Women & Sex Differences in Medicine
Stanford (WSDM) Center

Director, Marcia Stefanick, Ph.D.

Professor of Medicine
Stanford Prevention Research Center
Professor of Obstetrics & Gynecology
Stanford Univ. School of Medicine

Sonoo Thadaney, MBA
Managing Director

T.O. Preising, JD, MEd
Program Associate

Vision: Healthy women *and men* - from conception through the Life Course

Mission: Advancing human health across the lifespan through research and education in women's health, biology of sex differences, and gender medicine

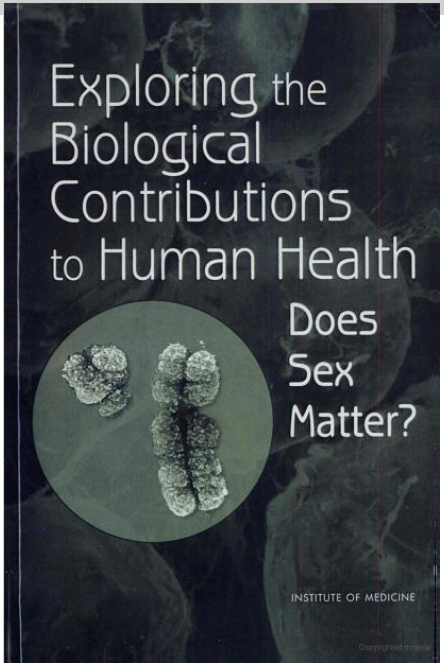
Special THANKS, WSDM Interns! **Ashley** Jowell, **Jasmine** Kyi
Sarah Roberts, **Baffour** Kyerematen

Stanford MEDICINE | School of Medicine

http://wsdm.stanford.edu

Stanford WSDM Center

2001: Institute of Medicine - IOM report on Sex Differences
2010: IOM – Women's Health Research



"Every Cell has Sex"

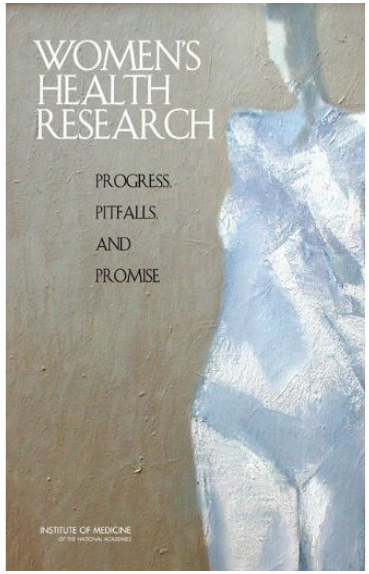
Proceedings of the Institute of Medicine (IOM) Committee on **Understanding the Biology of Sex and Gender Differences.**
Ed: T.M. Wizemann, M-L.Pardue

- **Sex – *Biological*** constellation of attributes that derive from **sex chromosome, reproductive organs,** and/or **specific hormones.** *(not interchangable with "gender")*
- **Gender - *Socio-cultural*** processes that interact with and influence biology. *IOM 2001: "*person's self-representation as male or female**."**

Gender norms: cultural attitudes that shape "feminine" and "masculine" behaviors

Gender relations: social interactions based on gender

Gender identities: how individuals or groups perceive and present themselves.



IOM Committee on **Women's Health Research. Progress, Pitfalls, and Promise**
National Academy Press, **2010**

http://genderedinnovations.stanford.edu/terms/gender.html

Women & Sex Differences in Medicine Stanford (WSDM) Center

WSDM Seed Grant Workshop (September 27, 2013)

Stanford University School of Medicine Departments (Basic Science & Clinical)

in alphabetical order

Department Mission Statements (in relation to WSDM Mission)
& WSDM-related Research Presentations by

***Chair-designated* Department Representatives**

SLIDES@ https://stanford.box.com/s/lobm8oxkga9onuwnp01g

Seed Grants Awarded to 5 Interdepartmental Teams

http://**wsdm.stanford.edu**

Stanford Women & Sex Differences in Medicine (WSDM) Center 2013 Seed Grand Awards

- **Sex-specific Recovery from Stroke: Differences in Clinical Trajectory**
  Rona Giffard, MD, PhD - *Anesthesia, Perioperative & Pain Medicine*
  Marion Buckwalter, MD, PhD - *Neurology & Neurological Sciences*
- **Sex differences in adipocyte responses to experimentally-induced weight gain**
  Tracey McLaughlin, MD, MS – *Medicine (Endocrinology, Gerontol, Metab)*
  Michael Snyder, MD - *Genetics*
- **Elucidating the APOE-by-Gender Interaction in Alzheimer's Disease**
  Michael D. Grecius, MD - *Neurology & Neurological Sciences*
  Hua Tang, PhD - *Genetics*
- **Effect of cell sex on smooth muscle cells and fibroblasts derived from human embryonic stem cells**
  Bertha Chen, MD - *Obstetrics & Gynecology*
  Jean Tang, MD, PhD - *Dermatology*
- **Androgen Signaling in Alcohol-Seeking Behavior between *Genders***
  Zijie Sun, PhD, MD- Urology
  Luis de Lecea, PhD - Psychiatry & Behavioral Sciences

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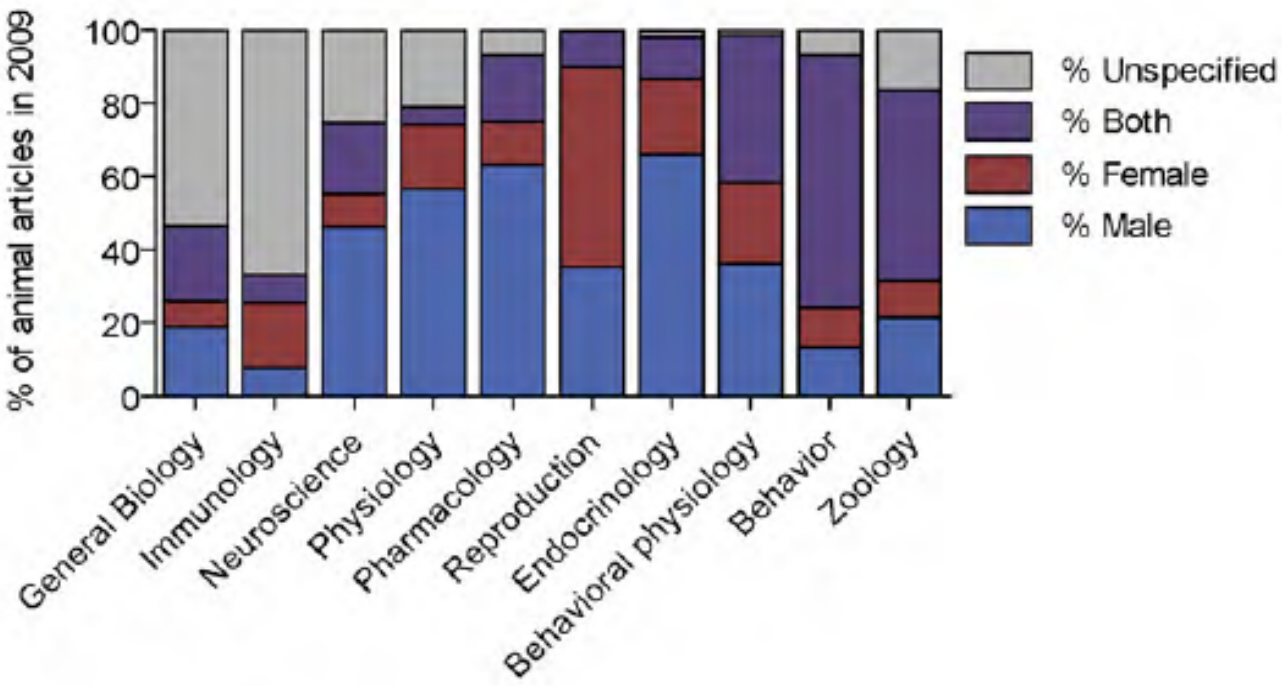
10 yrs later: Researchers not complying with guidelines

Distribution of non-human animal studies by sex and field in 2009



Percent of articles of **non-human animal research** using only male or female animals, both male & female, or did not specify sex of animals.

Beery, Zucker. *Sex bias in neuroscience and biomedical research.* **Neurosci Biobehav Rev 2010; 35: 565**.

Published online 16 March 2010 | *Nature* **464**, 332-333 (2010) | doi:10.1038/464332b

News

Sex bias blights drug studies

Omission of females is skewing results.

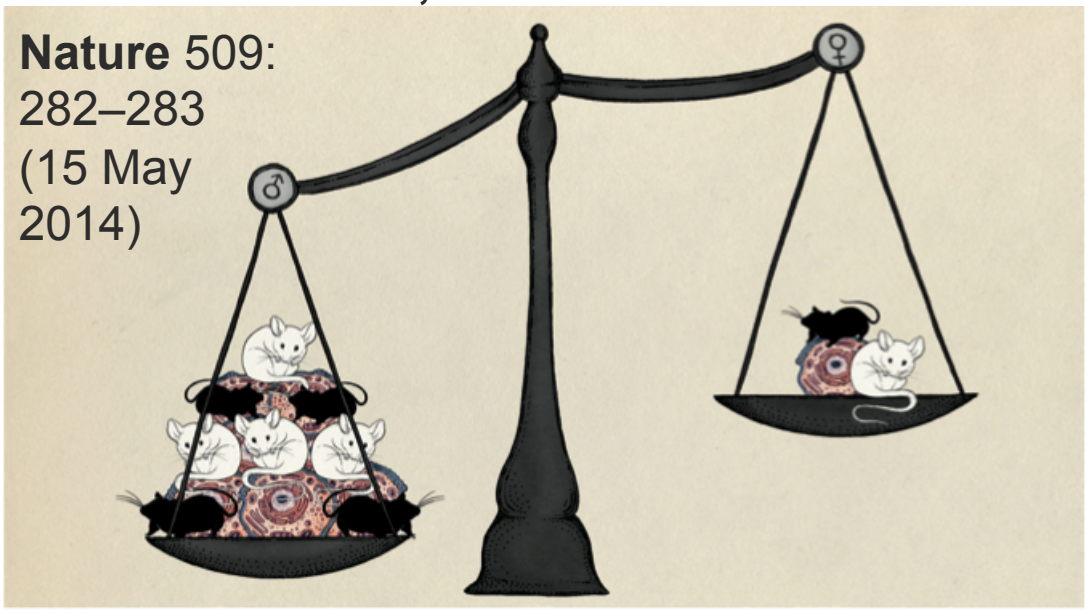
NATURE | EDITORIAL

Putting gender on the agenda

Nature **465**, 665 (10 June 2010) | doi:10.1038/465665a
Published online 09 June 2010

Women & Sex Differences in Medicine
Stanford (WSDM) Center

Janine A. Clayton, Director NIH Office of Research on Women's Health
Francis S. Collins, Director National Institutes of Health, Bethesda, Maryland, USA.



NIH to balance sex in cell and animal studies

Janine A. Clayton and **Francis S. Collins** unveil policies to ensure that preclinical research funded by the US National Institutes of Health considers females and males.

The New York Times

Health Researchers Will Get $10.1 Million to Counter Gender Bias in Studies

Including female mice in studies can drive up the costs, because his team must do extra experiments to control for the females' estrus cycles.
Stuart Wilson/Science Source

By RONI CARYN RABIN
SEPT. 23, 2014

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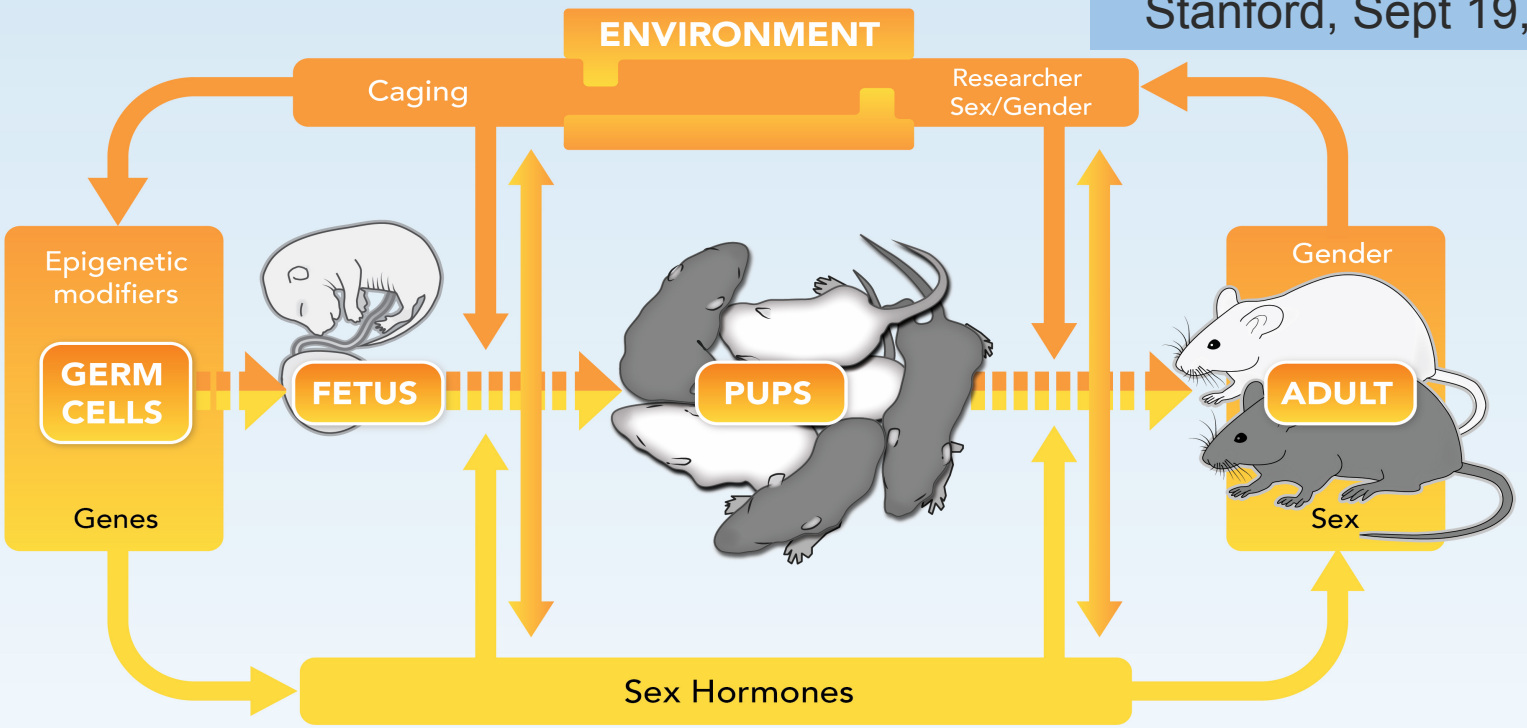
Women & Sex Differences in Medicine
Stanford (WSDM) Center

Analyzing Sex in Preclinical Research: FAQs

Sex and Gender Interact

NSF-funded Workshop
(Schiebinger, Stefanick)
Stanford, Sept 19, 2014



Adapted from Regitz-Zagrosek, V. (2012). Sex and Gender Differences in Health. EMBO Reports. 13(7). 596-603.

Stanford WSDM Center
Workshop (October 17, 2014)

Methods and Techniques for Integrating the Biological Variable "Sex" in Preclinical Research

A Workshop Sponsored by the Office of Research on Women's Health

National Institutes of Health
Office of Extramural Research

October 20, 2014
7:45 am to 5:30 pm
John Edward Porter Neuroscience Building
Building 35A, NIH, Main Campus, Bethesda, MD
#SexinScience

Request for Information: Consideration of Sex As a Biological Variable in Biomedical Research

http://grants.nih.gov/grants/rfi/rfi.cfm?ID=37

Background

In a May 14, 2014 Nature commentary (see Nature. 2014 May 15;509(7500):282-3.), NIH leadership stated an intention to develop and implement policies requiring applicants to consider sex as a biological variable in the design and analysis of NIH-funded research involving animals and cells. Although we have made major progress in achieving balance of sex in human studies — women now account for roughly half of the participants in NIH-funded clinical trials — we have not seen a similar pattern in biomedical research. Animal studies have typically focused on males, and investigators studying cell models have often ignored the sex of the individual from which the cells were obtained. Even if both sexes are included in a study design, resulting data may not be analyzed or disaggregated by sex. The failure to consider sex as a variable may leave critical knowledge gaps and undermine the quality and reproducibility of research findings (see *Nature*. 2014 Jan 30;505 (7485):612-3). Consideration of sex is a critical component of rigorous experimental design, just like randomization, blinding, sample-size calculations, or other basic design elements. By developing a policy to ensure that sex is considered in NIH-funded studies, NIH will ensure that sex and sex differences are examined in all aspects biomedical research. This will lead to a stronger foundation upon which to build clinical research and clinical trials.

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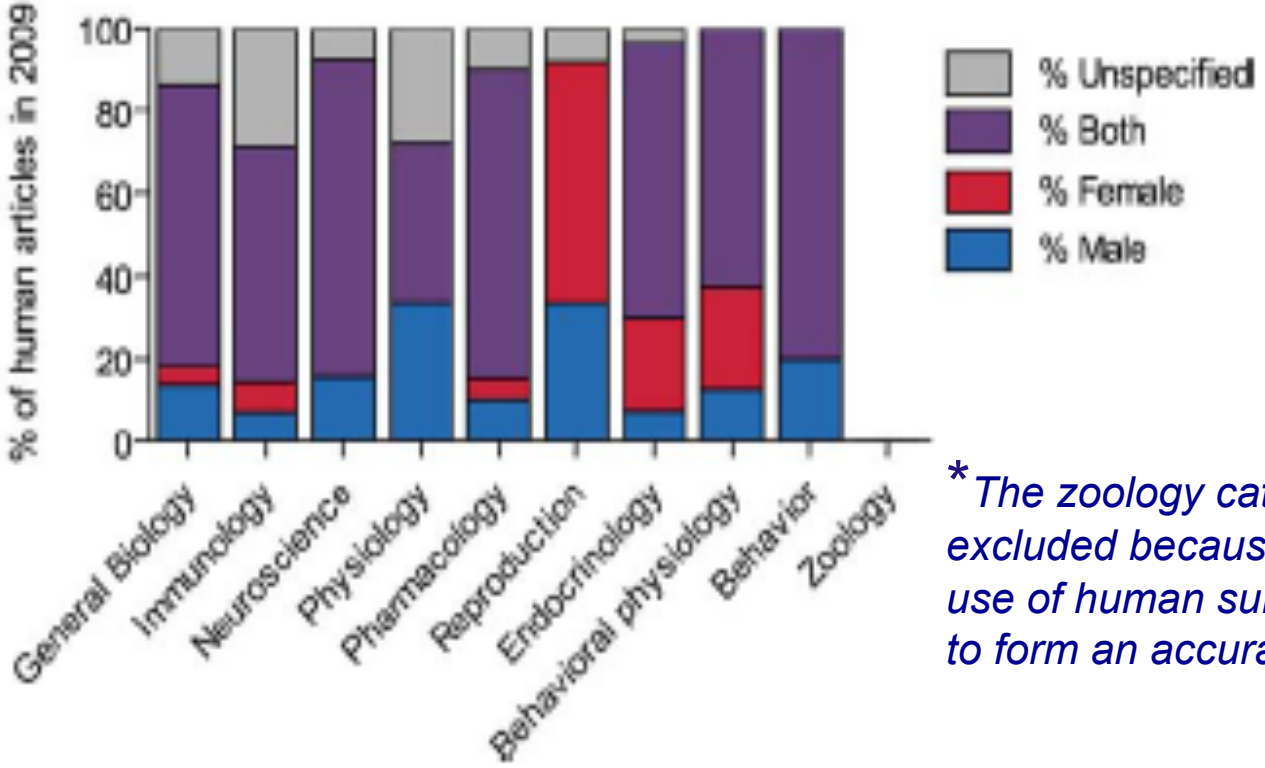
A higher percentage of articles reported on both sexes in human *than* non-human animal research (60% versus 26%).

Distribution of studies by sex and field in 2009



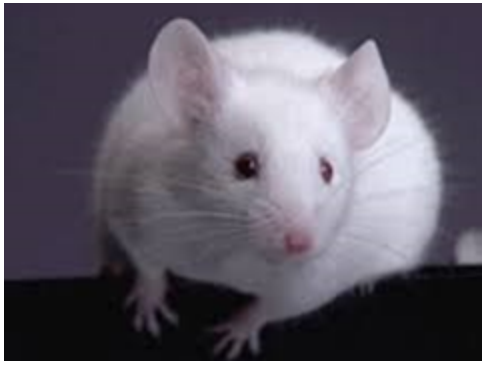
**The zoology category was excluded because of insufficient use of human subjects in this field to form an accurate estimate*

Percent of articles describing **human research** in the same categories.*

Beery, Zucker. *Sex bias in neuroscience and biomedical research.* **Neurosci Biobehav Rev 2010; 35: 565.**

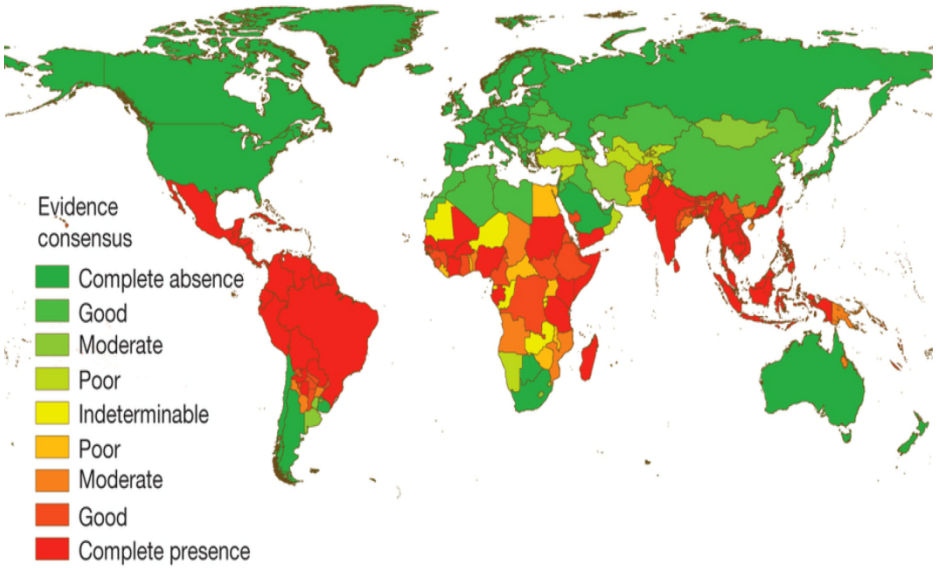
WSDM – 2014 Workshop

Rodent Sex Differences



Claude Nagamine,
DVM, PhD, DACLAM

Dengue Virus Mouse Models



AG 129

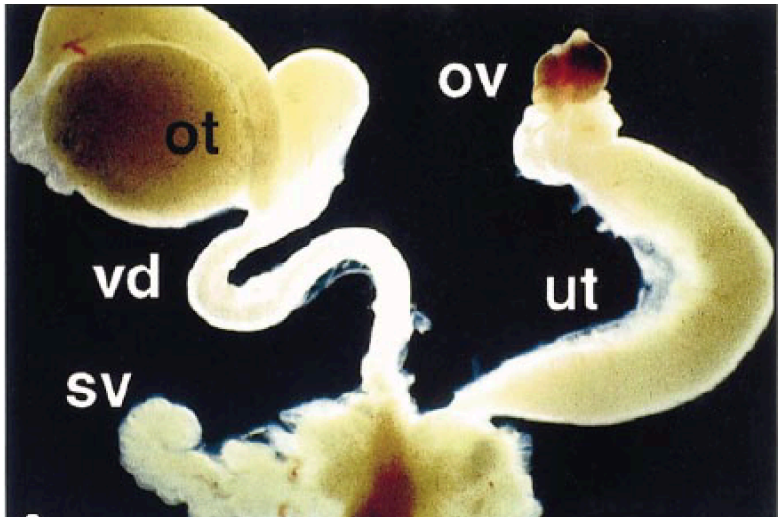
AG B6

- Karla Kirkegaard (Genet / Micro&Immun)
- Jan Carette (Micro&Immun)
- Shirit Einav (Med / Infect Dz)
- Jennifer Johns (Comp Med - *Anaplasma*)

Double knockout for receptors of Interferons Alpha/Beta and Gamma (Type I, II interferons) – susceptible to viruses / bacteria

Mouse Model of Abnormal Sex Determination

XY progeny develop as hermaphrodites or XY females



XY hermaphrodite gonads

MOUSE BIOLOGY

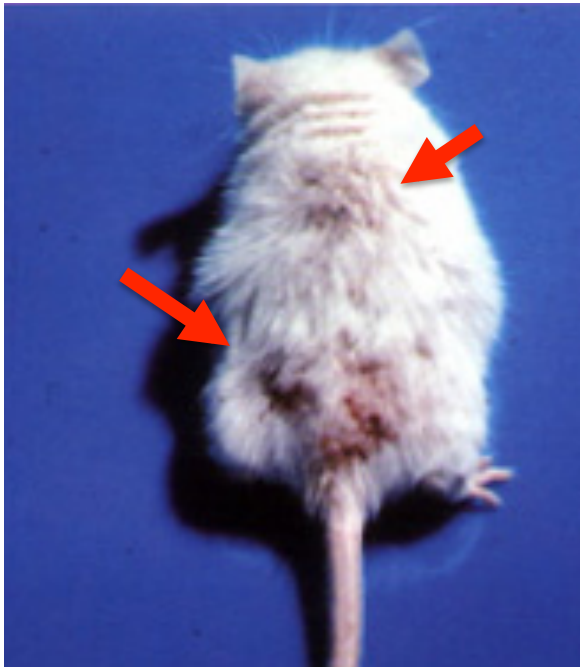
- Mice generally live ~ 2-3 years.
- Fecundity – decreases in females ≥ 6 mo; males ≥ 8 mo
- Estrous cycle = 4-5 days, gestation = 18-21 days; pups weaned at ~21 days (all varies with strains).
- Estrous can be controlled: crowd 5 females in cage w/o male → estrous prolonged → stops (anestrus; Lee-Boot Effect).
- Estrus can be synchronized: anestrus females + male dirty bedding → all females enter estrous (Whitten Effect).

STANDARD OF PRACTICE: BREEDING

- Once bred, do not house males together! They will fight. Females usually OK.

- When breeding, do not place two or more males with females - the males will fight.

- When breeding, place 1-2 females with 1 male.



MOUSE STRAIN FACTS

- Some are blind – C3H, FVB, SJL, SWISS
- Some have hearing loss – DBA/2J
- Some have neurological abnormalities – BALB/c (corpus callosum)
- Some have males that fight – BALB/c, FVB, SJL
- Some develop tumors – AKR (thymic lymphomas, >3 mo)

TAKE HOME MESSAGE: **STRAIN IS IMPORTANT! CHOOSE WISELY.**

SUBSTRAINS – WHY YOU SHOULD CARE

- Lines separated for 20 generations, e.g., 10 generations in parent colony, 10 in your colony

- BALB/cAnBy vs. BALB/cJ
  - weight
  - behavior
  - induction of plasmacytoma (relevant to mAb production)

TAKE HOME MESSAGE: **PAY ATTENTION TO SUBSTRAIN.**

Intrinsic Factors Affecting Animal Research

Sex

- Differences in pharmacologic and toxicologic responses has been demonstrated between male and female rodents.

- Sex-related differences in DMBA-induced mammary tumors:
  - Wistar-Furth rats: 100% in females; 19% in males.
  - Copenhagen rats: No difference

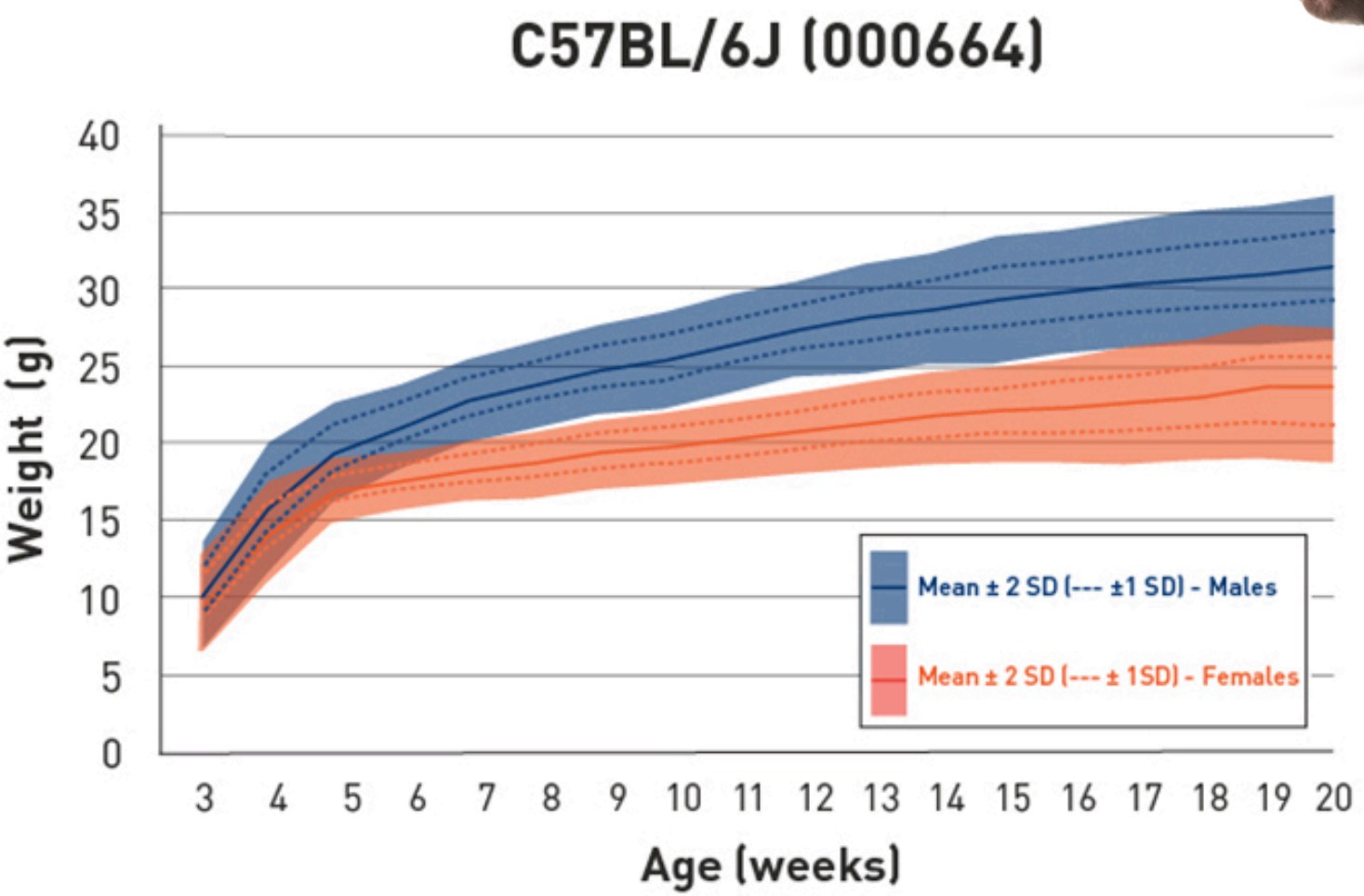
Take home message: **SEX IS IMPORTANT! CHOOSE WISELY.**

Intrinsic Factors Affecting Animal Research

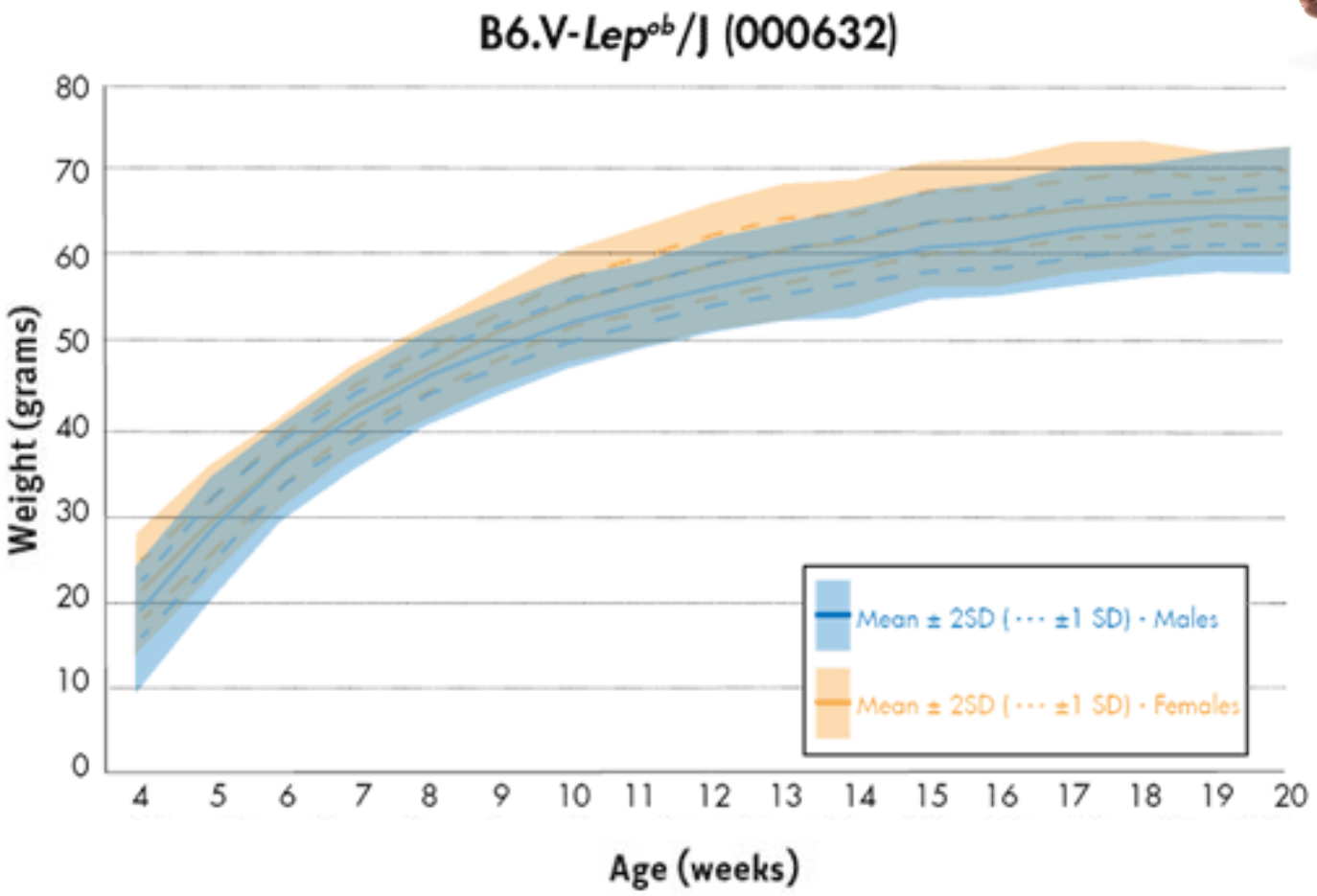
Endocrine Factors

- Sex-hormones are important determinants of cytochrome P450 enzyme activity.
- Testosterone administered to female rats increases their ability to biotransform xenobiotics.
- Castrating male rats decreases the ability to biotransform xenobiotics.

Male vs Female Mice: Age vs Weight in C57BL/6 (B6)



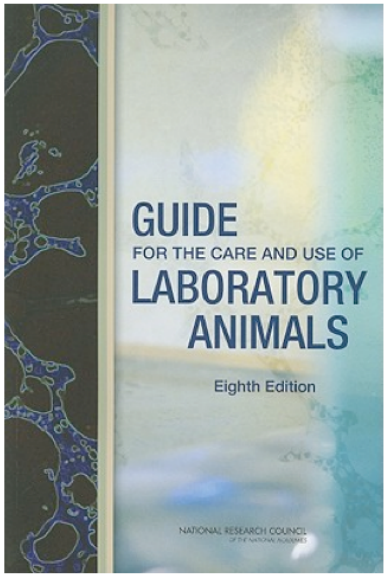
Male vs Female Mice: Age vs Weight in Obese mutation



Females equal to males.

HOUSING

- In general, no difference in housing between sexes … except if fighting.
- Must follow the *Guide for the Care and Use of Laboratory Animals*.



Weight	Floor area (in^2) / mouse	Height
<10 g	6 in^2 per mouse	**5 in**
10-15 g	8 in^2 per mouse	**5 in**
16-25 g	12 in^2 per mouse	**5 in**
>25g	≥ 15 in^2 per mouse	**5 in**

- 5 adult mice (25 g/ mouse) = ~75 in^2
- Typical cage floor area = 67-81 in^2

Housing Costs – Animal Research Is Expensive

- Major impact on sex difference mandate; mouse is cheapest.

Species	$ per day (2014)	$ / month (30 d)
NHP (rhesus)	$25.60	$768
Pig (<100 kg)	$63.75	$1913
Rabbit	$14.66	$440
Guinea pig	$8.25	$248
Hamster	$2.29	$69
Rat (2/cage)	$2.29	$69
Mouse (5/cage)	**$1.00**	**$30**

- Very important to monitor breeding / housing practices.

VSC Can Help

http://vsc.stanford.edu

- Mouse / Rat Handling and Basic Techniques Workshops
- Mouse Breeding Workshop
- Rodent Aseptic and Stereotaxic Surgery Workshops
- Colony Management Services
- APLAC Protocol Pre-review – get through regulations.

The NC3R's ARRIVE Guidelines

Sherril Green
10/17/2014
Veterinary Service Center

Acknowledgments: Daria Mochly-Rosen, and the National Center for the Replacement and Refinement & Reduction of Animals in Research (NC3R^{s})

The Arrive Guidelines: What Are They and What Are Their Purpose?

Animal **R**esearch: **R**eporting ***In Vivo*** **E**xperiments

The ARRIVE Guidelines were developed by NC3Rs (a UK government-sponsored scientific organization) to address the inconsistent/inadequate reporting of animal research in the publication process

ARRIVE guidelines are intended for researchers, editors and journal reviewers

Consists of 20 items describing the minimum information that all journals should include about animal experiments: species, strains, **SEX**, statistical and analytical methods, etc.

http://www.nc3rs.org.uk/ARRIVE

NC 3Rs

HOW BAD IS THE PROBLEM?

NC3Rs Survey (Kilkenny C., et al 2009)

59% of 271 randomly chosen articles stated the **sex**, age, species, strain *or* weight of the animals used

Most of the papers (87%) did not report using randomization or blinding (86%) to reduce bias in animal selection and outcome assessment

Only 70% of the publications used statistical methods which were fully described, and presented with a measure of precision and variability

OPEN ACCESS Freely available online

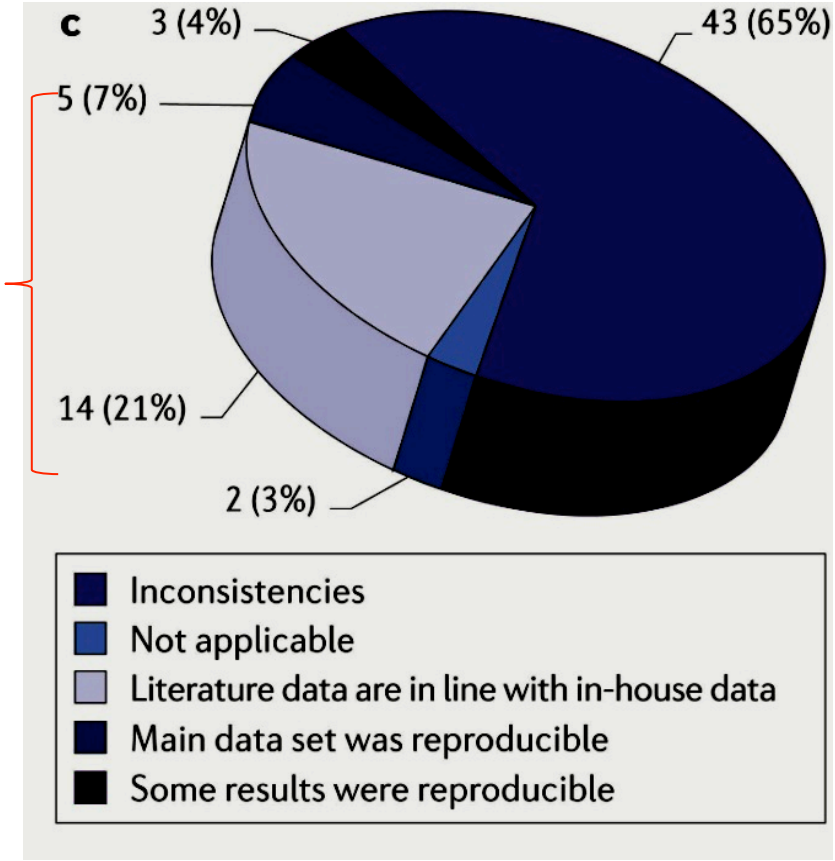
PLoS one

Survey of the Quality of Experimental Design, Statistical Analysis and Reporting of Research Using Animals

Carol Kilkenny[1]*, Nick Parsons[2], Ed Kadyszewski[3], Michael F. W. Festing[4], Innes C. Cuthill[5], Derek Fry[6], Jane Hutton[7], Douglas G. Altman[8]

Non-reproducibility of Academic Research

Bayer's study



- Survey of 23 scientists working on 67 projects.
- **ONLY 28%** of the published work was fully/mainly consistent.
- 65% inconsistencies requiring project termination.

WHAT ANIMALS USERS DON'T REPORT
WHAT JOURNALS/EDITORS/REVIEWERS FAIL TO ASK FOR

THE EXTRINSIC FACTORS AFFECTING ANIMAL RESEARCH

THE ITRINSC FACTORS AFFECTING ANIMAL RESEARCH

- **INTRINSIC FACTORS**: Inherent to the animal
  - Genetics, age, **sex**, nutrition, health status, immune status, circadian rhythms, **endocrine factors**

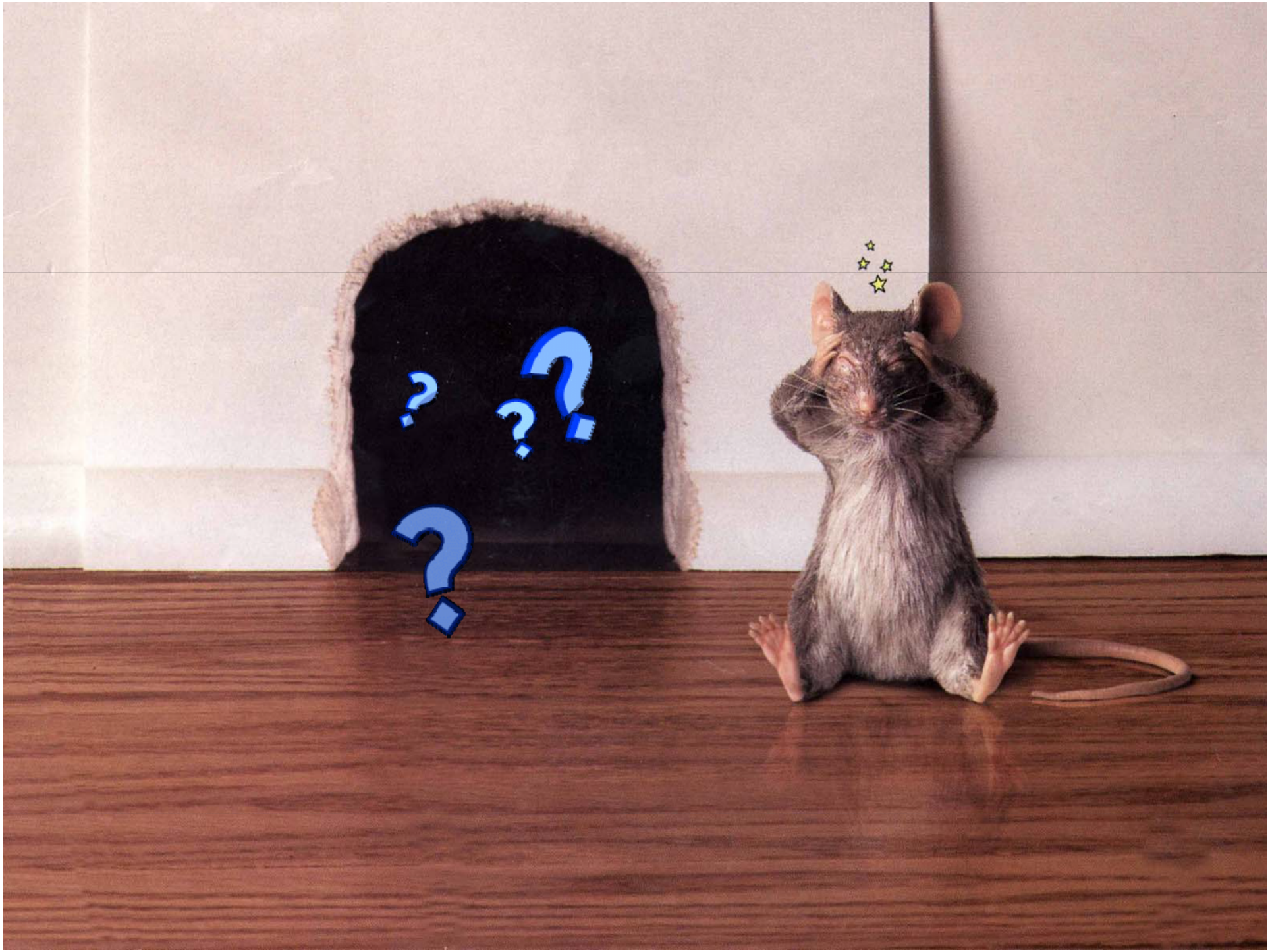
- **EXTRINSIC FACTORS**: External to the animal
  - Physical factors: housing, temperature, humidity, ventilation, feed, water, chemical factors, microbial agents, other stressors like housing density, chemicals, noise

INTRINSIC FACTORS AFFECTING RESEARCH

Sex/Endocrine Factors

- Marked differences in pharmacologic and toxicologic responses to xenobiotics has been demonstrated between male and female rats.

- Marked differences in response to endocrine disruptors has been demonstrated between males and females



The Arrive Guidelines: What Are They and What Are Their Purpose?

The objective of the ARRIVE Guidelines: to establish a standard for reporting animal studies, such that enough information about the animal experiments is included so that experiments are repeatable, reliable and valid.

Proven: reporting guidelines measurably improve the quality of reporting/ publications when instructions are included for authors:

- CONSORT guidelines for human clinical trials

- 90 plus other sets of guidelines (for genomics, metabolomics, etc.) developed for other health kinds of health research

ARRIVE

ARRIVE GUIDELINES

Endorsed by >300 peer reviewed Journals:

- Nature, PLOS, Science, including JAVMA, AJVR, Lab Animals
- 2013: all *Nature* journals require submission of a checklist with every manuscript. For research involving animals, this **includes reference to the ARRIVE guidelines** (Number 10 on the checklist).
- Summer 2014: *Science* requires reference to the ARRIVE guidelines
- But **NOT** endorsed by Comp Med or JAALAS

Endorsed by >25 Universities in the UK

- But **NOT** yet endorsed by many US universities (a handful at best).

WHAT'S UP WITH THAT?

The Bottom Line: This Has to Change

It is our **social responsibility to improve the quality** of animal research, so that others will not waste time, money and ANIMAL LIFE on useless studies. Include female animals in experiments.

My Opinion: Stanford should be leading the charge.

The **SEX** of animals should be reported. **Male AND females need to be included** in most, if not all, *in vivo* experiments.

So What Can We Do?

- ✓ Outreach/train scientists, reviewers, publishers
- ✓ Publicize the ARRIVE Guidelines
- ✓ Publish papers that follow ARRIVE Guidelines and state it
- ✓ Work with journal editorial review boards and push for a change
- ✓ Respond to critics who make sweeping generalizations.

"*Genomic Responses in Mouse Models Poorly Mimic Human Inflammatory Disease", Feb 2013, Proceedings National Academy of Sciences*

- Only one genetically identical inbred strain was used (C57BL/6).
- That's equivalent to using ONE individual human. **No female mice included.**

Winds of Change

- Reproducibility Initiatives

  www.sciencexchange.com

- Data Sharing
  - REACH
  - FIGSHARE
  - F1000 Research (open science journal)
- National/International Research Animal Registries
- Government Funded, Journal-Mandated MAs and SRs
- Systematic Review Center for Laboratory Animal Experimentation (SYRCLE)
- Meta-Research Innovation Center at Stanford

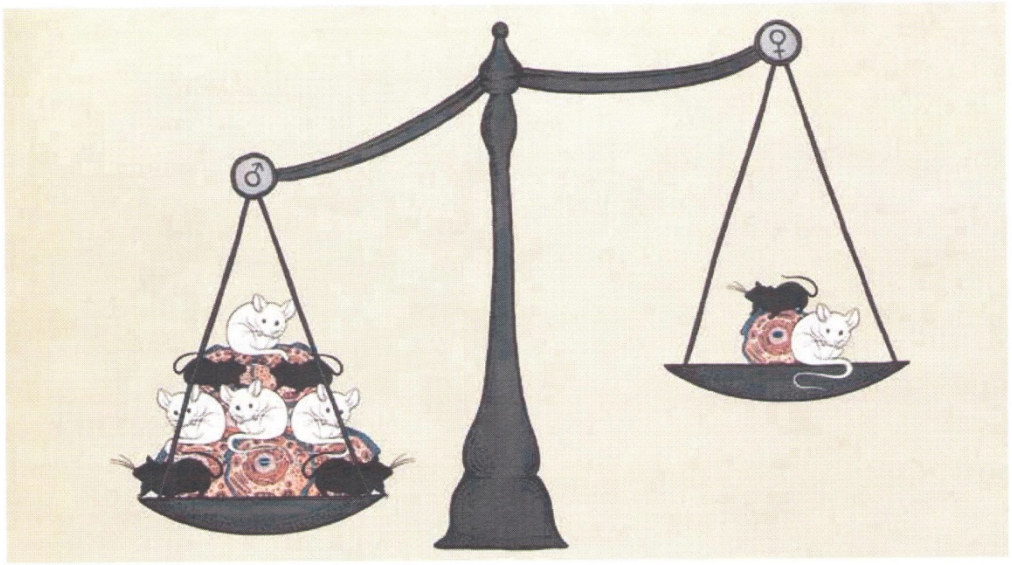


ILLUSTRATION BY KATIE SCOTT

NIH to balance sex in cell and animal studies

Janine A. Clayton and **Francis S. Collins** unveil policies to ensure that preclinical research funded by the US National Institutes of Health considers females and males.

More than two decades ago, the US National Institutes of Health (NIH) established the Office of Research on Women's Health (ORWH). At that time, the Congressional Caucus for Women's Issues, women's health advocacy groups and NIH scientists and leaders agreed that excluding women from clinical research was bad for women and bad for science. In 1993, the NIH Revitalization Act required the inclusion of women in NIH-funded clinical research.

Today, just over half of NIH-funded clinical-research participants are women. We know much more about the role of sex and gender in medicine, such as that low-dose aspirin has different preventive effects in women and men, and that drugs such as zolpidem, used to treat insomnia, require different dosing in women and men.

There has not been a corresponding revolution in experimental design and analyses in cell and animal research — despite multiple calls to action[1]. Publications often continue to neglect sex-based considerations and analyses in preclinical studies[2,3]. Reviewers, for the most part, are not attuned to this failure. The over-reliance on male animals and cells in preclinical research obscures key sex differences that could guide clinical studies. And it might be harmful: women experience higher rates of adverse drug reactions than men do[4]. Furthermore, inadequate inclusion of female cells and animals in experiments and inadequate analysis of data by sex may well contribute to the troubling rise of irreproducibility in preclinical biomedical research, which the NIH is now actively working to address[5,6].

The NIH plans to address the issue of sex and gender inclusion across biomedical research multi-dimensionally — through programme oversight, review and policy, as well as through collaboration with stakeholders including publishers. This move is essential, potentially very powerful and need not be difficult or costly.

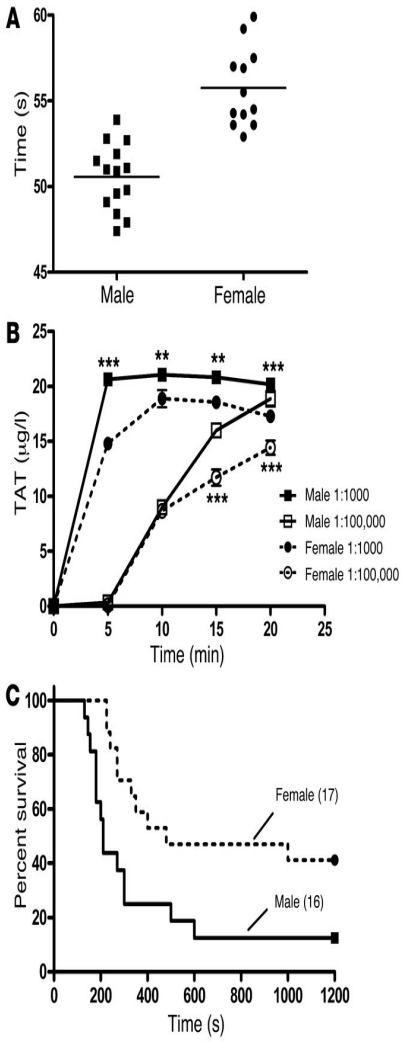
➲ NATURE.COM
Read about NIH reproducibility policy at:
go.nature.com/rcr1ef

BETTER WITH BOTH

Certain rigorous studies evaluating the effects of sex differences have been effective in bridging the divide between animal and human work. One example concerns multiple sclerosis (MS). Women are more susceptible to MS than men are, but develop less-severe forms of the disease. The most widely accepted MS animal model — rodent experimental autoimmune encephalomyelitis (EAE) — has revealed[7] that sex differences in MS are related to both reproductive and non-reproductive factors. Findings[8] that oestrogen therapy provided benefits in rodent EAE supported use of an oestrogenic ligand as a candidate neuroprotective agent for MS that is now being studied.

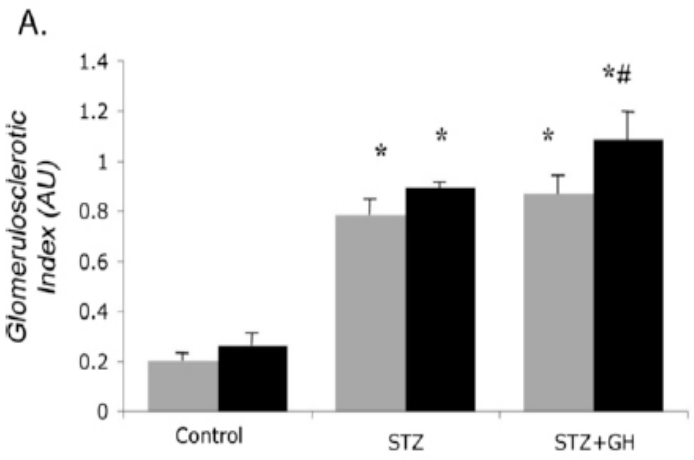
Moreover, differences between the sexes in both the animal model and human MS have

Stanford's Animal Diagnostic Laboratory Services Can Help

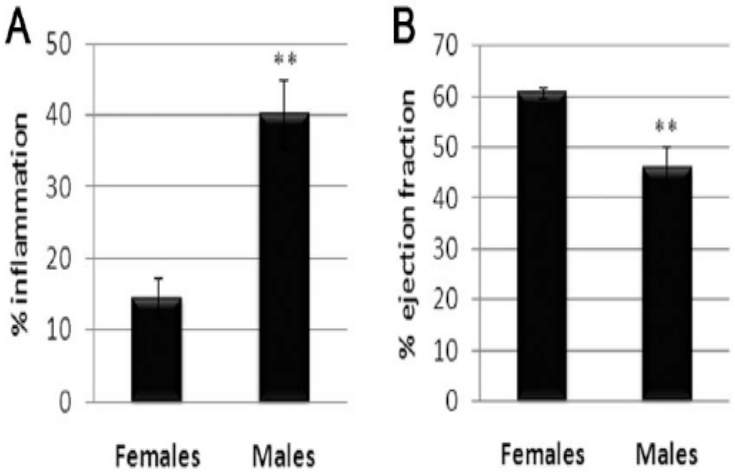


Sex differences in thrombosis in wild-type B6 mice.
J Clin Invest. 2008;118(8): 2969-2978.

- Sex-specific effects are noted in many species and include effects on:
  - Hematology parameters
  - Clinical chemistry parameters
  - Coagulation parameters
- Sex-specific differences are found in numerous animal models of human disease



Effect of sex on GSI in control and diabetic rats treated with or without GH.
Biol Sex Differ. 2013, 4:12 (6/27/13)

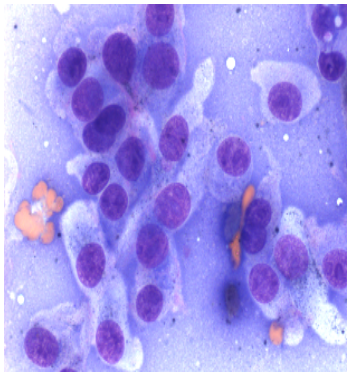


Effect of sex on coxsackievirus B3-induced cardiac inflammation in mice.
Biol Sex Differ. 2011, 2:2 (2/21/11)

Stanford's Animal Diagnostic Laboratory Service

- Necropsy service:

  Necropsy and histopathology services including in-house anatomic pathologist consultation and review



- Animal Diagnostic Lab (ADL):

  Clinical pathology services including:
  - Hematology
  - Clinical chemistry
  - Cytology/fluid analysis/bone marrow evaluation
  - Urinalysis
  - In-house microbiology and parasitology
  - Send-out specialized testing (molecular diagnostics, etc.)
  - In-house clinical pathologist consultation and review

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jljohns@stanford.edu

Website: http://vsc.stanford.edu/animaldiaglab/

STANFORD

SCHOOL OF
HUMANITIES AND SCIENCES

Londa Schiebinger
John L. Hinds Professor of History of Science

Director, EU/US Gendered Innovations in Science, Medicine, Engineering, and Environment

Gendered Innovations

in Science, Health & Medicine, Engineering, and Environment

What is **Gendered Innovations**?

SEX & GENDER ANALYSIS

- Methods
- Terms
- Checklists

CASE STUDIES

- Science
- Health & Medicine
- Engineering
- Environment

POLICY

INSTITUTIONAL TRANSFORMATION

- Sign up for updates
- How to cite website

SCIENCE

SCIENCE
HEALTH & MEDICINE
ENGINEERING
ENVIRONMENT

Sex and Gender Methods for Research | Gendered Innovations

FEATURED CASE STUDIES

Stem Cells: Analyzing Sex

Osteoporosis Research in Men: Breaking the Gender Paradigm

HIV Microbicides: Formulating Research Questions & Analyzing Academic Disciplines

Why Gendered Innovations?

"Gendered Innovations" employs methods of sex and gender analysis to create new knowledge.

Gendered Innovations…

- harness the creative power of sex and gender analysis to discover new things.

Now…

- **Researchers** need to learn how to do sex & gender analysis.
- **Universities** must integrate sex & gender analysis into the science, medicine, and engineering curriculum.

Doing Research Wrong Costs Lives and Money

- *Science* (26.3.2010): Between 1997 and 2000, 10 drugs were withdrawn from the U.S. market because of life-threatening health effects—8 of those showed greater severity in women.

Doing Research Right Saves Lives and Money

US WHI Hormone Therapy Trials

- Each $1 spent, returned $140

- Health Improvements
  * 76,000 fewer cases of cardiovascular disease
  * 126,000 fewer breast cancer cases
  * 145,000 more quality-adjusted life years

  * However: 263,000 more osteoporotic fractures

Joshua A. Roth et al., *Annals of Internal Medicine* (2014) 60, 9:594-602.

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Why Gendered Innovations?

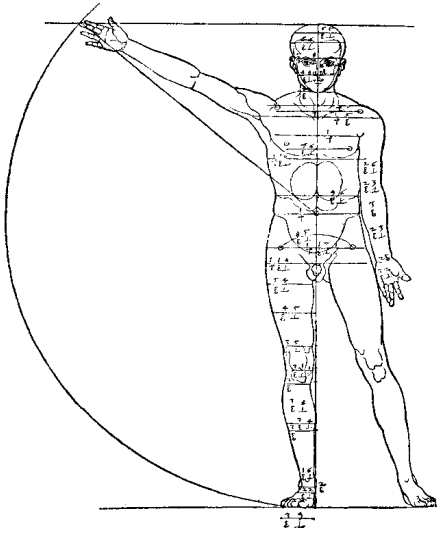
"Gendered Innovations" employs methods of sex and gender analysis to create new knowledge.

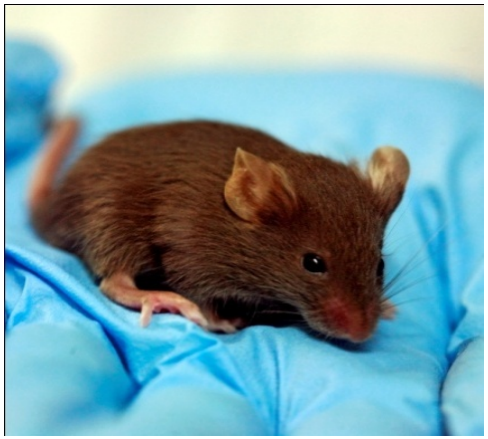
Gendered Innovations

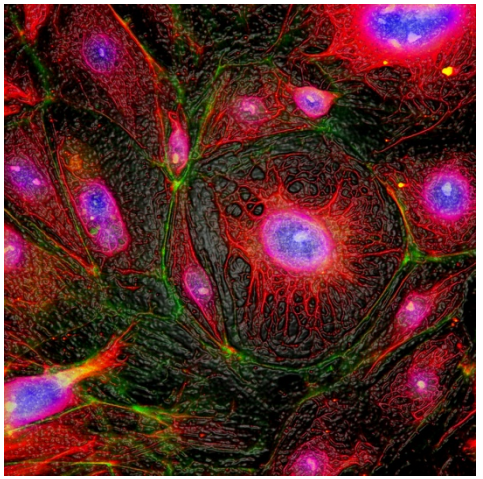
1) develop state-of-the-art Methods of sex and gender analysis

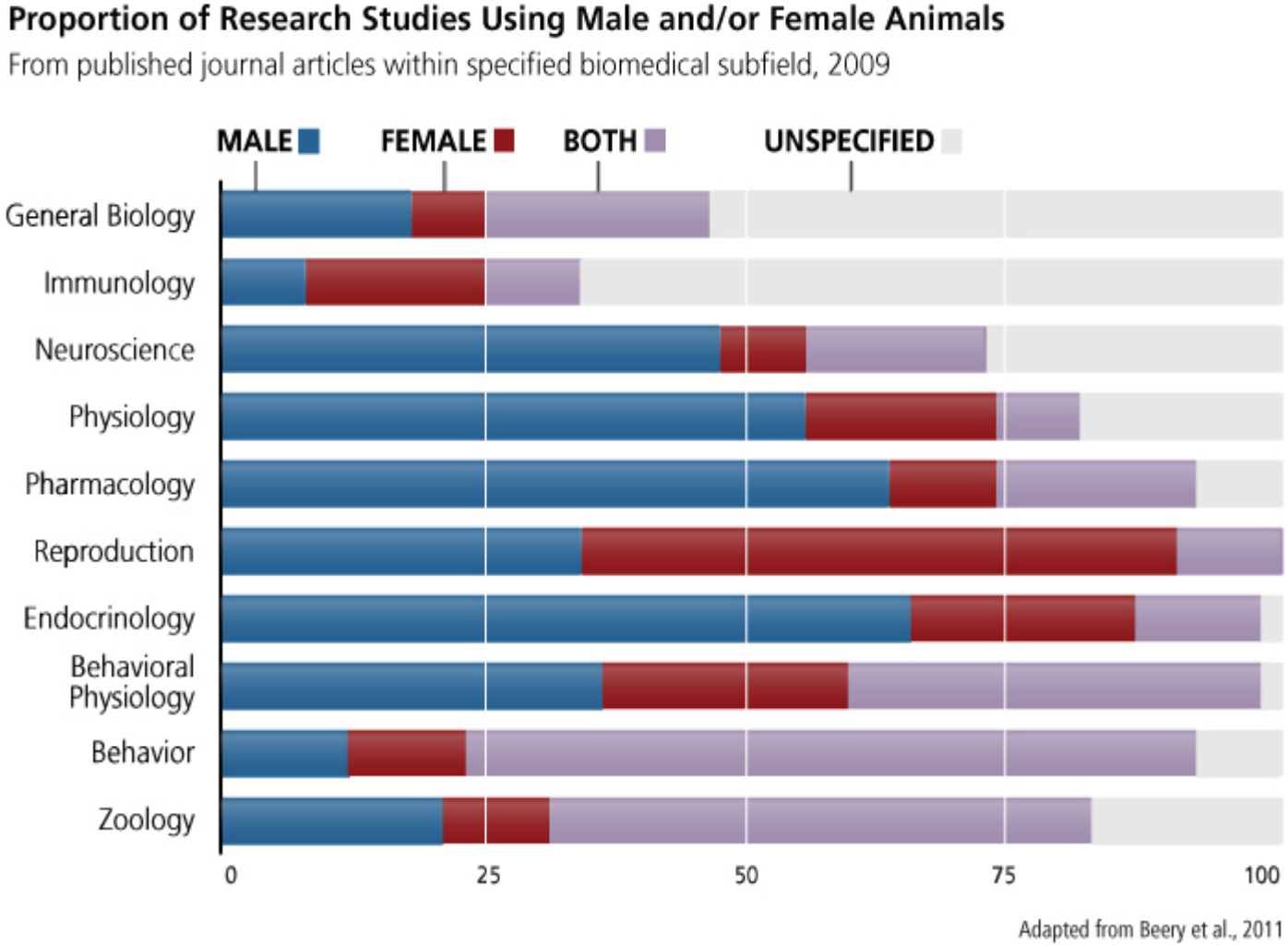
2) provide Case Studies to illustrate how gender analysis leads to innovative science and technology.

Most research is done in males





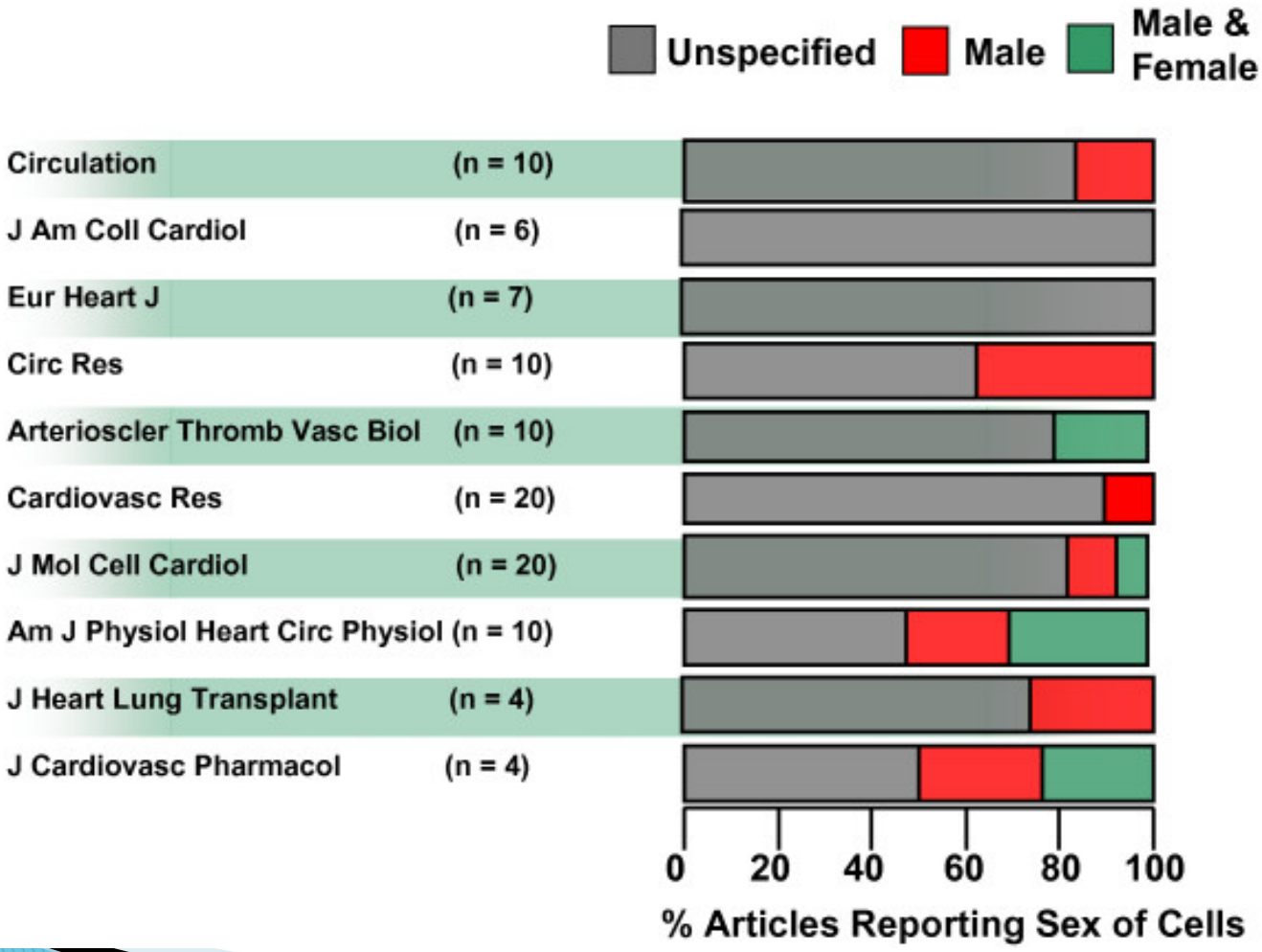




Beery, A., & Zucker, I. (2011). Sex Bias in Neuroscience and Biomedical Research. *Neuroscience and Biobehavioral Reviews, 35 (3)*, 565-572.

Taylor, K., Vallejo-Giraldo, C., Schaible, N., Zakeri, R., & Miller, V. (2011). Reporting of Sex as a Variable in Cardiovascular Studies using Cultured Cells. *Biology of Sex Differences, 2 (11)*, 1-7.

Percentage of articles reporting sex of cells used in the experiments



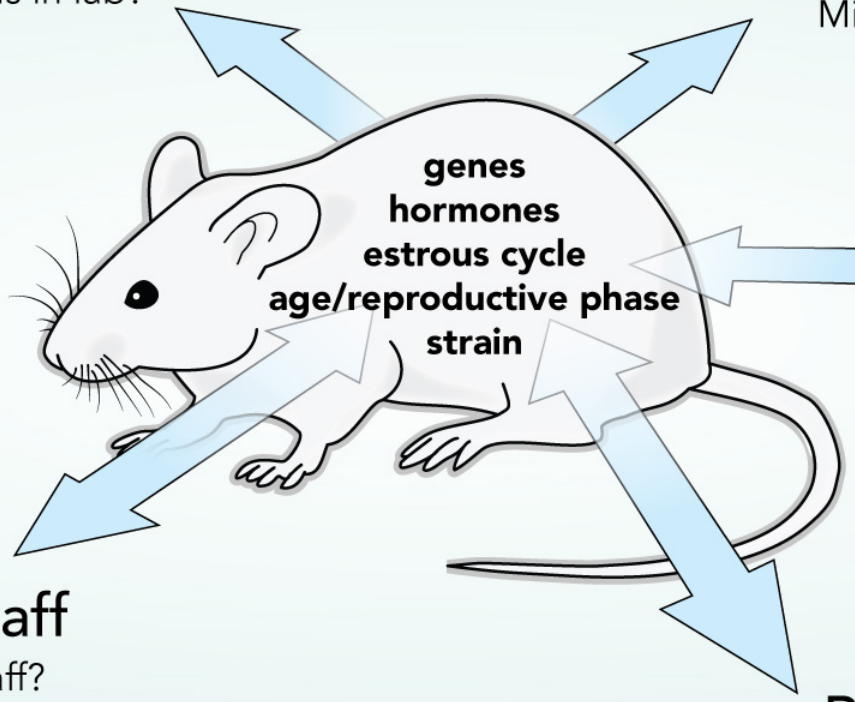
Integrating Sex & Gender into Animal Research

Social Dynamics

Sex-segregated or male/female mix?
Number and mix of animals in lab?

Caging

Individual or group? Size?
Complex environment vs. no enrichment?
Microbially sterile?



Diet

Researcher/Staff

Sex of researcher/staff?
Research/staff handling of male/female animals?

Room

Temperature?
Sound?
Lighting (circadian)?
Odor?

Prendergast, B. et al. (2014). *Neuroscience and Biobehavioral Reviews*. 40, 1–5.
Ritz, S. et al., (2014). *FASEB* J, 28(1), 4-13.
Sorge, R. et al. (2014). *Nature Methods*.

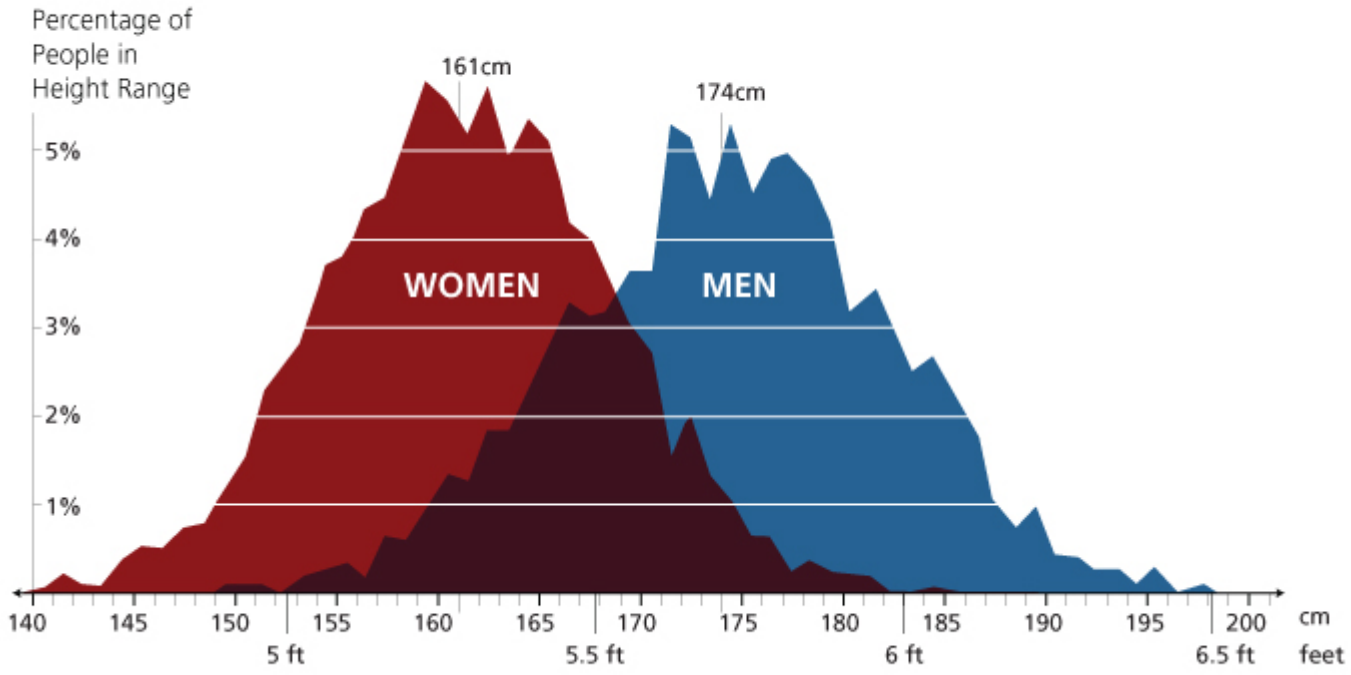
Method: Analyzing Sex

CAUTION: a male/female binary may obscure

- Sexual variation, such as intersex (1–2%)
- Overlap *between* groups
- Difference *within* groups

Height of Adult Women and Men

Within-group variation and between-group overlap are significant



Data from U.S. CDC, adults ages 18-86 in 2007

Method: Analyzing Factors Intersecting with Sex

Age
Estrous/Menstrual Cycle
Reproductive Phase
Body Composition
Comorbidities
Body Size
Disabilities
Ethnicity
Geographic Location
Socioeconomic Status
Educational Background
Sexual Identity
Religion
Lifestyle
Family Configuration
Environment …

Analyzing "gender" in animals

- **Gender Norms** refer to researchers' *attitudes* toward male or female animals.

- **Gender Relations** refer to the actual *interaction* between female and male animals and also between animals and men or women researchers.

[**Gender Identities** refer to how individual animals perceive and present themselves, and how they are perceived by others.]

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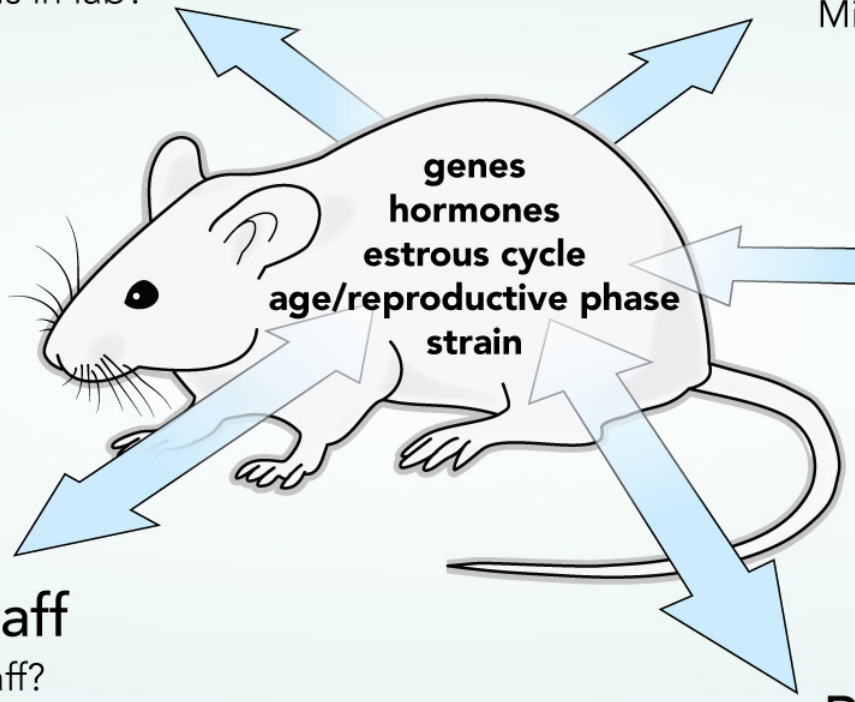

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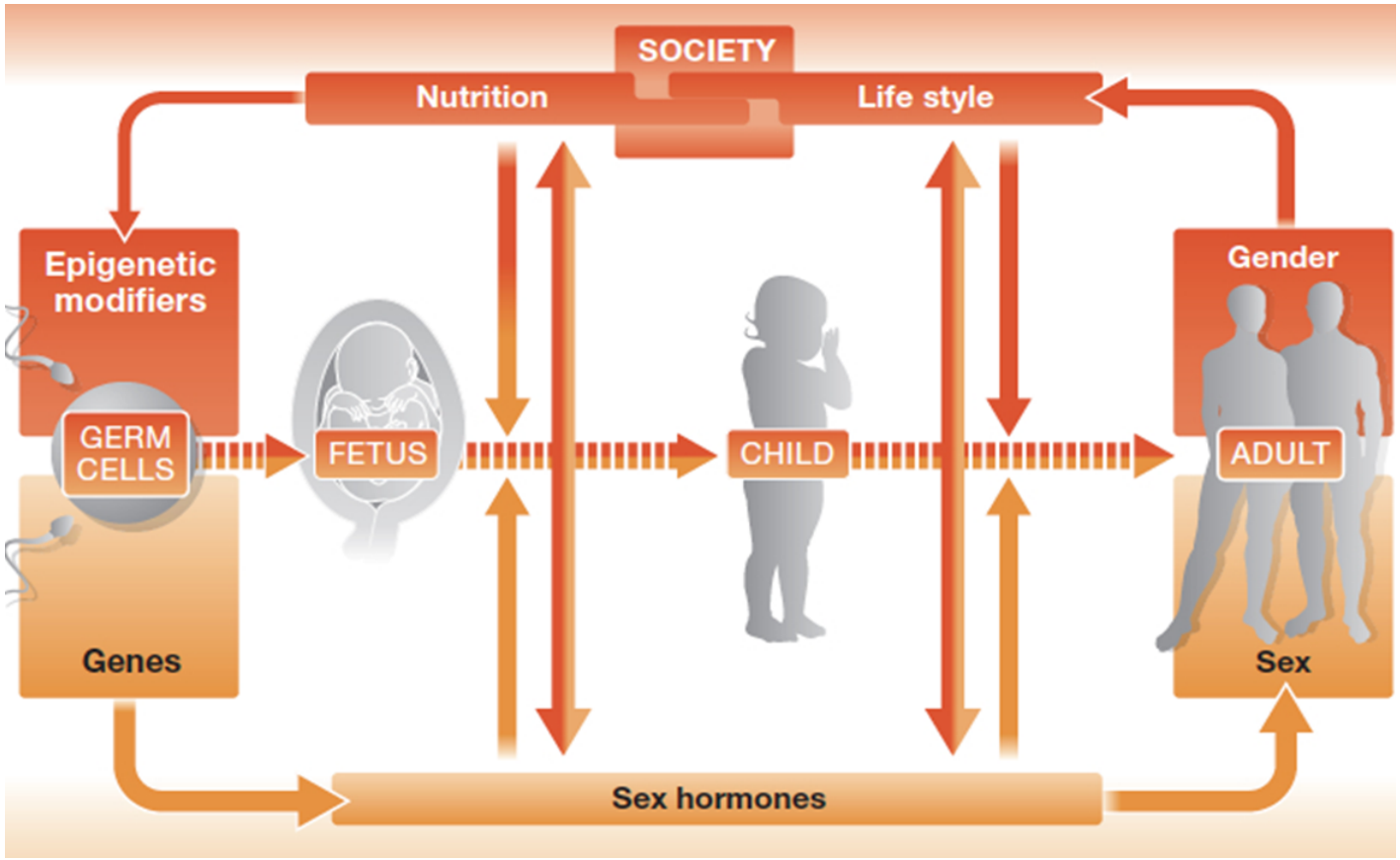
Ritz, S. et al., (2014). *FASEB* J, 28(1), 4-13.
Sorge, R. et al. (2014). *Nature Methods*.

How Sex & Gender Interact



Regitz-Zagrosek, V. (2012). Sex and Gender Differences in Health. *European Molecular Biology Organization Reports, 13 (7)*, 596-603.

Granting Agencies Require Sex & Gender Analysis

- European Commission Dec. 2013
- US National Institutes of Health May 2014
- Canadian IHR 2010

** We expect ERC and NSF to follow suit for the life sciences and engineering, i.e., any field with human endpoints.

European Commission

- Requires sex/gender analysis in 137calls for proposals

Economics
Psychology
Aeronautics
Nanotechnologies
Environmental biotechnologies
Oceanography
Archaeology
Transportation engineering
…

National Institutes of Health (~October 2014)

- "require applicants to report their plans for the balance of male and female cells and animals in preclinical studies."
- develop and deliver training on experimental design and analysis for NIH staff, trainees, and grantees.
- work with grant reviewers to enforce requirements for applicants.
- encourage publishers to promote rigorous reporting of sex and gender analyses.

Peer-Reviewed Journals

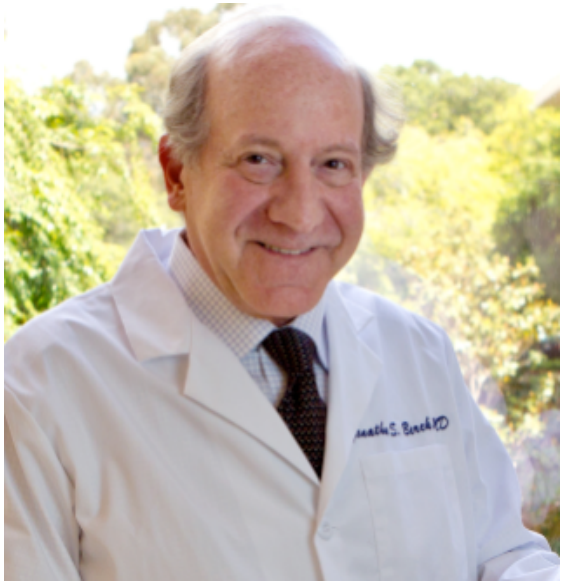
- *Science* and *Nature* will announce new guidelines for reporting sex and gender analysis.

Women & Sex Differences in Medicine Stanford (WSDM) Center

Jonathan Berek, MD, MMS, *Laurie Kraus Lacob Professor, Chair, Obstetrics & Gynecology* https://med.stanford.edu/profiles/jonathan-berek

Director, Stanford Women's Cancer Center

http://stanfordhealthcare.org/en/medical-clinics/womens-cancer-center.html



Plans for: **Stanford Women's Health Center** *(Comprehensive Women's Care)* ***in Redwood City***

WSDM as Clinical Interface *for Research and Education*

Stanford MEDICINE | School of Medicine

http://wsdm.stanford.edu

Stanford WSDM Center

STANFORD
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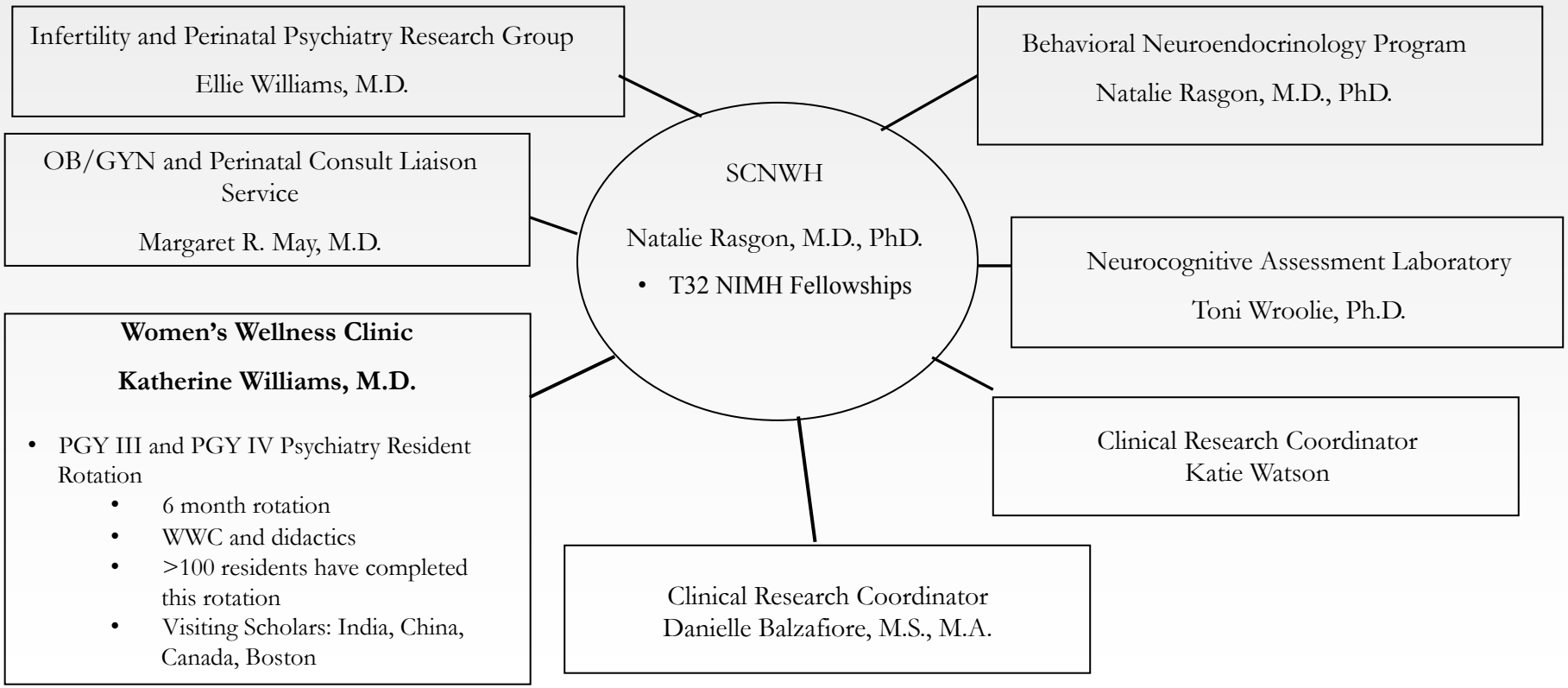
Stanford Center for Neuroscience in Women's Health

Stanford Center for Neuroscience in Women's Health (SCNWH)

- **Mission**:

  To provide innovative, state of the art treatment in the field of women's mental health through the integration of biomedical research, clinical practice, and clinical education

Stanford Center for Neuroscience in Women's Health (SCNWH) Organizational Structure



Referrals to Women's Wellness Clinic

- **Stanford University Clinicians**
  - Primary Care and Internal Medicine
  - Specialists: Endocrinology, Pain Clinic, Sleep Clinic
- **Gynecology Referrals**
  - PDD, PMDD, PCOS
  - PTSD related to Sexual Abuse
  - Chronic Pelvic Pain
  - Sexual Dysfunction
  - Adjustment and Mood Disorders Associated with Gynecological Conditions: Cancer, Endometriosis
- **Perinatal Referrals**
  - Unipolar Depression
  - Bipolar Disorder
  - Anxiety Disorders
  - Psychotic Disorders
  - Perinatal Grief
- **Community Clinicians**
  - Preconception Consultations
  - Treatment Resistant Mood and Anxiety Disorders
- **Self-Referral**

WWC Collaborations

Department of Obstetrics and Gynecology: Collaborative Research and Education

- Predictors of Postpartum Depression;
- Psychotropic Medication Exposure in Infants Admitted to the Neonatal Intensive Care Unit and Packard Special Care Nurseries
- Grand Rounds ; Resident Rounds

WWC and Stanford Fertility and Reproductive Medicine Center: Collaborative Research and Education

- Mood Disorders in Ovum Donor Candidates *Human Reproduction,* 26: 847-52, 2011
- Incidence of Moderate to Severe Depression in Recurrent Pregnancy Loss Patients Fertility and Sterility. 95 Issue 4, Supplement, 2011
- Stress and Anxiety Scores in Repeat IVF Cycles PLoS One. 2013 May 23;8(5
- Community Education Symposium: Recurrent Pregnancy Loss 2011
- American Psychiatric Association, 2013 "Evaluation and Treatment of Mood and Anxiety Disorders in Infertility Patients: An Integrated Approach" Katherine Williams, M.D., Penny Donnelly, MSW,RN and Natalie Rasgon, M.D., PhD.

WWC and Stanford Fertility and Reproductive Medicine Center

- Longstanding relationship (early 1990s)
- Early involvement in establishment of Psychological Support Program Current Director: Penny Donnelly, MSW
- Active involvement in Recurrent Pregnancy Loss Program (Ruth Lathi, MD Director)
  - Monthly interdisciplinary meetings
- Early involvement in establishment of psychological evaluation services for donor assisted reproduction
  - Ovum donor program
  - Sperm donor program

WWC Educational Programs

- T32 NIMH Fellowships
- Visiting Scholars
  - India, China, Canada, Boston
- PGY III and PGY IV Psychiatry Resident Rotation
  - 6 month rotation
  - WWC and didactics
  - >100 residents have completed this rotation

Proposed Extension of Clinical Services

- "Co-located Care"
  - Urgent referrals
  - Consultation services for patients from out of area
  - Consultation services for patients without insurance for psychiatric services who can be managed by primary physicians
  - Consultation/interventions for patients noncompliant with psychiatric referrals
  - Interdisciplinary case conferences

CNWH: Recently Completed Research Studies

- **Reproductive Endocrine Function and Mood in Women with Bipolar Disorder**
- **Lamotrigine in the Treatment of Bipolar Disorder in Women of Reproductive Age**
- **Estrogen Use in Protection from Cognitive Decline**
- **E-Citalopram in Midlife Depression in Women**
- **Prenatal Exposure to Antidepressants and Neonatal Adaptation**

SCNWH: Current Program Goals

- To investigate the intersection of reproductive hormones, mood, and brain function
- Research concentrations:
  - Reproductive endocrine status of women treated for mood disorders
  - Neuroendocrinology of aging
  - Role of insulin resistance in mood disorders, reproductive function, and cognitive decline

Effects of Liraglutide on Hippocampal Structure and Function in Aging Adults Across the Insulin Sensitivity Spectrum

- Industry sponsored study investigating the effects of liraglutide on the memory and attention of people with insulin resistance
  - Recruiting 80 men and women between the ages of 50 and 70; half of participants with have a family history of dementia
  - Evaluating whether 12 weeks of treatment will change performance on memory and attention tasks and the size and shape of the hippocampus
    - Neurocognitive battery
    - Oral glucose tolerance test (OGTT)
    - MRI

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    - MRI

Effects of Mifepristone on Biomarkers of Metabolic Function and Neuropsychological Performance among Middle-Aged and Older Individuals

- Department sponsored study investigating the effect of cortisol on memory and attention in people with a history of depression
  - Recruiting 40 men and women between the ages of 50 and 70 who do not meet criteria for current depressive episode
  - Evaluating
    - Relationship between glucose utilization and verbal memory and attention
    - Changes in neuropsychological test performance in people with insulin resistance
    - Interactions between changes in patients' glucose tolerance profiles and changes in verbal memory and attention

Future Goals

- Expansion of clinical services of WWC
- Increased collaborative research with other medical specialties
- Increased cross disciplinary education initiatives

RESEARCH AT WHH

Abha Khandelwal MD, MS
Clinical Assistant Professor
Cardiovascular Medicine
WHH

Sex Differences in Coronary Pathophysiology in Patients with Angina in the Absence of Obstructive Coronary Artery Disease (CAD)

- **Aim**: To investigate sex differences coronary pathophysiology in women and men presenting with angina in the absence of obstructive CAD on angiography
- **Project Status**: Data analysis
- **Corresponding author**: Dr. Jennifer Tremmel

The Diagnostic Value of Stress Echocardiography and Electrocardiography in Identifying Occult Coronary Abnormalities in Patients with Angina and No Obstructive Coronary Artery Disease

- **Aim**: To study the ability of stress echocardiogram and electrocardiogram to identify occult coronary abnormalities in patients with angina and no obstructive CAD
- **Project Status**: Data analysis
- **Corresponding author**: Dr. Jennifer Tremmel

The Effect of Mindfulness-Based Stress Reduction on Angina and Vascular Function in Women with Non-Obstructive Coronary Artery Disease

- **Aim**: To investigate whether mindfulness-based stress reduction (MBSR) would result in reduced anginal symptoms and improved vascular function in women with angina and no obstructive CAD.
- **Project Status**: Manuscript writing
- **Corresponding author**: Dr. Jennifer Tremmel

- **A Descriptive Study of Spontaneous Coronary Artery Dissection(SCAD) in a Tertiary Care Population**

  - **Aim**: To study clinical characteristics and outcome of patients with Spontaneous Coronary Artery Dissection presenting to tertiary care hospital
  - **Project Status**: Data collection
  - **Corresponding author**: Dr. Abha Khandelwal

Association between Lipoprotein(a) Levels and Risk of Coronary Artery Disease in South Asian Population

- **Aim**: To study risk association in elevated lipoprotein(a) levels and coronary artery disease in South Asian population
- **Project Status**: Study Design
- **Corresponding author**: Dr. Abha Khandelwal

HIP (Health-texting **I**n **P**ostpartum) **Moms Study**

Aim: RCT comparing usual care to mHealth behavior change intervention (outcome weight change 3 months)
Project status: Enrollment
Corresponding author: Dr. Sandra Tsai

Behavioral Health Research

- **Treatment of Insomnia in Women and Men with Cardiovascular Disease (pilot study)**
  - Aim: demonstrate feasibility of treating insomnia in a cardiac clinic using purely behavioral techniques
  - Status: ongoing, n=25 out of target=30 potential participants
  - Corresponding Author: Dr. Katherine Sears Edwards

- **Mindfulness Based Stress Reduction for Patients Referred to Cardiovascular Rehabilitation (pilot RCT)**
  - Aim: determine whether 8 week structured mindfulness intervention can benefit cardiac patients in terms of psychological, physiological and behavioral health outcomes
  - Status: completed, final n=26, manuscript in progress
  - Corresponding Author: Dr. Katherine Sears Edwards

Behavioral Health Research

- **Psychological Treatment for Cardiac Patients: Effects on Self-reported Symptoms and Health (pilot study)**
  - Aim: investigate outcomes of co-located, cardiac specific psychological care
  - Status: ongoing, n=5 out of target=35 potential participant
  - Corresponding Author: Dr. Katherine Sears Edwards

- **Sex Differences in Benefits and Barriers to Cardiac Rehabilitation (survey study)**
  - Aim: compare men and women on benefits of, and barriers to, participation in a local cardiac rehabilitation program
  - Status: completed, final n=128, 41% female
  - Corresponding Author: Dr. Katherine Sears Edwards

Sex-Specific Recovery from Stroke: Differences in Clinical Trajectory

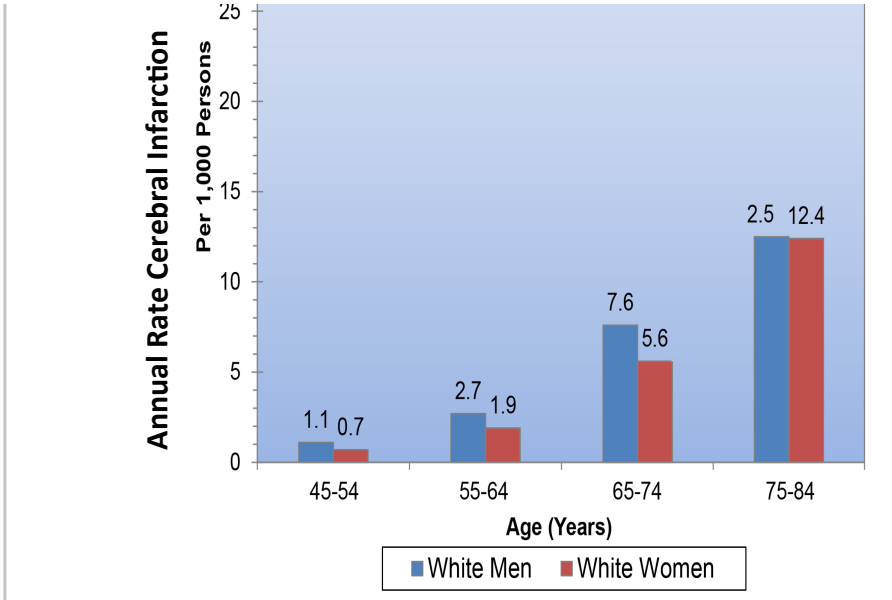
Rona Giffard
Marion Buckwalter
Maarten Lansberg

Immune Trajectory:
Martin Angst
Brice Gaudilliere
Nikolay Samusik

Depts of Anesthesiology, Periop and Pain Medicine and Neurology and Neurological Sciences

Sex differences in stroke

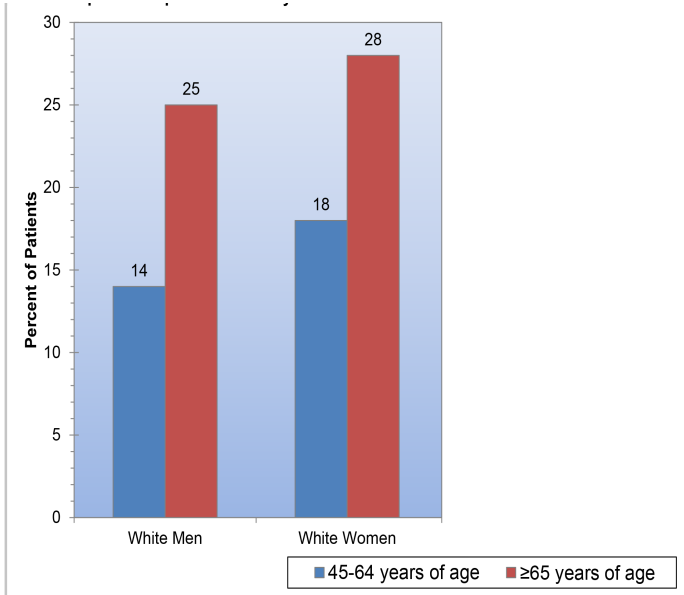
- Different incidence pattern: lower incidence in women than men until ~80, may then be higher
- Women have increased mortality, worse functional outcomes, more depression and fatigue after stroke



Greater Cincinnati/Northern Kentucky Stroke Study: 1999.
Source: Unpublished data from the Greater Cincinnati/Northern Kentucky Stroke Study.

Go AS et al. Published online

Patients dead 1 year after stroke



Source: pooled data from the FHS, ARIC, and CHS studies of the NHLBI.

Go AS et al. Published

Sex differences in immune response may contribute

- Little human data on how sex affects clinical or immune trajectories of recovery after stroke
- Immune response is important to stroke outcome, and sex differences in immune function are known
- Determining clinical and immune trajectories could provide biomarkers and clues to mechanisms underlying sex differences

WSDM funded pilot study

Recruit 16 adult male and female acute stroke patients-

- **Aim 1:** Determine sex-specific differences in time course and degree of **motor-function recovery**
  - days 1,3,7, 14, 28 and 90
- **Aim 2:** Determine sex-specific differences in time course and degree of **cognitive recovery**
  - days 1,3,7, 14, 28 and 90
- **Aim 3:** Determine sex-specific differences in **delayed cognitive decline**
- Analysis of immune phenotype to be performed as a subsequent analysis

Initial problems

- Time to IRB approval and revision
- Lower Recruitment than anticipated

Revised Plan

- Continue to recruit acute subjects
- Use a chronic population to determine if changes over time in NIHSS over longer periods differ by sex
  - New arms 3 and 4

- Arm 3: Chronic stroke. Goal 60 adult men and women
  - previous diagnosis of ischemic stroke; able to participate in cognitive assessments; known size, location, and date of stroke; known NIHSS at time of stroke
- Arm 4: Healthy volunteer group. Goal 40 age and sex matched subjects recruited from spouse, significant others in clinic

- Retrospective look at Stanford Stroke Clinic Patients for sex differences in recovery in prior Stanford studies (Maarten Lansberg)

Funding and Future Directions

- Big Idea funding from the Neurosciences Institute

study immune, motor, and cognitive trajectories in 50 recovering stroke patients (SCAN: Stroke Collaborative Action Network, led by Drs. Buckwalter and Lansberg).

  - Extends initial WSDM pilot, adds additional assessments
    - Immune phenotyping (Angst/Gaudilliere)
    - Innovative motor measures (Cutkosky/Okamura/Delp),
    - OT/PT evaluations (Flavin)
    - PET/MRI and resting state MRI (James/Zaharchuk/Greicius)

- Data from the new, chronic arms of the WSDM study will provide preliminary data for an R01 in late Jan, NIA/NINDS RFA on vascular causes of dementia
  - One arm will compare men vs. women
  - Hypothesis: auto-immune responses to brain antigens are associated with post-stroke dementia.

Thanks WSDM!

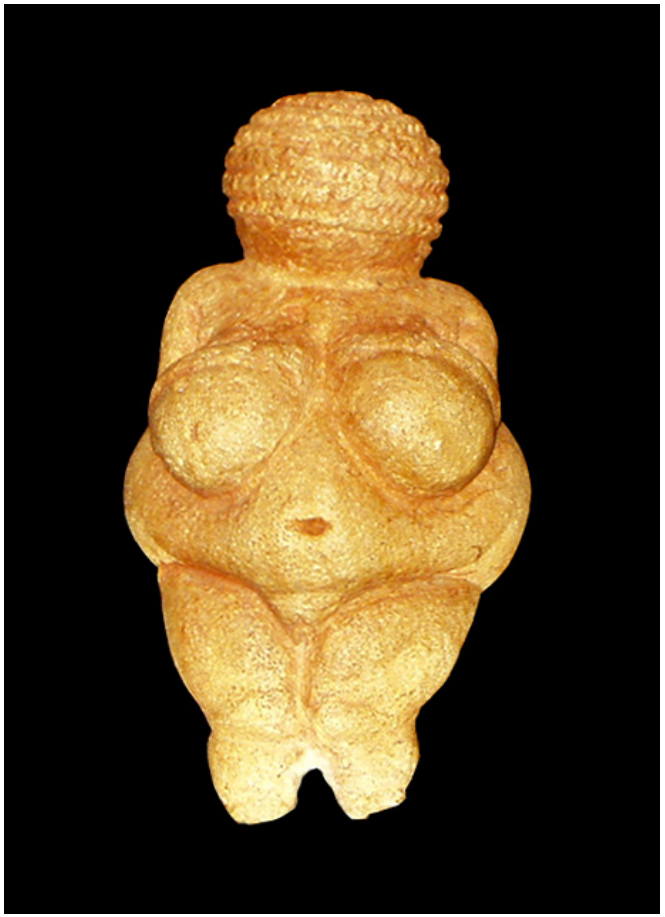
Sex differences in adipocyte responses to experimentally-induced weight gain

Tracey McLaughlin, MD: Department of Medicine
Michael Snyder,PhD: Department of Genetics
Brian Piening, PhD: Department of Genetics

WSDM Pilot Project

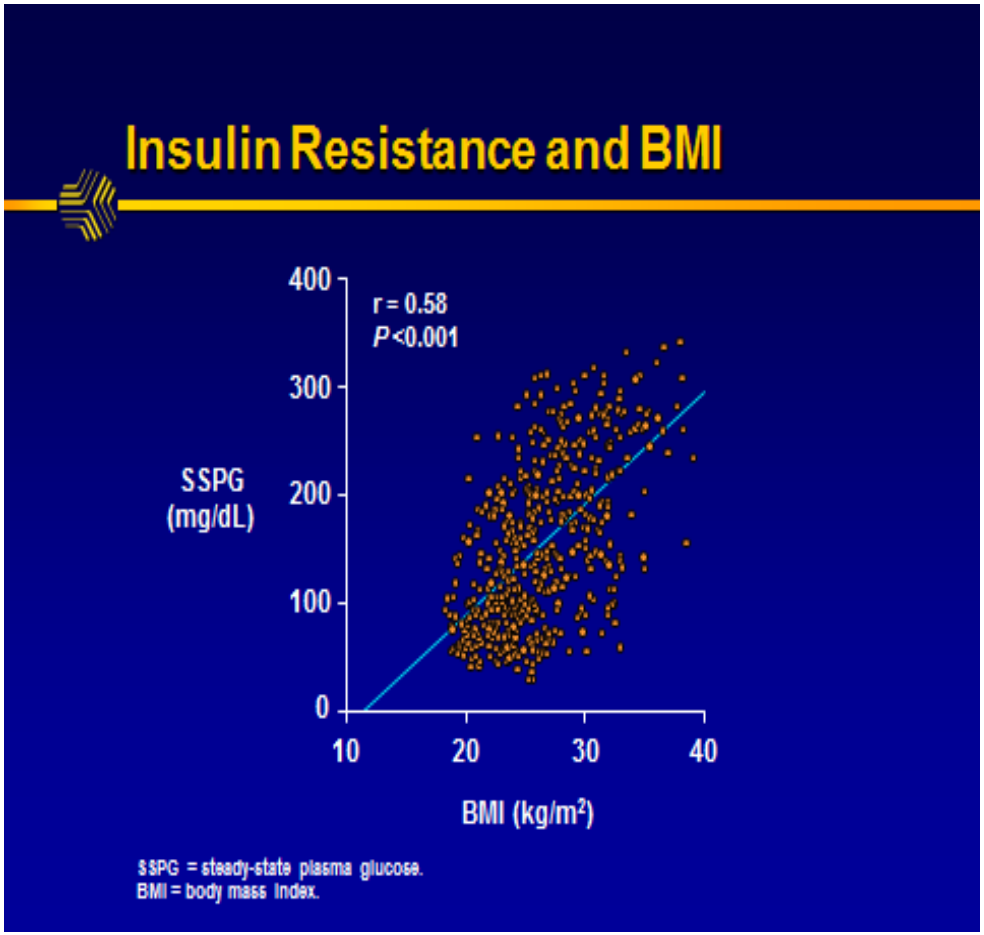
Background

- Women are known to have a different fat distribution than men
- Greater total body fat
- Greater lower body fat
- Lower visceral fat
- Larger fat cells



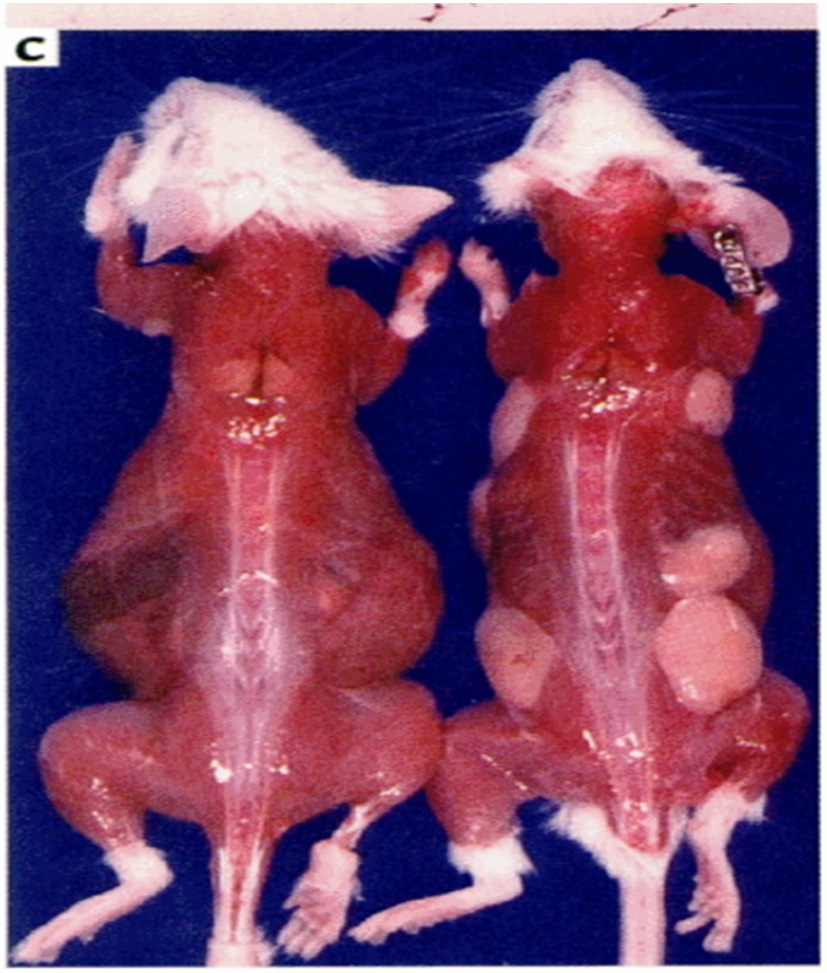
Excess body fat and insulin resistance

- Increasing body fat is associated with insulin resistance
- Not all overweight/ obese individuals are insulin resistant (IR)
- Those who are not IR may have adaptations in adipose tissue that prevent insulin resistance



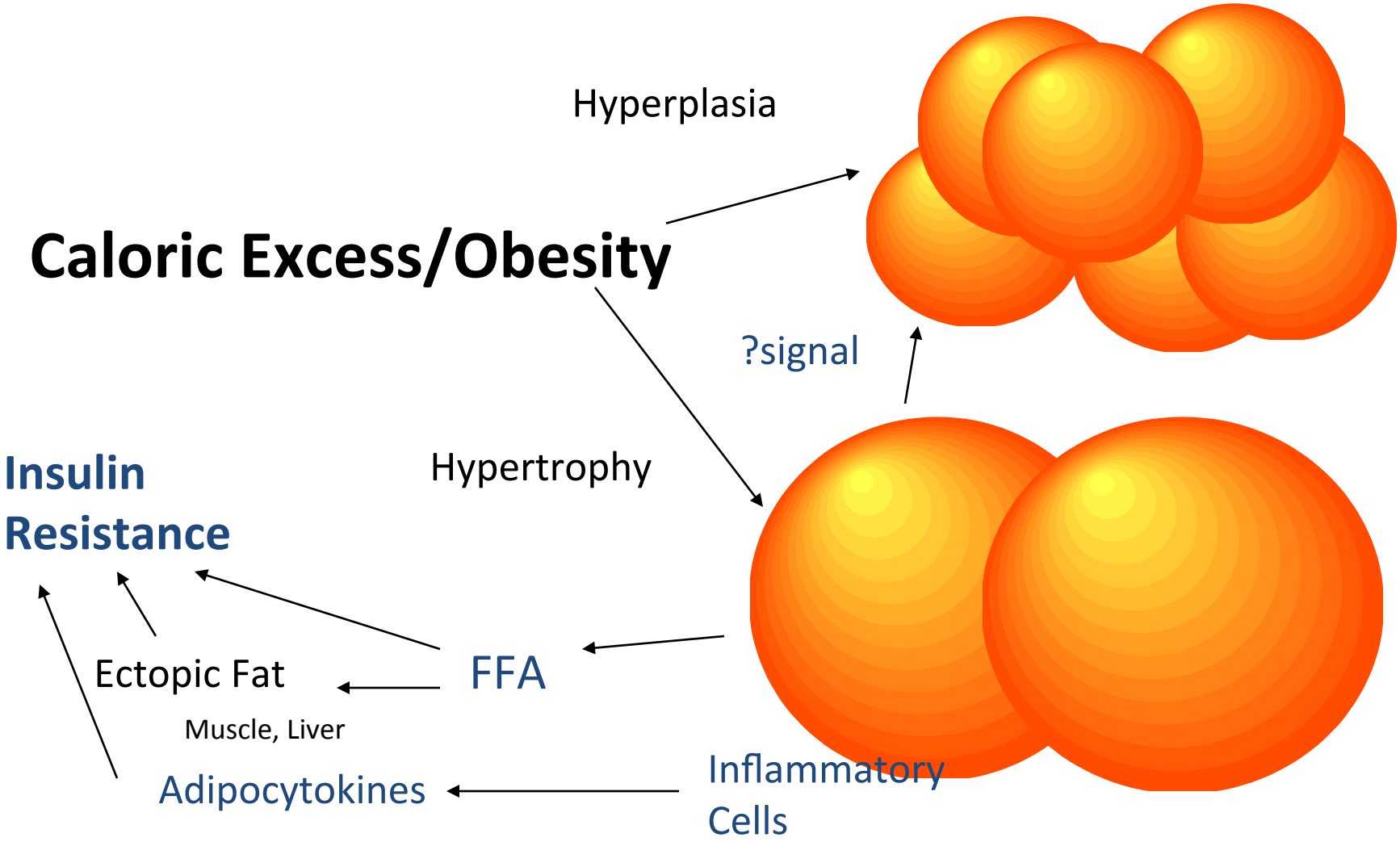
Adipose Tissue "Expandability" Hypothesis

- In the setting of caloric excess, individuals who can more readily store TG in SAT will be protected from insulin resistance via less need to store fat in visceral cavity and ectopic sites (liver, muscle)
- Adipocyte enlargement is finite: thus continued weight gain requires recruitment of new cells for TG storage
- Recruitment of new adipocytes prevents hypertrophy and stress in existing already-mature adipocytes: individuals who cannot recruit well develop IR



Kim and Shulman, JBC, 2000

Response to Continued Caloric Excess

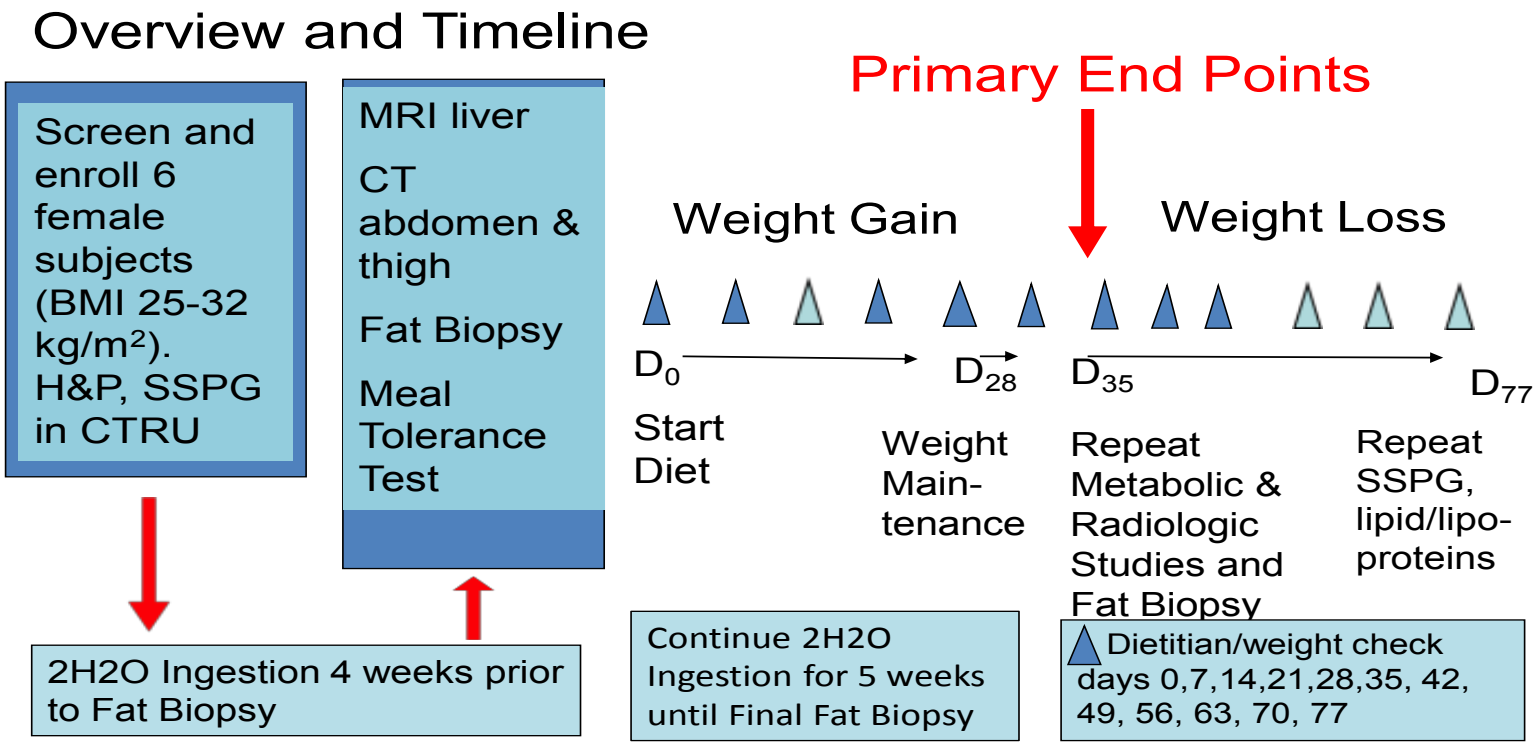


WSDM Project: Adipose Tissue Expandability in Women versus Men

Specific Aims: Test hypothesis that premenopausal women respond differently than men to weight gain, with greater capacity to store fat in the subcutaneous adipose tissue and less metabolic deterioration. We aim to test the following responses to overfeeding/weight gain intervention:

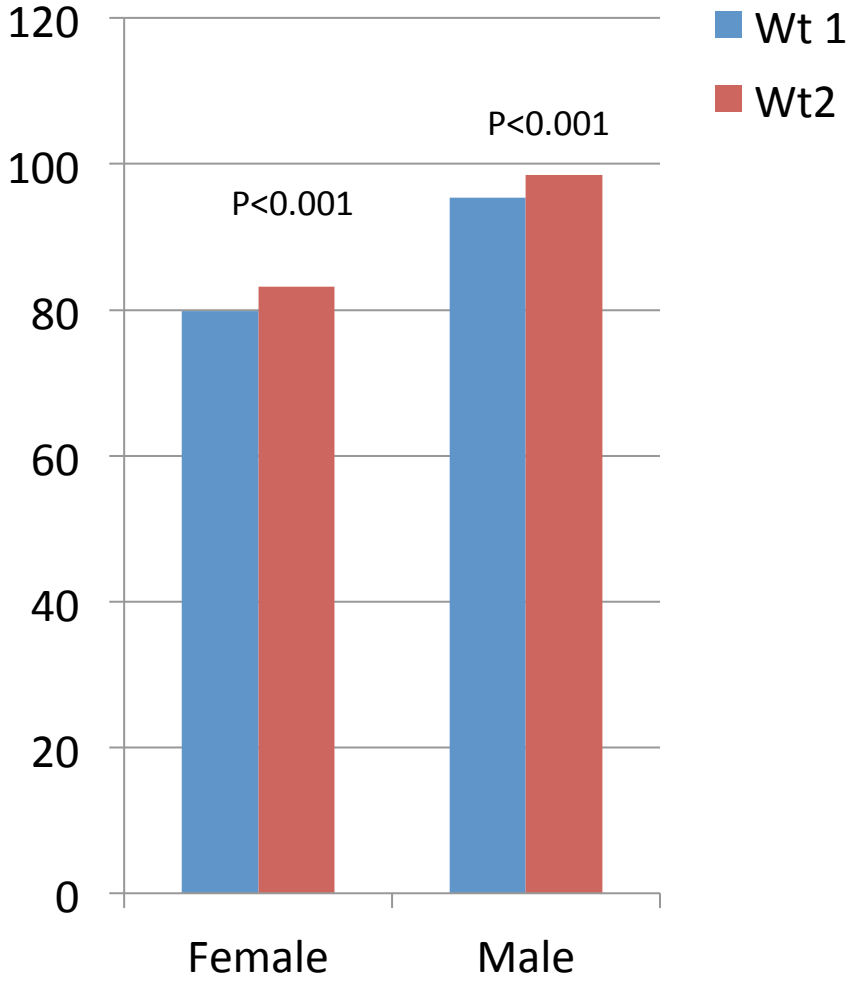
1. Test hypothesis that total TG uptake and de novo lipogenesis in subcutaneous adipose cells, quantified by 2H2O incorporation into glycerol and palmitate, will be increased in women as compared with men
2. Test hypothesis that fat deposition will favor SAT and thigh over visceral and liver in women as compared with men
3. Test the hypothesis that change in adipose cell size distribution with weight gain will differ by sex
4. Test the hypothesis that with weight gain, lipogenic genes, measured by RNA sequencing, will be upregulated to a greater degree in women than men, and change in adipocyte stress markers, such as hypoxia, inflammation, and ER stress, will be downregulated in women as compared to men.

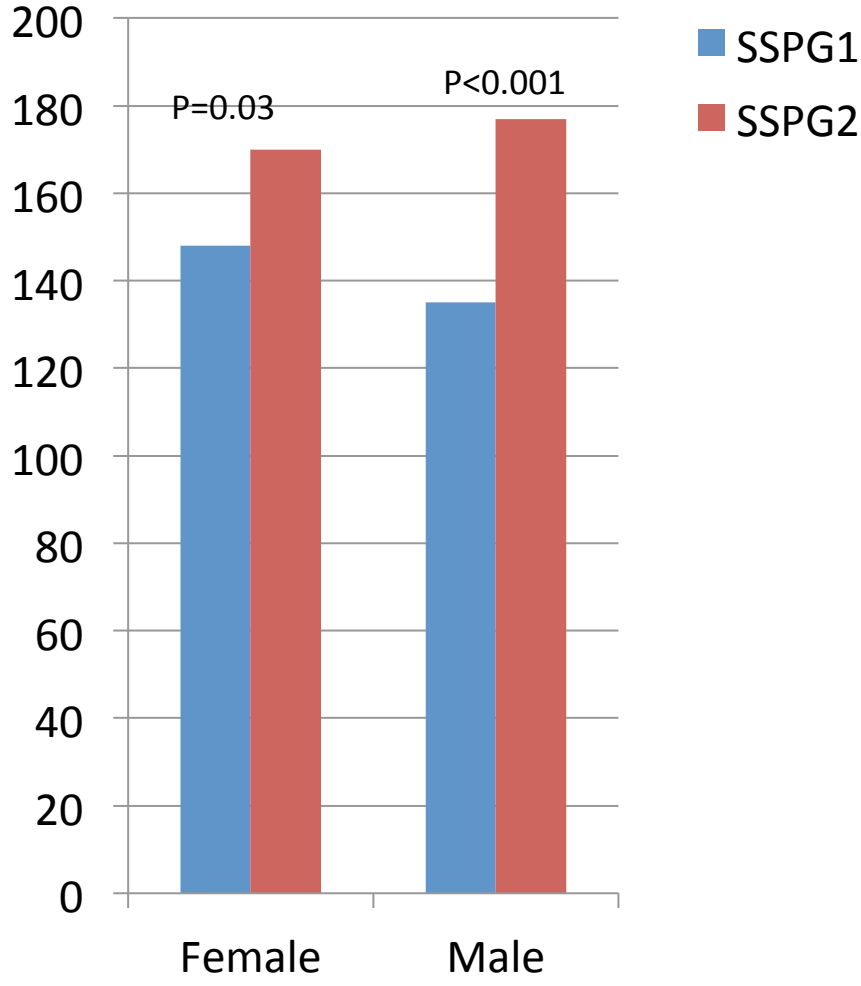
Pilot Nested Within Larger Study of 26 Males and Females



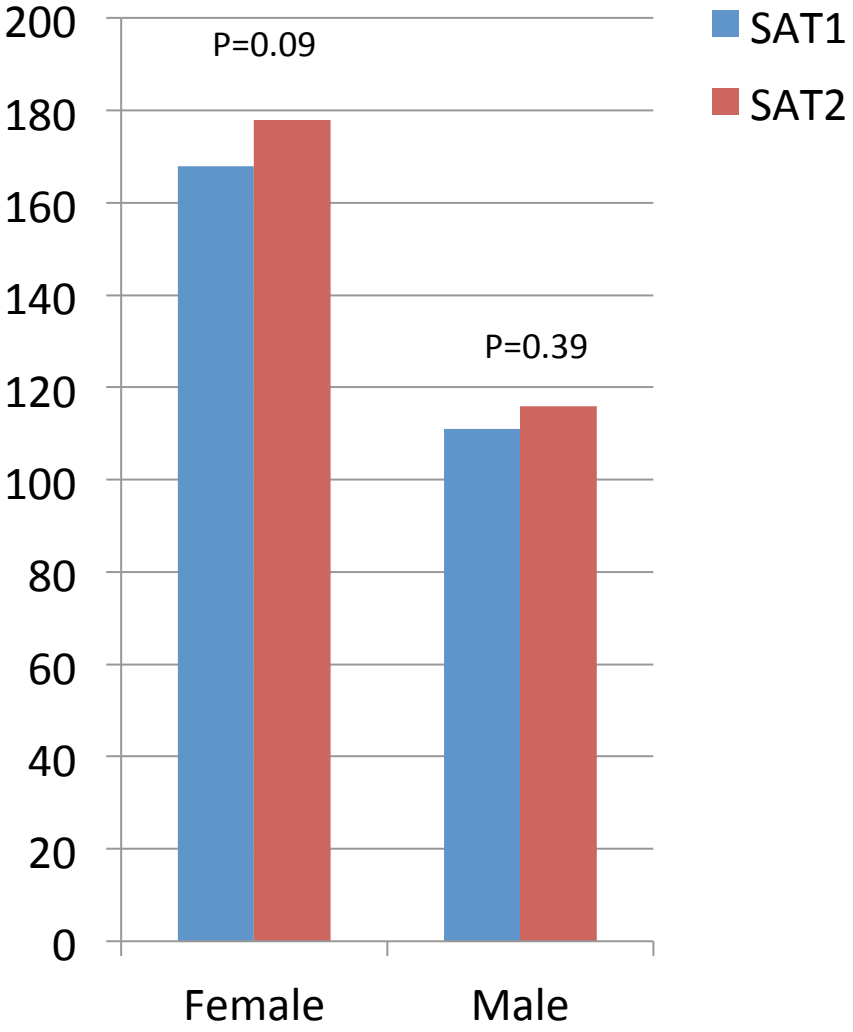
*SSPG: Steady-state plasma glucose derived from modified insulin suppression test
Recruiting over 6 months: completion by 8 months; RNAseq and lipid dynamics (stable isotope) analysis by 9 months

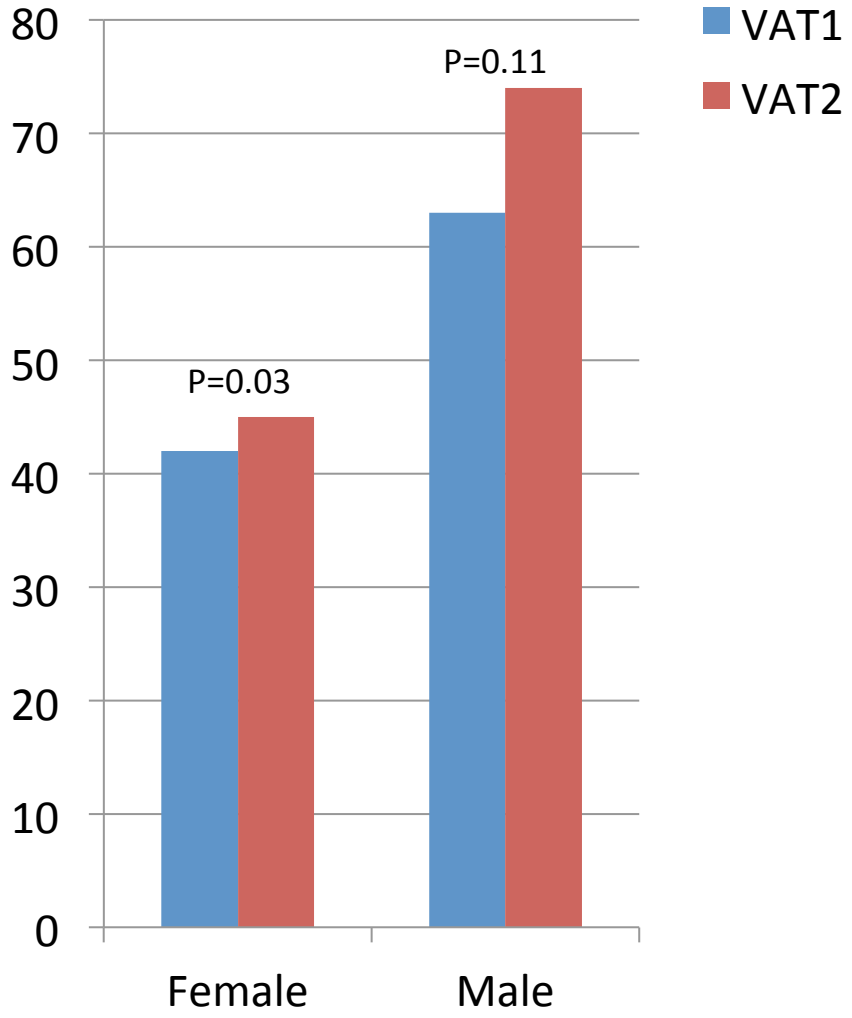
Results (13 females; 15 males)



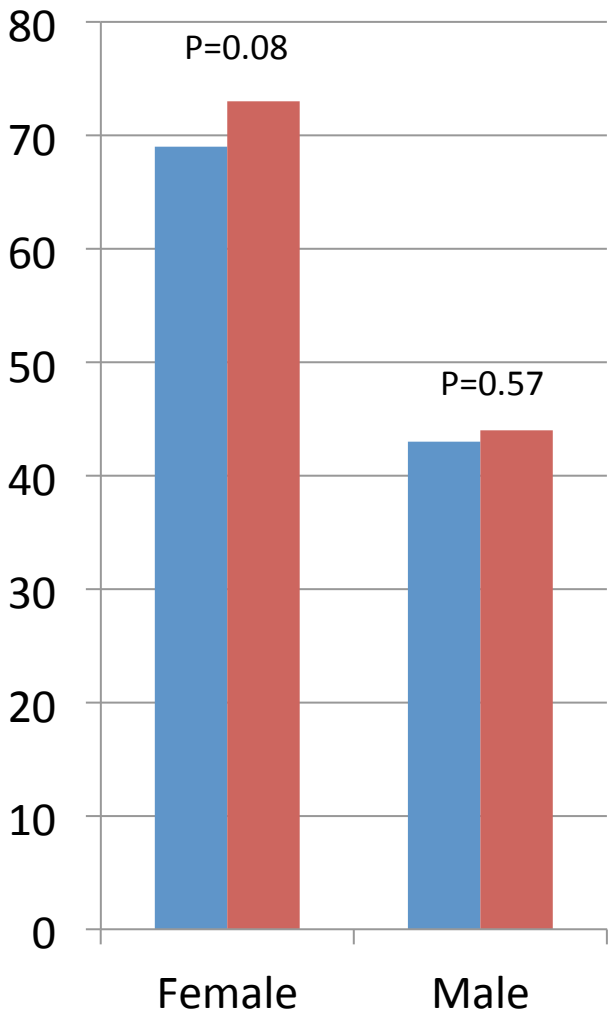


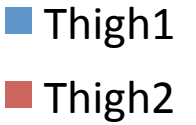
Results

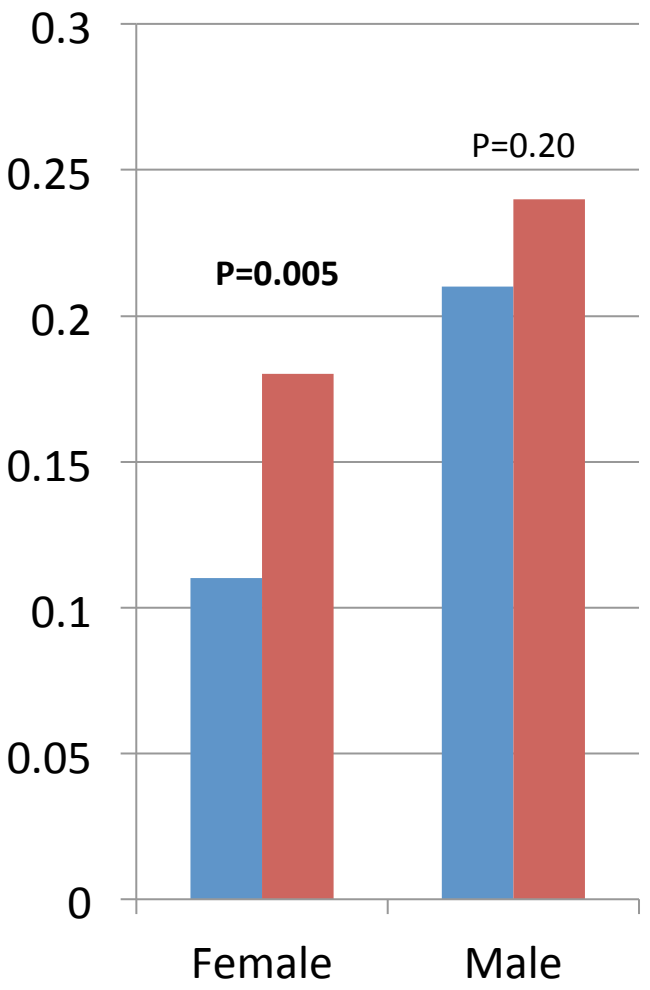

200
180
160
140
120
100
80
60
40
20
0
P=0.09
P=0.39
SAT1
SAT2
Female
Male

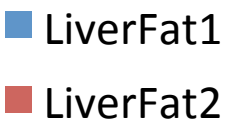

80
70
60
50
40
30
20
10
0
P=0.03
P=0.11
VAT1
VAT2
Female
Male

Results


80
70
60
50
40
30
20
10
0
P=0.08
P=0.57
Female
Male

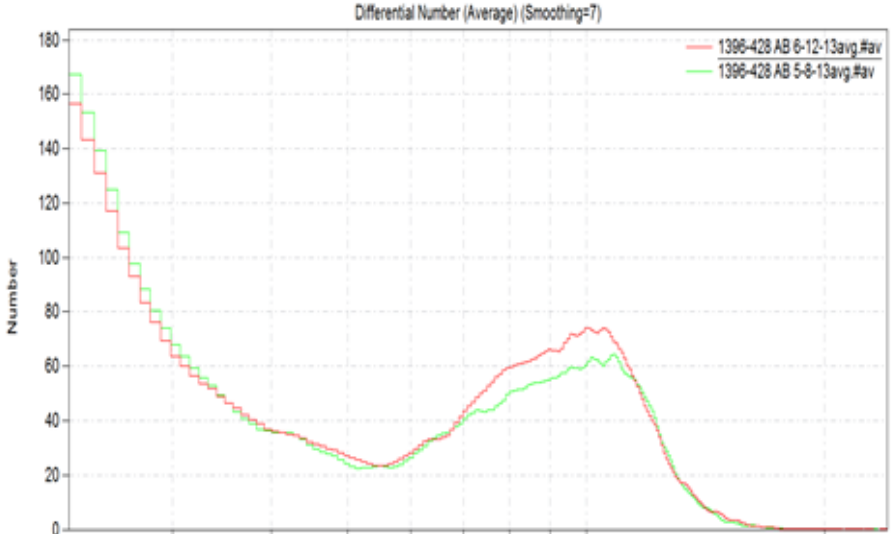

Thigh1
Thigh2


0.3
0.25
0.2
0.15
0.1
0.05
0
P=0.20
P=0.005
Female
Male


LiverFat1
LiverFat2

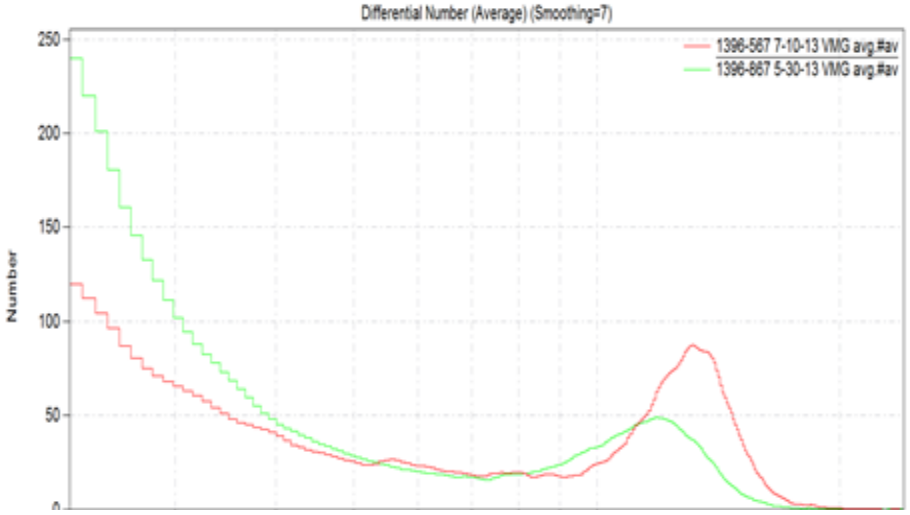
Female IS no increase SSPG

Green PRE
Red Post



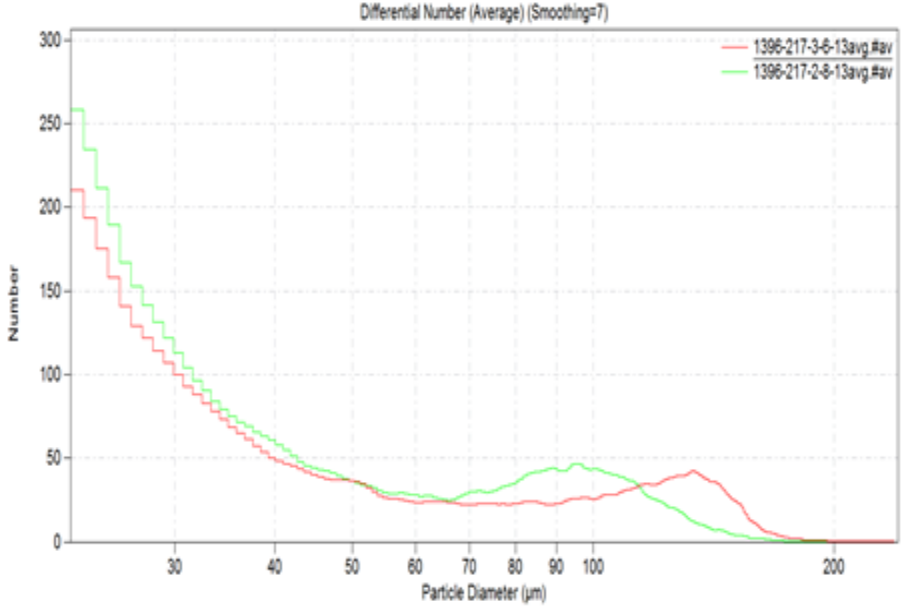
Female IS increased SSPG

Green PRE
Red POST



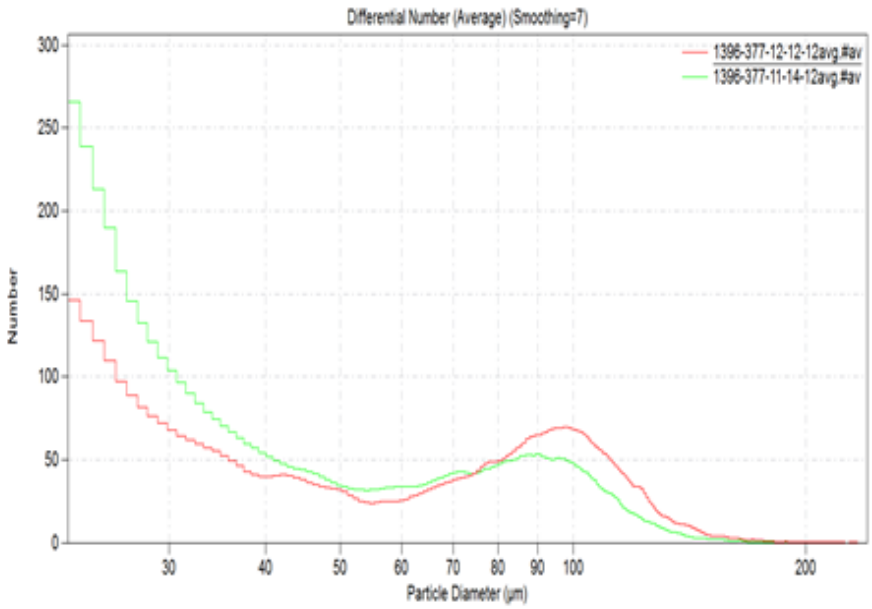
Male IR no change SSPG

Green PRE
Red Post

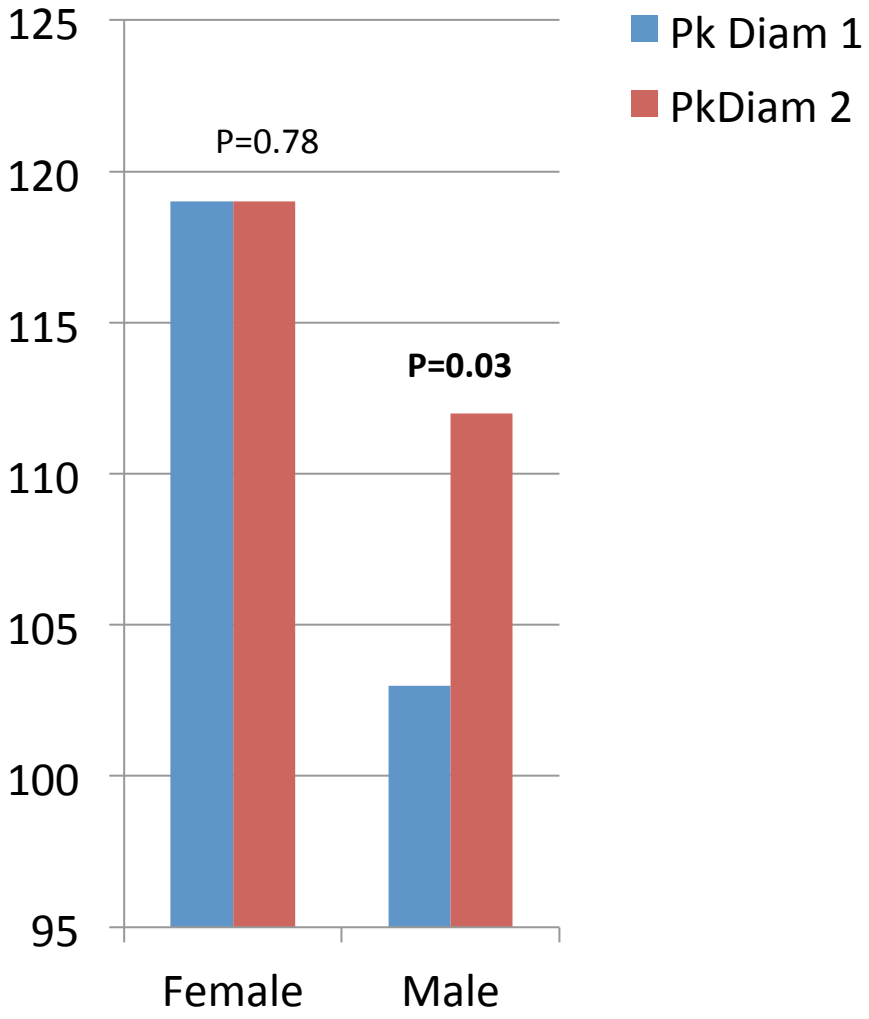


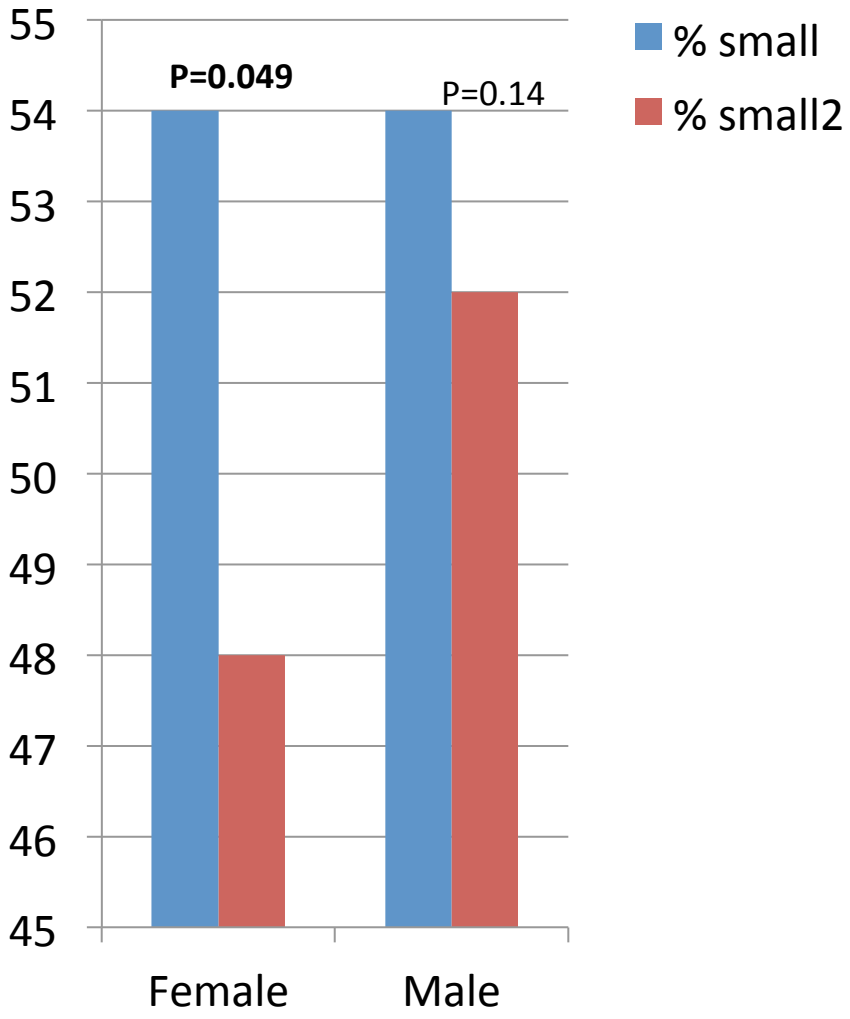
Male IS increased SSPG

Green PRE
Red Post

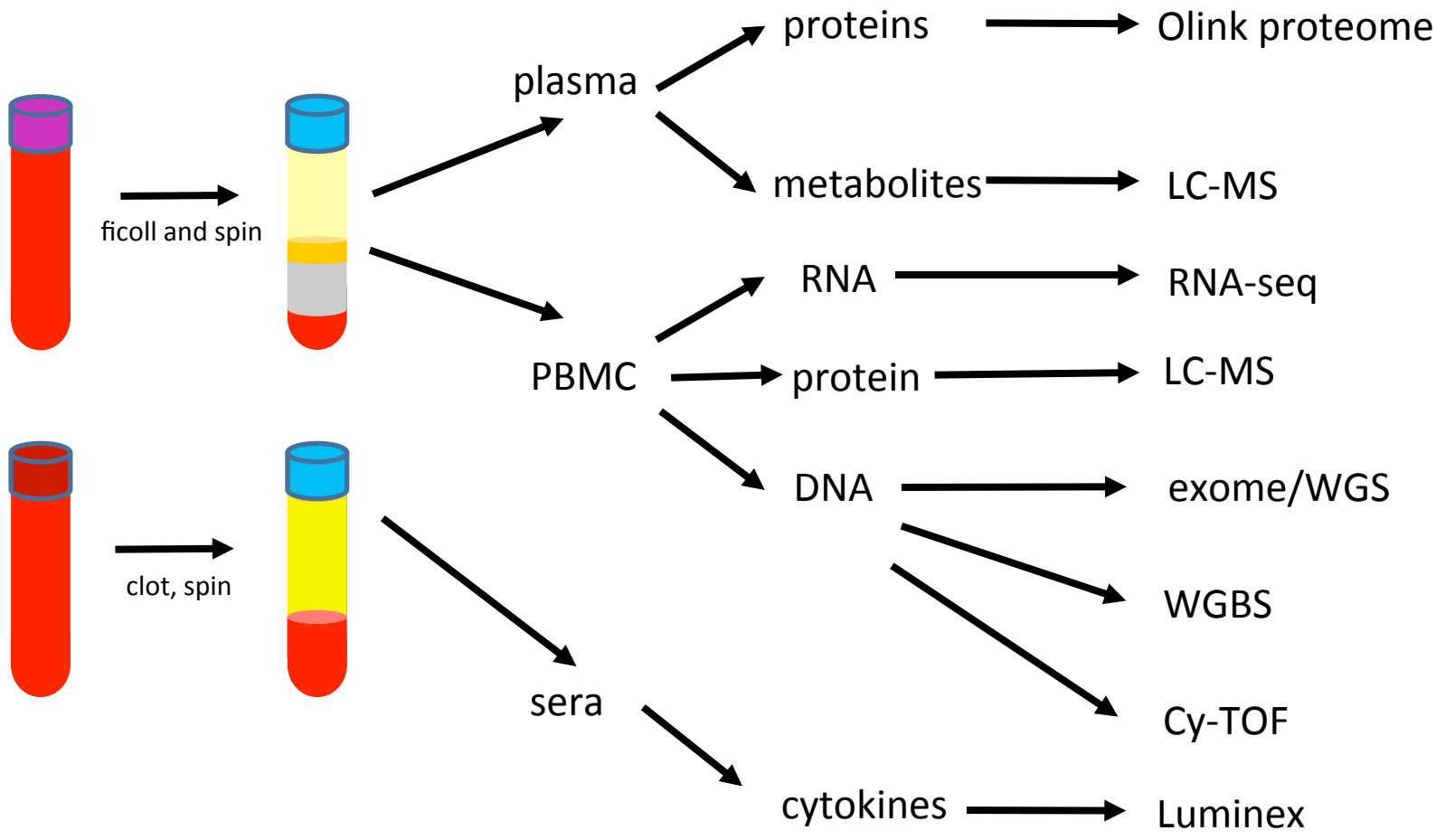


Results

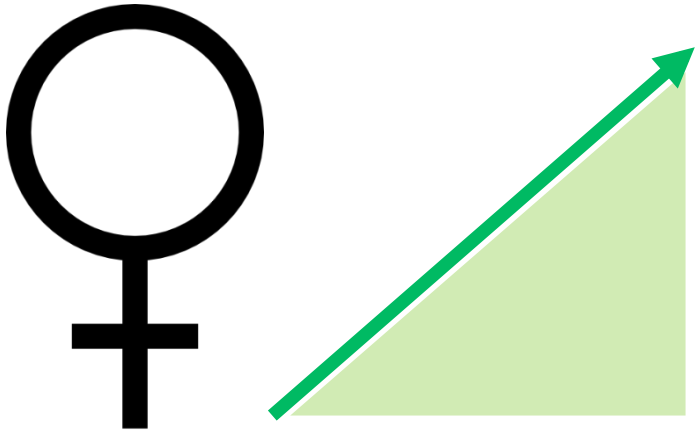

125
120
115
110
105
100
95
P=0.78
P=0.03
Pk Diam 1
PkDiam 2
Female
Male


55
54
53
52
51
50
49
48
47
46
45
P=0.049
P=0.14
% small
% small2
Female
Male

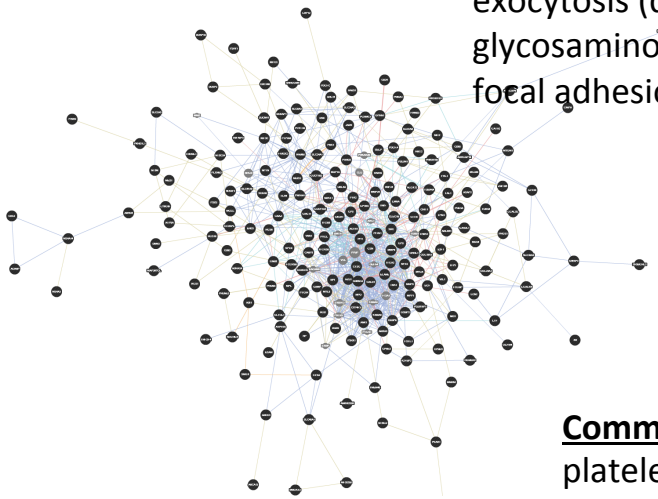
A wealth of omics data can be extracted from blood.



Preliminary RNA-seq reveals sex differences in response to overfeeding

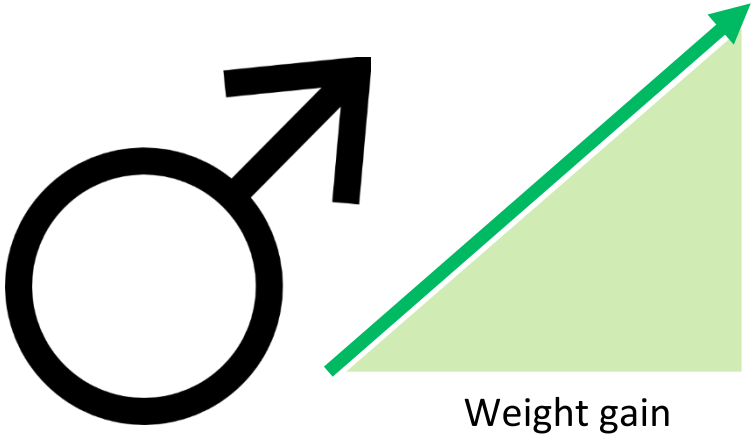


Weight gain

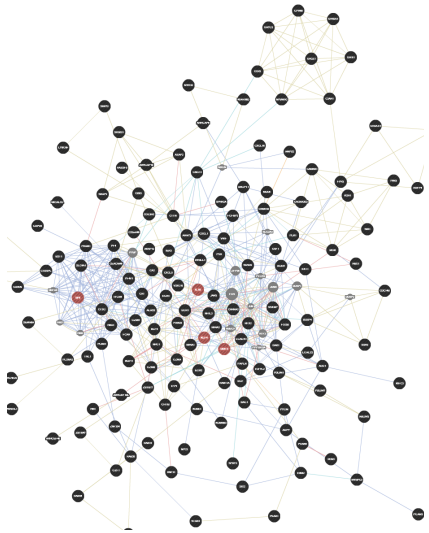


Female-specific pathways include
exocytosis (q<4e-9)
glycosaminoglycan binding (q<1e-4)
focal adhesion (q<0.05)

Common pathways include
platelet activation (q<1e-13)
vesicle lumen (q<1e-7)
inflammatory response (q<0.005)



Weight gain



Male-specific pathways include
response to gonadotrophin (q<0.05)

Future

- Still recruiting premenopausal women for the 2H2O evaluation to measure TG storage in subcutaneous adipose cells. Males = 11; Females = 4 and recruiting
- Increase power for the between-sex comparisons in entire group – missing some cell size and CT/MRI data
- Omics evaluations in greater detail – perform omics analyses in adipose tissue (future grant)

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Elucidating the APOE-by-Gender Interaction in Alzheimer's Disease

WSDM: project update 10/17/2014

André Altmann

with Michael D Greicius and Hua Tang

Stanford University
Department of Neurology and Neurological Sciences

altmann@stanford.edu

Alzheimer's Disease and APOE

- The e4 allele of the APOE gene is the strongest common genetic risk factor for late onset AD
- In 1997 Farrer et *al.* showed an APOE-by-sex effect
  - e3/e4 women show OR ~ 4
  - e3/e4 men show OR ~ 1
- Recently, we confirmed the APOE-by-sex effect in longitudinal data provided by the NACC …

  (Altmann et al., 2014, Annals of Neurology)
- … and showed an APOE-by-sex effect on imaging biomarkers

  (Damoiseaux et al., 2012, J Neuroscience)

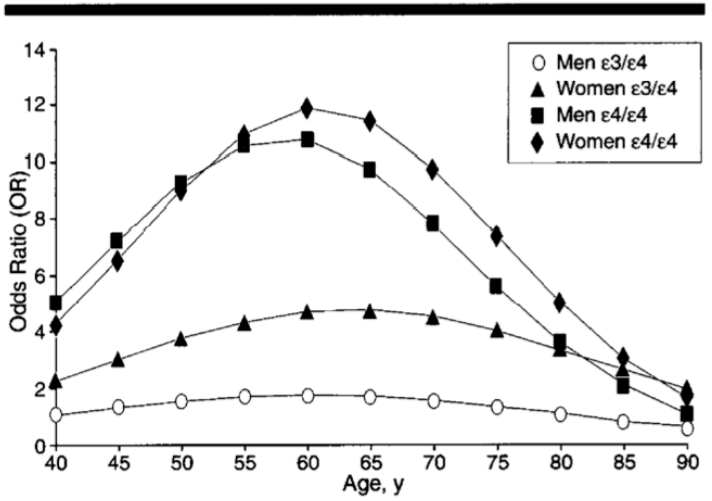
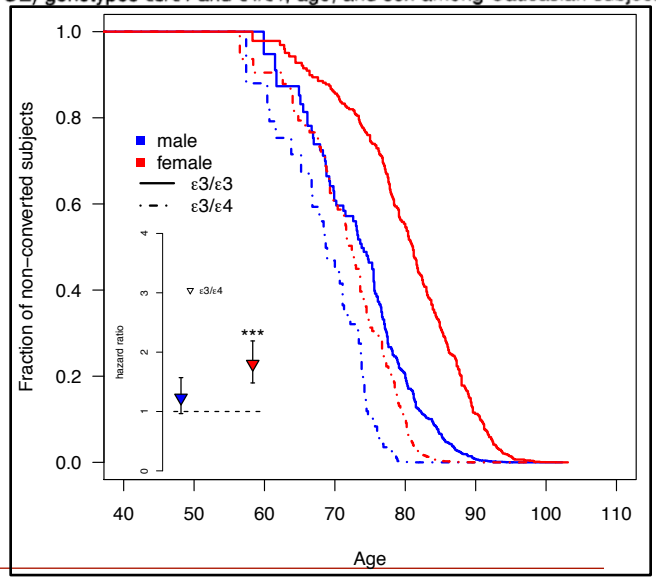


Figure 2.—Relative odds of Alzheimer disease according to apolipoprotein E (*APOE*) genotypes ε3/ε4 and ε4/ε4, age, and sex among Caucasian subjects



Are there any SNPs with Sex-interaction on AD risk?

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- Based on results by Farrer et al. (1997; JAMA) and our group's recent results we had following questions:
  1. Are there other SNPs that affect AD risk in a sex-dependent fashion?
  2. Are there SNPs that show an APOE-by-sex-by-SNP effect?

- The AD Genetics Consortium data
  - 15 AD case/control GWA studies (three are family-based)
  - 21,940 subjects
  - HC: 10,491; AD: 11,449
  - Women: 13,079; men: 8,801 -> 59% women
  - Studies analyzed separately
  - Results combined using methods for meta analysis

Classic analysis – SNP main effect

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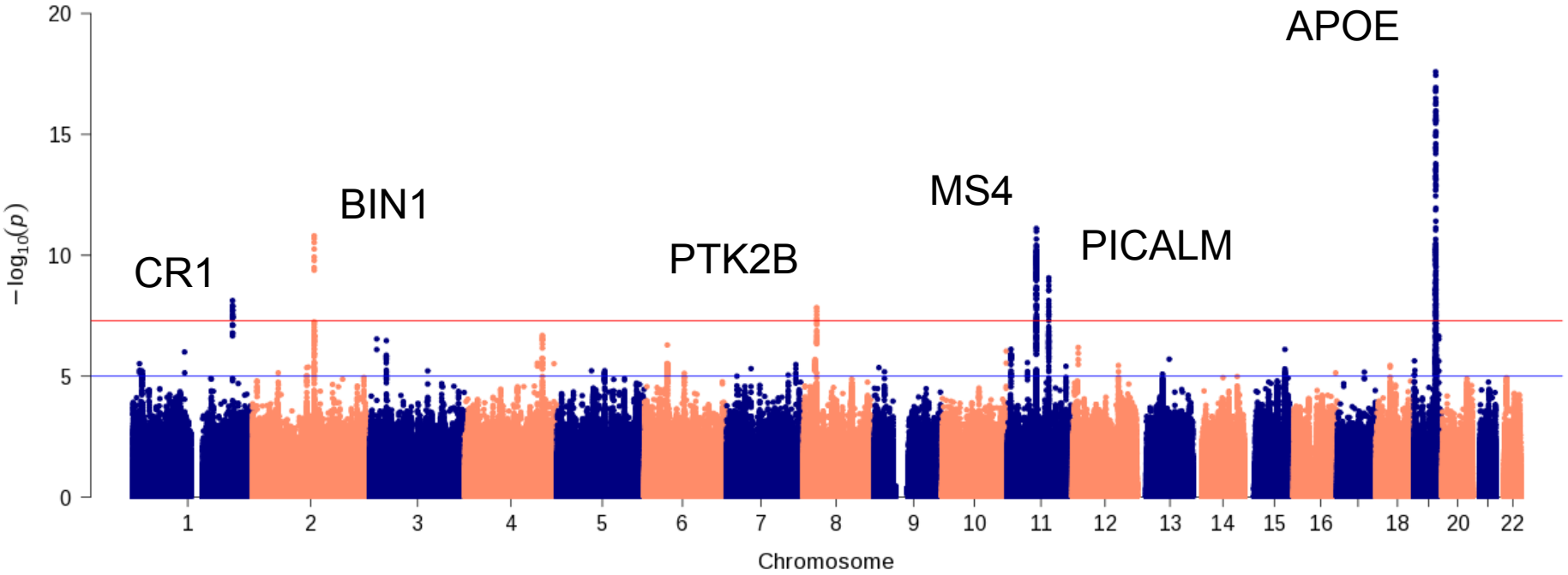
- "Sanity" check
- Classic analysis reveals a number of known AD genes



SNP-by-SEX effect

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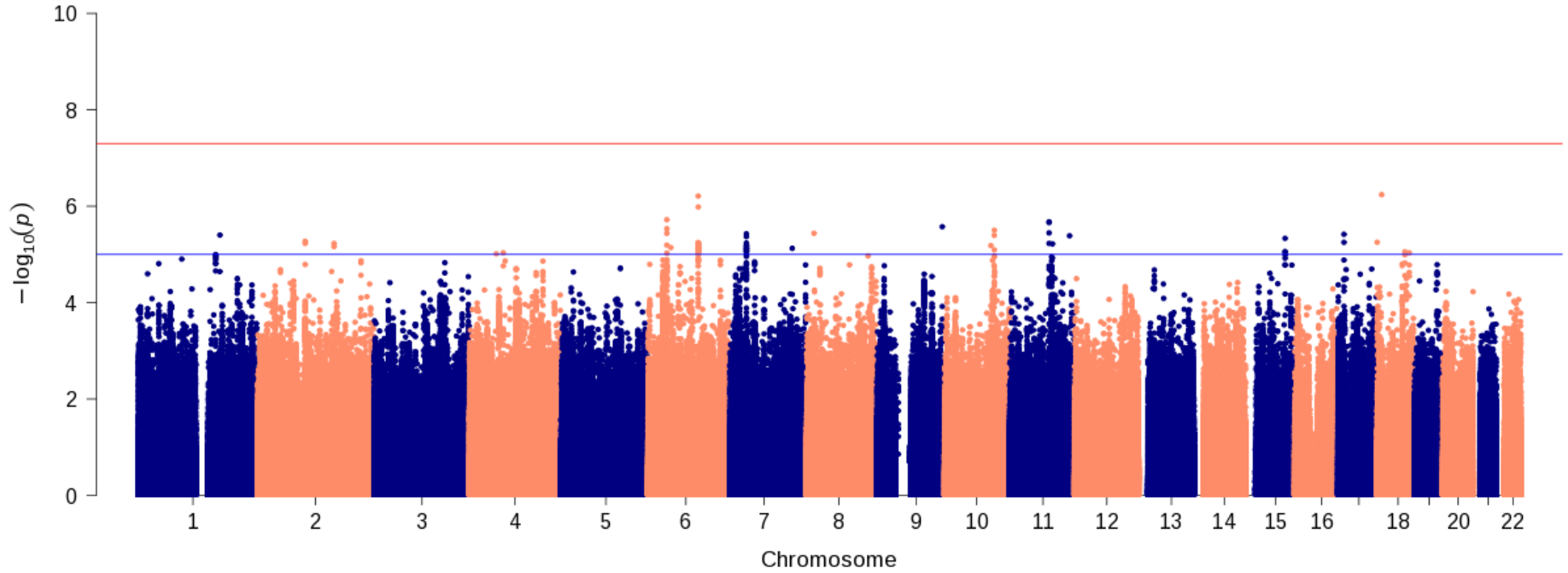
- No SNP passes the genome-wide threshold for the SNP-by-sex interaction
- Hardly any SNP passes the “suggestive” threshold



SNP-by-SEX effect – II

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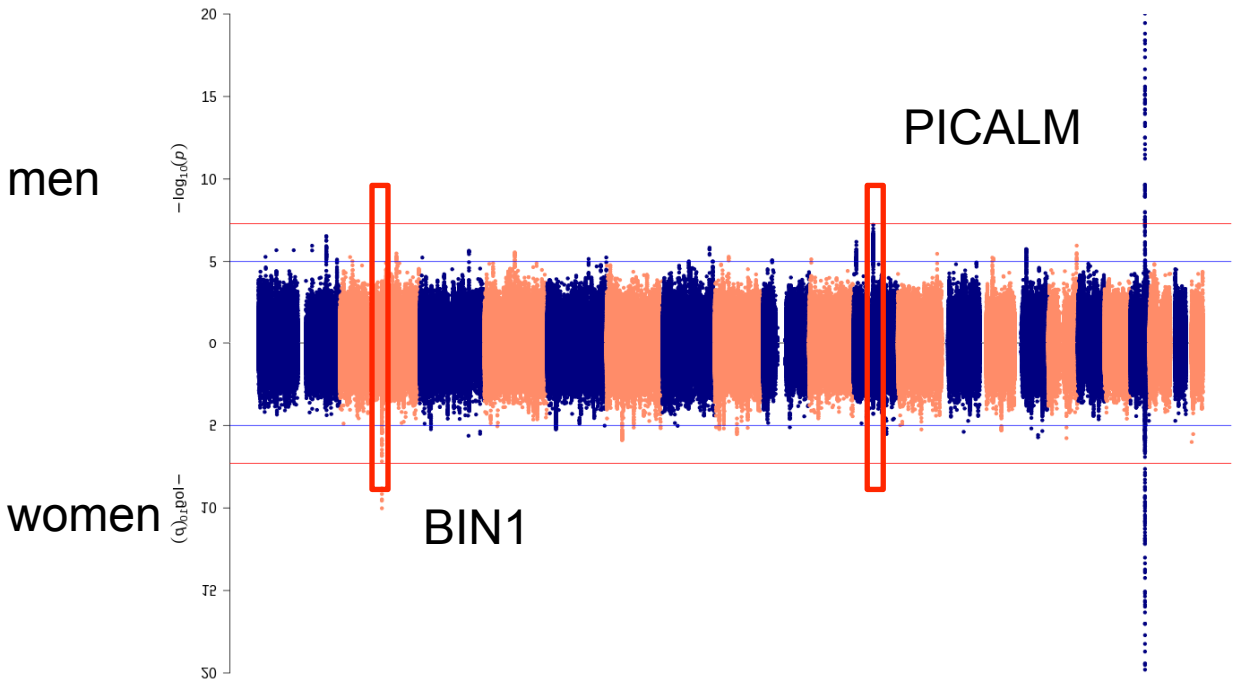
- Looking separately at men and women



- Corresponding P-value in the interaction models are P>0.25

SNP-by-SEX-by-APOE4

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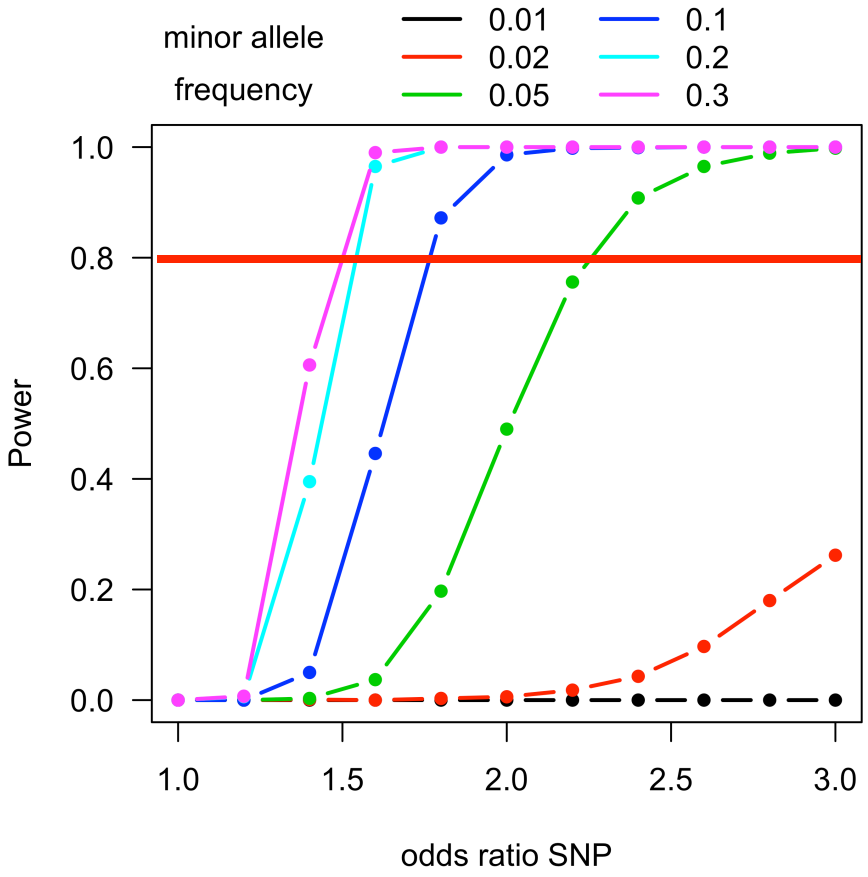
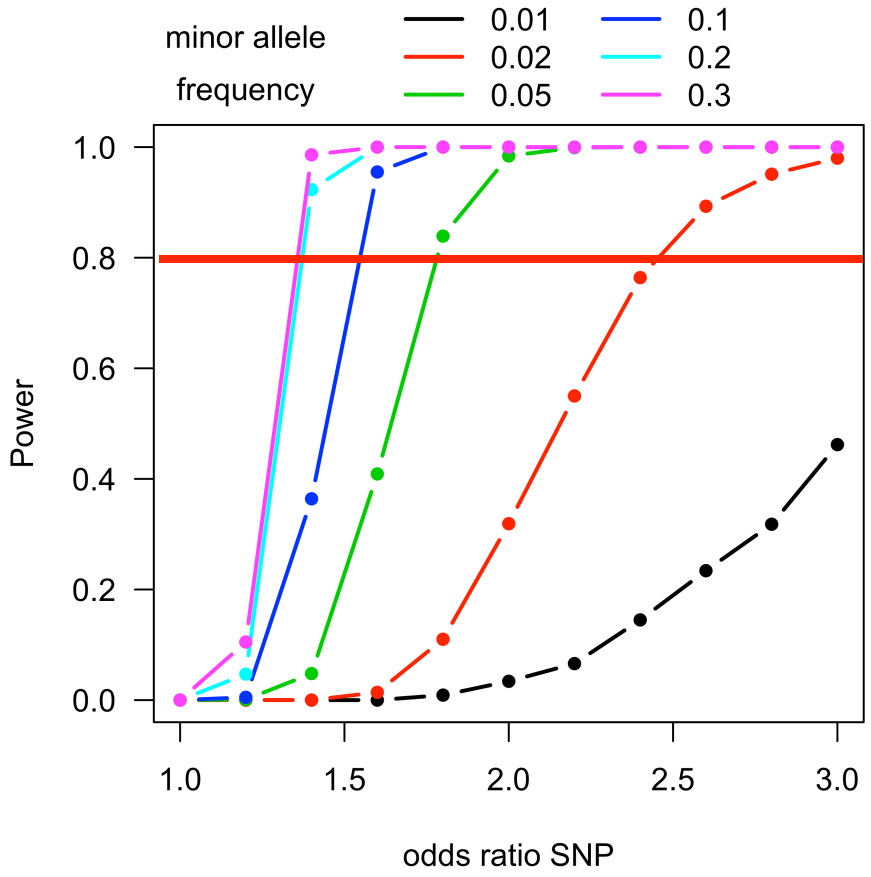
3-way interaction is still work in progress





Conclusion

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- Possible SNP-by-sex interaction in *PICALM* and *BIN1*
- SNP-by-sex-by-APOE still in progress
- Explore Gene Set Enrichment analysis
- Preliminary results were used in a grant application
  - McKnight grant (1 of 12 finalists; 70+ letters of intent)

Acknowledgements

Michael D Greicius

Hua Tang

Funding:

Stanford WSDM Center

Data:

ADGC Alzheimer's Disease Genetics Consortium

Effect of cell sex on smooth muscle cells and fibroblasts derived from human embryonic stem cells

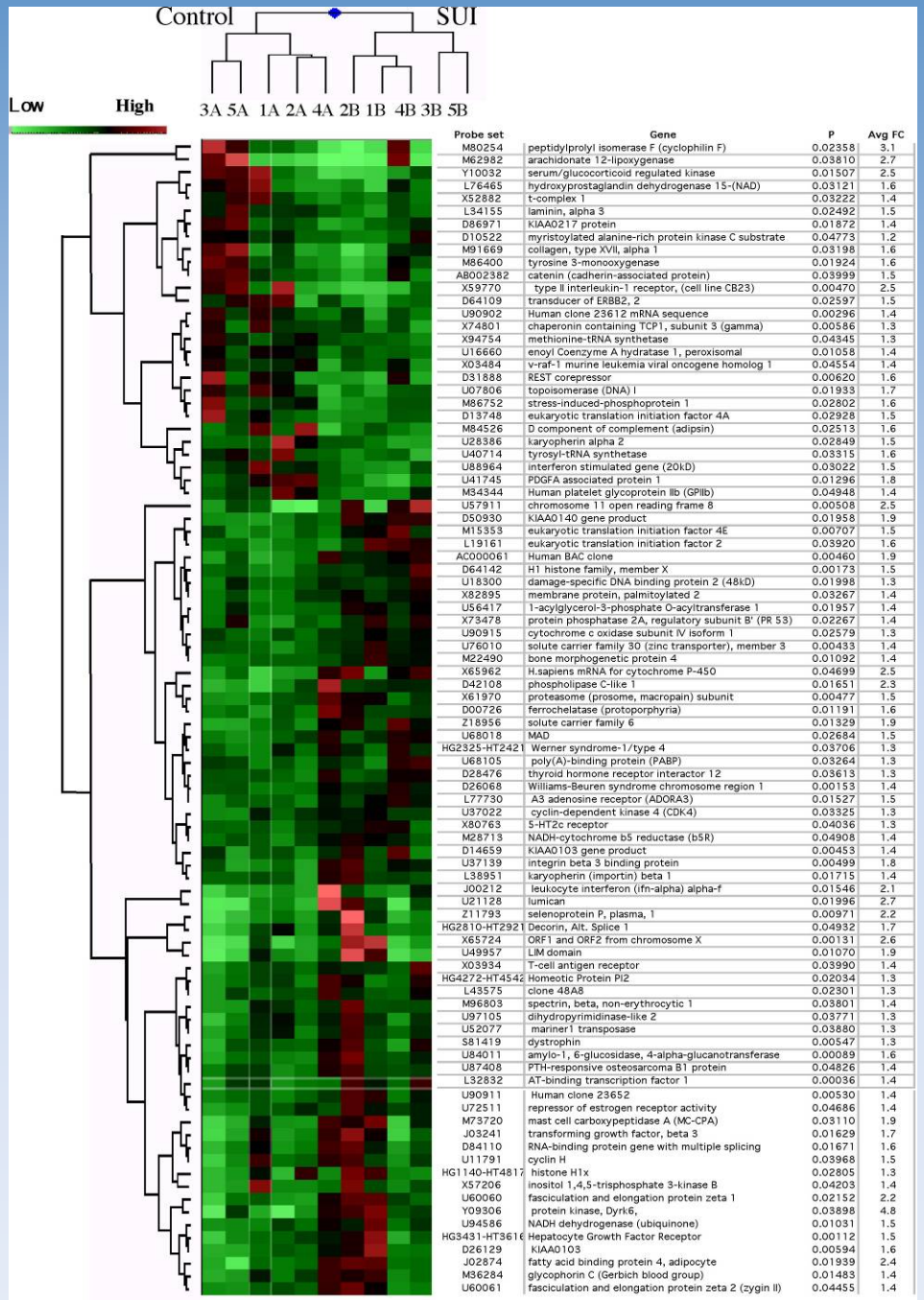
Bertha Chen, MD
Department of Ob/Gyn
Jean Tang, MD
Department of Dermatology

Sex-related differences in stem cells

- Sex differences observed in progenitor and stem cell behavior both *in vitro* and *in vivo* after transplant. Observed in cells differentiated from embryonic stem cells (ESC), mesenchymal stem cells (MSC), and neural stem cells.
- The efficiency of *in vivo* skeletal muscle regeneration appears to be dependent on cell sex. Compared with the transplantation of male MSCs, female MSCs leads to substantially more regeneration of the diseased skeletal muscle.
- These sex-related differences do not appear to be exclusively related to hormonal environment.

Sex differences in disease

- gender dimorphisms have been linked to:
  - sex steroid exposure
  - sex of the cells
- **We hypothesize that sex of cells may be a significant determinant of function of cells derived from ESC and that this may be modulated by sex steroids.**



Probe set	Gene	P	Avg FC
M80254	peptidylprolyl isomerase F (cyclophilin F)	0.02358	3.1
M62982	arachidonate 12-lipoxygenase	0.03810	2.7
Y10032	serum/glucocorticoid regulated kinase	0.01507	2.5
L76465	hydroxyprostaglandin dehydrogenase 15-(NAD)	0.03121	1.6
X52882	t-complex 1	0.03222	1.4
L34155	laminin, alpha 3	0.02492	1.5
D86971	KIAA0217 protein	0.01872	1.4
D10522	myristoylated alanine-rich protein kinase C substrate	0.04773	1.2
M91669	collagen, type XVII, alpha 1	0.03198	1.6
M86400	tyrosine 3-monooxygenase	0.01924	1.6
AB002382	catenin (cadherin-associated protein)	0.03999	1.5
X59770	type II interleukin-1 receptor, (cell line CB23)	0.00470	2.5
D64109	transducer of ERBB2, 2	0.02597	1.5
U90902	Human clone 23612 mRNA sequence	0.00296	1.4
X74801	chaperonin containing TCP1, subunit 3 (gamma)	0.00586	1.3
X94754	methionine-tRNA synthetase	0.04345	1.3
U16660	enoyl Coenzyme A hydratase 1, peroxisomal	0.01058	1.4
X03484	v-raf-1 murine leukemia viral oncogene homolog 1	0.04554	1.4
D31888	REST corepressor	0.00620	1.6
U07806	topoisomerase (DNA) I	0.01933	1.7
M86752	stress-induced-phosphoprotein 1	0.02802	1.6
D13748	eukaryotic translation initiation factor 4A	0.02928	1.5
M84526	D component of complement (adipsin)	0.02513	1.6
U28386	karyopherin alpha 2	0.02849	1.5
U40714	tyrosyl-tRNA synthetase	0.03315	1.6
U88964	interferon stimulated gene (20kD)	0.03022	1.5
U41745	PDGFA associated protein 1	0.01296	1.8
M34344	Human platelet glycoprotein IIb (GPIIb)	0.04948	1.4
U57911	chromosome 11 open reading frame 8	0.00508	2.5
D50930	KIAA0140 gene product	0.01958	1.9
M15353	eukaryotic translation initiation factor 4E	0.00707	1.5
L19161	eukaryotic translation initiation factor 2	0.03920	1.6
AC000061	Human BAC clone	0.00460	1.9
D64142	H1 histone family, member X	0.00173	1.5
U18300	damage-specific DNA binding protein 2 (48kD)	0.01998	1.3
X82895	membrane protein, palmitoylated 2	0.03267	1.4
U56417	1-acylglycerol-3-phosphate O-acyltransferase 1	0.01957	1.4
X75478	protein phosphatase 2A, regulatory subunit B' (PR 53)	0.02267	1.4
U90915	cytochrome c oxidase subunit IV isoform 1	0.02579	1.3
U76010	solute carrier family 30 (zinc transporter), member 3	0.00433	1.4
M22490	bone morphogenetic protein 4	0.01092	1.4
X65962	H.sapiens mRNA for cytochrome P-450	0.04699	2.5
D42108	phospholipase C-like 1	0.01651	2.3
X61970	proteasome (prosome, macropain) subunit	0.00477	1.5
D00726	ferrochelatase (protoporphyria)	0.01191	1.6
Z18956	solute carrier family 6	0.01329	1.9
U68018	MAD	0.02684	1.5
HG2325-HT2421	Werner syndrome-1/type 4	0.03706	1.3
U68105	poly(A)-binding protein (PABP)	0.03264	1.3
D28476	thyroid hormone receptor interactor 12	0.03613	1.3
D26068	Williams-Beuren syndrome chromosome region 1	0.00153	1.4
L77730	A3 adenosine receptor (ADORA3)	0.01527	1.5
U37022	cyclin-dependent kinase 4 (CDK4)	0.03325	1.3
X80763	5-HT2c receptor	0.04036	1.3
M28713	NADH-cytochrome b5 reductase (b5R)	0.04908	1.4
D14659	KIAA0103 gene product	0.00453	1.4
U37139	integrin beta 3 binding protein	0.00499	1.8
L38951	karyopherin (importin) beta 1	0.01715	1.4
J00212	leukocyte interferon (ifn-alpha) alpha-f	0.01546	2.1
U21128	lumican	0.01996	2.7
Z11793	selenoprotein P, plasma, 1	0.00971	2.2
HG2810-HT2921	Decorin, Alt. Splice 1	0.04932	1.7
X65724	ORF1 and ORF2 from chromosome X	0.00131	2.6
U49957	LIM domain	0.01070	1.9
X03934	T-cell antigen receptor	0.03990	1.4
HG4272-HT4542	Homeotic Protein Pl2	0.02034	1.3
L43575	clone 48A8	0.02301	1.3
M96803	spectrin, beta, non-erythrocytic 1	0.03801	1.4
U97105	dihydropyrimidinase-like 2	0.03771	1.3
U52077	mariner1 transposase	0.03880	1.3
S81419	dystrophin	0.00547	1.3
U84011	amylo-1, 6-glucosidase, 4-alpha-glucanotransferase	0.00089	1.6
U87408	PTH-responsive osteosarcoma B1 protein	0.04826	1.4
L32832	AT-binding transcription factor 1	0.00036	1.4
U90911	Human clone 23652	0.00530	1.4
U72511	repressor of estrogen receptor activity	0.04686	1.4
M73720	mast cell carboxypeptidase A (MC-CPA)	0.03110	1.9
J03241	transforming growth factor, beta 3	0.01629	1.7
D84110	RNA-binding protein gene with multiple splicing	0.01671	1.6
U11791	cyclin H	0.03968	1.5
HG1140-HT4817	histone H1x	0.02805	1.3
X57206	inositol 1,4,5-trisphosphate 3-kinase B	0.04203	1.4
U60060	fasciculation and elongation protein zeta 1	0.02152	2.2
Y09306	protein kinase, Dyrk6,	0.03898	4.8
U94586	NADH dehydrogenase (ubiquinone)	0.01031	1.5
HG3431-HT3616	Hepatocyte Growth Factor Receptor	0.00112	1.5
D26129	KIAA0103	0.00594	1.6
J02874	fatty acid binding protein 4, adipocyte	0.01939	2.4
M36284	glycophorin C (Gerbich blood group)	0.01483	1.4
U60061	fasciculation and elongation protein zeta 2 (zygin II)	0.04455	1.4

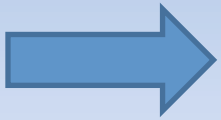
Chen et al, Am J Obstet Gynecol 2003

Sex-related differences in fibroblasts derived from pluripotent stem cells

Fibroblasts derived from male hESC

Fibroblasts derived from female hESC

Fibroblasts derived from female iPSC



TIMP-1

TIMP-3

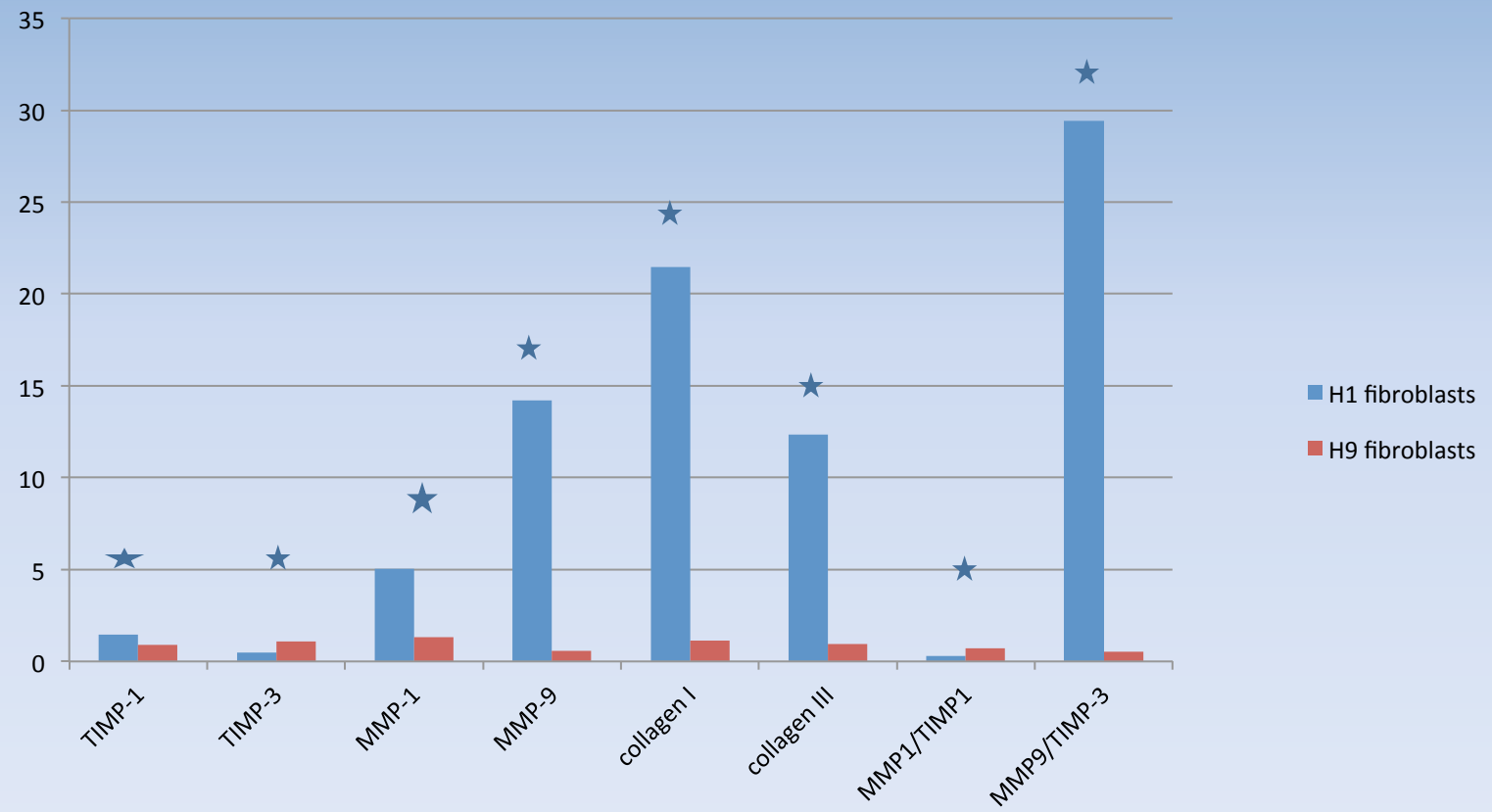
MMP-1

MMP-9

collagen I

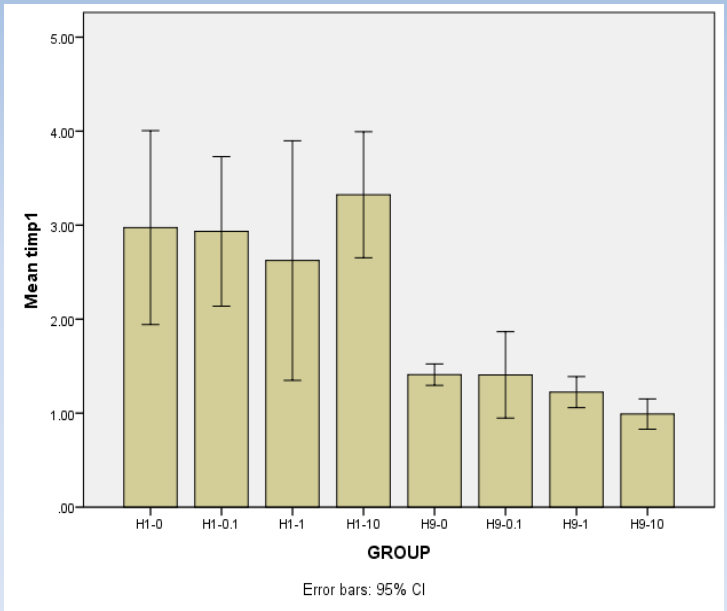
collagen III

Gene expression of extracellular matrix proteins in fibroblasts derived from male and female hESCs

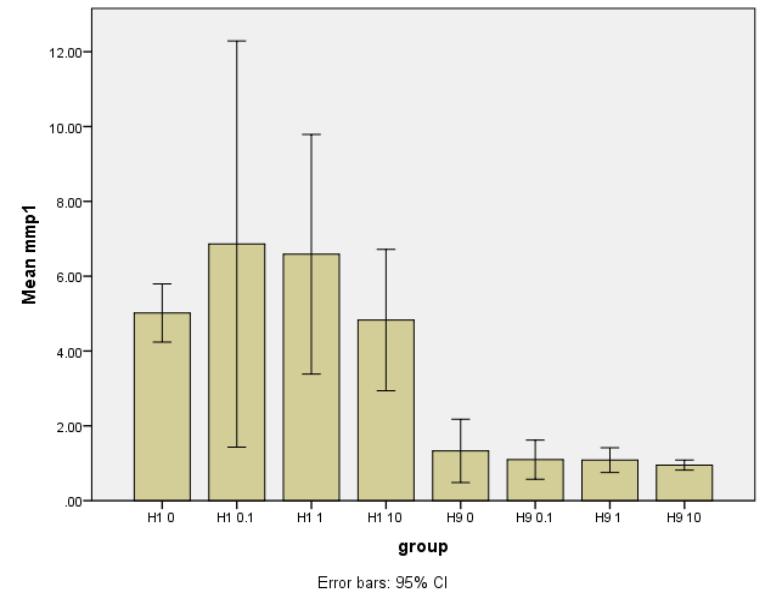


Effect of estrogen on differentiation of fibroblast from hESC

- TIMP-1



MMP-1

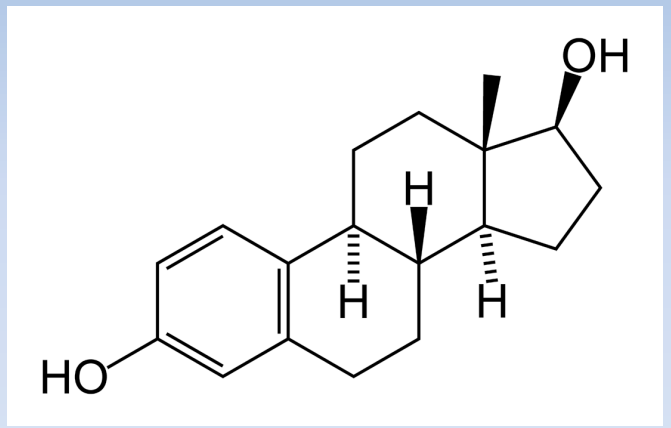


Estrogen stimulation of SMC progenitor cells derived from hESC

H9 Progenitor Smooth Muscle Cells

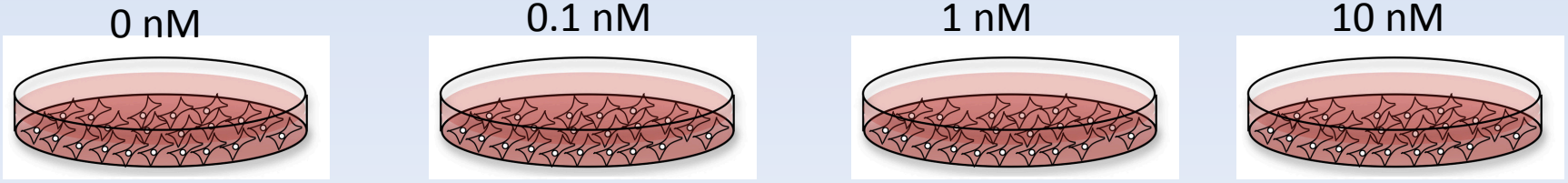
10 day differentiation →

Mature Smooth Muscle Cells

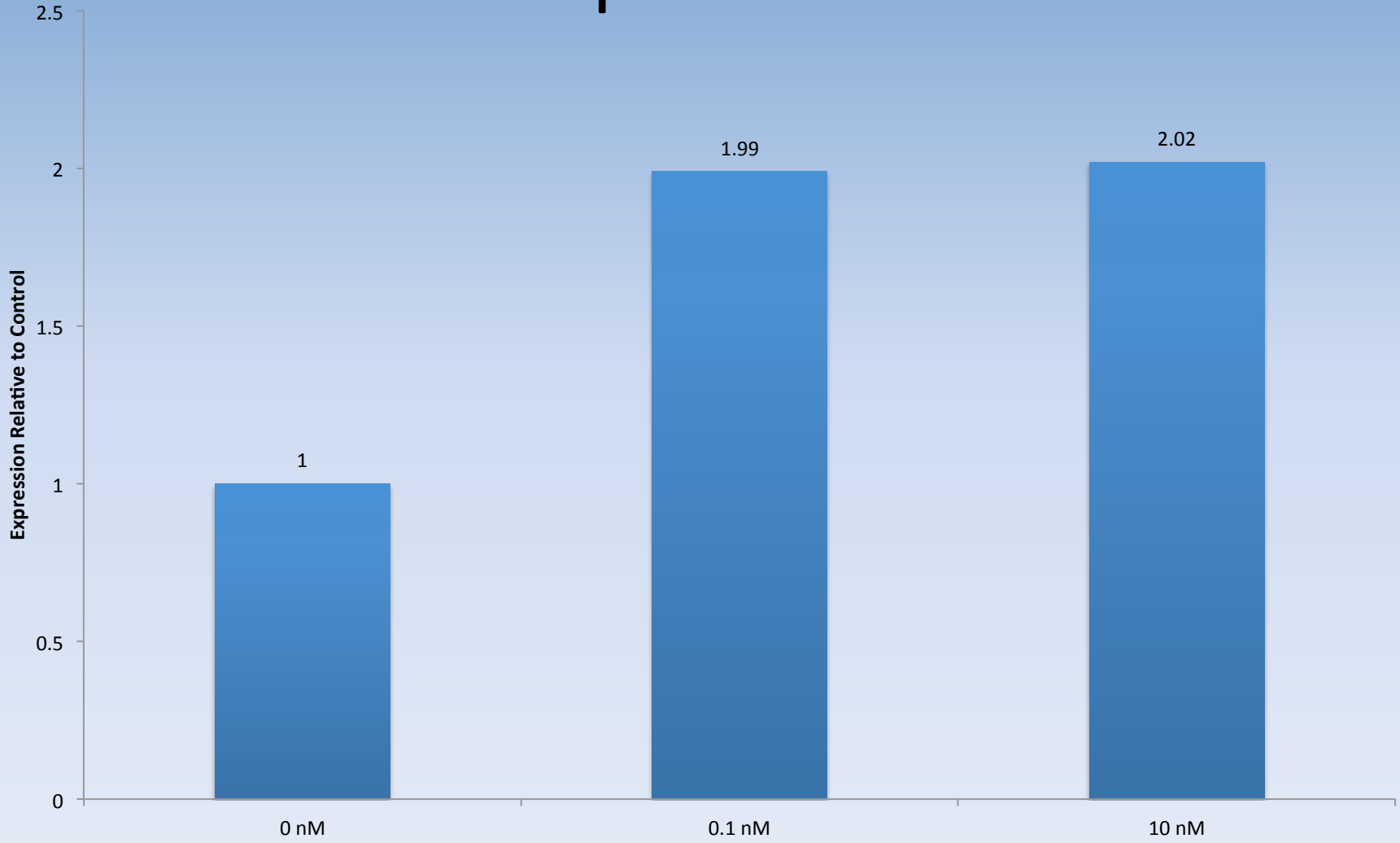


Estrogen

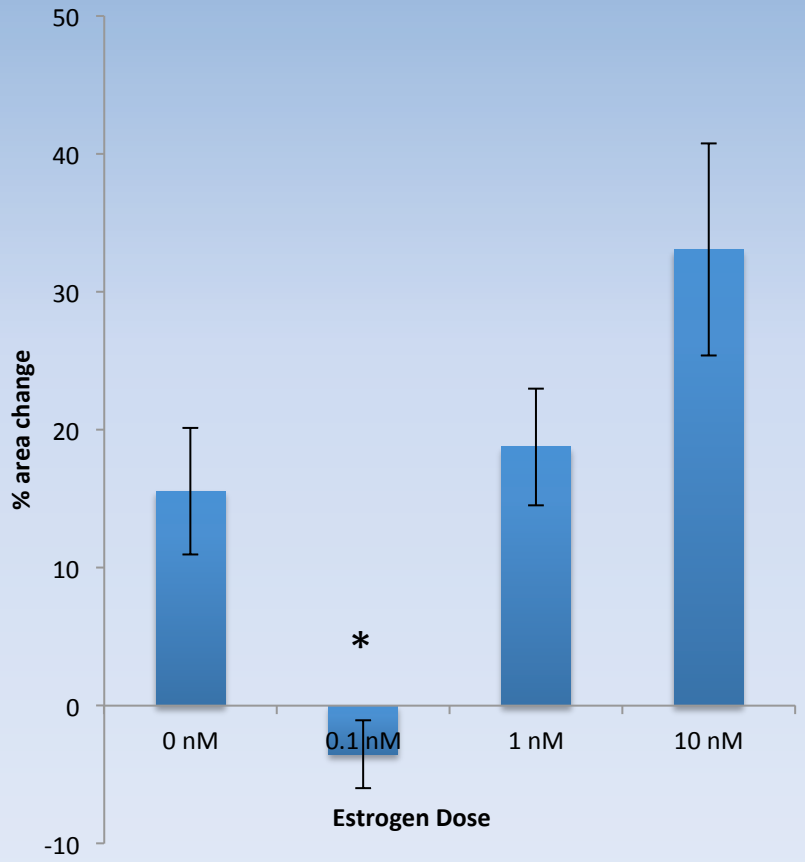
Physiological range:
0.25 nM- 2 nM



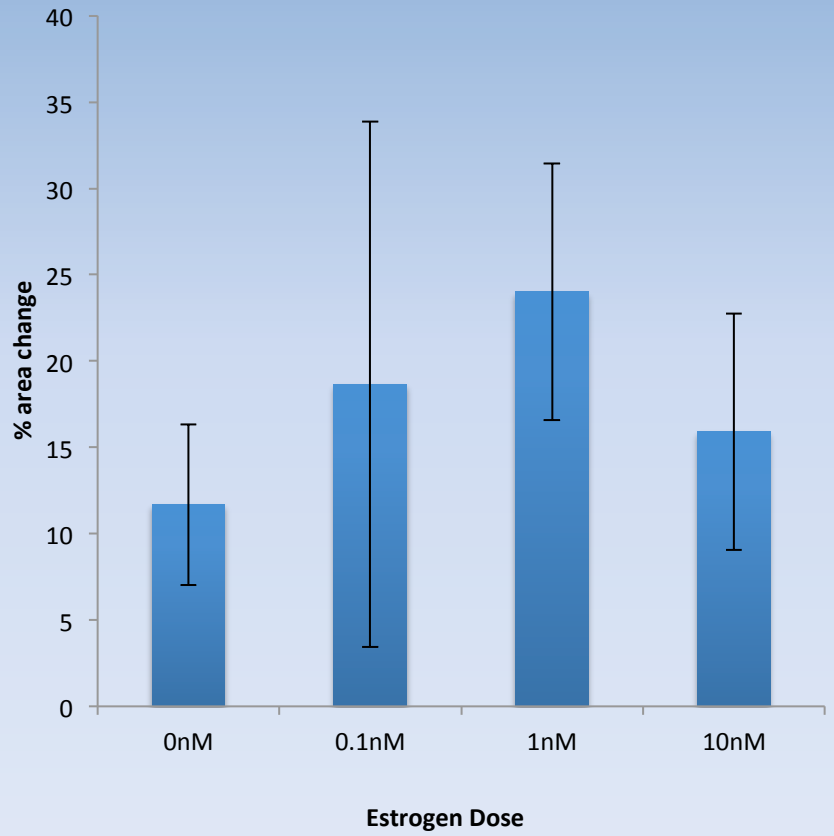
Estrogen doubles SM22 alpha expression



Effect of estrogen on SMC contractility



Day 6



Day 10

Summary

- Phenotypical differences in progenitor cells are related to sex of the embryonic stem cell
- Exposure to sex steroids during specific stages of differentiation from ESC into smooth muscle cells or fibroblasts can modulate these differences.
- We await data from iPSC-derived cells to examine epigenetic effects.
- These pilot studies will direct us to specific areas for studies in stem cell sex differences, as well as whether attention to sex steroid environment is needed to improve transplantation outcomes.

Sex Differences in Androgen Signaling and Alcohol-Seeking Behavior

William Giardino, Ph.D.
Postdoctoral Fellow
de Lecea Laboratory
Department of Psychiatry & Behavioral Sciences
Stanford University School of Medicine

Alcohol Abuse

Table 2. Actual Causes of Death in the United States in 1990 and 2000

Actual Cause	No. (%) in 1990*	No. (%) in 2000
Tobacco	400 000 (19)	435 000 (18.1)
Poor diet and physical inactivity	300 000 (14)	400 000 (16.6)
Alcohol consumption	100 000 (5)	85 000 (3.5)

Mokdad et al., 2004 (*J.A.M.A.*)

DSM-IV PREVALENCE:

- Alcohol Abuse: 17.8%
- Alcohol Dependence: 12.5%

Hasin et al., 2007 *Arch. Gen. Psych.*

BINGE DRINKING (NIH DEFINITION):

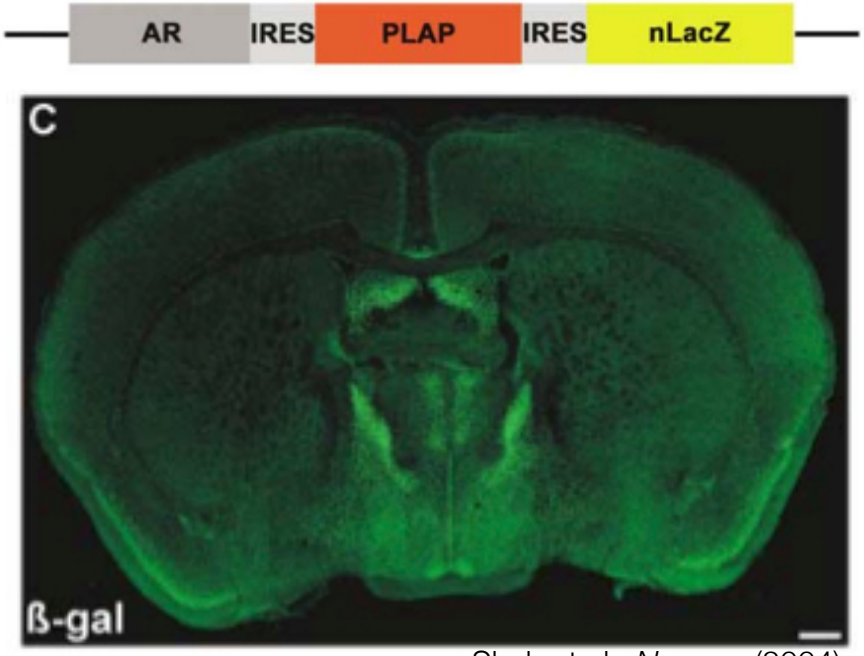
- Blood alcohol concentrations above 80 mg/dl (".08 percent")
  - ~4-5 "drinks" within 2 hours

Sex Differences in Alcohol's Effects

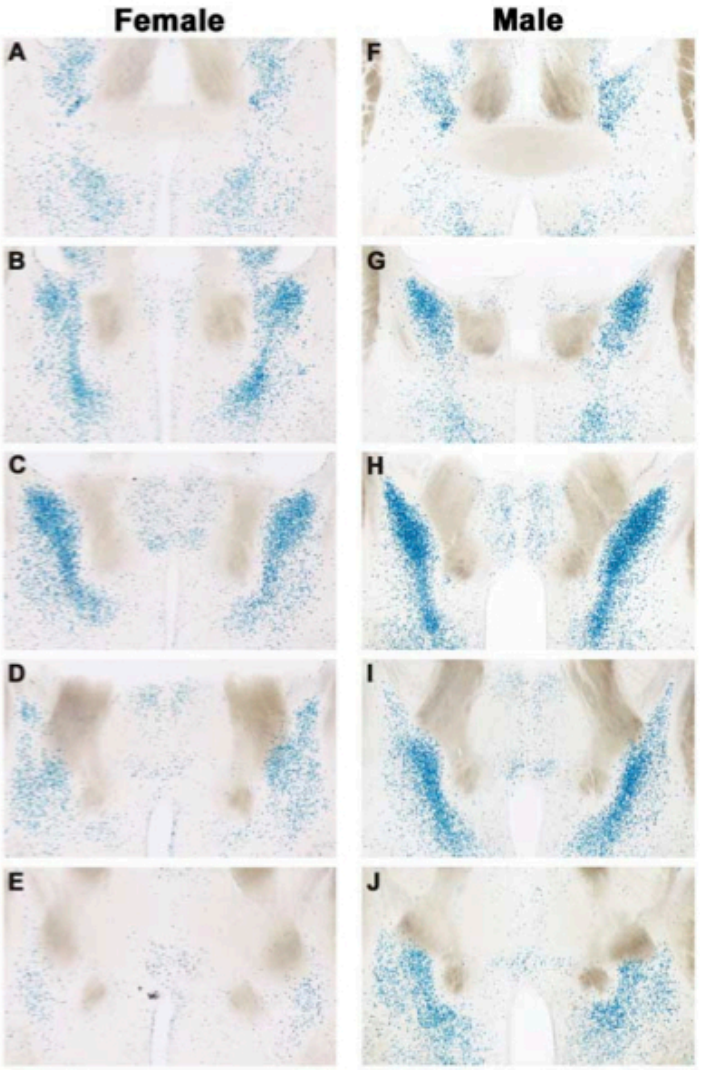
- Alcohol abuse and dependence more prevalent in M>F
- Deleterious physiological effects can be greater in F>M
- High serum testosterone levels can enhance alcohol consumption, alcohol-related aggression
- Polymorphism in the *Androgen Receptor* (AR) gene associated with craving during alcohol withdrawal
- AR acts as a transcription factor in response to binding by testosterone (T) and dihydrotestosterone (DHT)

AR-Reporter: Visualizing Neural Sex Differences

- *LacZ* expressed under control of AR promoter
- β-galactosidase staining reveals AR distribution
- M>F AR in extended amygdala, hypothalamus

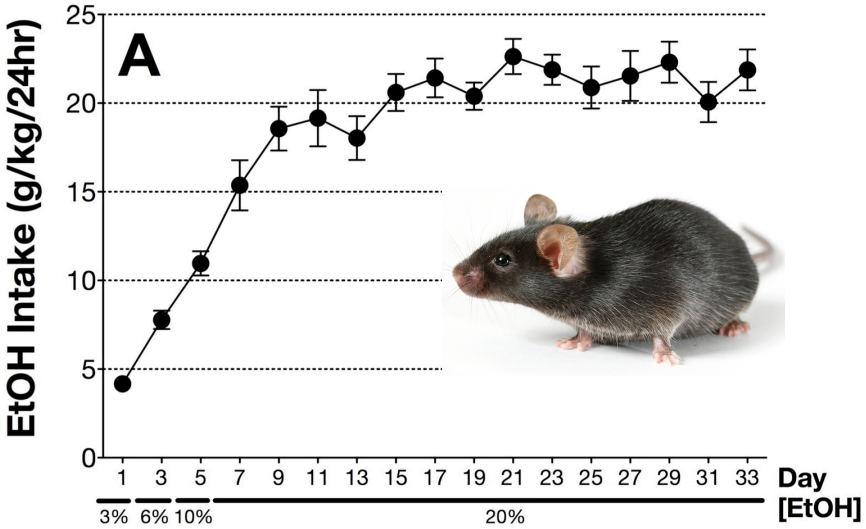


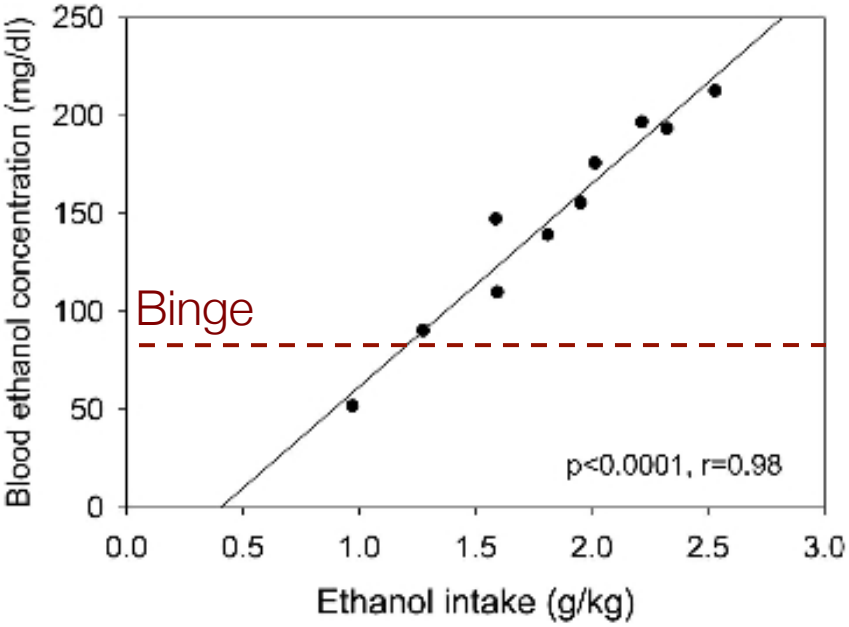
Shah et al., *Neuron* (2004)



Shah et al., *Neuron* (2004)

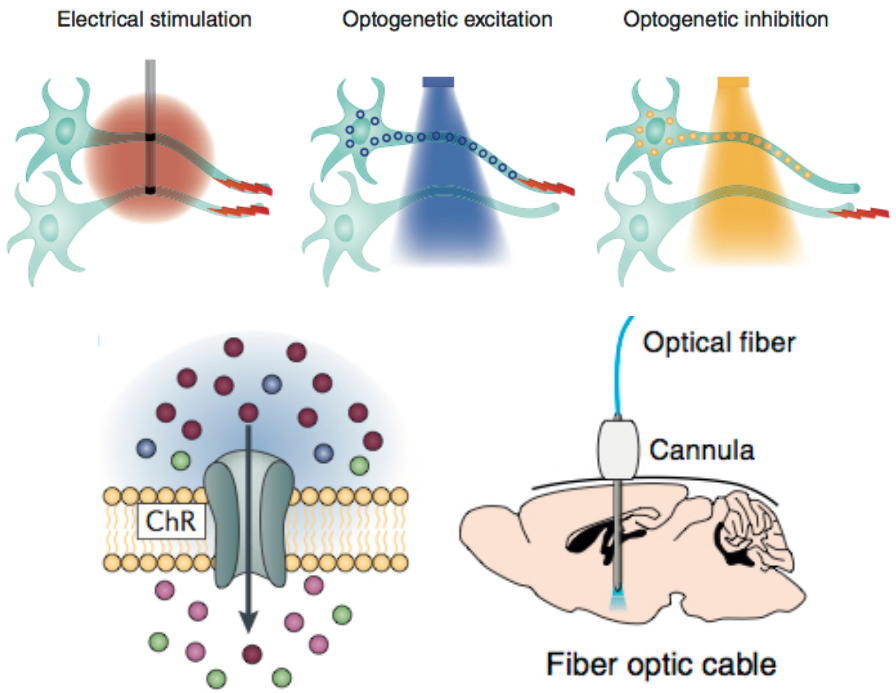
Mouse Models of Binge Alcohol Drinking

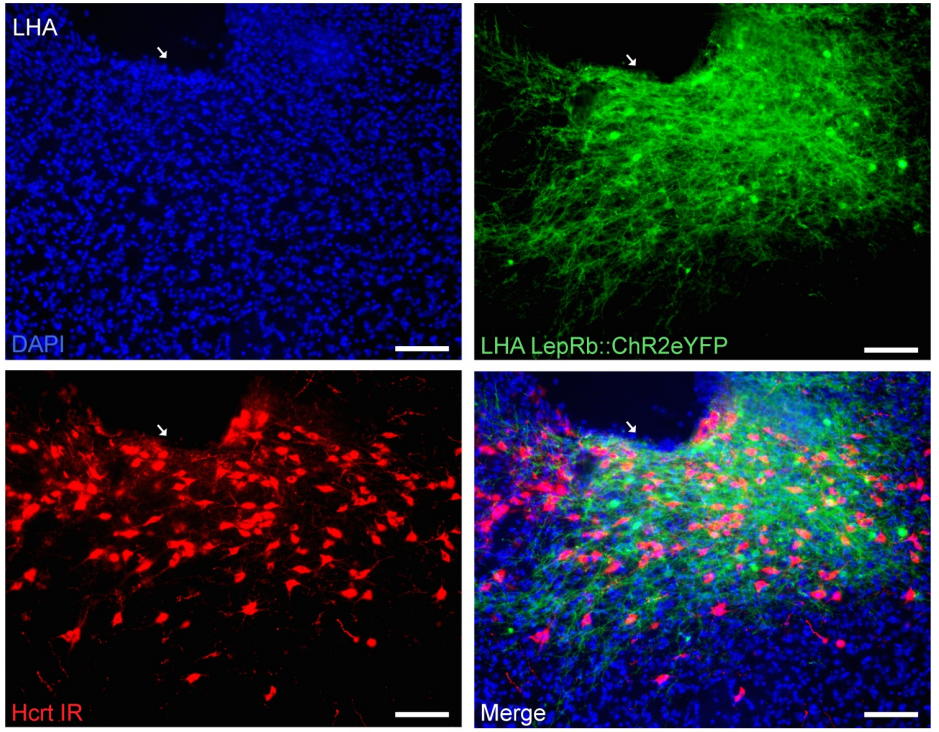




Optogenetic Stimulation of Hypothalamic Subpopulations

- Hypocretin (Hcrt) neurons of the Lateral Hypothalamus (LH)
  - Major projections to midbrain dopamine neurons encoding reward
  - Optogenetic stimulation increases wakefulness, arousal, HPA activity
- Leptin receptor (LepRb) neurons of the LH
  - Inhibitory cells intermingled with LH-Hcrt neurons
  - Optogenetic stimulation dampens LH-Hcrt activity and stress response





P. Bonnavion, L. de Lecea, et al. (in press)

Specific Aims

AIM 1

- Optogenetic stimulation of Hcrt or LepRb hypothalamic neurons
- Assess effects of stimulation on alcohol-seeking and AR expression

x

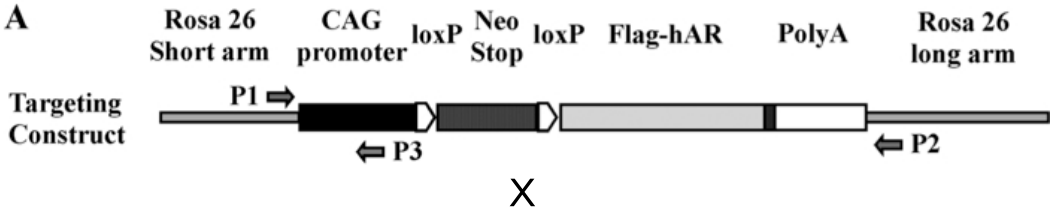
Hcrt::Cre, LepRb::Cre

Hypotheses: photostimulation of LH-Hcrt neurons will drive increased alcohol consumption and reward via interactions with midbrain dopamine neurons. Increased alcohol consumption will be associated with changes in endogenous AR expression throughout distinct forebrain nuclei

Specific Aims

AIM 2

- Conditional AR transgene expression in Hcrt and LepRb neurons
- Determine impact of AR activation on alcohol drinking behavior



X

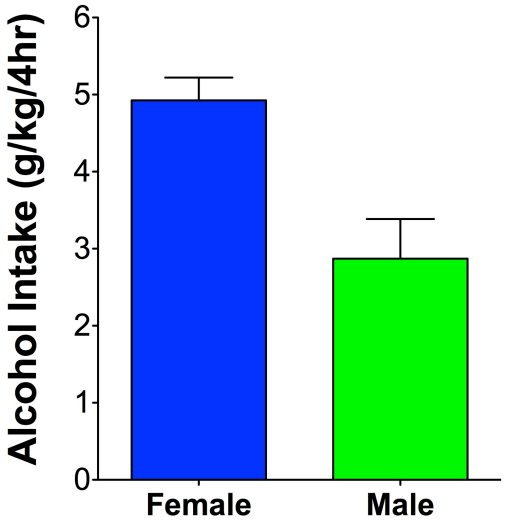
Hcrt::Cre, LepRb::Cre

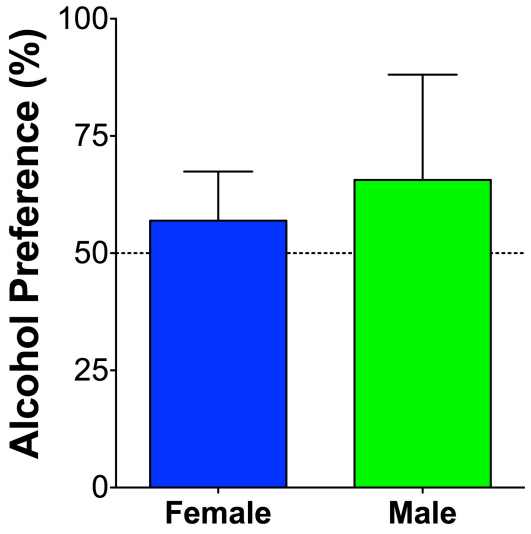
Hypotheses: long-term transgenic activation of AR signaling in LH-Hcrt or LH-LepRb neurons will produce opposing effects on binge alcohol drinking and alcohol-conditioned reward

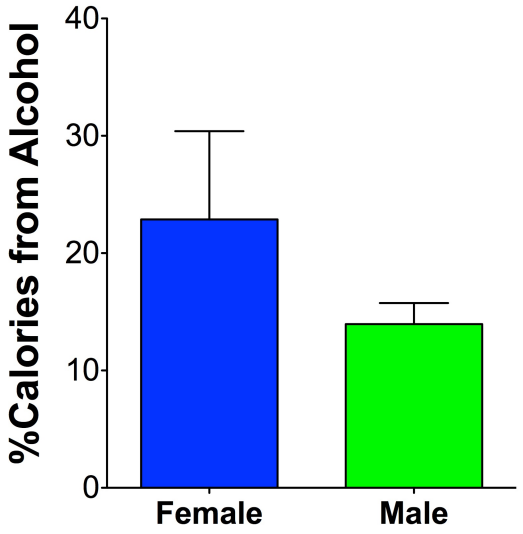
Binge Alcohol Drinking in AR-Reporter Mice

Pilot Studies

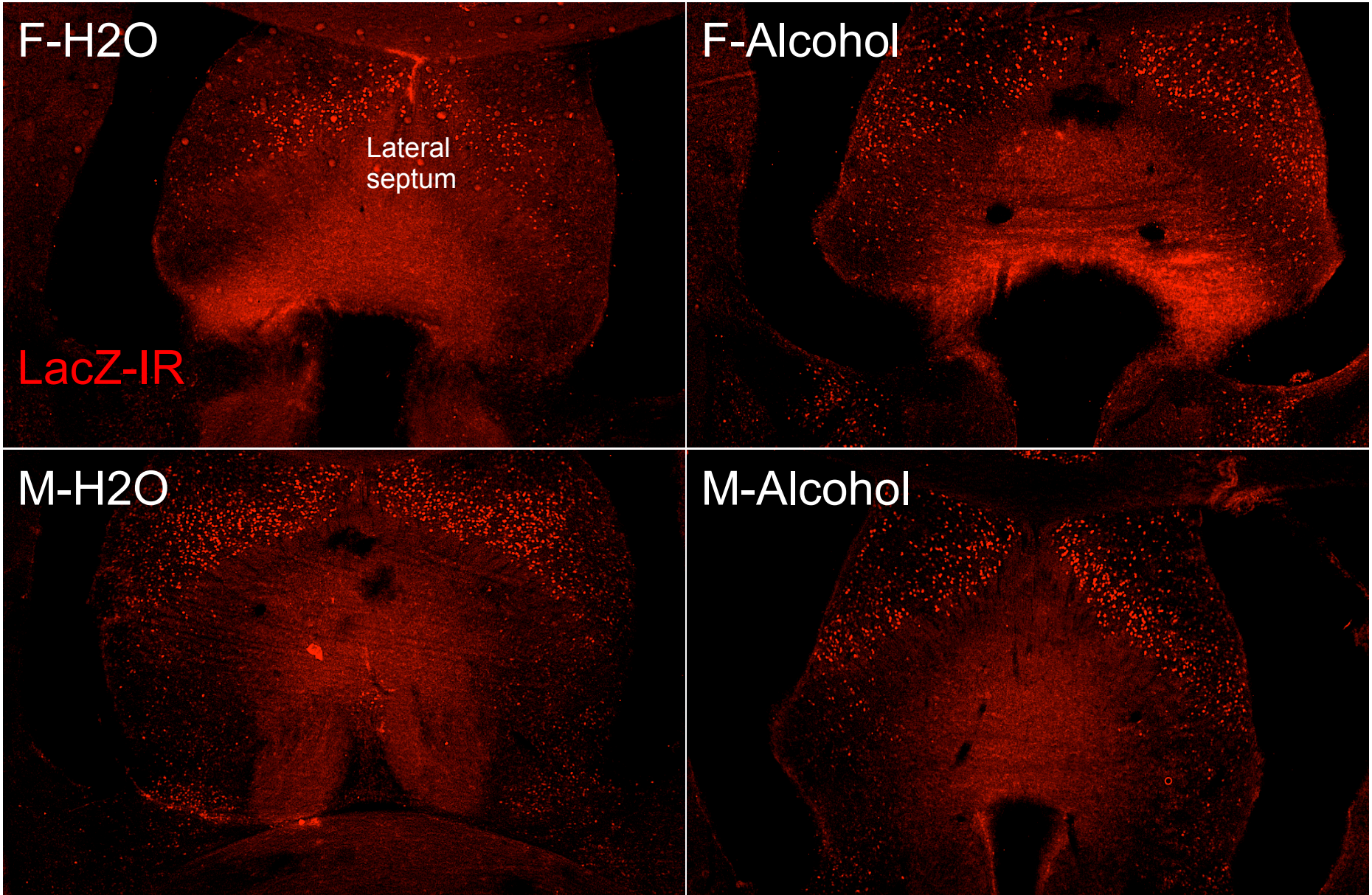
- Do female and male mice differ in voluntary alcohol consumption?
- How does alcohol exposure alter AR expression in the brain?



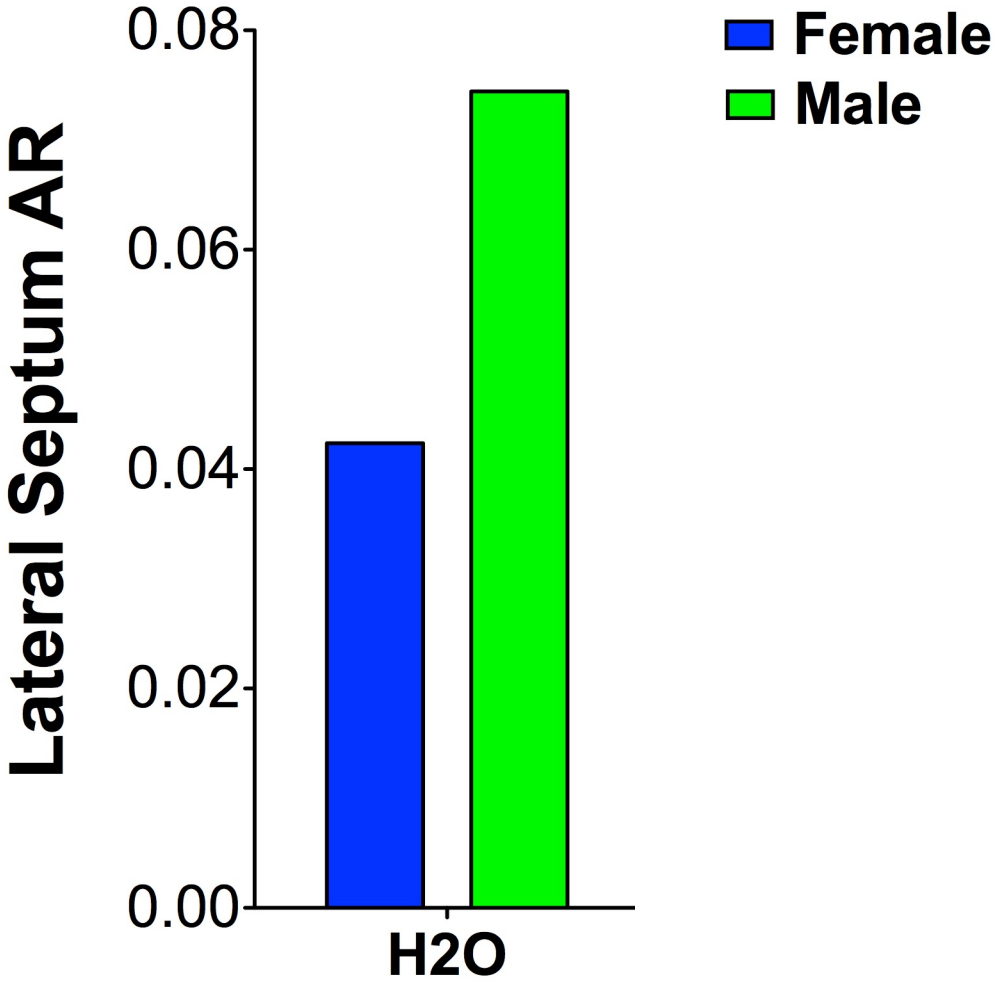




Lateral Septum AR Expression



Lateral Septum AR Expression



Summary and Conclusions

- Sex differences are important factors mediating the psychological and physiological consequences of pathological alcohol consumption

- Differences in androgen sensitivity related to AR signaling and distribution may underlie naturally-occurring variations in alcohol-seeking behavior

- Manipulations of discrete hypothalamic subpopulations have the potential to alter alcohol consumption and/or changes in AR expression throughout the brain

Acknowledgments

Luis de Lecea Laboratory
Department of Psychiatry & Behavioral Sciences

Zijie Sun Laboratory
Department of Urology

NIAAA
National Institute on Alcohol Abuse and Alcoholism

Stanford WSDM Center

STANFORD
SCHOOL OF MEDICINE
Stanford University Medical Center

Systems Biology Approaches to Immune Sexual Dimorphism

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Laboratory of Mark M Davis
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101714

The bad

1. Often observational
   - Identification of mechanisms may be challenging

2. Big data is noisy -> curse of dimensionality

3. Expensive for most labs
   average cost for deep baseline immuno-phenotyping >$1,000

4. Requires substantial infrastructure

The good

1. Lightly invasive >> rapid IRB approval (expd.IRB)

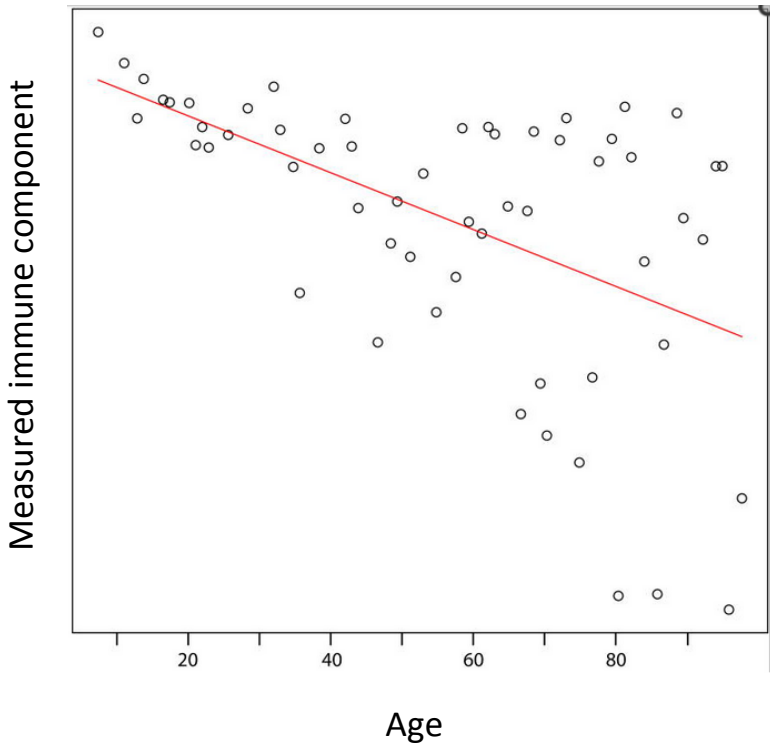
2. Broader understanding (look at more things simultaneously <-- technology)

3. Allows us to observe different immune “structures” or “states” which depend upon the angle of the observer
   - Largely relies on variation

4. Force us to be flexible

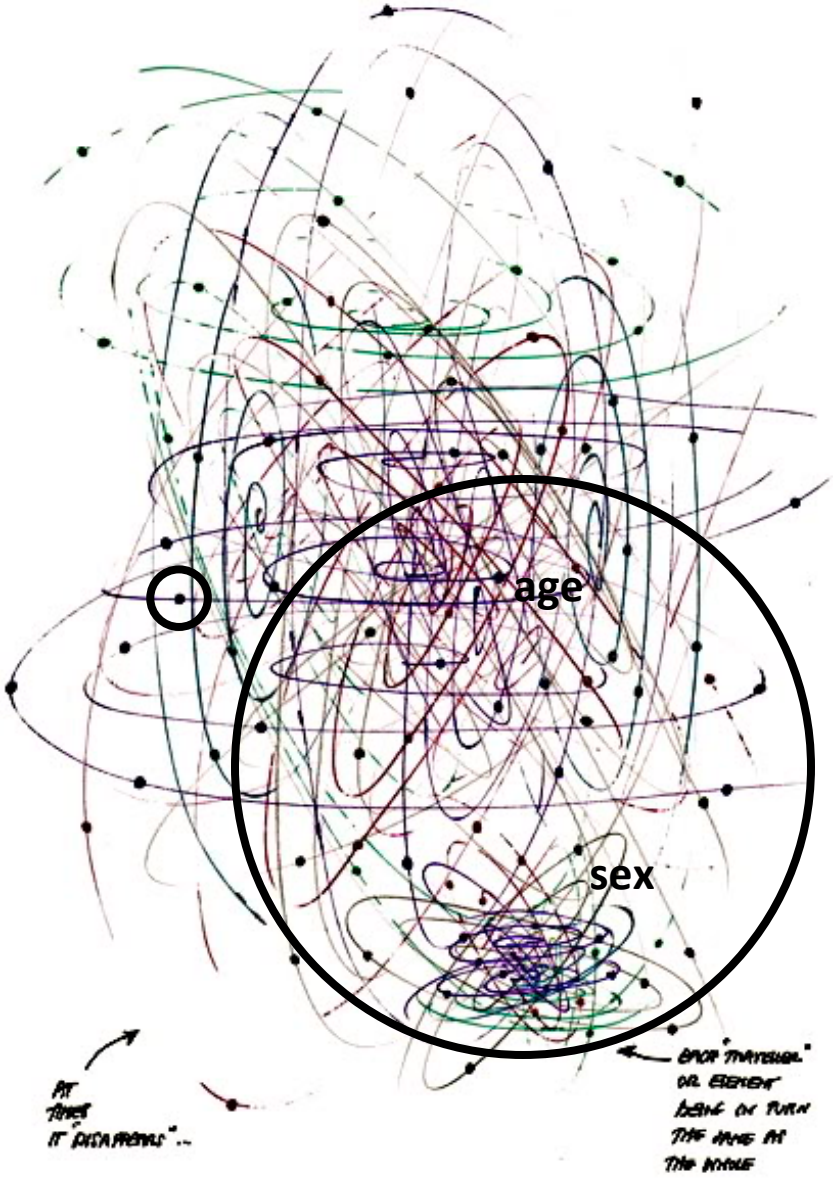
5. Increasing interest

Variance increases with age



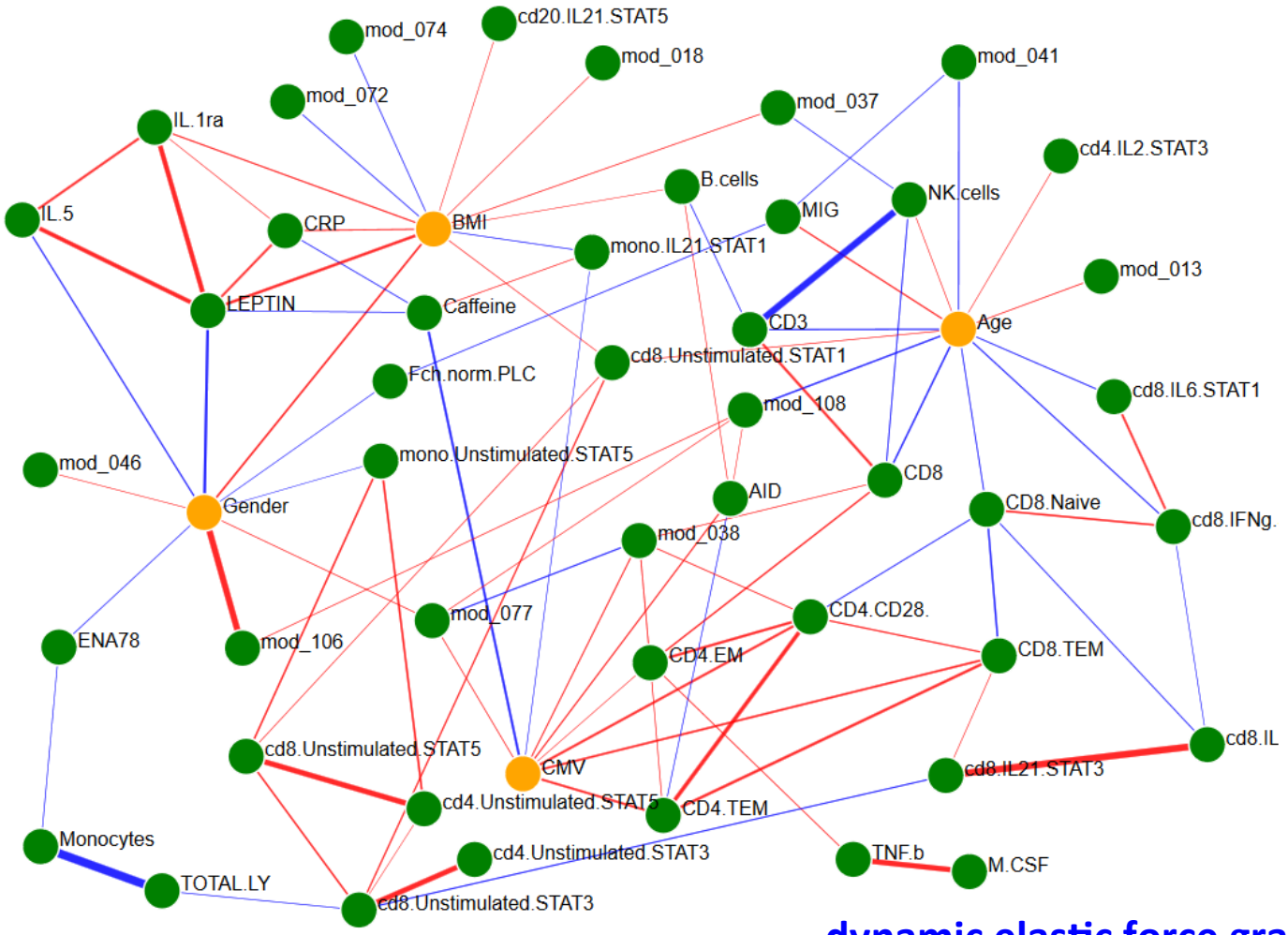
Shen-Orr S. and Furman D., (2013) *Curr Opin Immunol* 25(4): 542–547

N-dimensional immune space



Credits: Catherine Armant

Conditional independence analysis: Partial correlation



[dynamic elastic force graph]

Molecular Systems Biology 9; Article number 659; doi:10.1038/msb.2013.15
Citation: Molecular Systems Biology 9:659
www.molecularsystemsbiology.com

OPEN ACCESS TRANSPARENT PROCESS

Apoptosis and other immune biomarkers predict influenza vaccine responsiveness

David Furman[1,8,9], Vladimir Jojic[2,8,10], Brian Kidd[3], Shai Shen-Orr[4], Jordan Price[1], Justin Jarrell[5], Tiffany Tse[3], Huan Peder Lund[1], Holden T Maecker[3], Paul J Utz[3,5], Cornelia L Dekker[3,6], Daphne Koller[2] and Mark M Davis[1,3,7,*]

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[8] T
[9] F
[10]
* (
Pa

Persistent expression of inflammatory genes links hyperte with arterial stiffness and longevity in older adults

David Furman[1*], Christopher R Bolen[1], François Haddad[2], Vladimir Jojic[3], François Moreau[4], Julie Déchanet-Merville[4], Cornelia L Dekker[5], Mark M I Benjamin Faustin[4*]

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[6]*Howard Hughes Medical Institute, Stanford University School of Medicine, 279 Campus Beckman Center, 94305, Stanford, California, USA*

PNAS

Systems analysis of sex differences reveals an immunosuppressive role for testosterone in the response to influenza vaccination

David Furman[a,1,2,3], Boris P. Hejblum[b,1], Noah Simon[c], Vladimir Jojic[d], Cornelia L. Dekker[e], Rodolphe Thiébaut[b], Robert J. Tibshirani[c,f], and Mark M. Davis[a,g,h,3]

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Improved immune responses to influenza in cytomegalovirus infection

David Furman[1*], Vladimir Jojic[2*], Shalini Sharma[3*], Shai Shen-Orr[4], Cesar J Lopez Angel[1], Suna Onengut-Gumuscu[5], Brian Kidd[6], Holden T Maecker[6], Patrick Concannon[5,7¶], Cornelia L Dekker[8], Paul G Thomas[3] and Mark M Davis[1,6,9]

[1]*Department of Microbiology & Immunology, School of Medicine, Stanford University, Stanford, 94305, USA*
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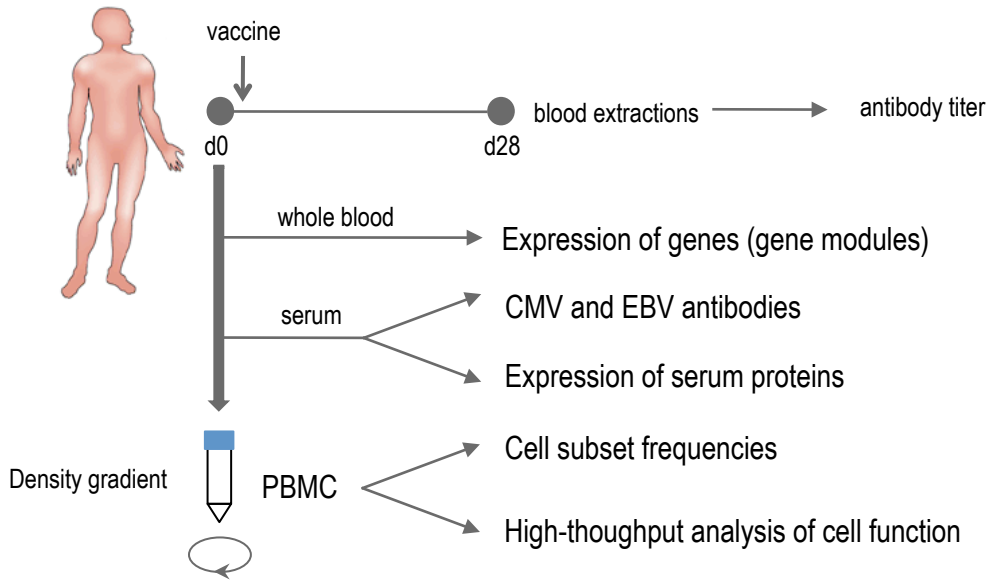
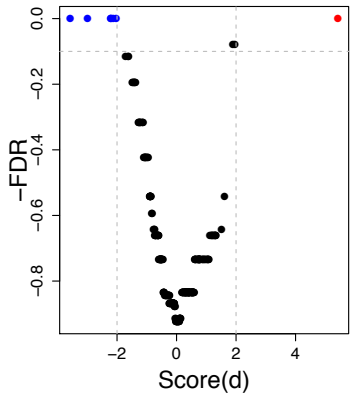
·*Current address: The Icahn School of Medicine at Mount Sinai, New York City, New York, 10457, USA*
[¶]*Current address: Genetics Institute and Department of Pathology, Immunology and Laboratory Medicine, University of Florida, Gainesville, Florida, 32605, USA*

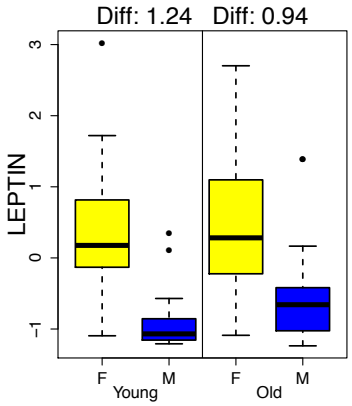
Study design

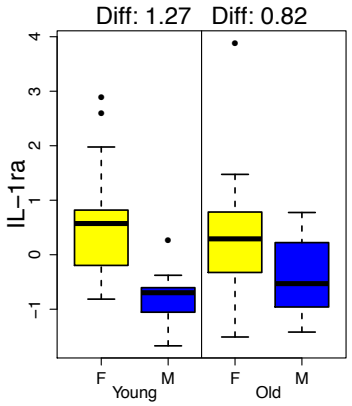
Cohort characteristics (N= 90)
young, older, cmv-, cmv+, females and males

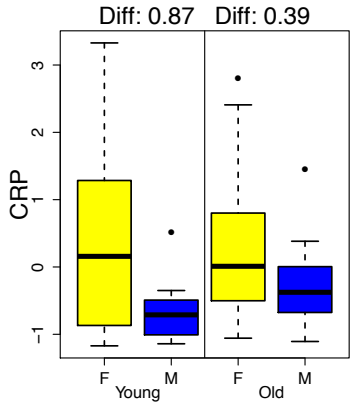


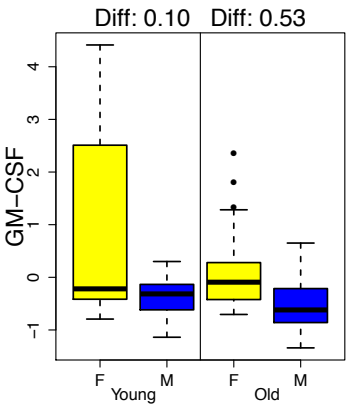
Sex differences in inflammatory markers

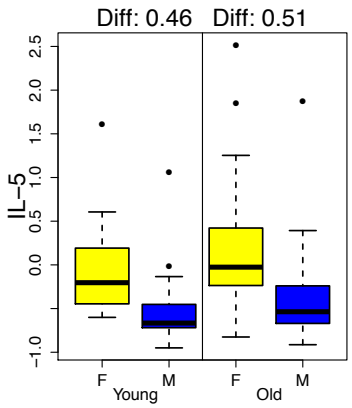


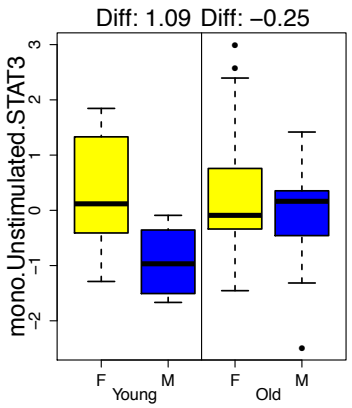


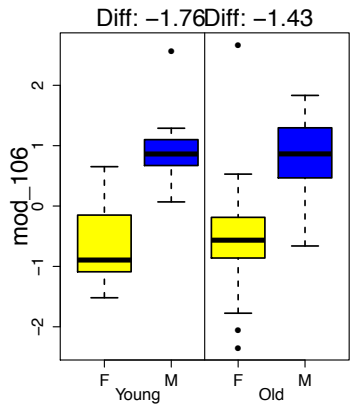








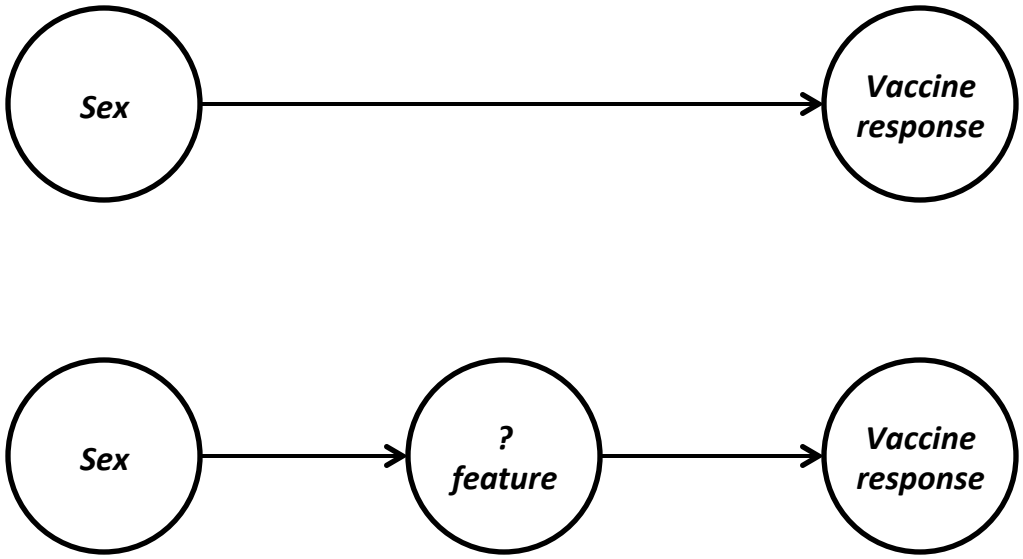




Dimorphism in the antibody neutralizing activity

		Beta	*Std. Error*	*z value*	*P-value*
H1N1	(Intercept)	-0.272	0.229	-1.190	0.234
	Age	-0.690	0.236	-2.919	0.004
	Gender	-0.011	0.234	-0.047	0.962
H3N2	(Intercept)	-0.038	0.228	-0.166	0.868
	Age	-0.190	0.236	-0.804	0.421
	Gender	-0.716	0.239	-2.992	0.003
B	(Intercept)	-0.502	0.236	-2.128	0.033
	Age	-0.583	0.246	-2.367	0.018
	Gender	-0.594	0.256	-2.324	0.020

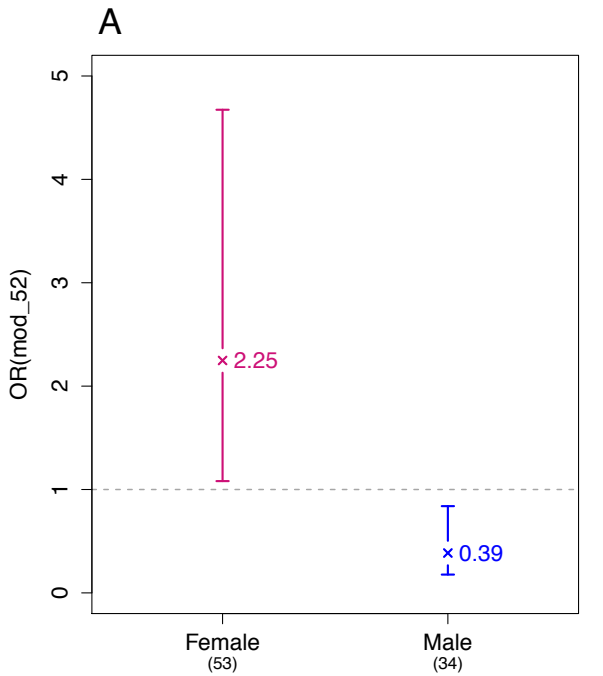
Is there a feature that can explain the differences?



Search for interactions with sex that explain vax response

1. Test for significant interactions (FDR < 0.1)
2. Fit model to estimate OR

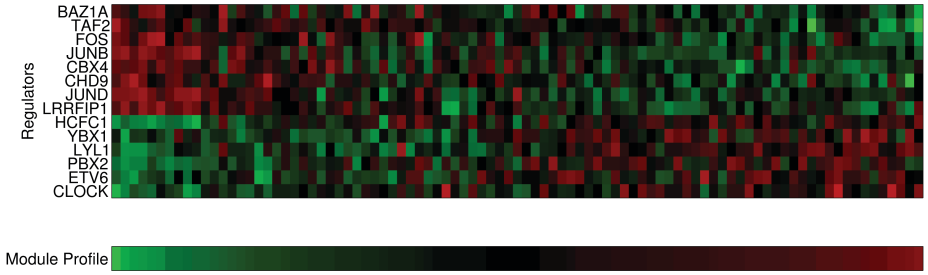
$$\text{logit}(y_i) = \mu + \beta_g \cdot male_i + \beta_c \cdot crp_i + \beta_{m42} \cdot mod\_42_i + \beta_{m52} \cdot mod\_52_i + \boxed{\beta_{g:m52} \cdot male_i : mod\_52_i} + \varepsilon_i$$



Module 52 – immunosuppression*

35 genes
Lipid biosynthesis ($P < 0.0001$)
Interesting genes: LTA4H, PDSS2

RP > BAZ1A, FOS, JUNB (repressors)

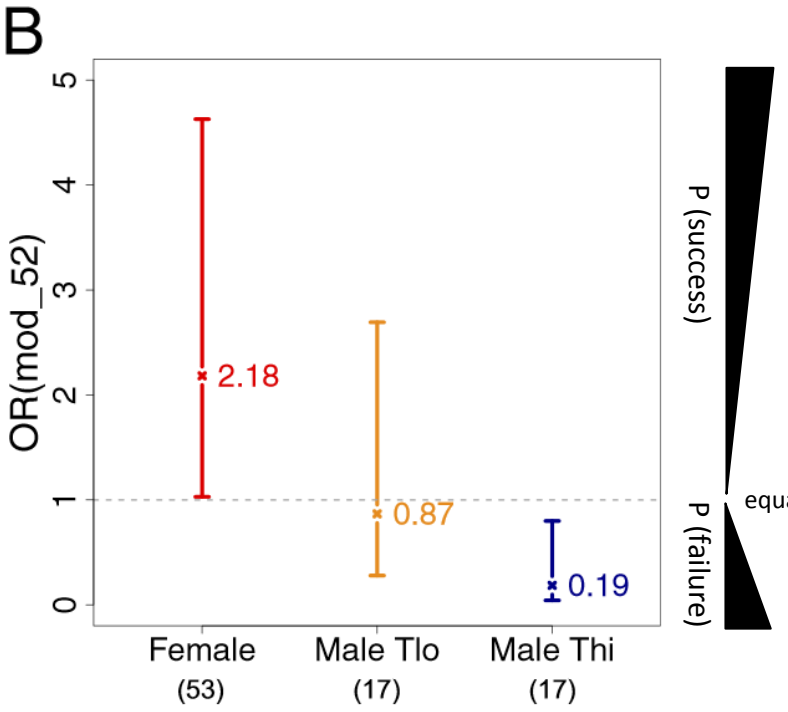


Gene-chemical association for testosterone $P = 0.0003$

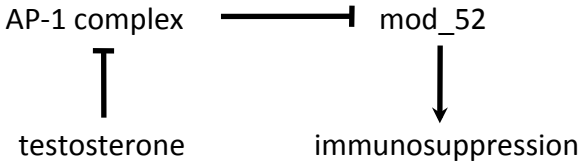
* Yokota Y, *et al.* (2012) *Blood* 120(17):3444-3454.
Juzan M, Hostein I, & Gualde N (1992) *Prostaglandins Leukot Essent Fatty Acids* 46(4):247-255.
Kanneganti TD & Dixit VD (2012) *Nat Immunol* 13(8):707-712.

Simon, N. and Tibshirani, R. (2012) A Permutation Approach to Testing Marginal Interactions in Many Dimensions

High testosterone in males with lowest responses



equal probability of being a good or poor responder



Furman, D. et al. (2014) *PNAS* 111(2):869-74

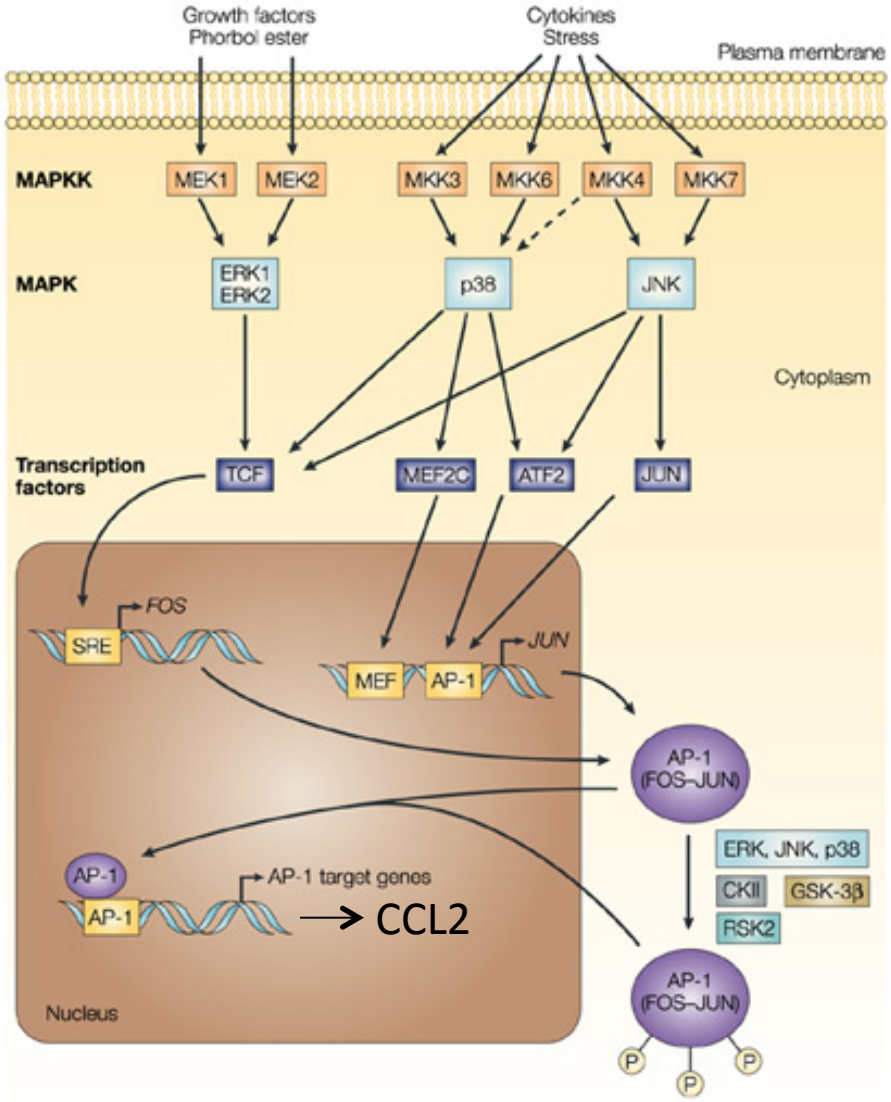
Testosterone-associated gene modules are connected to AP-1 genes

ID	Functional annotation	p-value*	JUN	JUNB	JUND	FOS	FOSB
mod_005	GSE29614_CTRL_VS_TIV_FLU_VACCINE_PBMC_2007_DN	6.80E-04	0	1	0	0	0
mod_007	GSE14000_UNSTIM_VS_4H_LPS_DC_UP	5.40E-03	0	1	0	0	1
mod_008	GSE2706_UNSTIM_VS_2H_LPS_AND_R848_DC_UP	1.50E-03	0	1	0	0	0
mod_037	KEGG_RIBOSOME	2.30E-15	0	0	1	1	0
mod_038	GSE26495_NAIVE_VS_PD1LOW_CD8_TCELL_DN	1.90E-13	0	1	0	0	1
mod_039	REACTOME_TRANSLATION	1.10E-16	0	0	0	0	0
mod_040	GSE29618_BCELL_VS_MDC_UP	4.40E-16	0	0	0	0	0
mod_041	GSE22886_NAIVE_TCELL_VS_MONOCYTE_UP	5.80E-07	0	1	0	1	0
mod_043	GSE22886_UNSTIM_VS_IL15_STIM_NKCELL_DN	4.00E-04	0	1	0	0	1
mod_044	KEGG_LONG_TERM_DEPRESSION	4.90E-04	0	1	0	1	1
mod_045	GSE25087_FETAL_VS_ADULT_TCONV_UP	7.10E-05	0	0	0	1	1
mod_046	GSE9006_HEALTHY_VS_TYPE_2_DIABETES_PBMC_AT_DX_UP	8.10E-04	0	0	0	0	1
mod_047	PDGF_ERK_DN.V1_UP	1.80E-04	0	0	0	0	0
mod_048	GOLDRATH_NAIVE_VS_EFF_CD8_TCELL_UP	2.20E-04	0	1	1	0	1
mod_049	ACTIN_FILAMENT_ORGANIZATION	3.60E-04	0	0	0	0	0
mod_050	GSE29618_PRE_VS_DAY7_FLU_VACCINE_BCELL_UP	1.50E-03	0	1	0	0	0
mod_052	FATTY_ACID_BIOSYNTHETIC_PROCESS	6.00E-04	0	1	1	1	0
mod_055	REGULATION_OF_ACTION_POTENTIAL	2.00E-04	1	1	1	1	0
mod_083	KEGG_ALDOSTERONE_REGULATED_SODIUM_REABSORPTION	2.00E-03	0	0	0	0	0
mod_084	REACTOME_INFLUENZA_VIRAL_RNA_TRANSCRIPTION_AND_REPLICATION	3.90E-13	0	0	1	1	0
mod_086	REACTOME_CHOLESTEROL_BIOSYNTHESIS	8.50E-04	0	0	0	0	0
mod_091	GSE22886_NAIVE_TCELL_VS_MONOCYTE_UP	2.50E-04	0	0	0	0	0
mod_096	KEGG_PRIMARY_BILE_ACID_BIOSYNTHESIS	5.30E-03	0	0	0	0	0
mod_097	GSE17721_POLYIC_VS_CPG_0.5H_BMDM_UP	7.90E-04	0	0	0	0	0
mod_098	MITOCHONDRIAL_MEMBRANE_PART	2.00E-03	0	0	0	0	0
mod_101	CARBOHYDRATE_TRANSPORT	4.60E-04	0	0	1	1	0
mod_108	GSE26495_NAIVE_VS_PD1HIGH_CD8_TCELL_UP	5.20E-04	0	0	0	0	0
		TRM[1]	1	11	6	8	7
		ARM[2]	2	17	13	12	14
		p-value**	0.37	0.0001	0.048	0.0012	0.021

TRM = number of gene modules likely regulated by each AP-1 gene in testosterone-associated gene modules; ARM = number of gene modules likely regulated by each AP-1 gene in all gene modules. 1/0 = present/absent in module regulatory program.

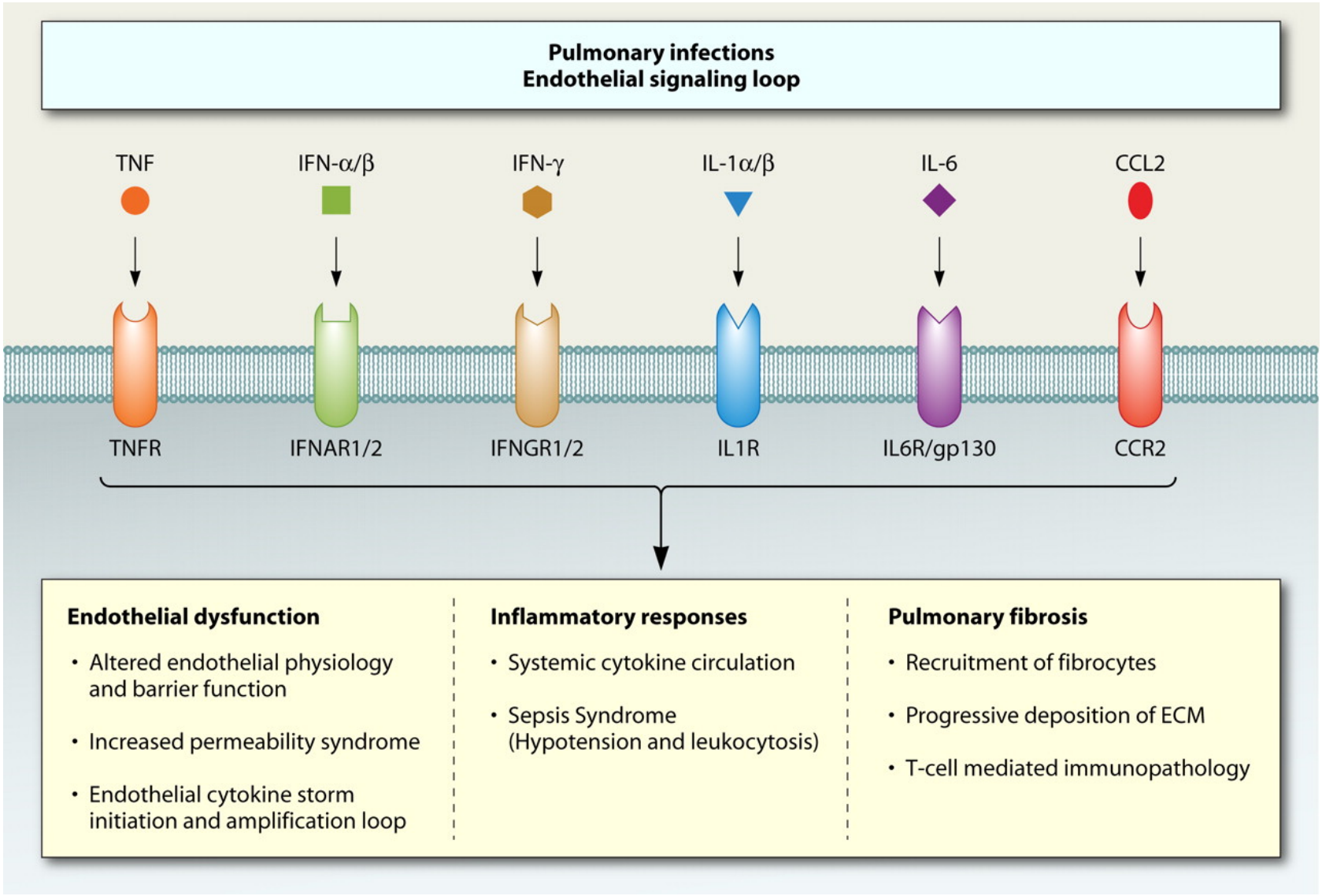
Furman, D. (2014) *Exp Rev Vaccines* Oct 2:1-11

AP-1 is induced by and activates transcription of cytokines



Nature Reviews | Cancer

CCL2 is involved in influenza-induced cytokine storm



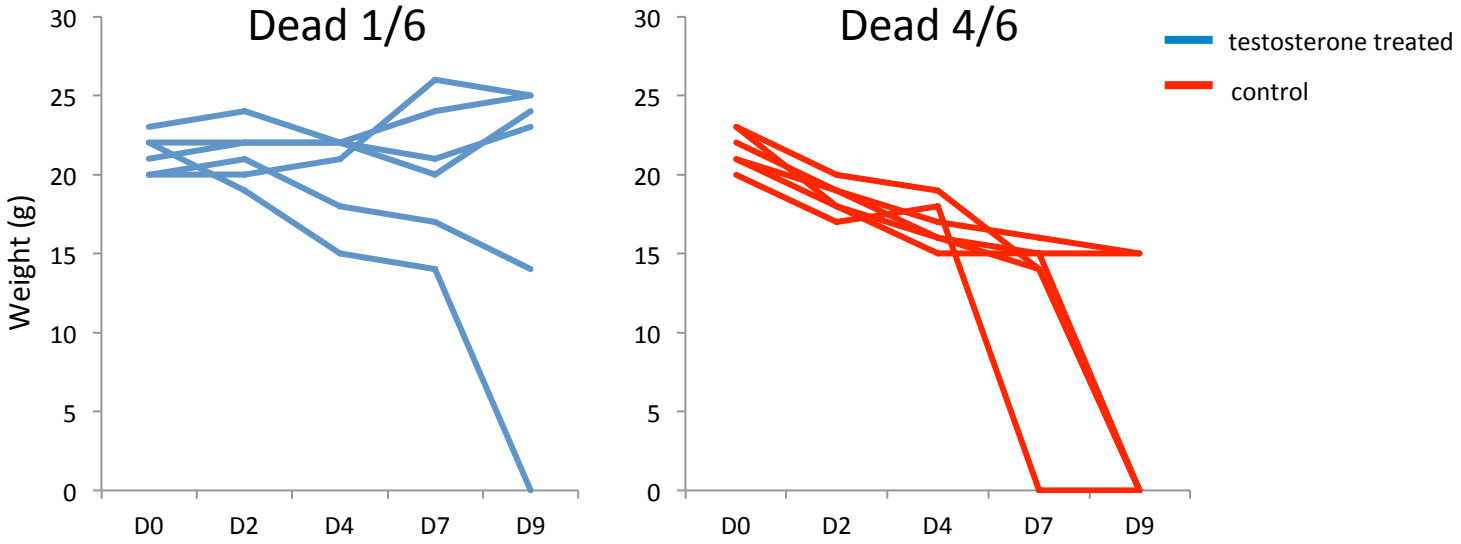
Tisoncik J R et al. (2012) *Microbiol Mol Biol Rev* 76:16-32

i.p. testosterone administration may prevent dead in infected mice

Pilot study

Male mice treated i.p. with T daily

Mouse-adapted influenza infection



P = 0.24

Next : increase samples size!

Conclusions

- Testosterone is important in the regulation of the immune response to vaccination
  - AP-1 could be implicated in the mechanism

- Influenza-induced cytokine storm may be controlled by testosterone >> through AP-1 and CCL2?

Acknowledgements

Mark M Davis
Helen McGuire
Erick Schieda

HIMC
Holden Maecker
Patty Lovelace
Yael Rosenberg-Hasson
Xuhuai Ji

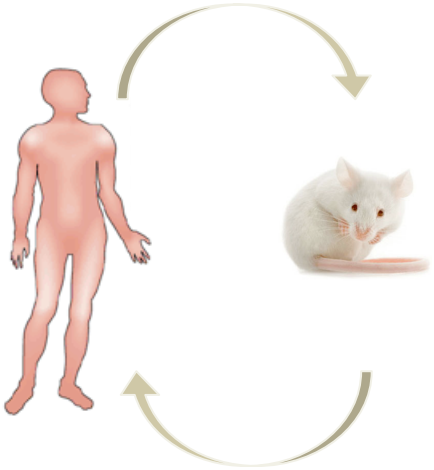
Clinical core
Corry Dekker
Sally Mackey

Daphne Koller
Vladimir Jojic

The Ellison Medical Foundation

SCL Fellowship

HIPC - U19 Grant



Sexual Dimorphism in Melanoma Outcomes

John B. Sunwoo, M.D.
Susan M. Swetter, M.D.

Is Melanoma More Malignant in Men?

- Concept that melanoma behaves "somewhat less malignant" in females proposed by Dr. Wallace Clark in 1969
- Is it differences in behavior, detection, diagnostic delays, and screening?
- Or is there a biologic gender difference?
  - Munich and Dutch cancer registries
  - Females have a consistent 30% advantage in OS, disease-specific survival, time to LN and distant mets compared to men
  - True regardless of thickness, subtype, anatomic site

Joosse A. et al. *J Clin Oncol* 2012 and *J Invest Dermatol* 2011

Melanoma Survival Among Adolescent And Young Adult Caucasian Males

(Gamba C, Swetter S, Tao L, Keegan, T, Clarke C)

- Several studies have shown that male sex is a predictor of poor survival
  - Few analyses stratified by age and none adjusted for covariates (body site, thickness, LN/distant mets, subtype, etc.)
- CPIC/Stanford collaboration:
  - SEER 1989-2009 (to allow for >10 year f/u – mean 7.5 years), non-Hispanic whites
  - Assessed melanoma-spec mortality, 15-39 y
    - 26,107 invasive melanomas, 1561 deaths
    - Males accounted for 40% of overall melanoma cases, but comprised 64% of melanoma-specific deaths

Melanoma Survival Disadvantage in AYA Males Compared with Females

SEER 1989-2009

Non-hispanic Whites aged 15-39 years

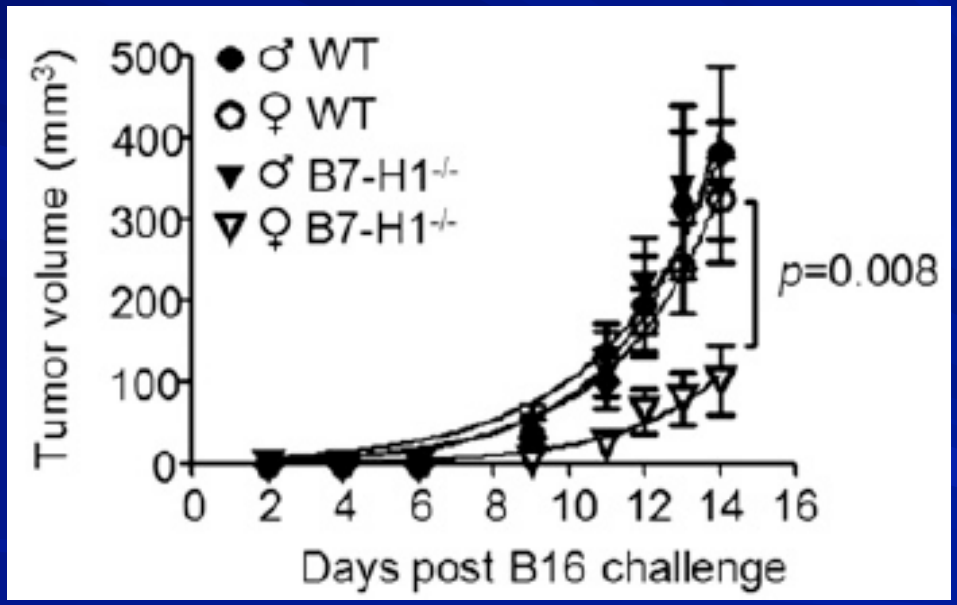
Risk of Death at up to 20 years F/U

Gender	No. of deaths	Fully adjusted HR (95% CI)
Female	331	1.00 (reference)
Male	518	**1.55 (1.39-1.73)**

**Adjusted for age at diagnosis, other primary cancer, body site, tumor thickness, regional lymph nodes, distant metastasis, histologic subtype*

Gamba CS, et al. *JAMA Dermatol*. 2013;149:912-20.

Potential Immune Mechanism Underlying Sexual Dimorphism in Melanoma Control in Mice



Lin et al. *J. Immunology* 2010

Immune Studies Planned

- Assess differences in immune cell subset profiles and function between male and female melanoma patients
  - Human Immune Monitoring Core (Holden Maecker)
- Determine epigentic signatures of tumor-specific cytotoxic T lymphocytes and Tregs between male and female melanoma patients
  - ATAC-Seq (Michelle Longmire and Howard Chang)

Acknowledgments

- Susan Swetter (co-PI)
- Robert Haile
- Theresa Keegan (CPIC)
- Christina Clarke (CPIC)
- Holden Maecker
- Serena Chang
- Howard Chang
- Michelle Longmire
- Li Tao
- Holbrook Kohrt
- Mark Davis
- CCTO
  - Amanda Rajapaksa
  - Risa Jiron
- Funding:
  - Stanford Translational Developmental Cancer Research Award 2014
  - NCI/NIH

Conclusions

- Adolescent and young adult Caucasian males with invasive cutaneous melanoma were 55% more likely to die than age-matched females
- Additionally, when comparing tumor characteristics, males had significantly poorer survival according to:
  - Age at diagnosis: across all age ranges
    - (15-24, 25-29, 30-24, and 35-39 years)
  - Anatomic location:
    - 67% more likely to die of lower extremity melanoma
  - Thickness: ≤1.0 mm or 2.01- 4.0 mm, >4 mm
    - Twice as likely to die with the thinnest T1 melanoma!
  - Histologic subtype: superficial spreading or nodular
  - Presence and extent of metastasis
    - Cutaneous only: 52% more likely to die
    - Regional nodal disease: 74% more likely to die

Sex and Gender Studies in Microbiology & Immunology

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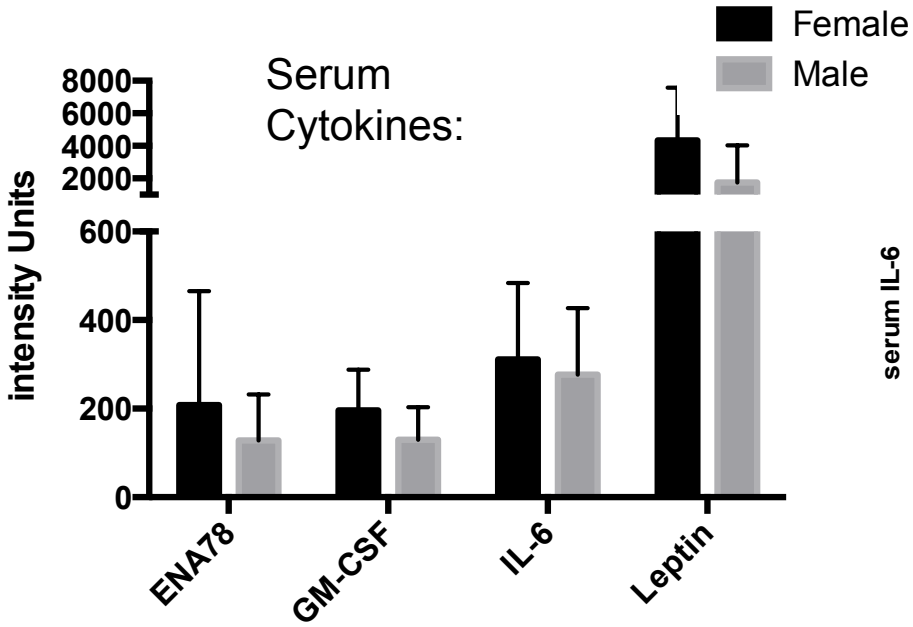
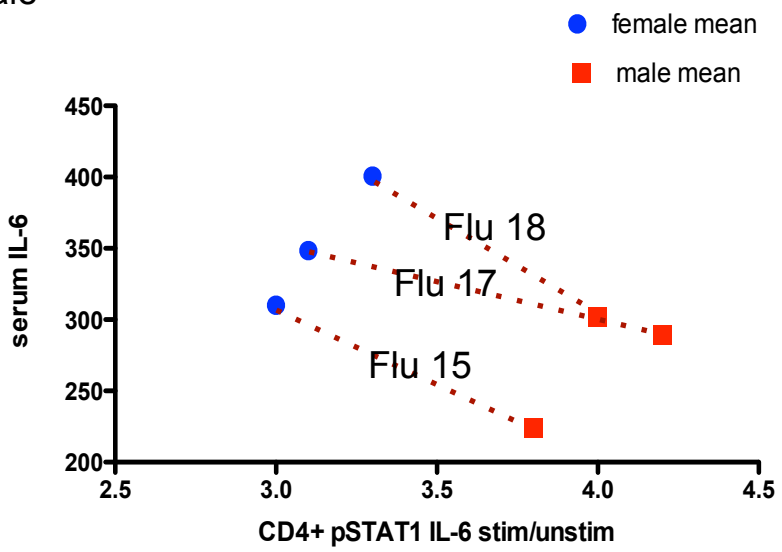
Institute for Immunity, Transplantation and Infection

- Historically, immunologists try NOT to study sex differences (i.e., use all female mice)
- Human immune studies allow us to study sex effects at a system level





Serum cytokine outlier patterns in aging study (Garry Fathman)

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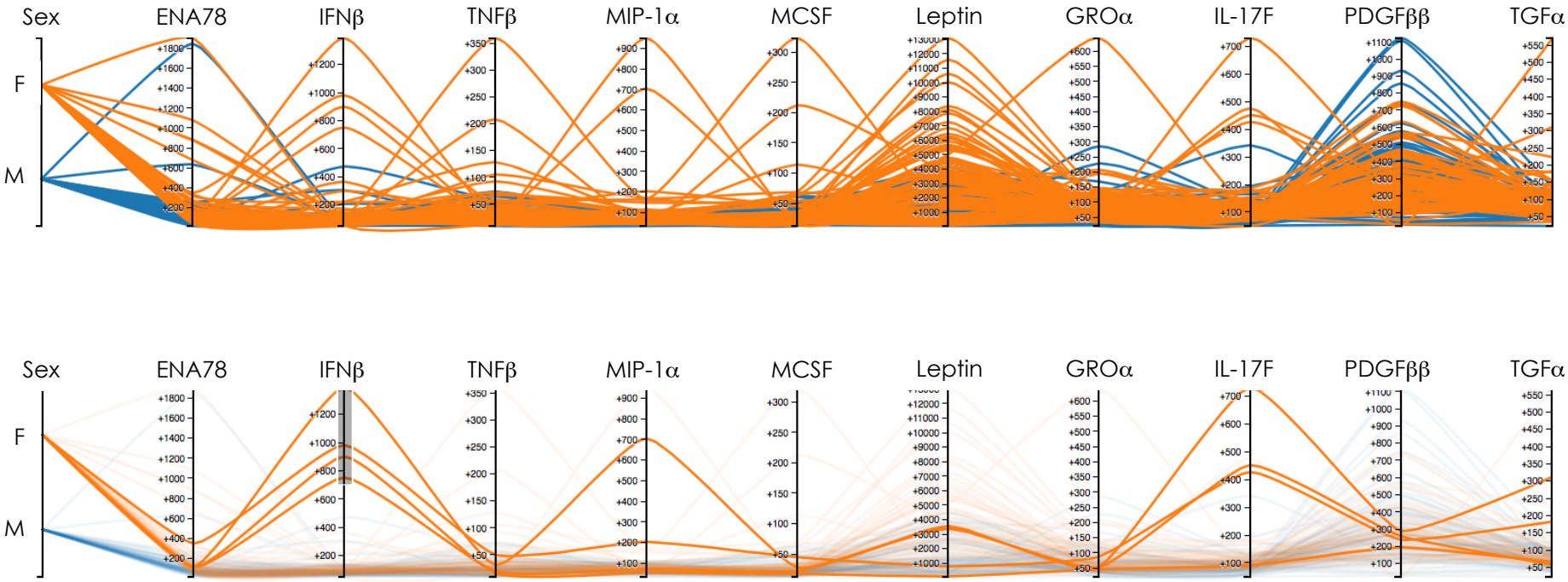
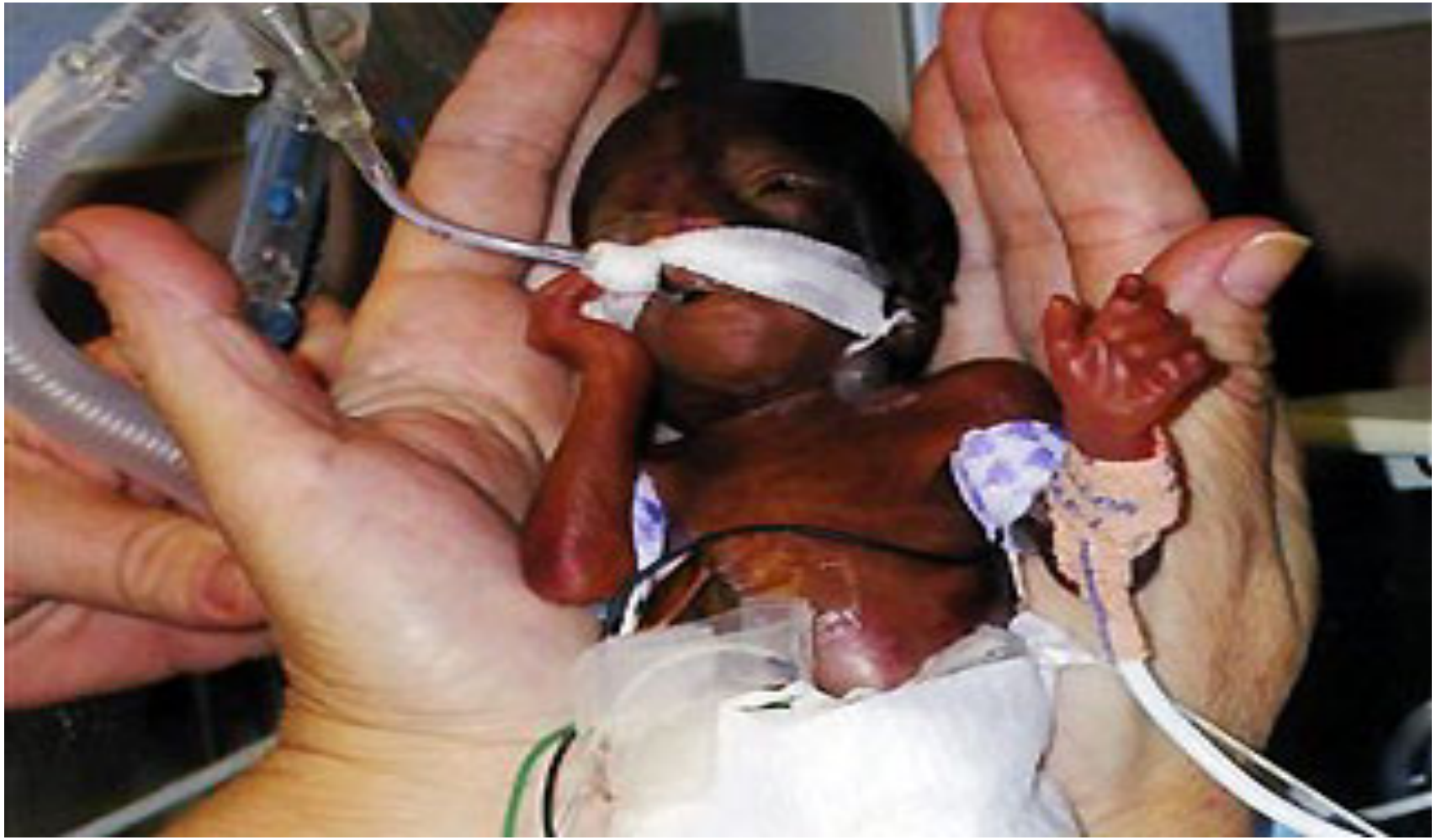




Why preterm birth?

- 1 in 8 babies born early in US
- leading cause of newborn death in US
- Substantial morbidity
  - respiratory problems;
  - underdeveloped organs;
  - cerebral palsy;
  - life-threatening infections; and
  - developmental disabilities later in life
- costs US more than $26 billion annually
- nearly 30 percent higher than it was in early 1980s

march of dimes®
prematurity research center
at STANFORD University School of Medicine

DAVID STEVENSON - PI

GARY SHAW
co PI

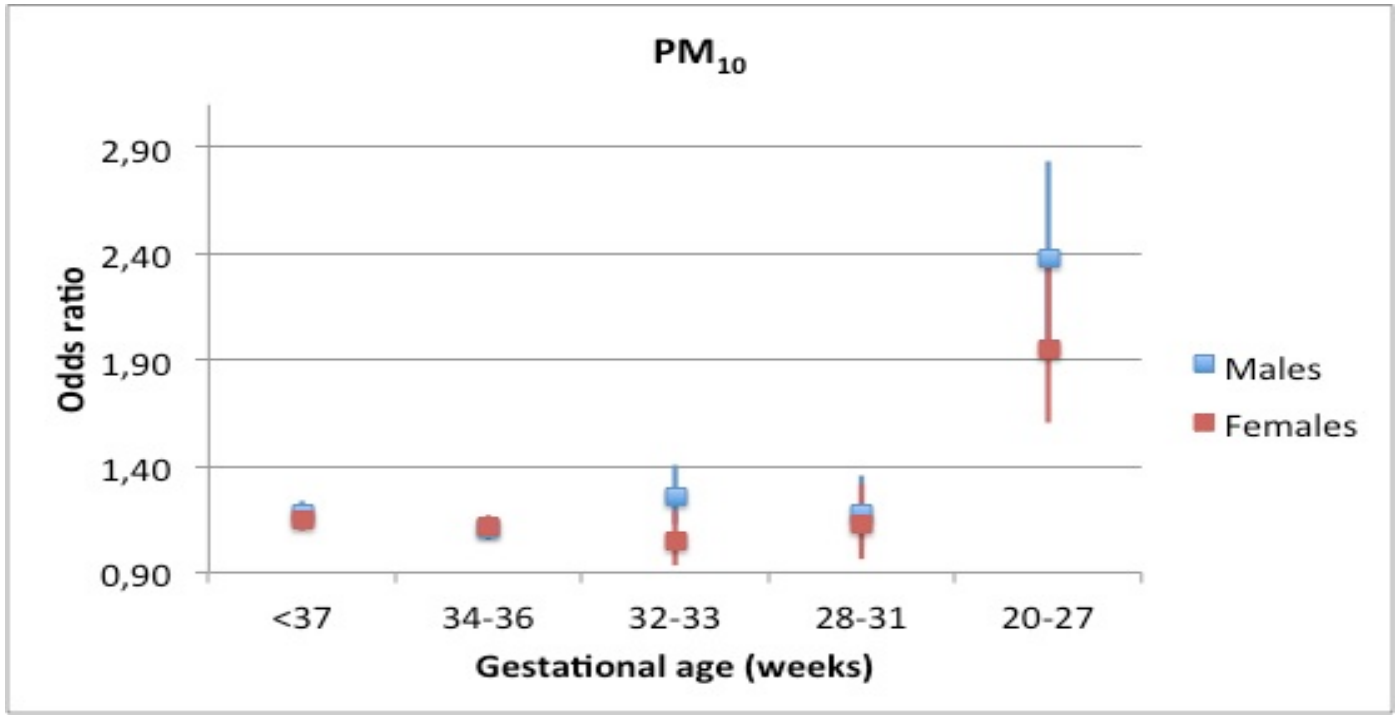
MAURICE DRUZIN
co PI

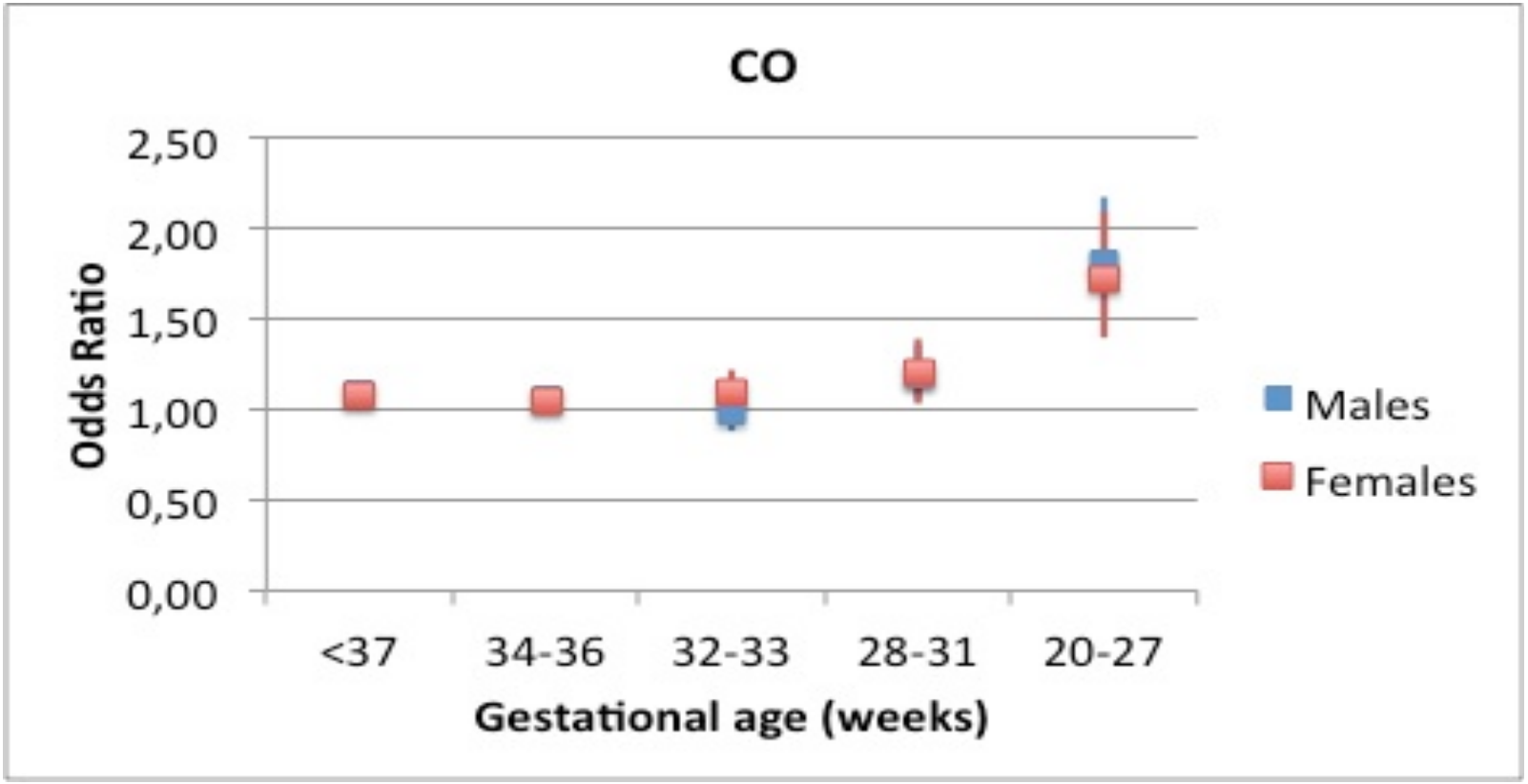
PAUL WISE
co PI

TRANSDISCIPLINARY:
>100 INVESTIGATORS

SCIENTIFIC AREAS
- NEONATOLOGY
- MATERNAL – FETAL MEDICINE
- PULMONOLOGY
- BIOLOGY
- MICROBIOLOGY
- IMMUNOLOGY
- GENETICS
- EPIDEMIOLOGY
- MEDICAL INFORMATICS
- SYSTEMS ENGINEERING & ROBOTICS
- BIOENGINEERING AND STATISTICS
- HEALTH POLICY
- ECONOMICS
- EDUCATION
- ETHICS
- SOCIOLOGY


Figure 2.
1,35
1,30
1,25
1,20
1,15
1,10
1,05
1,00
Sex ratio
37-42
<37
34-36
32-33
28-31
20-27
Gestational age (weeks)

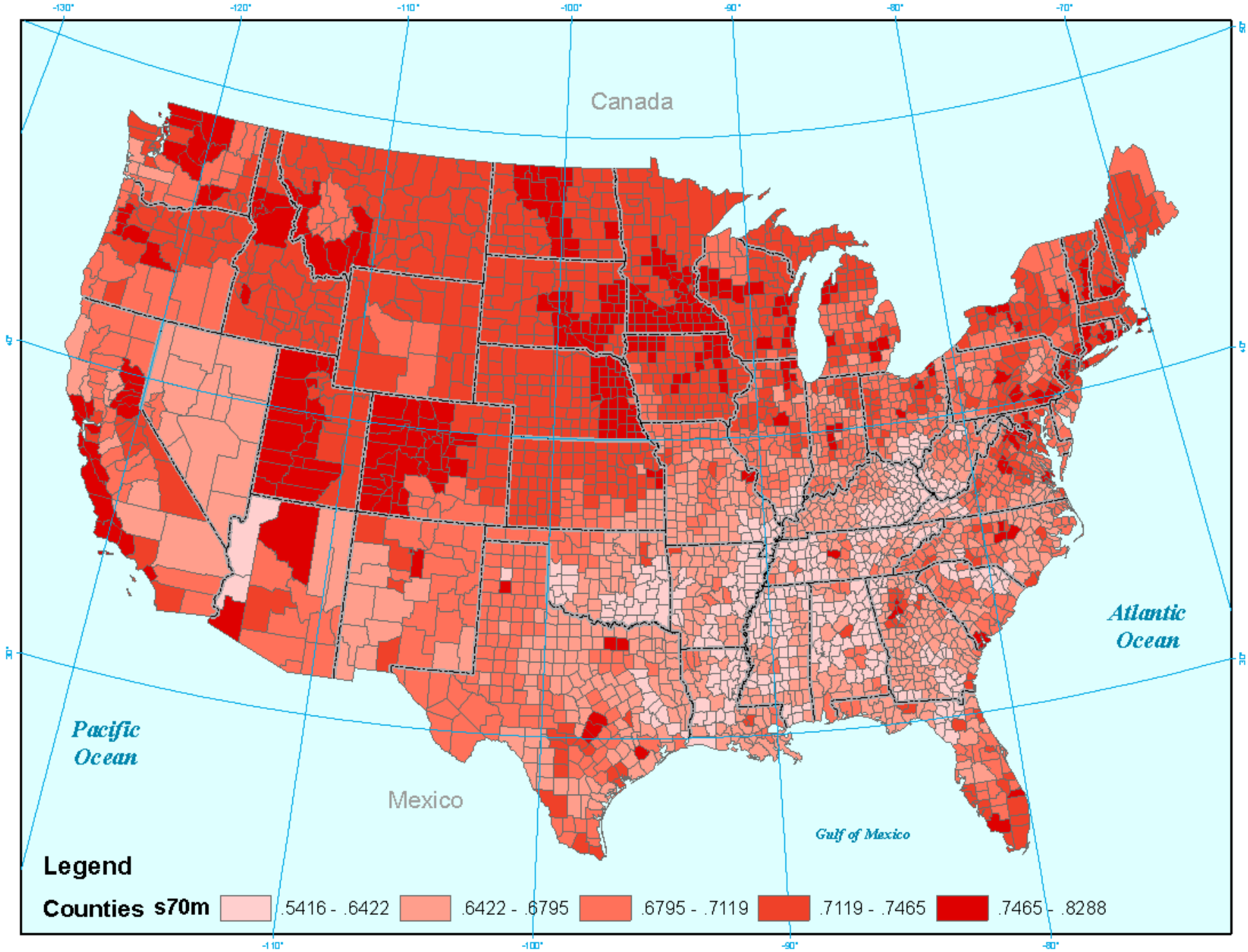

PM10
2,90
2,40
1,90
1,40
0,90
Odds ratio
<37
34-36
32-33
28-31
20-27
Gestational age (weeks)
Males
Females


CO
2,50
2,00
1,50
1,00
0,50
0,00
Odds Ratio
<37
34-36
32-33
28-31
20-27
Gestational age (weeks)
Males
Females

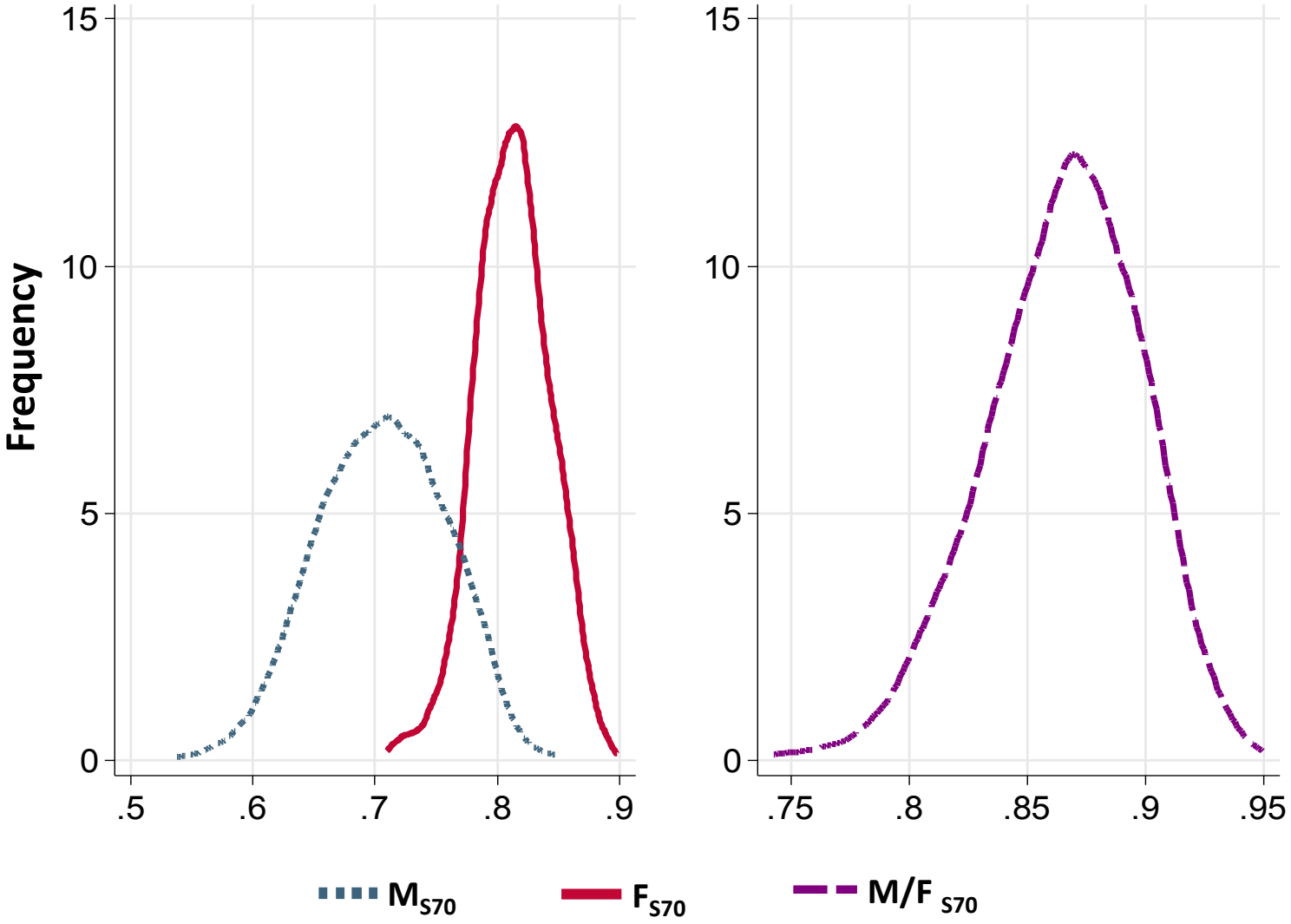
Mechanisms?

- Increase M:F, based on intrauterine inflammation/infection response
- Decrease M:F, based on environmental stressors

Figure 2 County Map of S70, White Males, 2000

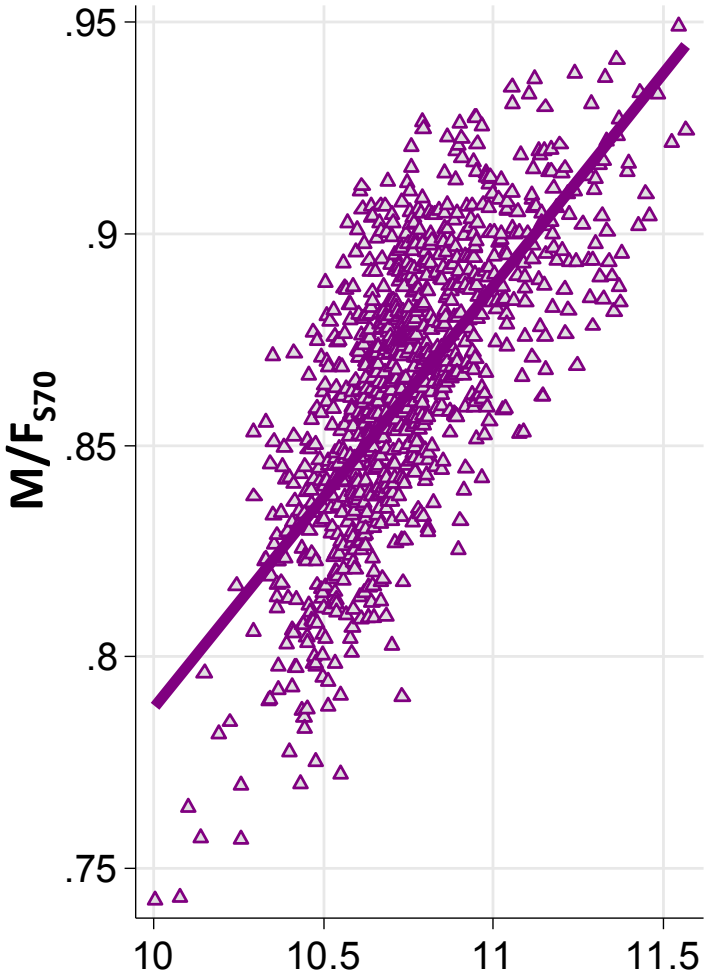


Frequency Distribution of S70 for US PUMACS for Whites, Males and Females, and M/F_{S70}



Male, Female S70 vs Log Household Income, US PUMACS, 2010

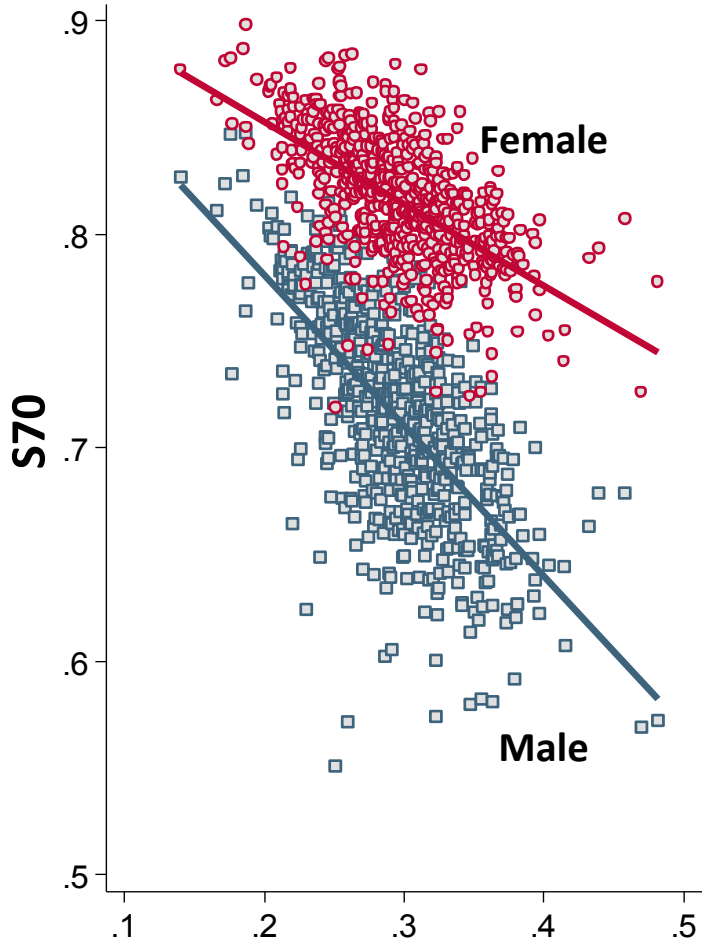
M/F_{S70} vs Log Household Income, US PUMACS, 2010



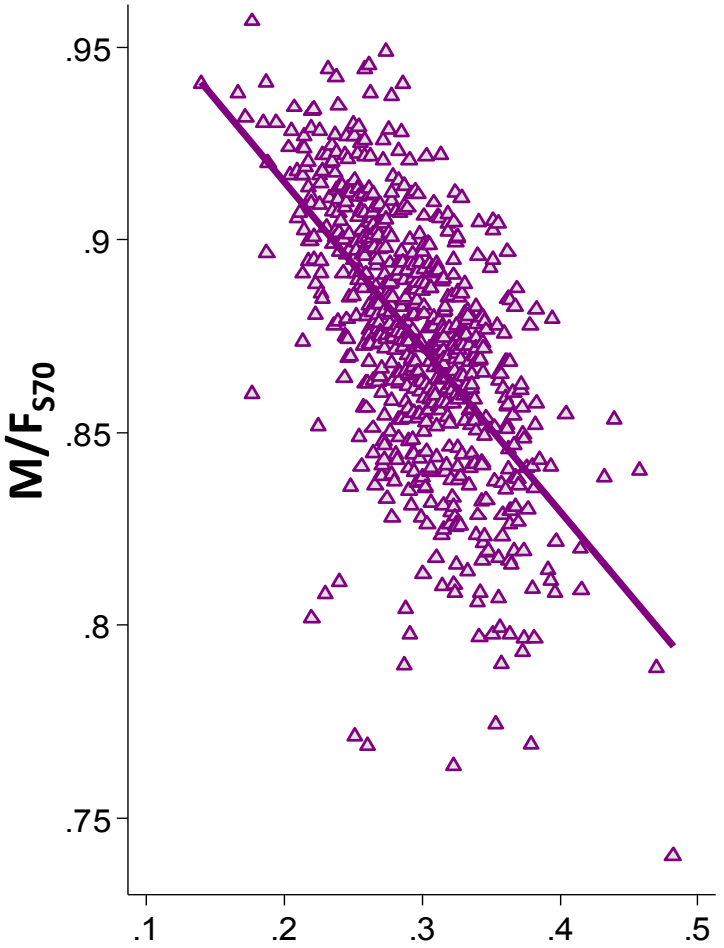
Log Income per Household

Data Unit: 957 PUMACS from 2010 Census, all Whites

Male, Female S_{70} vs Occupational Dissimilarity Index, US PUMACS, 2010



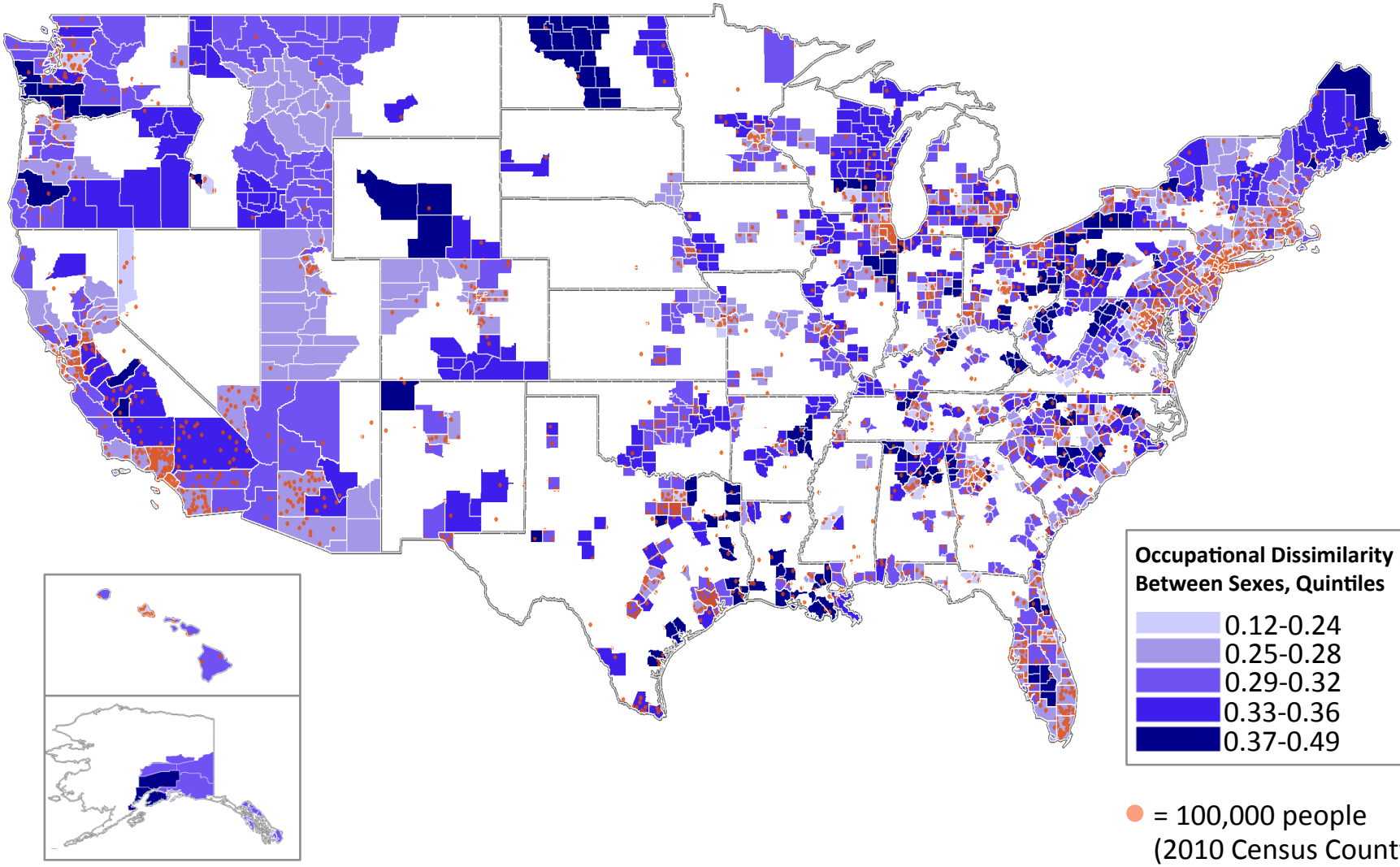
M/FS70 vs Occupational Dissimilarity Index, US PUMACS, 2010



Occupational Dissimilarity Index

Var	Coef.	Std Err	t	p-value	ρ
M	-0.712	0.029	-23.55	<0.001	-0.72
F	-0.382	0.030	-18.05	<0.001	-0.67
M/F	-0.428	0.023	-18.83	<0.001	-0.68

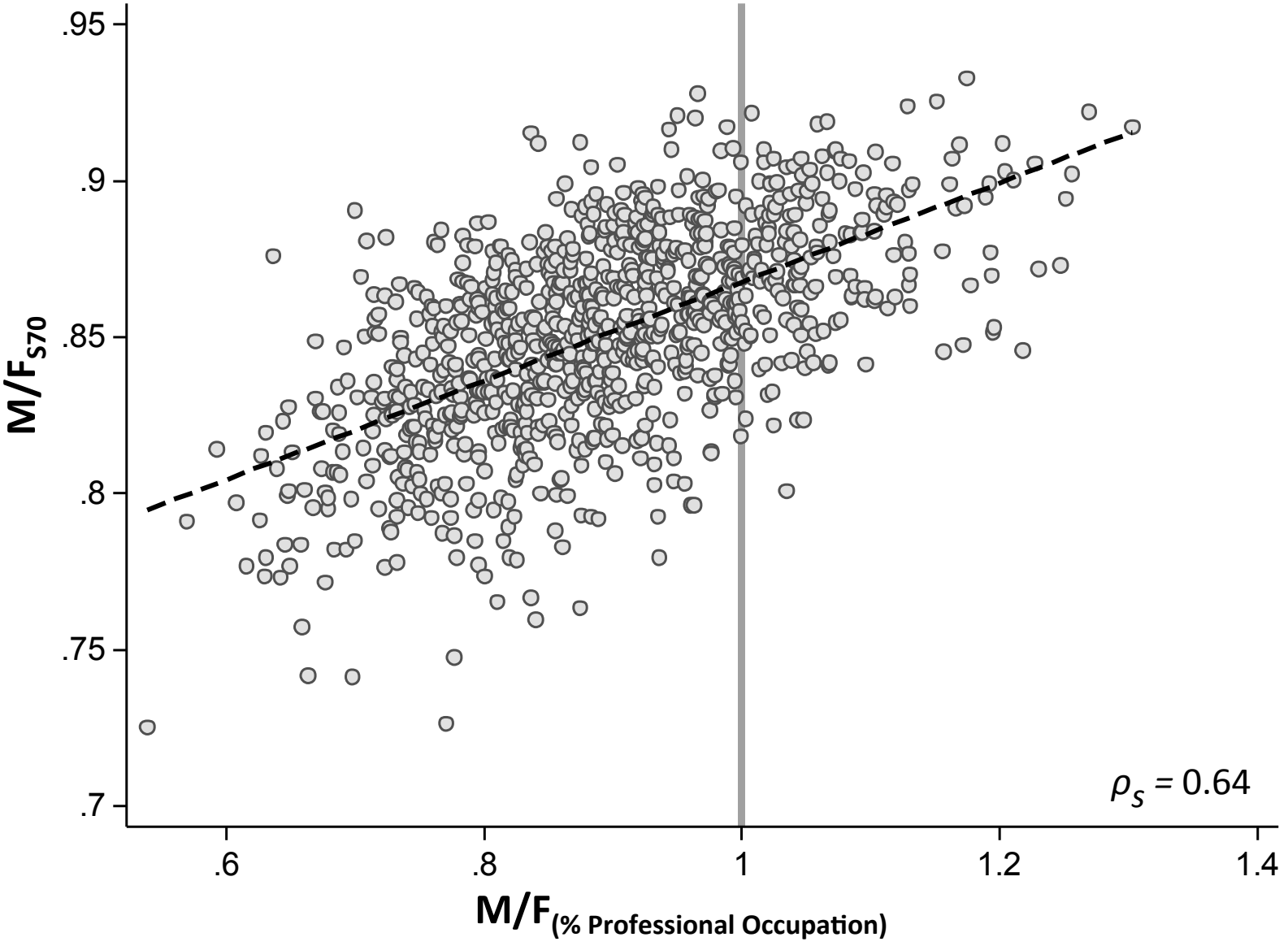
Occupational Dissimilarity Index, by US County, 2010



*2010 ACS, 5-Year Estimates, Occupations by Sex, for non-Hispanic Whites, ages 24-64

*Census does not provide occupation data for counties with low populations (<20,000 applicable residents)

M/F_{S70} vs $M/F_{\%\text{ in Professional Occupations}}$



→ *Relatively more men in professional jobs*

Census, 2000

M/F_{S70} vs SES Metrics, OLS Model

SES - M/F_{S70} Gradients, N=892		ρ_s
Occupational Dissimilarity Index	-0.190*** (0.011)	-0.680
% HS Grad	0.081*** (0.014)	0.614
Log Average Income	0.079*** (0.006)	0.775
% Poverty	-0.067*** (0.000)	-0.760
% Obese	0.033** (0.000)	0.643
% Smokers, Male	-0.002 (0.004)	-0.300
% Smokers, Female	0.001 (0.004)	0.310

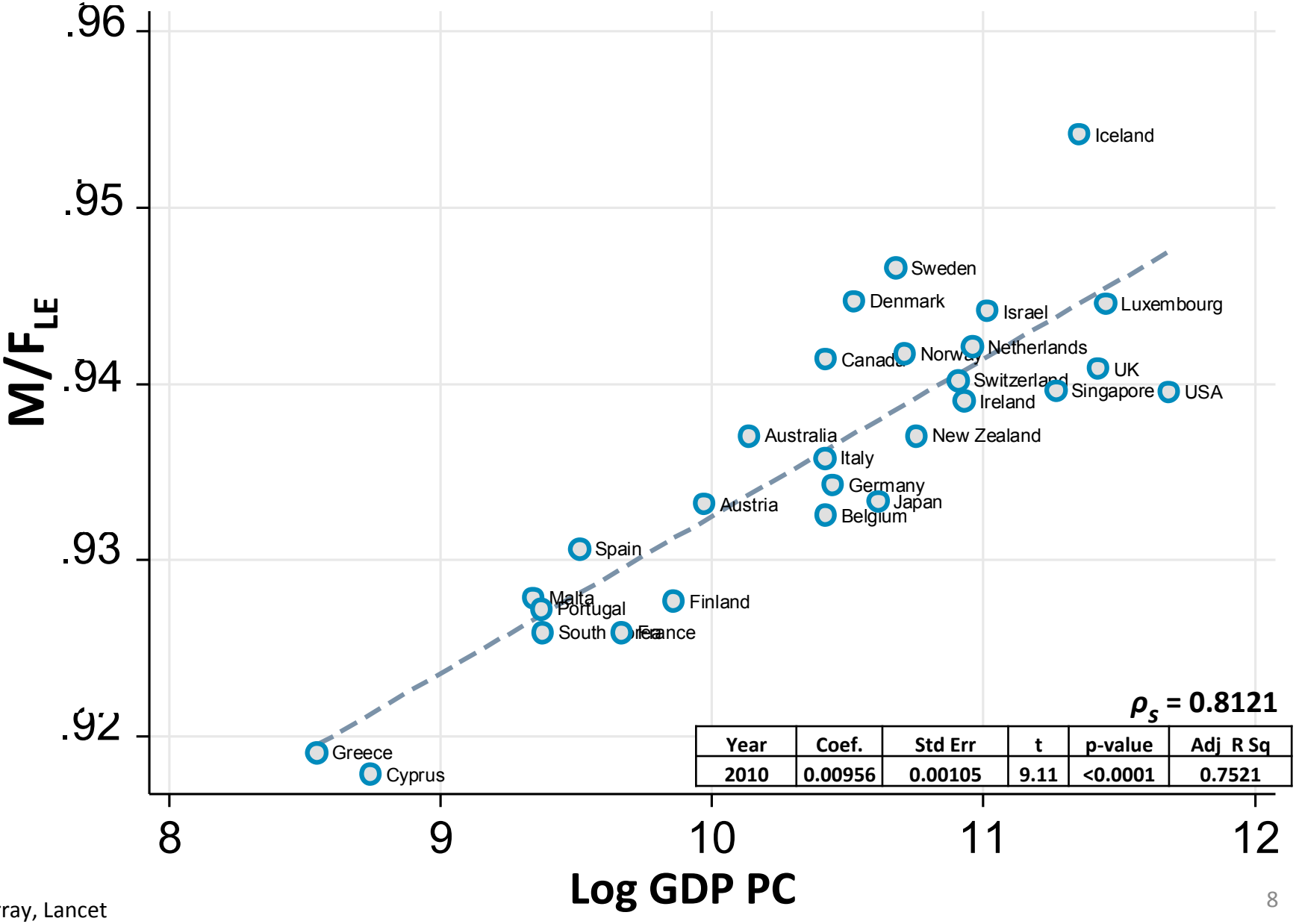
(†p ‹ .10; *p ‹ .05; **p ‹ .01; ***p ‹ .001)

Standard Errors are shown in parentheses

2010 census; HS grad data for ages 35-55

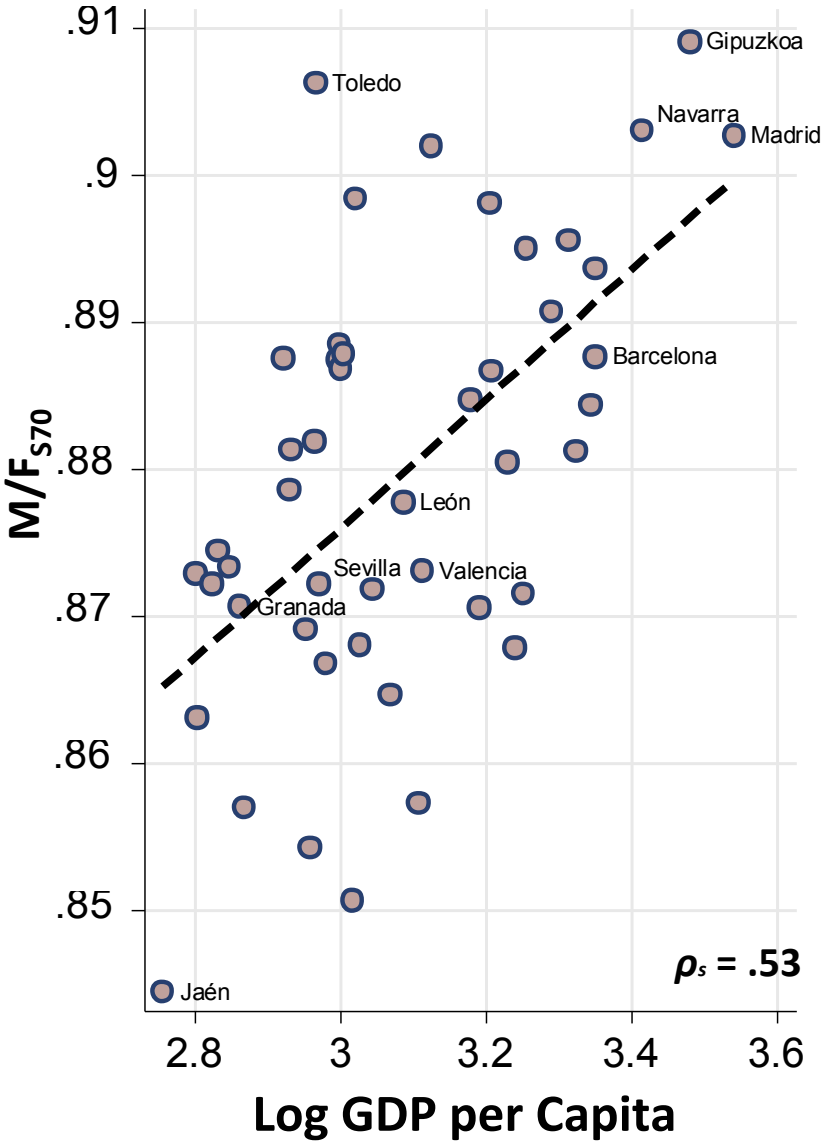
ρ_s denotes Spearman's Correlation Coefficient

M/F_{LE} v. log per cap GDP for Group 1 countries, 2010

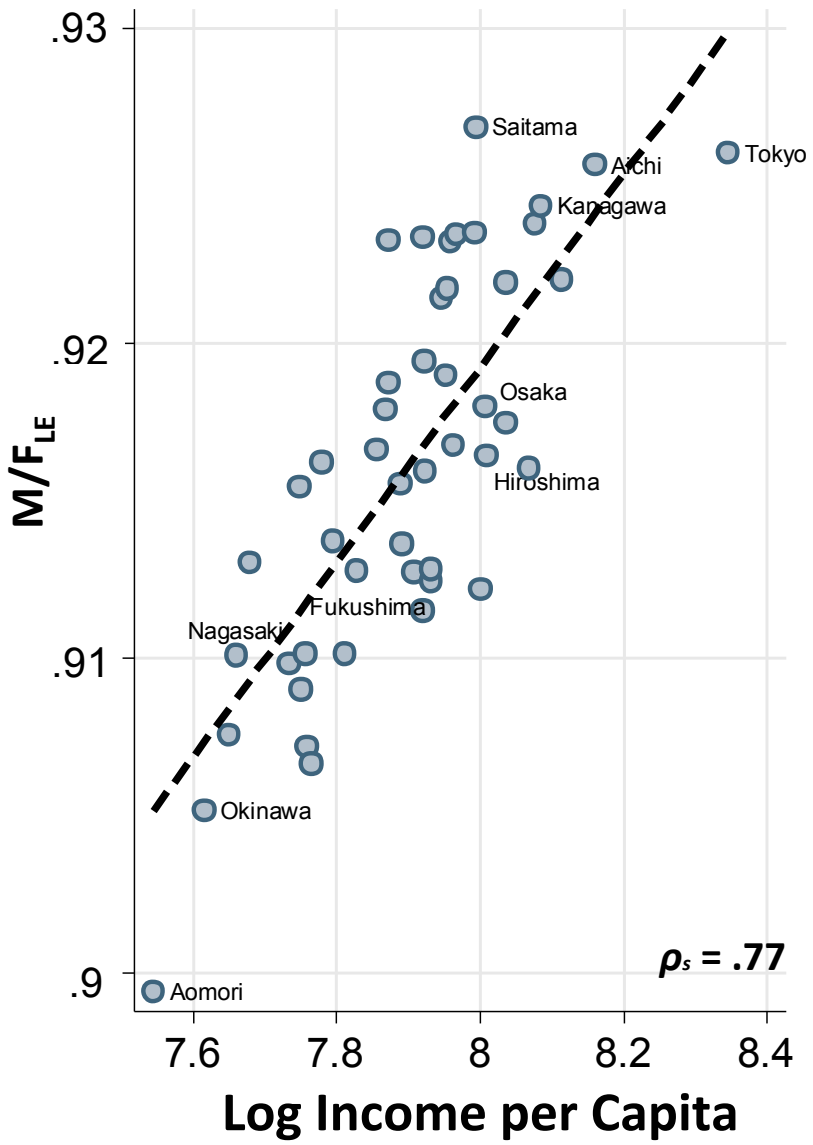


Murray, Lancet

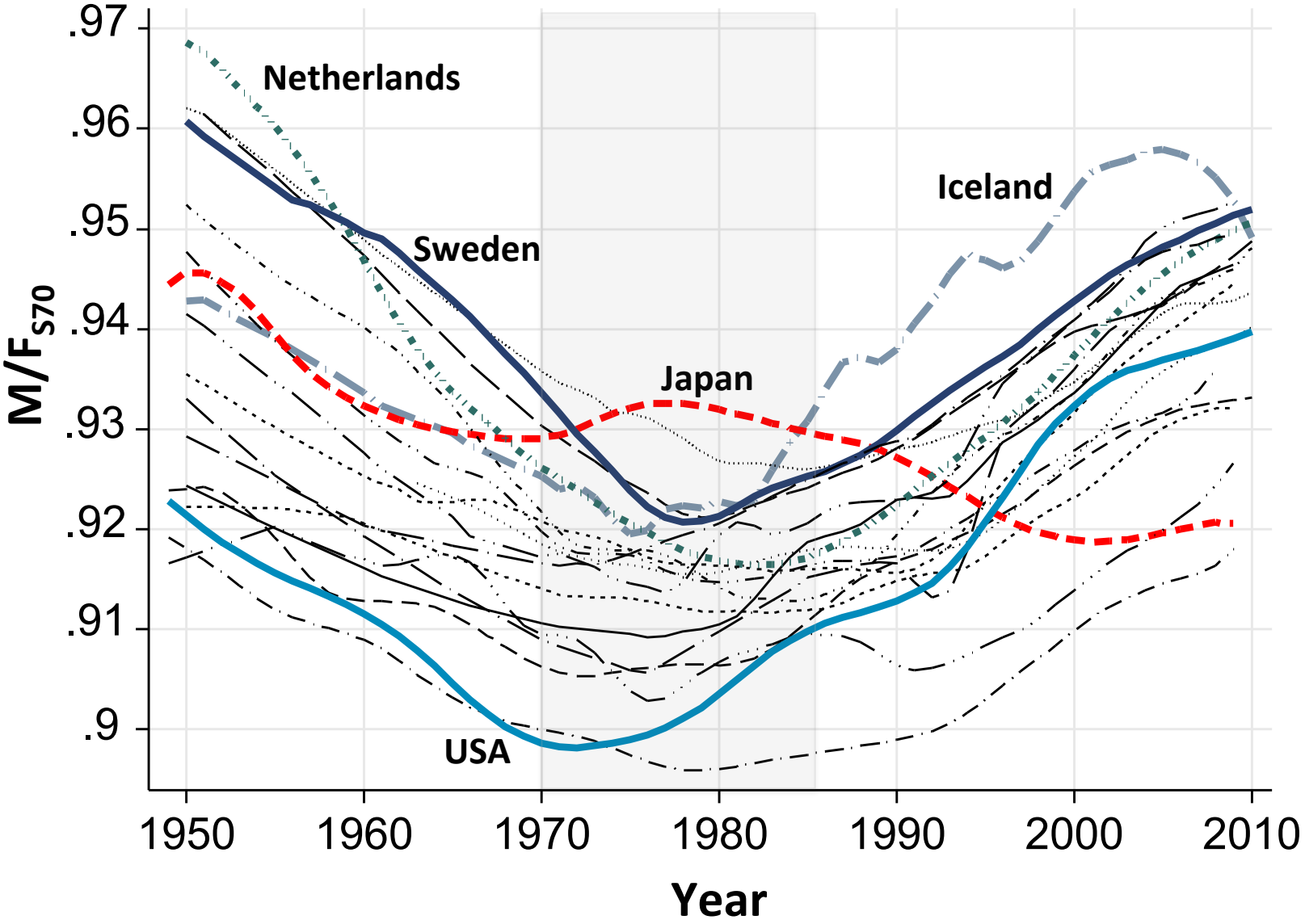
M/F_{S70} vs Log GDP PC, Spain, 2005 by Region



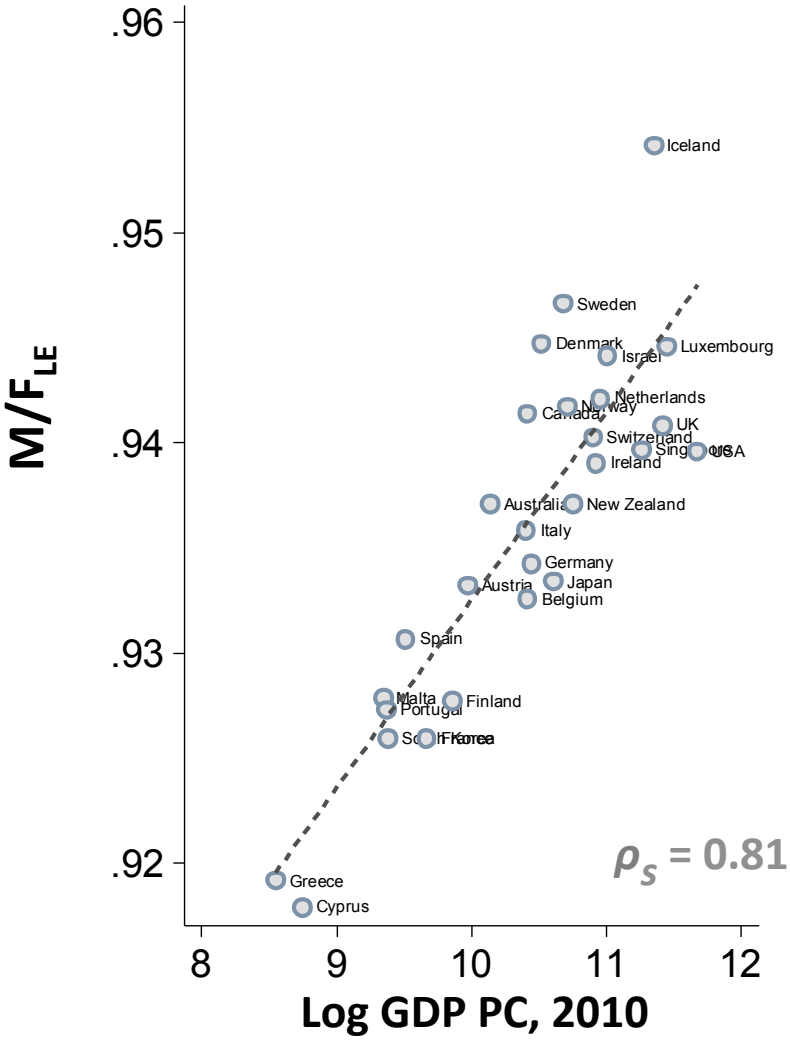
M/F_{LE} vs Log Income PC, Japan, 2005, by Prefecture



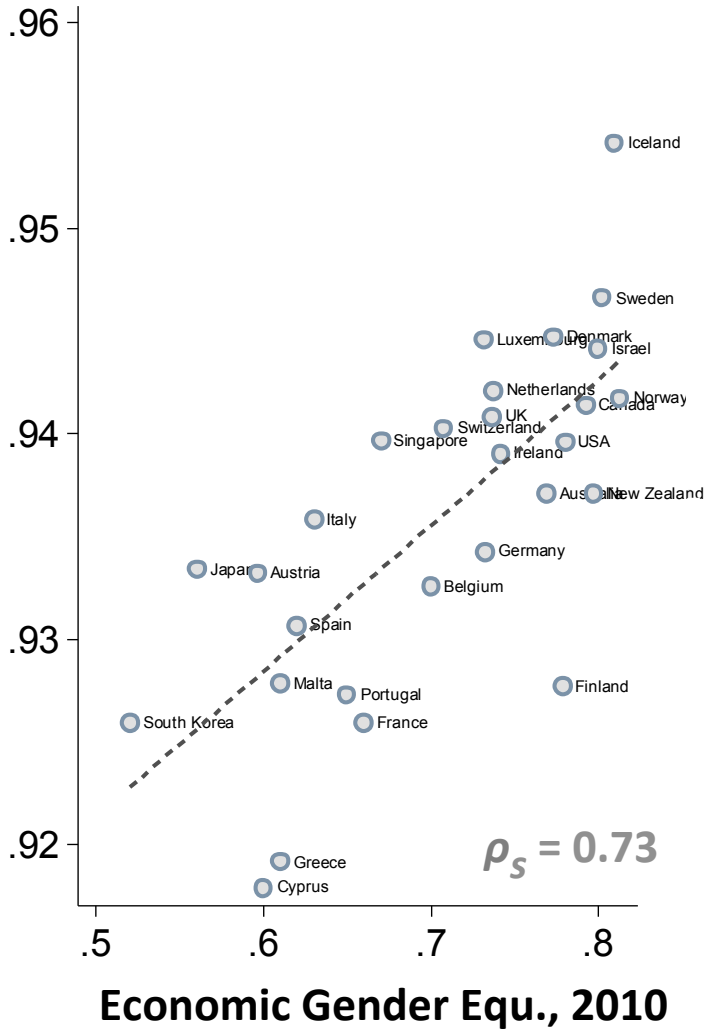
M/F_{S70} over Years 1950-2010, 20 Wealthiest OECD Countries

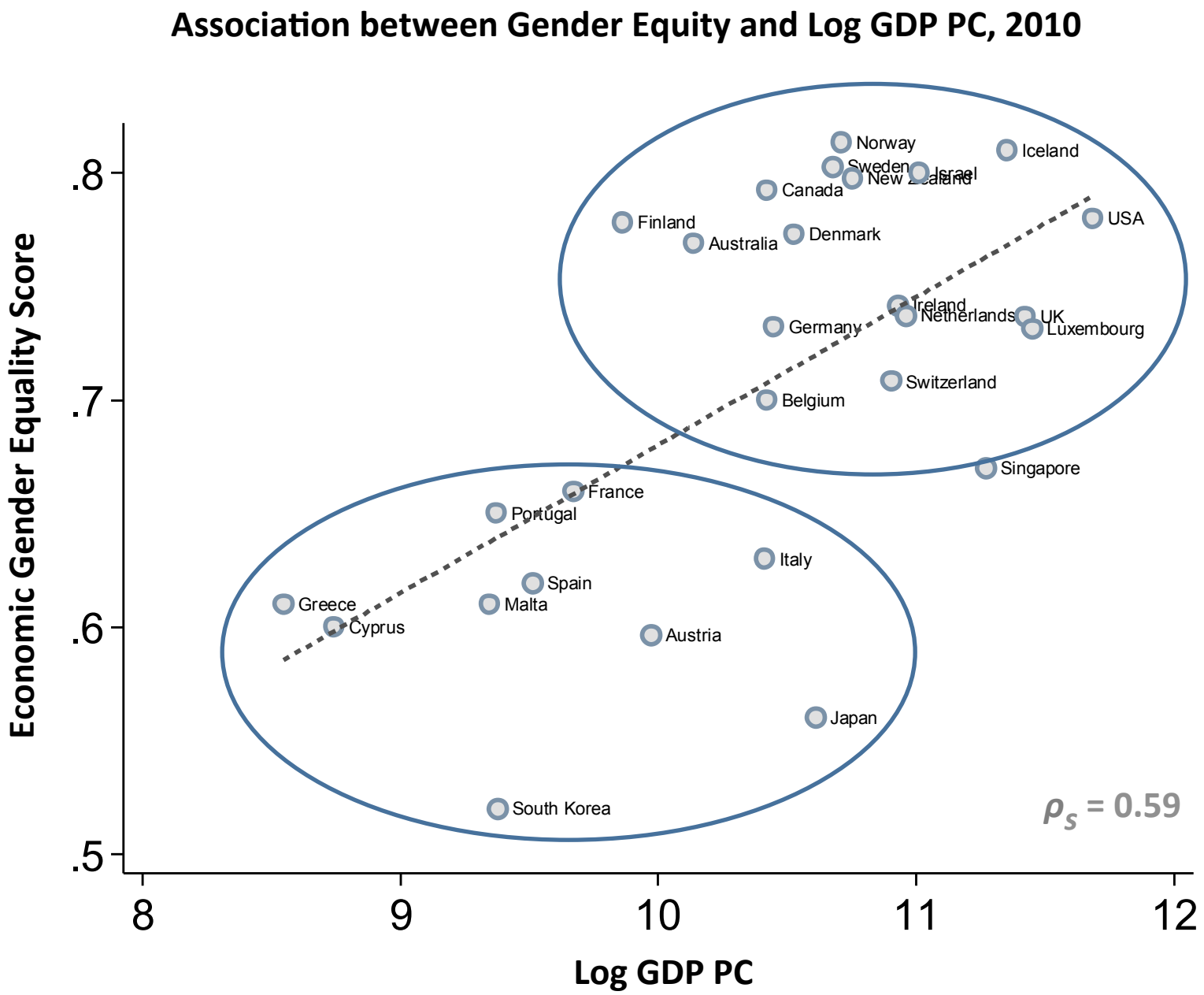


M/F_{LE} vs Log GDP PC, 2010



M/F_{LE} vs Gender Equity, 2010




Association between Gender Equity and Log GDP PC, 2010
Economic Gender Equality Score
.5
.6
.7
.8
8
9
10
11
12
Log GDP PC
Norway
Iceland
Sweden
Israel
New Zealand
Canada
Finland
USA
Australia
Denmark
Ireland
Netherlands
UK
Germany
Luxembourg
Switzerland
Belgium
Singapore
France
Portugal
Italy
Spain
Greece
Malta
Cyprus
Austria
Japan
South Korea
$\rho_s = 0.59$

Clinical Department
Orthopaedic Surgery

Department Chair: **William J. Maloney, M.D.,** Elsbach-Richards Professor

Dept. Representative: **Amy Ladd, M.D.**, Professor (Hand Surgery)

Mission/Vision

- advancing knowledge related to care of musculoskeletal system conditions through basic science and clinical research

Research Areas (subspecialties): adult reconstruction & joint replacement; sports medicine; spine surgery; surgery of hand & wrist, shoulder & elbow, foot & ankle; musculoskeletal tumor surgery; orthopaedic trauma surgery; pediatric orthopaedics; physical medicine and rehabilitation

Education: 5-yr comprehensive residency. **Fellowships**: **Foot & Ankle; Hand & Upper Limb; Joint Replacement/Adult Reconstruction; Spine; Orthopaedic Trauma; PM&R Intervention Spine; PM&R Sports Medicine**

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Stanford University Medical Center

http://ortho.stanford.edu

Stanford WSDM Center

Clinical Department
Orthopaedic Surgery

Dept. Representative: **Amy Ladd, M.D.,** Professor (Hand Surgery)

- Sex and gender in musculoskeletal science
  - Disproportionate incidence and manifestations men vs. women
    - Osteoarthritis
    - Osteoporosis
    - Injuries – ACL (anterior cruciate ligament)
    - Scoliosis
  - Disproportionate surgical treatment, usually favoring men
  - High incidence of breast cancer in women orthopaedic surgeons
- Collaboration needed
  - Advance research to understand demographics, incidence, prevention, and treatment

Clinical Department
Orthopaedic Surgery

Dept. Representative: **Amy Ladd, M.D.,** Professor (Hand Surgery)

- Desired collaborations
  - Engineering sciences
    - *Mechanical engineering, Bioengineering, Tissue Engineering*
  - Clinical and medical sciences
    - *Radiology – imaging disease, injury, and disorder*
    - *OB/GYN – breast cancer*
    - *Endocrine – thyroid cancer, osteoporosis*
    - *Psychiatry – mental health among*
  - Campus wide
    - Bioinformatics, Statistics
      - *Demographics of disease*
    - Anthropology
    - Humanities & Sciences – Music (CCRMA), dance, arts

Gendered Innovations in Orthopaedic Science

Following Londa Schiebinger's GI lead

- Manuscript changes to orthopaedic journals
  - Journal of Bone & Joint Surgery (JBJS)
  - Clinical Orthopaedics & Related Research (CORR)
    - *"Fairness to all: gender and sex in scientific reporting" 2014*

- Quarterly column in CORR 2014 – Ladd
  - "Let's talk about sex, baby: gendered innovations in orthopaedic science."
  - ***Title IX pieces:***
    - "The sports bra, the ACL, and Title IX--the game in play." - Athletics
    - "Title IX education: book learnin' and bone mendin'." - Education
  - "From Access to Zygote: A Gender and Orthopaedics Lexicon."

STANFORD SCHOOL OF MEDICINE
Stanford University Medical Center

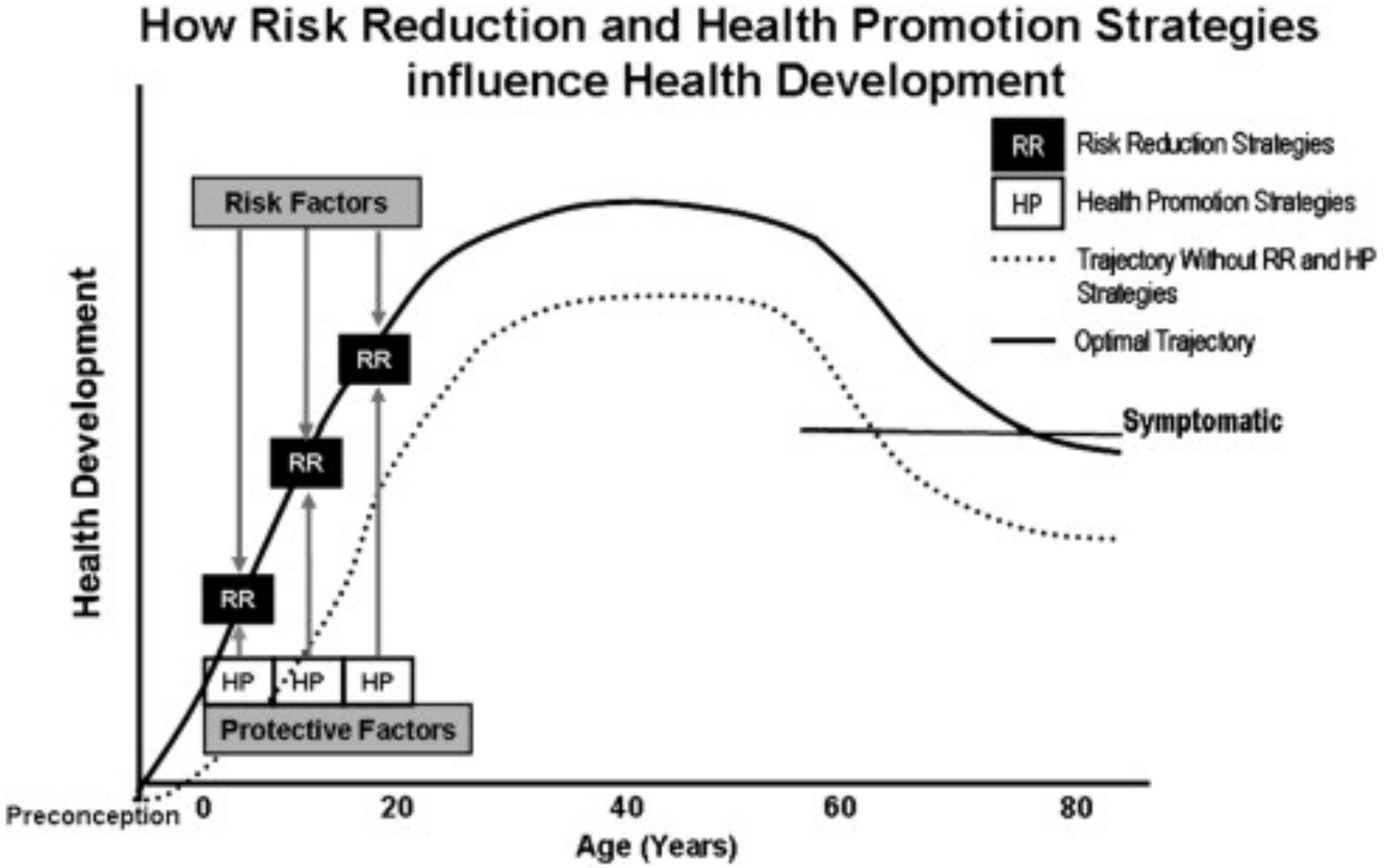
Stanford WSDM Center

Stanford MEDICINE

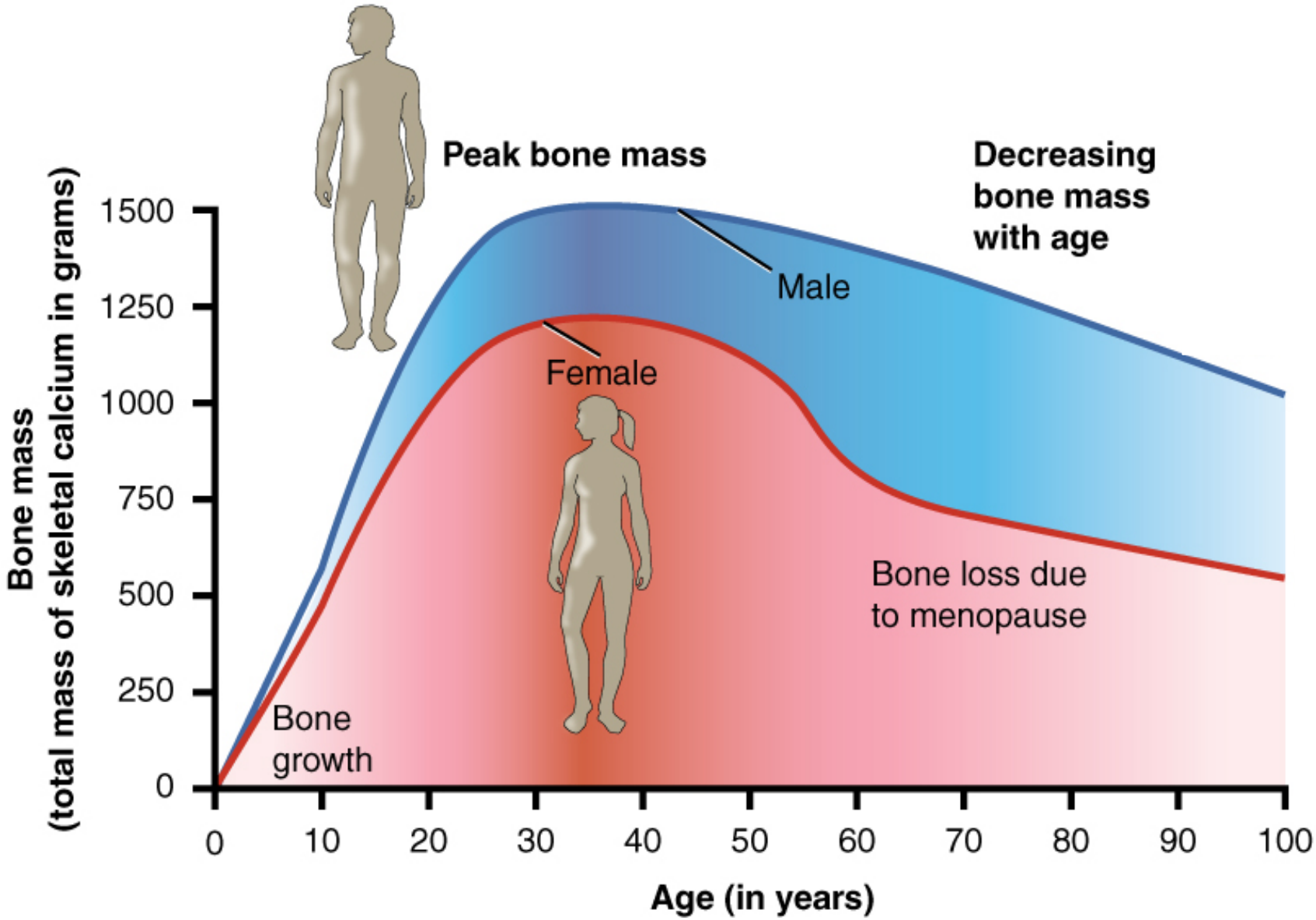
Bone Health and Nutrition Assessment Center

Mary B. Leonard, MD, MSCE
Professor of Pediatrics & Medicine

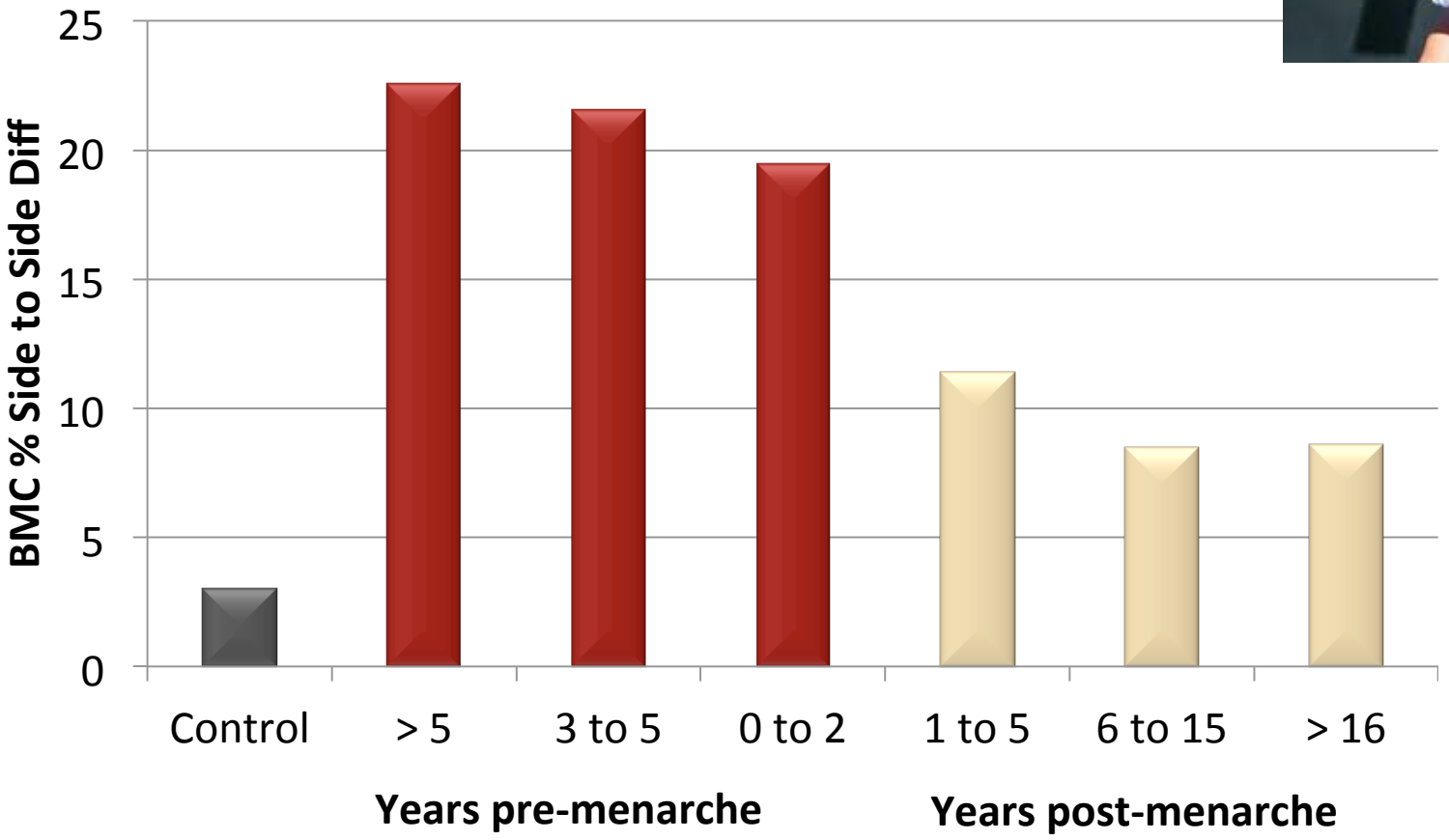
Stanford Children's Health | Lucile Packard Children's Hospital Stanford



From Neal Halfon

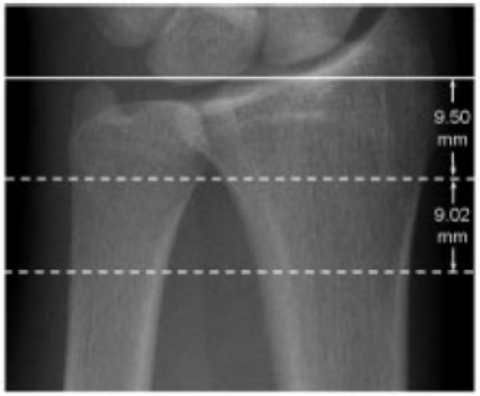

Peak bone mass
Decreasing bone mass with age
Male
Female
Bone loss due to menopause
Bone growth
Bone mass (total mass of skeletal calcium in grams)
1500
1250
1000
750
500
250
0
0
10
20
30
40
50
60
70
80
90
100
Age (in years)

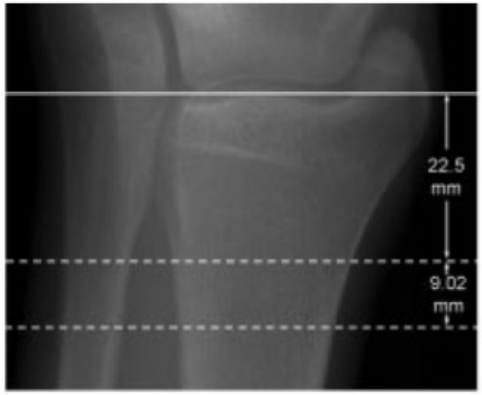
Window of Opportunity

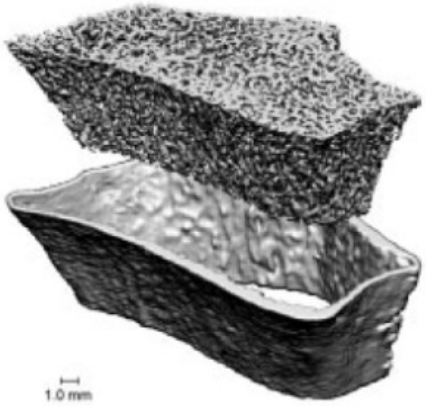


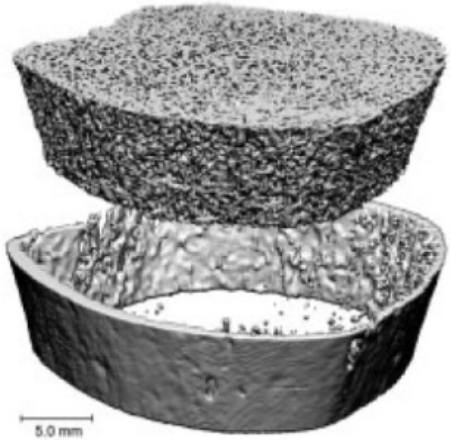
Kannus,et al. Ann Internal Med 1995

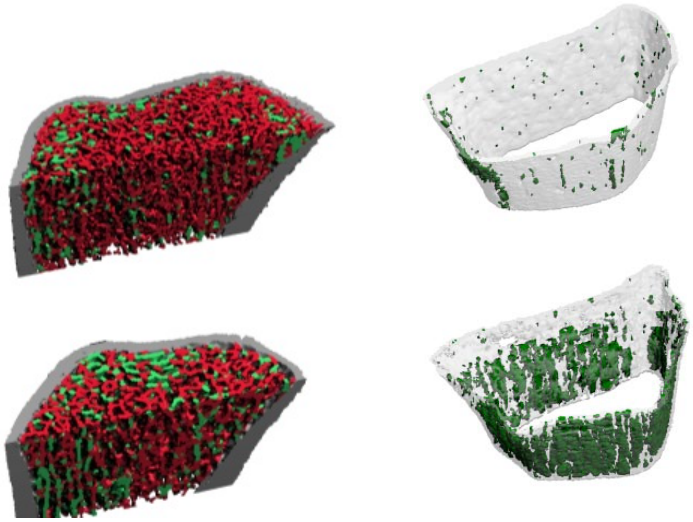
HR-pQCT: Xtreme CT I

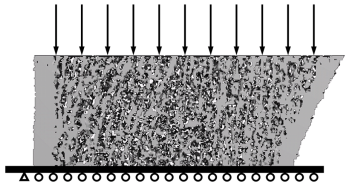


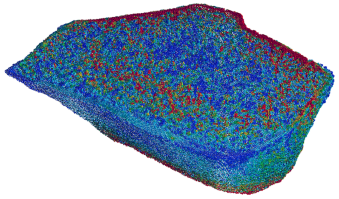




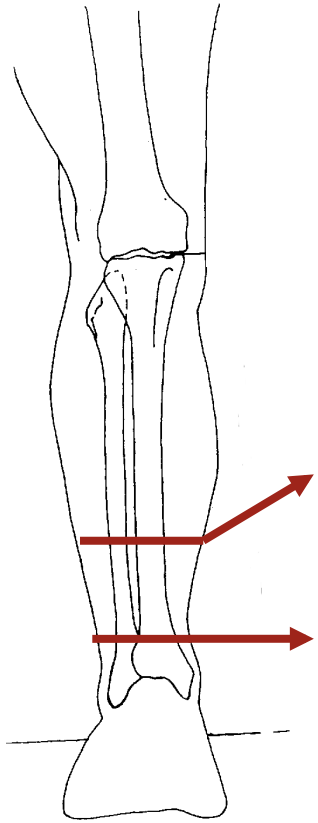


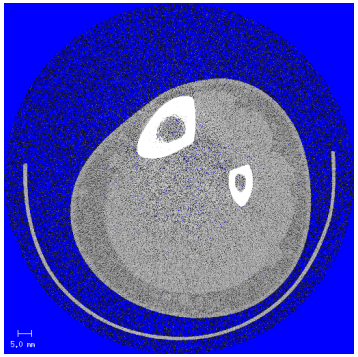


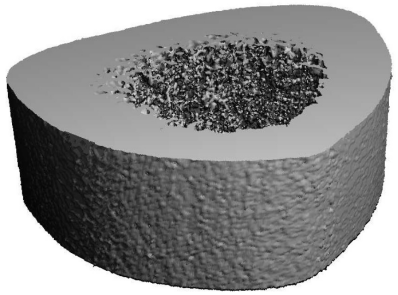


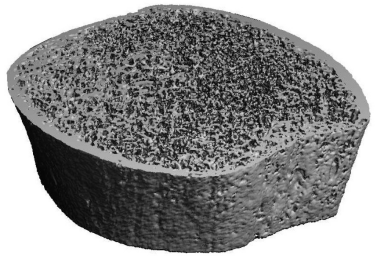


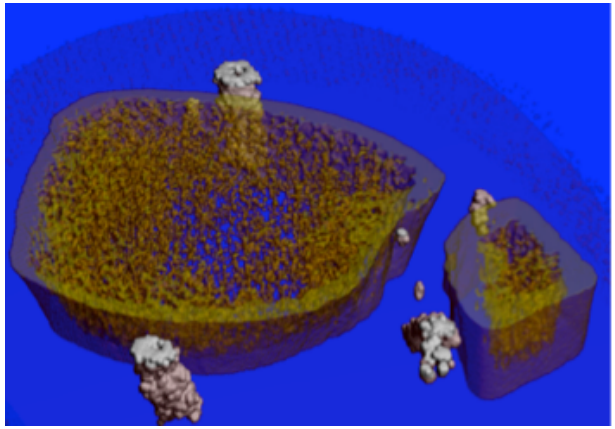
Stanford Bone Health & Nutrition Assessment Center





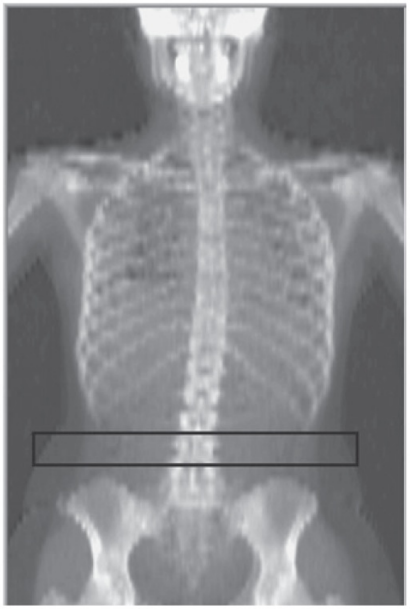


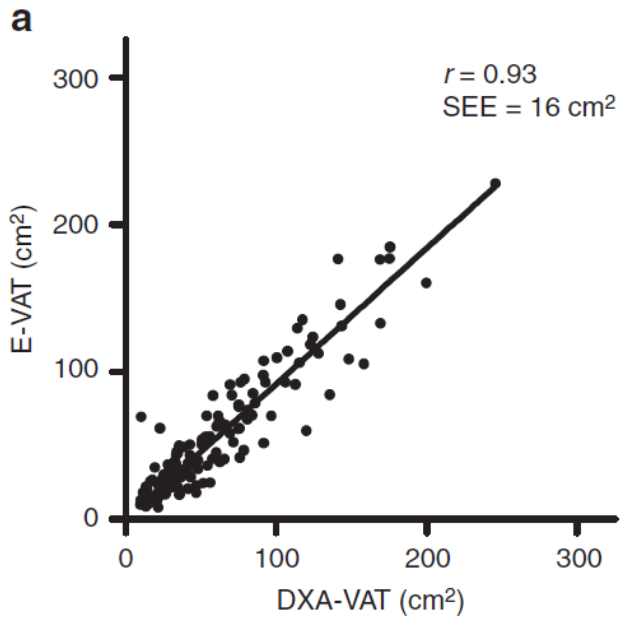




Stanford Bone Health & Nutrition Assessment Center

Stanford Children's Health





Acknowledgements

NIDDK
NATIONAL INSTITUTE OF DIABETES AND DIGESTIVE AND KIDNEY DISEASES

NIAMS
National Institute of Arthritis and Musculoskeletal and Skin Diseases

NATIONAL CANCER INSTITUTE

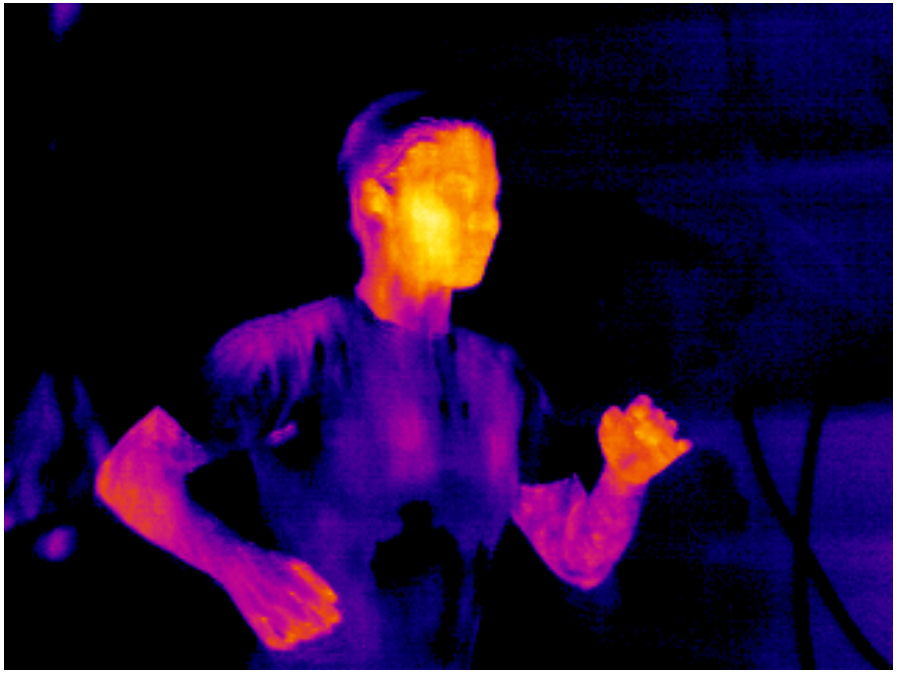
Eunice Kennedy Shriver NICHD
National Institute of Child Health & Human Development

Sex Differences in Thermal Enhancement of Physical Conditioning

Craig Heller

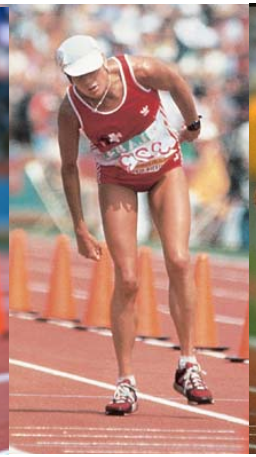
Biology Department

Stanford University



Temperature is a Limiting Factor in Conditioning and in Physical Performance

Los Angeles August 5, 1984 *USA Today*
"…a lasting image of the race was of Switzerland's Gabrielle Andersen-Scheiss staggering the final 400 meters around the Coliseum track in an agonizing 5 minutes and 44 seconds, suffering from heat prostration."



Benoit wins first women's Olympic marathon!

History repeats

Paula Radcliffe bails out of 2004 Olympic marathon.

Paula Radcliffe wins NY marathon 2004, 2007, 2008.

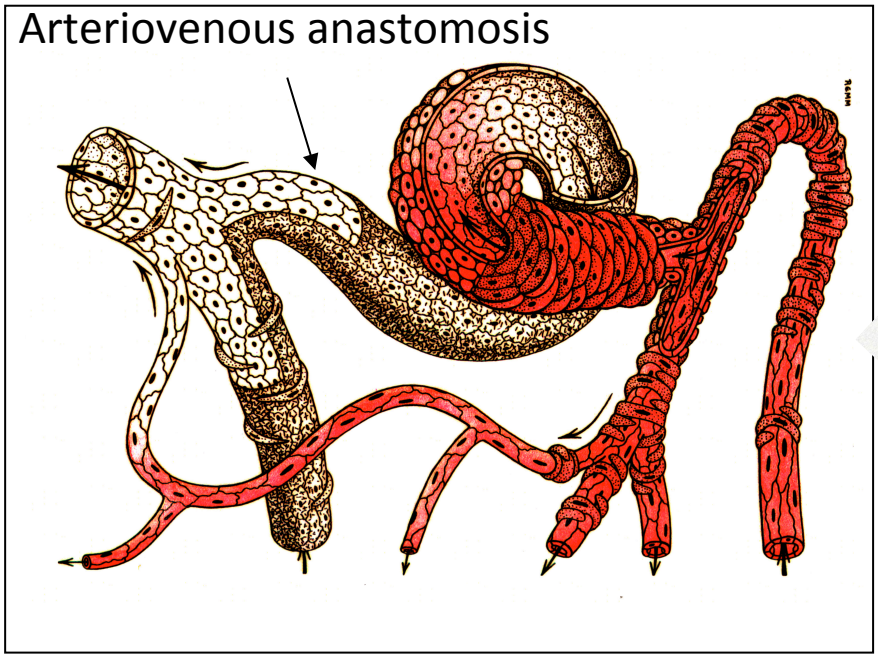
2:23:10, 2:23.9, 2:23.56

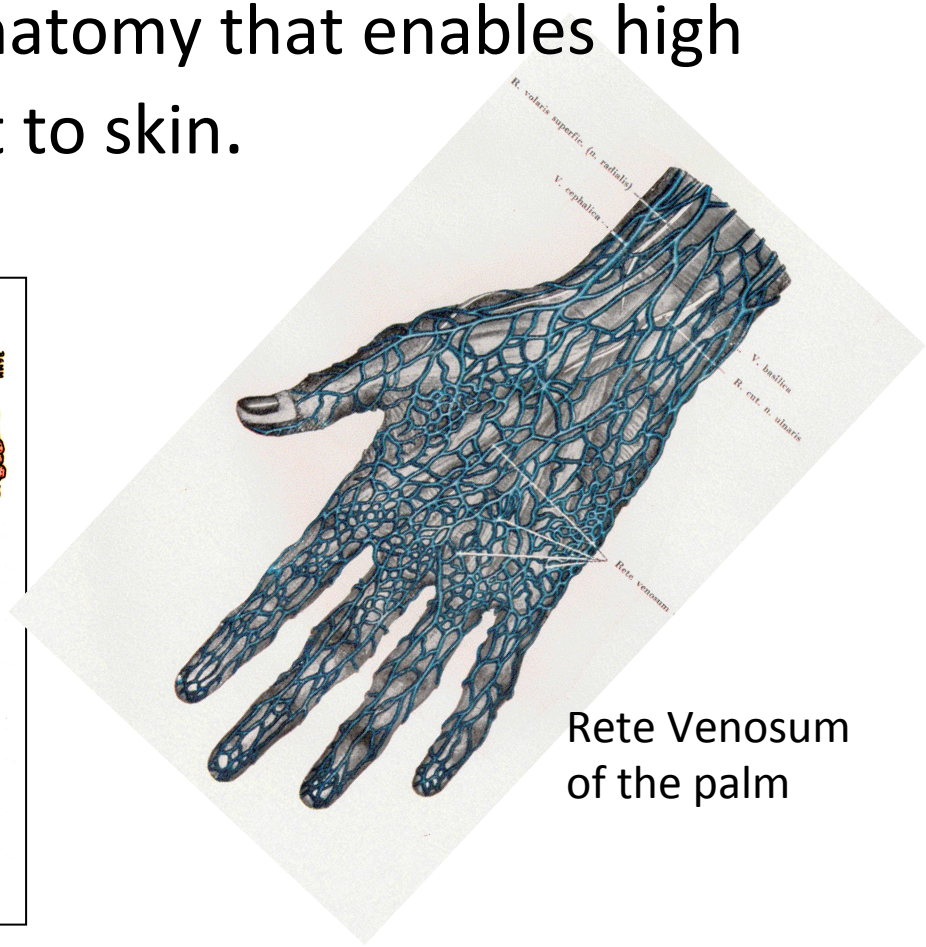
Difference?
Athens - 95ºF
Ave. NY - 45ºF

Controlling core temperature extends work capacity -- focus on glabrous skin areas.

For furred mammals, glabrous skin is the major path for heat dissipation.

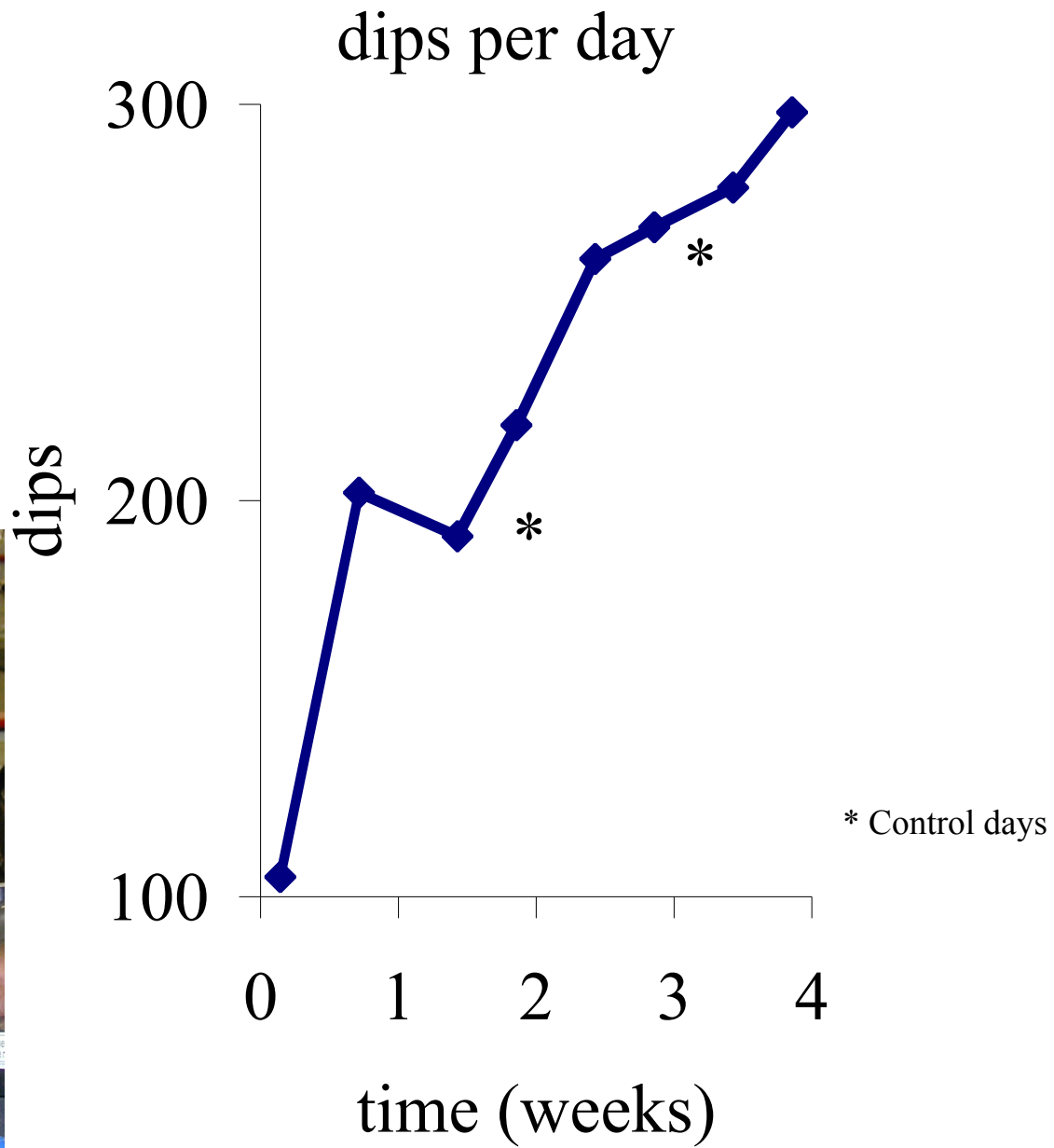
Glabrous skin has vascular anatomy that enables high blood flow for heat transport to skin.





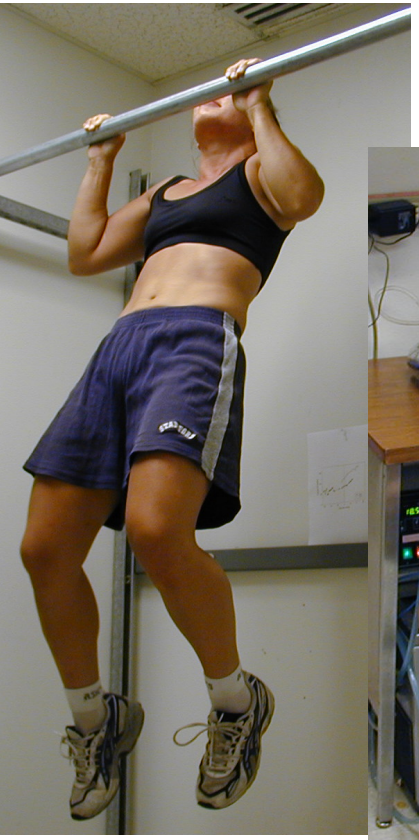
Rete Venosum of the palm

Four weeks of training with cooling

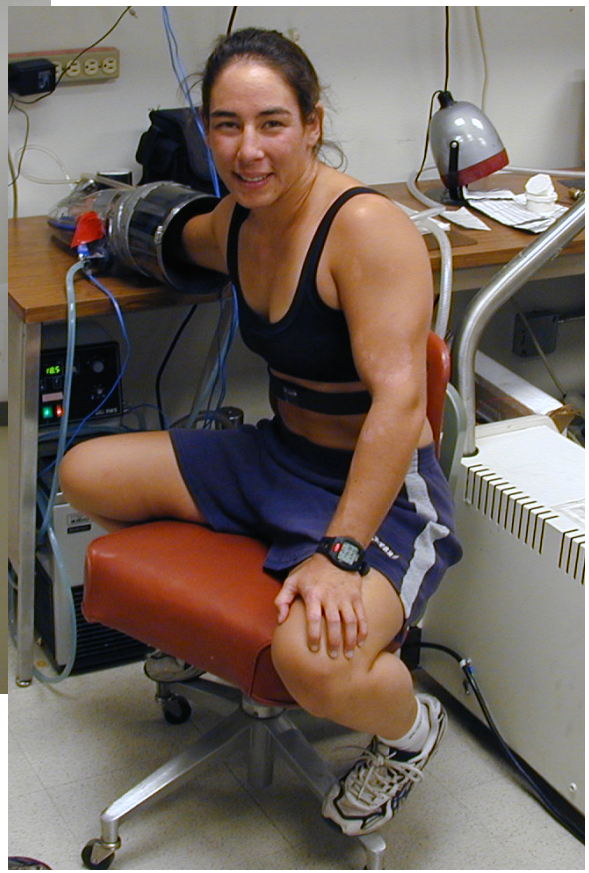


Experiment repeated in reverse order to rule out priming effect.

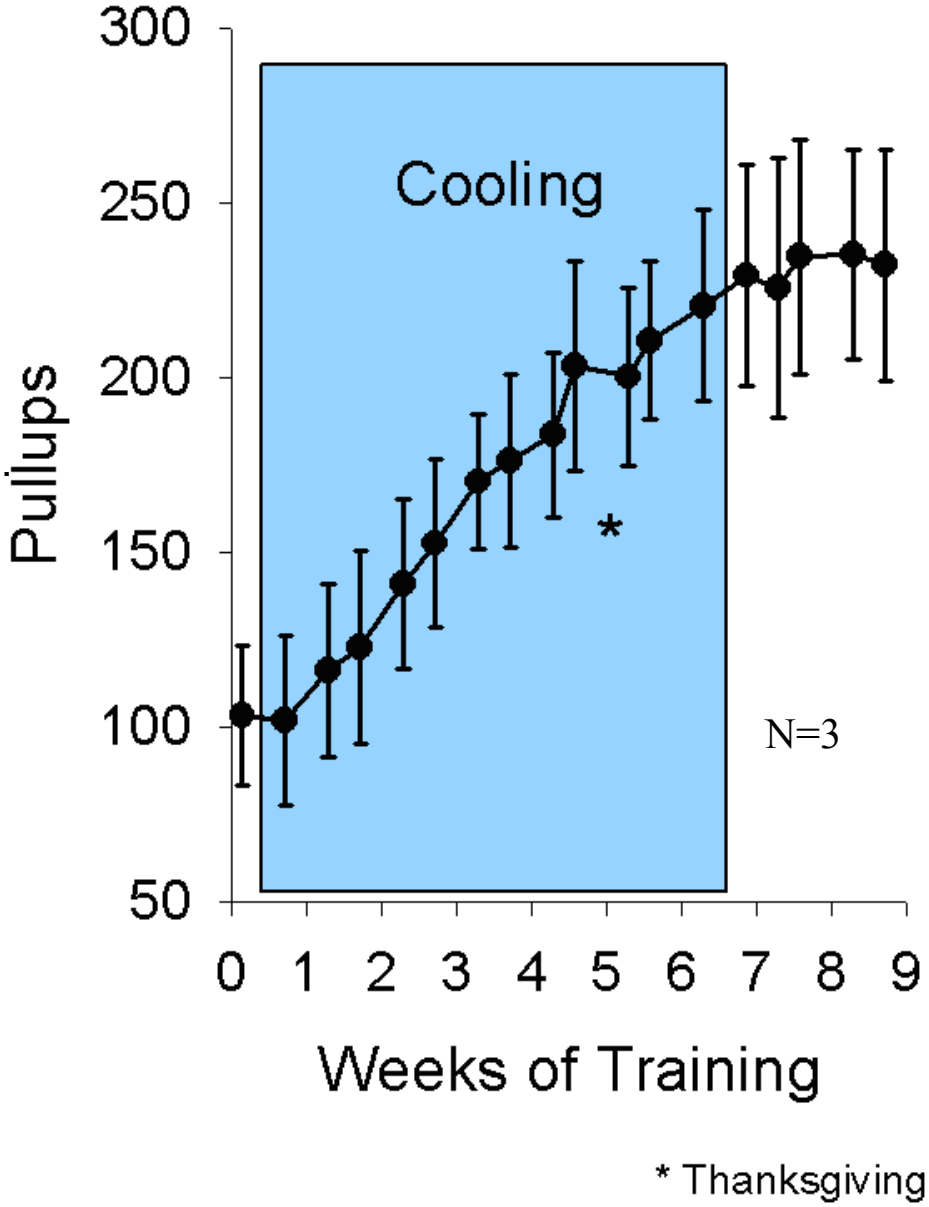
3 min of cooling between sets for 6 weeks followed by two weeks of controls with out cooling.



Subjects: Stanford wrestlers.



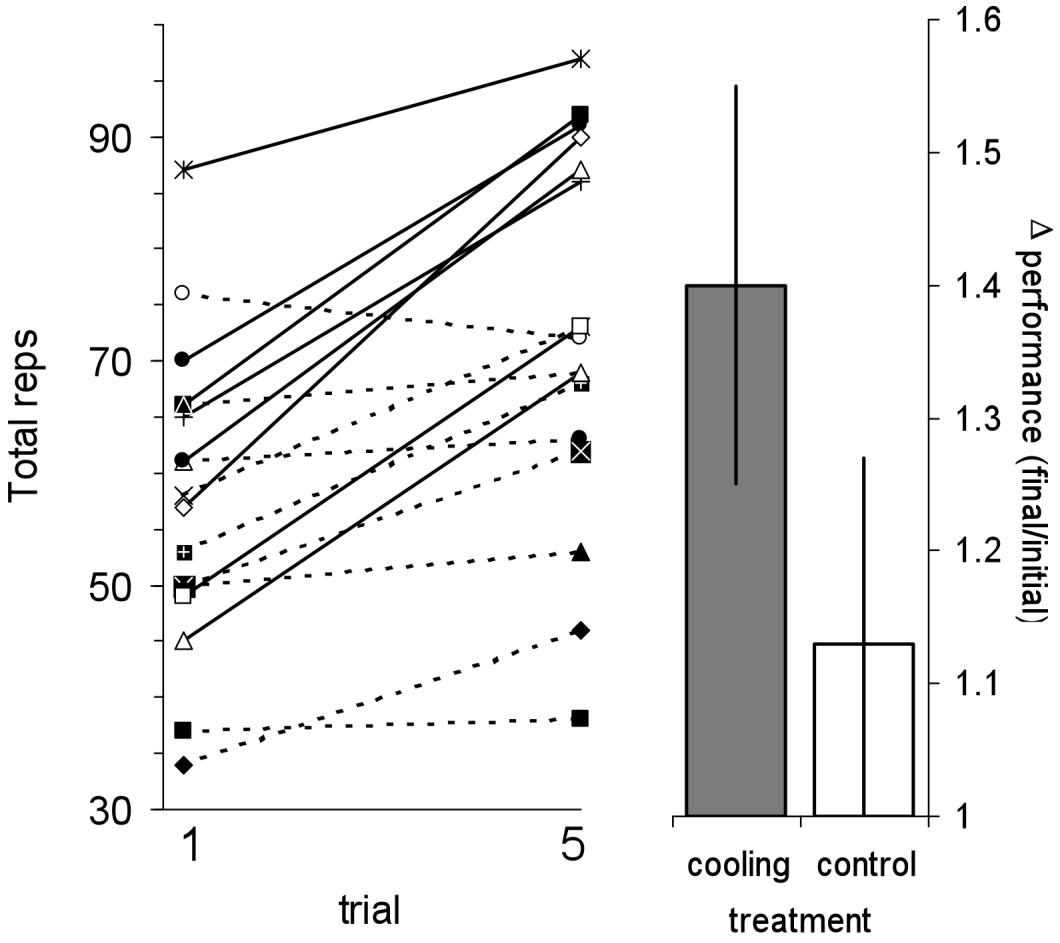
Patty Ramirez
Olympic bronze
Athens, 2004



2-3 X increases in workout capacity.

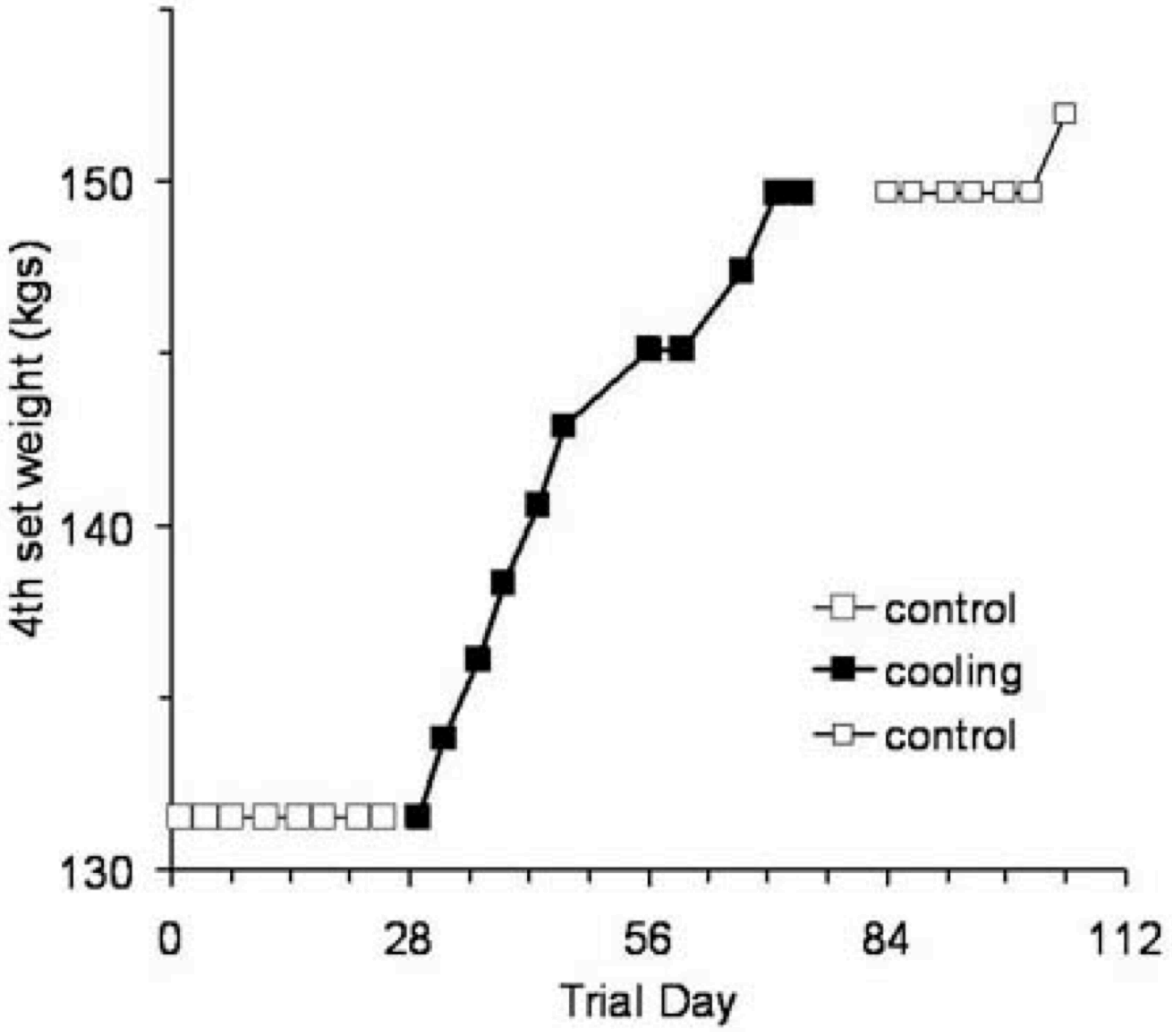
Bench Press Experiment

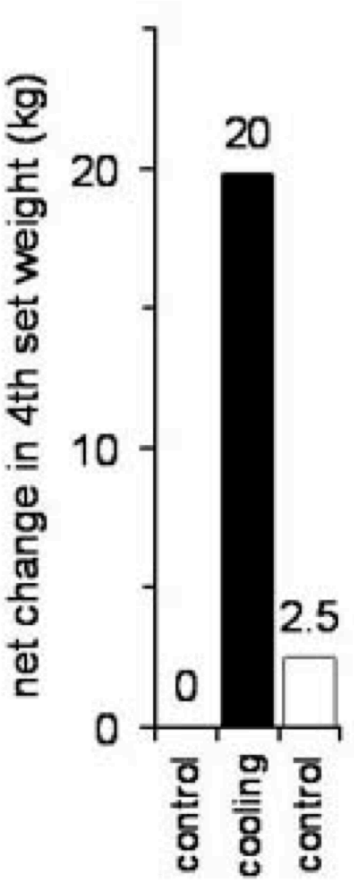
80% 1 rep max – 10 sets to muscle failure



Strength Gains

Pyramid conditioning routine



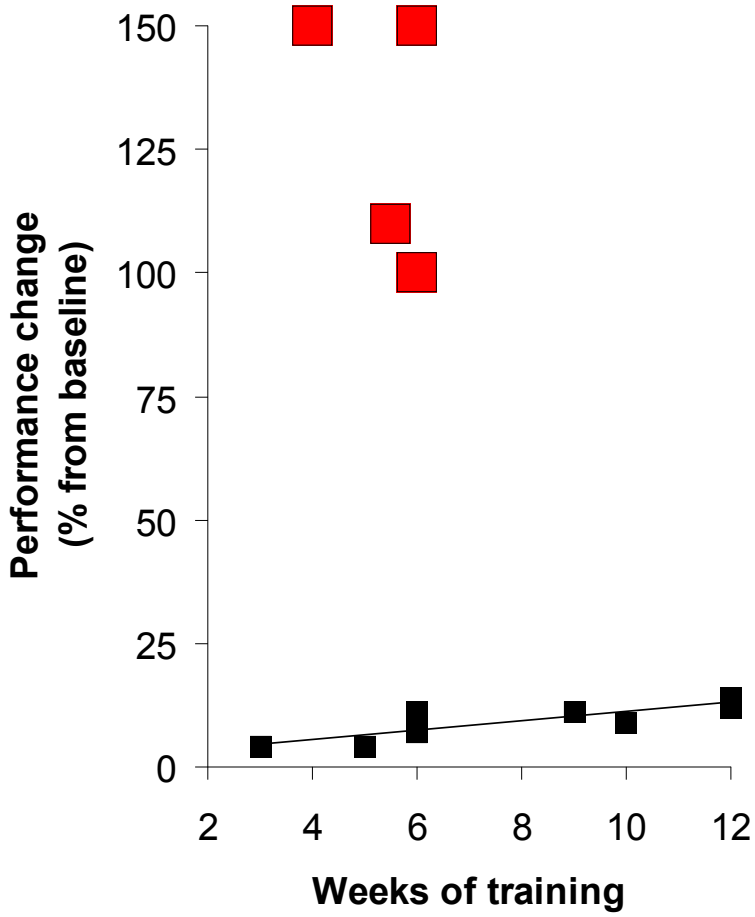


What about endurance?

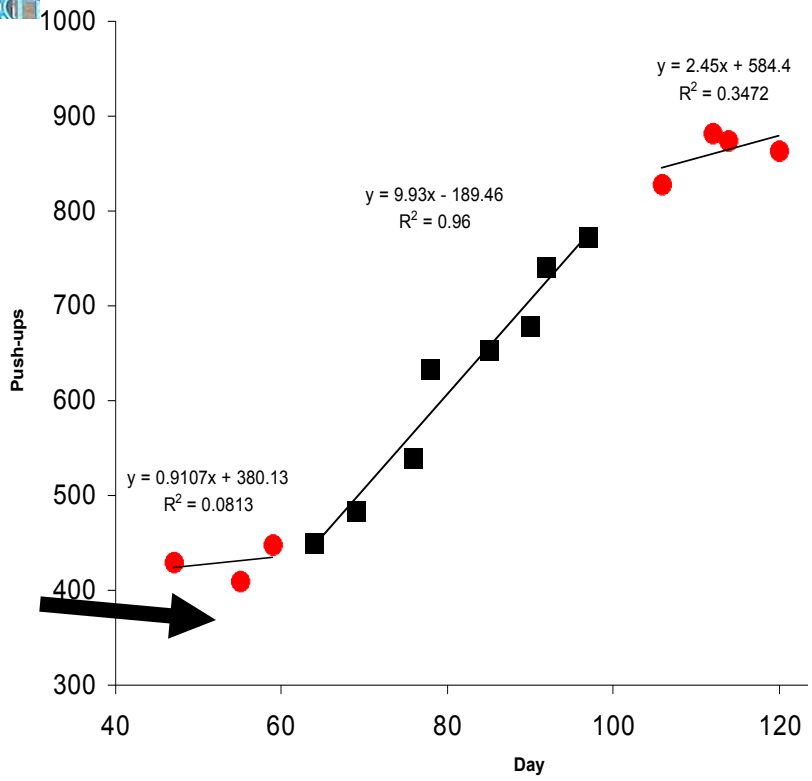
How do the results from cooling compare to experiments with anabolic steroids?

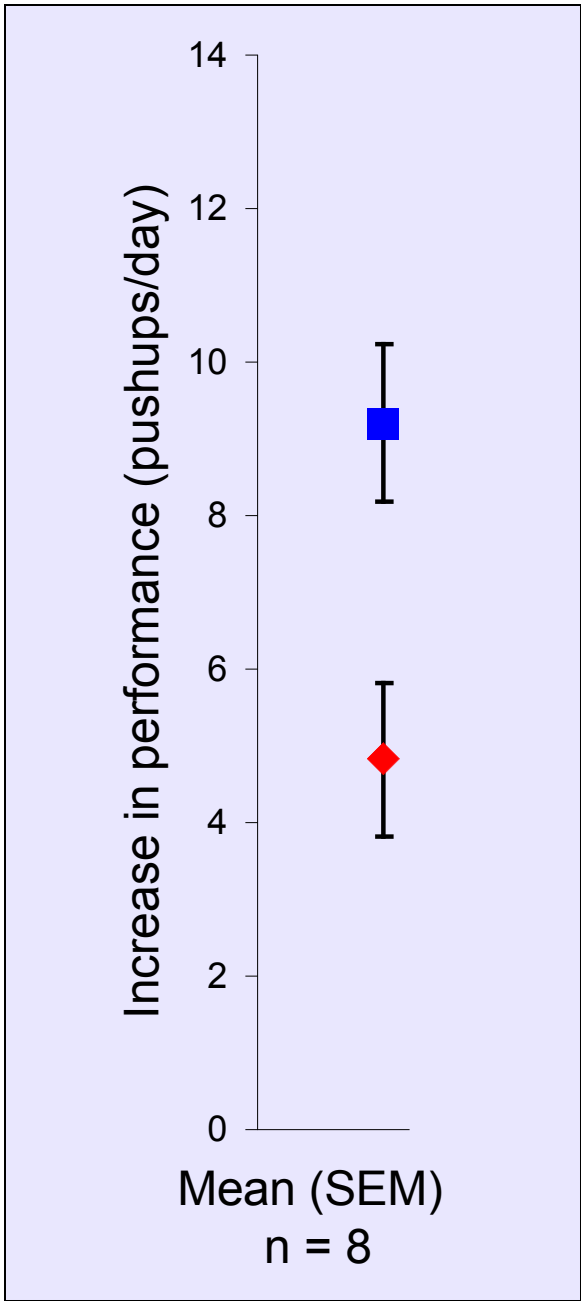
Studies showing effects of anabolic steroids on bench press conditioning

Authors	weeks of training	improvement (%)
Johnson & O'Shea 1989	**3**	**4**
Ward 1973	**5**	**4**
Rogerson, et al. 2007	**6**	**11**
King et al. 1999	**6**	**7**
Fahey & Brown 1973	**9**	**11**
Bhasin, et al. 1996	**10**	**9**
Giorgi, et al. 1999	**12**	**12**
Blazevich & Giorgi 2001	**12**	**14**



Pushups -- 10 sets, 3 min rests:



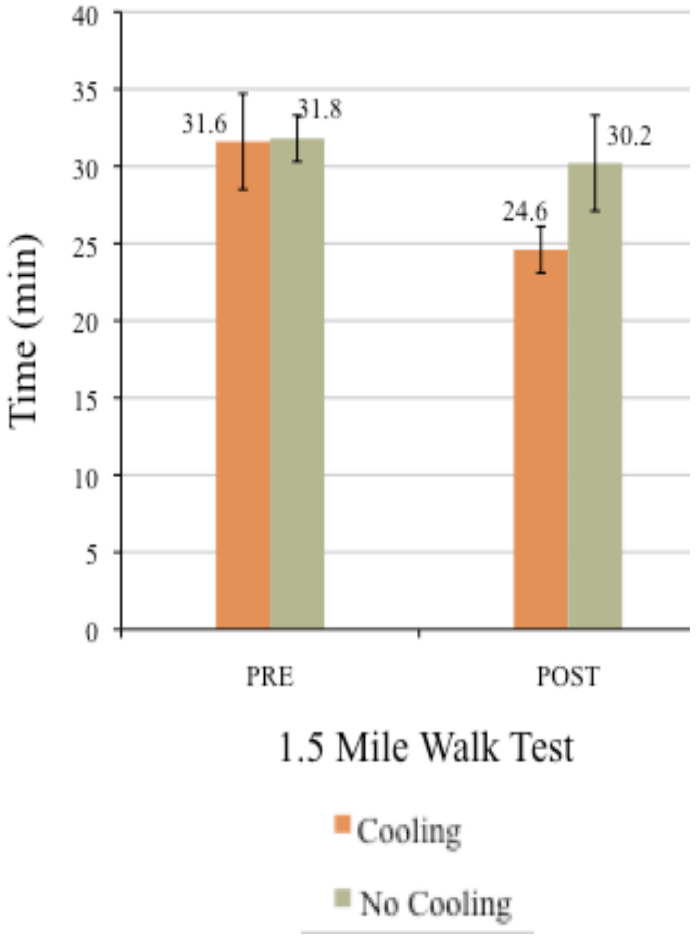


Innovation in Exercise: Increasing Capacity of Sedentary Obese Women with Palm Cooling

Stacy T Sims, MSc PhD[1]; Sandra Tsai, MPh MD[1]; H. Craig Heller, PhD[2]; Marcia L Stefanick, PhD[1]

[1]Stanford Prevention Research Center, Stanford School of Medicine, Stanford, California, [2]Biological Sciences, Stanford University, California

Funded by: The Cardiovascular Institute, Women's Health, Stanford University



Thanks to CVI Seed Grant