic fibrosis)

1992
Fertility Clinic Success Rate and Certification Act calls for uniform definition of success; to take effect October 1994

1993
Supreme Court decides frozen embryos cannot be implanted against the father’s will
In Vitro Fertilization (IVF) - 2014

- SART Data: 61,740 IVF babies born in 2012 in U.S.
- IVF babies now constitute almost 2% of U.S. births
- Estimated 400,000 IVF babies born in 2012 in world
- IVF births now almost 4% of births in Europe
- Estimated 5,000,000 IVF births by Oct. 2013

- Who Knew ?????
The world's first IVF baby Louise Brown (2nd right) posing with her son Cameron, her mother Lesley Brown and IVF pioneer Professor Robert Edwards in 2008