What A Difference an X Makes: The Role of Sex Hormones and Sex Chromosomes in All Aspects of Life

Deborah J. Clegg
Vice President for Research
Professor Internal Medicine
Paul L. Foster School of Medicine
Texas Tech Health Sciences Center El Paso, TX
What A Difference an EX Makes:

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Males And Females Are Different?
Males And Females Are Different!!!!!!
Outside the gonads XY and XX cells are functionally equivalent....

NOT True!!!!

X ≠ Y

In females, the majority of the cells on one of the X chromosomes are silenced

However, some genes on the inactive X chromosome are not silenced, leading to higher levels of their products in female cells

The Y chromosome carries genes that are involved in basic cellular functions
Cardiovascular Disease is the Leading Killer of US Adults

CVD Mortality 1910-2015

Sex Differences In Cardiovascular Disease

The graphs illustrate the cumulative incidence of various cardiovascular diseases in men and women across different age groups. The diseases include coronary heart disease, cerebrovascular disease, heart failure, and other cardiovascular death. The incidence rates are presented against age in years.
Estrogens Regulate Metabolism

Basic Science

UTSW Medical Center
Estrogens: Critical Regulators of Metabolic Function
Estrogens Bind to One of Many Different Receptors
• Obesity (BMI: 30.5)
• Insulin Resistance
• Glucose Intolerance
• CVD
Estrogens: Critical Regulators of Metabolism
ERα\textsuperscript{loxlox}/Nestin-Cre

Reduction of ERα expression in the whole brain

Xu et al., Cell Metabolism, 2011
Hypothalamic ERα Influences Body Weight

Distinct Hypothalamic Neurons Mediate Estrogenic Effects on Energy Homeostasis and Reproduction

Yong Xu,1,2,5 Thekkethil P. Nedungadi,3,5 Liangru Zhu,1 Nasim Sobhani,3 Boman G. Irani,3 Kathryn E. Davis,3 Xiaorui Zhang,1 Fang Zou,1 Lana M. Gent,3 Lisa D. Hahner,3 Sohaib A. Khan,4 Carol F. Elias,2 Joel K. Elmquist,2 and Deborah J. Clegg3,*

Xu Y. et al., Cell Metabolism, 2012
HFD-Induced Obesity

Western diet

<table>
<thead>
<tr>
<th></th>
<th>% by weight</th>
<th>% kcal from</th>
</tr>
</thead>
<tbody>
<tr>
<td>Protein</td>
<td>17.3</td>
<td>15.2</td>
</tr>
<tr>
<td>Carbohydrate</td>
<td>48.5</td>
<td>42.7</td>
</tr>
<tr>
<td>Fat</td>
<td>21.2</td>
<td>42.0</td>
</tr>
</tbody>
</table>

Typical Fatty Acid Profile

<table>
<thead>
<tr>
<th></th>
<th>Mean</th>
<th>SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Saturated fat</td>
<td>61.8</td>
<td>2.0</td>
</tr>
<tr>
<td>Monounsaturated fat</td>
<td>27.3</td>
<td>2.1</td>
</tr>
<tr>
<td>Polyunsaturated fat</td>
<td>4.7</td>
<td>0.8</td>
</tr>
<tr>
<td>4:0</td>
<td>2.1</td>
<td>1.1</td>
</tr>
<tr>
<td>6:0</td>
<td>1.5</td>
<td>0.7</td>
</tr>
<tr>
<td>8:0</td>
<td>1.1</td>
<td>0.3</td>
</tr>
<tr>
<td>10:0</td>
<td>2.6</td>
<td>0.5</td>
</tr>
<tr>
<td>12:0</td>
<td>3.3</td>
<td>0.5</td>
</tr>
<tr>
<td>14:0</td>
<td>10.6</td>
<td>0.9</td>
</tr>
<tr>
<td>16:0</td>
<td>28.9</td>
<td>1.3</td>
</tr>
<tr>
<td>16:1</td>
<td>1.5</td>
<td>0.2</td>
</tr>
<tr>
<td>18:0</td>
<td>12.5</td>
<td>0.8</td>
</tr>
<tr>
<td>18:1 (Oleic)</td>
<td>29.9</td>
<td>2.6</td>
</tr>
<tr>
<td>18:1 Isomers²</td>
<td>4.0</td>
<td>1.2</td>
</tr>
<tr>
<td>18:2 (Linoleic)</td>
<td>2.3</td>
<td>1.0</td>
</tr>
<tr>
<td>18:3 Isomers³</td>
<td>1.3</td>
<td>0.5</td>
</tr>
<tr>
<td>18:3 (Linolenic)</td>
<td>0.7</td>
<td>0.2</td>
</tr>
</tbody>
</table>

Body weight

Morselli E, *Cell Reports*, 2014

TD. 88137 Harlan
HFD-Induces Inflammation in the Hypothalamus

Morselli E, *Cell Reports*, 2014

*: p< 0.005 (chow to HFD)
†: p< 0.005 (chow M to chow F)
#: p< 0.005 (HFD M to HFD F)
Does the HFD-Induced Inflammation Occur in Females??

Morselli E, Cell Reports, 2014

* : p< 0.005 (chow to HFD)
†: p< 0.005 (chow M to chow F)
#: p< 0.005 (HFD M to HFD F)
Females Do Not Have HFD-Induced Hypothalamic Inflammation

Morselli E, *Cell Reports*, 2014
**In Vitro: N43 cells, Hypothalamic Neurons**

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**Palmitic Acid (PA)**

- Most common fatty acid found in animals
- Palmitoyl-CoA is accumulated in the hypothalamus of animals fed on HFD
- Induces inflammasome activation
Palmitic Acid (PA) is Elevated **ONLY** in the Male Brain Following HFD Exposure

Morselli E, *Cell Reports*, 2014
Lipidomics: Males and Females Differ

Sex As a Biological Variable Policy

NIH Mandate: January 25, 2016
Sex Differences In Cardiovascular Disease

![Cumulative incidence graphs showing differences between men and women for various cardiovascular conditions.](image)
‘Estrogens’ and CVD Risk

• The incidence of CVD and resultant morbidity and mortality is lower in premenopausal women compared to age-matched men.

• Female protection from CVD disappears after menopause, implicating both age and estrogens as the primary sources of cardioprotection.

• Hypoestrogenemia (HypoE), such as Turner’s syndrome and primary ovarian insufficiency, increases CVD risk

• Early menopause is associated with accelerated atherosclerosis, a 2.6 fold increase in CVD risk, and increased risk of CVD mortality.
Menstrual Cycle and Risk of CHD in the Nurses Health Study

Hazard Ratio for CHD

- Regular (n=810)
- Usually Regular (n=327)
- Usually Irregular (n=184)
- Very Irregular (n=96)

p<0.001 for trend

Solomon et al, J Clin Endocrinol Metab, 2002
Increased Cardiovascular Disease in Women as They Age
Estrogen Levels in Women Across the Lifespan
Trend for ↓ risk of heart disease when hormone therapy initiated w/in 10yr compared to ↑ risk in women > 20yr past menopause
Cardiometabolic Effects of Estradiol

Modified from Mendelsohn & Karas. NEJM. 340:1801-1811, 1999

Modified from Traish. Steroids. 88:117-126, 2014
A Basic Science Approach for Determining the Mechanisms for Sexual Dimorphisms In Humans

Differentiation of hypothalamic-like neurons from human pluripotent stem cells

Liheng Wang, Karla Meece, Damien J. Williams, Kingui Alice Lu, Matthew Ziemienow, Garrett Heinrich, Jayne Martin Cardi, Charles A. Laduc, Lei Sun, Lori M. Zeltzer, Matthew Plessky, Robin Collard, Stephen H. Tsang, Sharon L. Wardlaw, Dieter Egli, and Randolph G. Laibe
Scatter Plots
(negative value indicates downregulation)
What??

• These data are opposite from our *pre-clinical* observations because:
  
  • Humans aren’t mice?
  
  • Is there something that happens during the culturing of cells which augments results????

• *Does the cell culture media influence the results?*
Cell Culture And *Cell Sex*

- Cell culture medium is the most important component of the culture environment because it provides:
  - Nutrients
  - Growth Factors
  - *Hormones for cell growth*
  - Factors to facilitate regulating the pH of the culture
Sex Chromosomes And Sex Hormones

Estrogen > Testosterone  
Testosterone > Estrogen

Cis Female  
Cis Male
Transgender Individuals

Testosterone > Estrogen

Transgender men

Estrogen > Testosterone

Transgender woman
Sex and Gender

Morselli et al., *Cell Metabolism*, 2016
Gender Identity

• In the United States, there is an estimated 1.4 million people living with gender identity disorder

![Figure 1. Percentage of Individuals Who Identify as Transgender by Age](image)

• Transgender individuals opt for interventions and procedures aimed at alleviating the incongruence between their gender identity and their biological chromosomal and gonadal sex
Table 4. Criteria for Gender-Affirming Hormone Therapy for Adults

1. Persistent, well-documented gender dysphoria/gender incongruence
2. The capacity to make a fully informed decision and to consent for treatment
3. The age of majority in a given country (if younger, follow the criteria for adolescents)
4. Mental health concerns, if present, must be reasonably well controlled

Table 11. Hormone Regimens in Transgender Persons

<table>
<thead>
<tr>
<th>Transgender females</th>
<th>Transgender males</th>
</tr>
</thead>
<tbody>
<tr>
<td>Estradiol Oral</td>
<td>Testosterone</td>
</tr>
<tr>
<td>Transdermal Estradiol transdermal patch (New patch placed every 3-5 d)</td>
<td>Parenteral testosterone</td>
</tr>
<tr>
<td>Parenteral Estradiol valerate or cypionate</td>
<td>Transdermal testosterone</td>
</tr>
<tr>
<td>Anti-androgens</td>
<td>Testosterone enanthate or cypionate</td>
</tr>
<tr>
<td>Spironolactone</td>
<td>Testosterone undecanoate</td>
</tr>
<tr>
<td>Cyproterone acetate</td>
<td>Transdermal testosterone</td>
</tr>
<tr>
<td>GnRH agonist</td>
<td>Testosterone gel 1.6%</td>
</tr>
<tr>
<td></td>
<td>Testosterone transdermal patch</td>
</tr>
<tr>
<td>2.0-6.0 mg Id</td>
<td>100-200 mg SQ (IM) every 2 wk or SQ (SC) 50% per week</td>
</tr>
<tr>
<td>0.025-0.2 mg Id</td>
<td>1000 mg every 12 wk</td>
</tr>
<tr>
<td>5-30 mg IM every 2 wk</td>
<td>50-100 mg Id</td>
</tr>
<tr>
<td>2-10 mg IM every week</td>
<td>2.5-7.5 mg Id</td>
</tr>
</tbody>
</table>
Sex Differences In Cardiovascular Disease
Increased Cardiovascular Morbidity/Mortality In Transgender Women

Remarkably, the second leading cause of death in transgender women is CVD

Dhejne et al. PLoS ONE. 2011, E16885
Death Rates in Transgender Individuals

Dhejne et al. Plos One. 2011
Aging Population in the US: Is CVD a Future Epidemic

• ~40 million adults >65 yrs in 2010
• increase to 2020 to 55 million
• projected to increase to ~72 million by 2030
Is the Trajectory of Cardiometabolic Health Different in Transgender vs Cisgender Individuals Across the Lifespan?
Transgender Women Dying Faster Than General Population — Biggest drivers were HIV, suicide, and CVD

In a retrospective, observational study spanning from 1972 to 2018, a total of 10.8% (317 of 2,927) of transgender women using hormonal therapy included in the analysis died during follow-up. This number was starkly higher than the number of cisgender men and women in the general population that died over the same period.

*The Lancet Diabetes & Endocrinology* September 2, 2021
Acute CV Events in Transgender Individuals Using Hormone Therapy

<table>
<thead>
<tr>
<th>Acute Cardiovascular Events</th>
<th>Women (IRs)</th>
<th>Using Women as Reference</th>
<th>Using Men as Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>ECs</td>
<td>SIR (95% CI)</td>
<td>ECs</td>
</tr>
<tr>
<td>Transwomen</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Stroke</td>
<td>29 (127)</td>
<td>12.01</td>
<td>16.08</td>
</tr>
<tr>
<td>Myocardial infarction</td>
<td>30 (131)</td>
<td>11.38</td>
<td>38.03</td>
</tr>
<tr>
<td>Venous thromboembolism</td>
<td>73 (327)</td>
<td>13.22</td>
<td>16.04</td>
</tr>
<tr>
<td>Transmen</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Stroke</td>
<td>6 (55)</td>
<td>3.49</td>
<td>4.10</td>
</tr>
<tr>
<td>Myocardial infarction</td>
<td>11 (229)</td>
<td>2.98</td>
<td>10.99</td>
</tr>
<tr>
<td>Venous thromboembolism</td>
<td>2 (18)</td>
<td>0.84</td>
<td>5.56</td>
</tr>
</tbody>
</table>

ECs indicates expected cases; IR, incidence rate; OCIs, observed cases; and SIR, standardized incidence ratio.
*Per 100,000 person-years.
†Significant finding.

Nota, et al. Circulation, 2019;139:00-00. DOI: 10.1161/CIRCULATIONAHA.118.038584
Cardiometabolic Health in Transgender Women
Cardiometabolic Health/Risk in Transgender Individuals With \textit{Gender Affirming Surgery}???
Study Design

12 MtoF:
- 8 testes (+)
- 4 testes (-)

Two visits:
1) Oral glucose tolerance testing
   Blood Chemistry
2) MRS - Liver

Nelson et al., 2016
Nelson et al., 2016
Lower serum testosterone is independently associated with insulin resistance in non-diabetic older men: the Health In Men Study

Bu B Yeap¹,², S A Paul Chubb¹,³, Zoë Hyde⁴, Konrad Jamrozik⁵, Graeme J Hankey¹, Leon Flicker¹,⁴ and Paul E Norman⁶
Testosterone Levels

Nelson et al., 2016
Insulin Resistance and Testosterone Concentration

Nelson et al., 2016
Hepatic Steatosis

Hepatic TG Content

% F/W

Testes -

Testes +

Hepatic TG Content

Hepatic Water

Hepatic Fat

Hepatic Tissue Metabolites

3.5 2.5 1.5 ppm

20

10

0

Nelson et al., 2016
Hepatic Steatosis and Testosterone Concentration

Nelson et al., 2016
High Testosterone Levels Associated with Obesity

Nelson et al., 2016
Conversion of Testosterone to Estrogen, Tissue Levels or Circulating Levels?
Is There An *Optimal Ratio* Of Androgens To Estrogens And Sex Chromosomes To Protect Against Disease Risk???
Testosterone and Estrogen Production in Men and Women Across the Lifespan
Elevated T/E_2 Ratio Is Associated with an Increased Risk of Cerebrovascular Disease in Elderly Men

Yanping Gong¹, Hailing Xiao¹, Chunlin Li¹, Jie Bai², Xiaoling Cheng¹, Mengmeng Jin¹, Boruo Sun¹, Yanhui Lu¹, Yinghong Shao¹, Hui Tian¹

Imbalance of testosterone/estradiol promotes male CHD development.
Zheng HY¹, Li Y, Dai W, Wei CD, Sun KS, Tong YQ
## Testosterone to Estradiol Ratio Calculator

<p>| | |</p>
<table>
<thead>
<tr>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Testosterone</strong></td>
<td><strong>43 ng/dL</strong></td>
</tr>
<tr>
<td><strong>Estradiol</strong></td>
<td><strong>101 pg/mL</strong></td>
</tr>
<tr>
<td><strong>Ratio</strong></td>
<td><strong>4.02</strong></td>
</tr>
</tbody>
</table>
Is the *Ratio* Between T and E2 More Important Than Their Absolute Quantities?

Nelson et al., 2016

**T/E2**

53x HIGHER!!
Sex differences in skeletal muscle revealed through fiber type, capillarity, and transcriptomics profiling in mice

Juliana O’Reilly1 | Kikumi D. Ono-Moore2 | Sree V. Chintapalli2,3 | Jennifer M. Rutkowski4,5 | Todd Tolentino5,6 | K. C. Kent Lloyd5,6,7 | I. Mark Olfert1 | Sean H. Adams7

Physiological Reports. 2021;9:e15031
TRANSCRIPTS EXPRESSED IN FEMALES AT LEVELS 200% OF THE LEVEL IN MALES, IN BOTH MUSCLES

![Venn Diagram]

- **GASTROC - UP**
  - 88 (49.4%)
  - Xist
  - Akr1cl
  - Mrgprg
  - Slc39a12
  - Adrb3
  - Dhrs9

- **SOLEUS - UP**
  - 16 (9%)
  - Ces2g
  - Car12
  - H2-Q10
  - AW551984
  - Fasn
  - Scg3

- **GASTROC & SOLEUS - UP**
  - 74 (41.6%)
  - Greb1
  - Elovl6
  - Ngfr
  - Otoa
Ever wonder how much faster (or slower) you'd run if you were the opposite sex?

Janet Furman Bowman may be the only runner in America who knows

Runners World
May 4, 2005
• **Jim Furman, a 5’11”, 148-pound** middle-aged man in excellent physical shape, extremely fast runner.

• As **Janet Furman, a 5’11”, 148-pound** middle-aged woman in excellent physical shape. When Janet sprints across the finish line- and checks her watch for her time, 23:27, she knows instantly how it compares to her PR for the 5-K: **six minutes, 25 seconds slower**, or more than two minutes per mile.
Matching donors improve survival odds

Survival rates increase if heart transplants are from donors of the same sex. Last year, 71 percent of recipients were of the same gender.

Heart transplant survival estimates:
- Male donor/Male recipient: 59%
- Female/Female: 54%
- Male/Female: 56%
- Female/Male: 61%

Annual transplants:
- 2.5 thousand

Donor and recipient same gender
- Mixed gender

Years: 1, 3, 5, 9

SOURCE: United Network for Organ Sharing

Summary of Our Findings

• Our preliminary data suggest transgender women who elected for bi-lateral orchietomy had improved metabolic health when compared to those transgender women who retained their testes.

• Transgender women who retained their testes were stratified according to circulating T levels, those with the highest T also had the greatest level of hepatic steatosis and insulin resistance.
Is Health Influenced by Sex?
“Personalized” by Sex??
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Noel Bairey Merz MD

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NIH: P01 088761-01
Society for Womens Health Foundation
Klarman Foundation
2001 U.S. Institute of Medicine Declared That Every Cell Has A Sex

Cell Sex Underreported In Medicine And Science
Males And Females Have Partially Different Genomes

• Male and female germ cells differentially imprint the genetic information to be transmitted to their progeny

• In females, the majority of the cells on one of the X chromosomes are silenced

• However, some genes on the inactive X chromosome are not silenced, leading to higher levels of their products in female cells

• The Y chromosome carries genes that are involved in basic cellular functions
X Chromosome Excluded From > 90% of GWAS

• Not ‘genome wide association’

• ‘Neutrome’ wide association – virtually devoid of the X chromosome