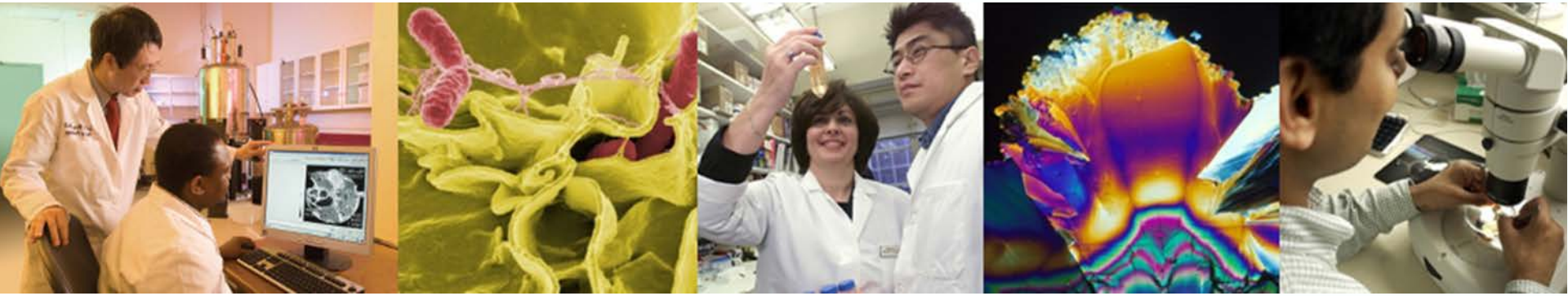
Methods and Techniques for Integrating the Biological Variable "Sex" in Preclinical Research

The Importance of Reproducibility:
Studying Both Sexes in Preclinical Research



October 20, 2014

Lawrence A. Tabak, DDS, PhD
Principal Deputy Director, NIH
Department of Health and Human Services

Every System Is Exquisitely Tuned to Get the Results It Gets

- What is “default” biology?
  - In the lab: male cells and animals
  - In the clinic: 70-kg male
- But, female is not equal to non-male
  - Just like minorities are not “non-whites”
- Rigor and reproducibility
  - Every experiment is part of larger system, the quest to understand fundamental basic living systems
  - Approaches and results should be consistent and free of bias at the outset

COMMENT

NIH plans to enhance reproducibility

Francis S. Collins and **Lawrence A. Tabak** discuss initiatives that the US National Institutes of Health is exploring to restore the self-correcting nature of preclinical research.

A growing chorus of concern, from scientists and laypeople, contends

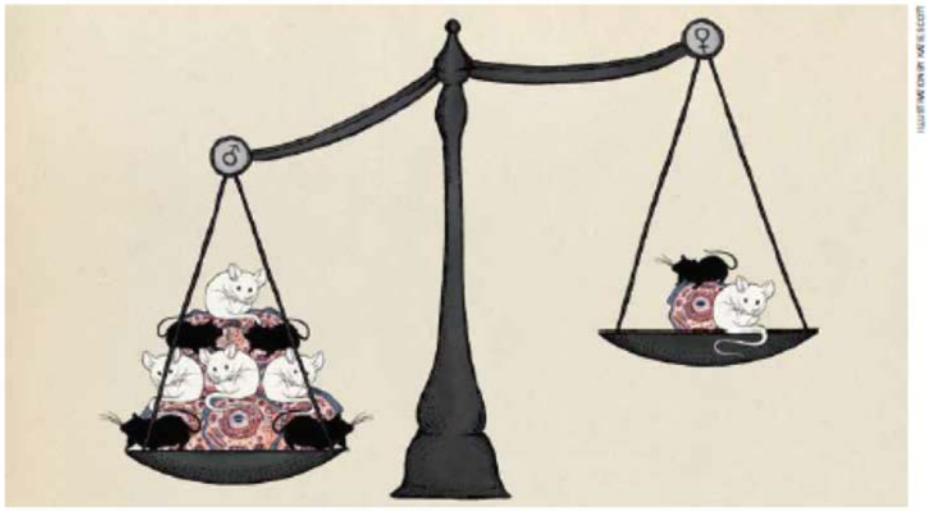
shorter term, however, the checks and balances that once ensured scientific fidelity

Crucial experimental design elements that are all too frequently ignored include blinding, randomization, replication, sample-size calculation and the effect of sex differences.

nature

COMMENT

May 14, 2014



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.

Clayton JA, Collins FS.
Nature. 2014 May 15;509(7500):282-3.

NIH Takes Steps to Address Sex Differences in Preclinical Research

May 14, 2014

Over the past two decades, we have learned a great deal about how men and women respond differently to medications. This knowledge came after a concerted effort in the early '90s to increase the number of women in NIH-funded clinical research. Today, just over half of NIH-funded clinical research participants are women. Unfortunately, experimental design in cell and animal research has not always followed suit. An over-reliance on male animals, and neglect of attention to the sex of cells, can lead to neglect of key sex differences that should be guiding clinical studies, and ultimately, clinical practice. NIH is taking action to address this shortfall as outlined by Janine A. Clayton, M.D., Director of the NIH Office of Research on Women's Health, and me in the *Nature* Comment below.

Francis S. Collins, M.D., Ph.D.
Director, National Institutes of Health

Director's Page

Filling the Gaps: NIH Enacts New Policies to Address Sex Differences

Posted May 14, 2014

Janine Austin Clayton, M.D.

Today in *Nature*, National Institutes of Health (NIH) Director Dr. Francis Collins and I announce that NIH will be requiring applicants to report their cell and animal inclusion plans as part of preclinical experimental design. By developing this policy, we are promoting a balanced approach to addressing male and female differences in cells and animals – just as we did years ago with women and men in NIH-funded clinical trials.

The New York Times

HEALTH

Labs Are Told to Start Including a Neglected Variable: Females

By RONI CARYN RABIN MAY 14, 2014

Slate

THE XX FACTOR | WHAT WOMEN REALLY THINK | MAY 15 2014 12:11 PM

Why Are All the Lab Rats Boys? NIH Tells Drug Researchers to Stop Being Sexist Pigs.

By Amanda Marcotte

Science

NEWS & ANALYSIS

NATIONAL INSTITUTES OF HEALTH

Needed: More Females in Animal and Cell Studies

Jennifer Couzin-Frankel

Vox

US government to require affirmative action for female lab mice

NATURE NEWS BLOG

NIH to require sex-reporting in preclinical studies

14 May 2014 | 18:00 BST | Posted by Sara Reardon | Category: Biology & Biotechnology, Drug discovery, Institutions, Policy, Publishing

PBS NEWSHOUR

Chicago Tribune

The Washington Post

USA TODAY

Bloomberg

npr

WISCONSIN PUBLIC RADIO

npr

ALJAZEERA

Bio·IT World

THE CHRONICLE OF HIGHER EDUCATION

BioTechniques

The International Journal of Life Science Methods

genomeweb

PLOS | BLOGS

POPULAR SCIENCE

SHAPE

GLAMOUR

NIH in 2014: Studying Sex to Strengthen Science

Problem

Looking for female/male differences is like a blind spot in biomedical research, leaving gaps in our knowledge.

Solution

Sex is a fundamental biological variable that must be considered throughout the biomedical research continuum.

Action

NIH is requiring a deliberate approach in considering sex in cells and animals to make sure men and women get the full benefit of medical research.

Outcome

When researchers consider sex as a fundamental biological variable, NIH continues to deliver rigorous science that drives medical advances.

NIH cannot do this alone and is working on multiple fronts with stakeholders in the public and private sectors.

NIH OFFICE OF RESEARCH ON WOMEN'S HEALTH (ORWH)

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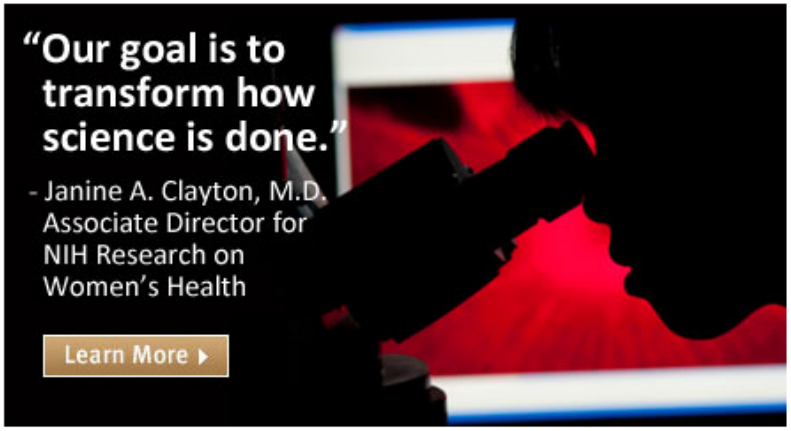
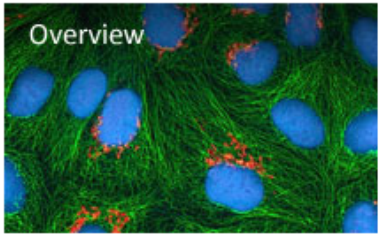
Studying Sex to Strengthen Science (S4)





Latest News

NIH Takes Steps to Address Sex Differences in Preclinical Research, NIH Director's Statement

Filling the Gaps: NIH to Enact New Policies to Address Sex Differences, ORWH Director's Blog

Rock Talk: More on Addressing Sex Differences in Preclinical Studies, OER Director's Blog

Highlights

Studying Sex to Strengthen Science: Questions & Answers

Nature Comment: NIH to balance sex in cell and animal studies

Media Contact

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Communications Director
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anne.rancourt@nih.gov

Specialized Centers of Research (SCOR) on Sex Differences

- ORWH/FDA Collaboration
- Interdisciplinary collaborations
- Research on sex/gender factors underlying a priority women's health issue
- Bridges basic and clinical research
- $113.7 million investment from FY 2002 to 2013

11 Funded SCOR Centers		
Sex-Specific Risk for Vascular Dysfunction and Cognitive Decline (NIA)	Center for Neurovisceral Sciences and Women's Health (NIDDK)	Prepubertal Stress, Windows of Risk, and Sex Bias for Affective Disturbance (NIMH)
Sex and Gender Differences in Addictions and Stress Response (NIDA)	Metabolic Consequences of Loss of Gonadal Function (NICHD)	Molecular and Epidemiologic Basis of UTI in Women (NIDDK)
Genes, Androgens, and Intrauterine Environment in PCOS (NICHD)	Birth, Muscle Injury, and Pelvic Floor Dysfunction (NICHD)	Gender-Sensitive Treatment for Tobacco Dependence (NIDA)
Sex Differences in Musculoskeletal Conditions Across the Lifespan (NIAMS	Sex Differences and Progesterone Effects on Impulsivity, Smoking, and Cocaine (NIDA)	

ORWH Administrative Supplements for Research on Sex/Gender Differences

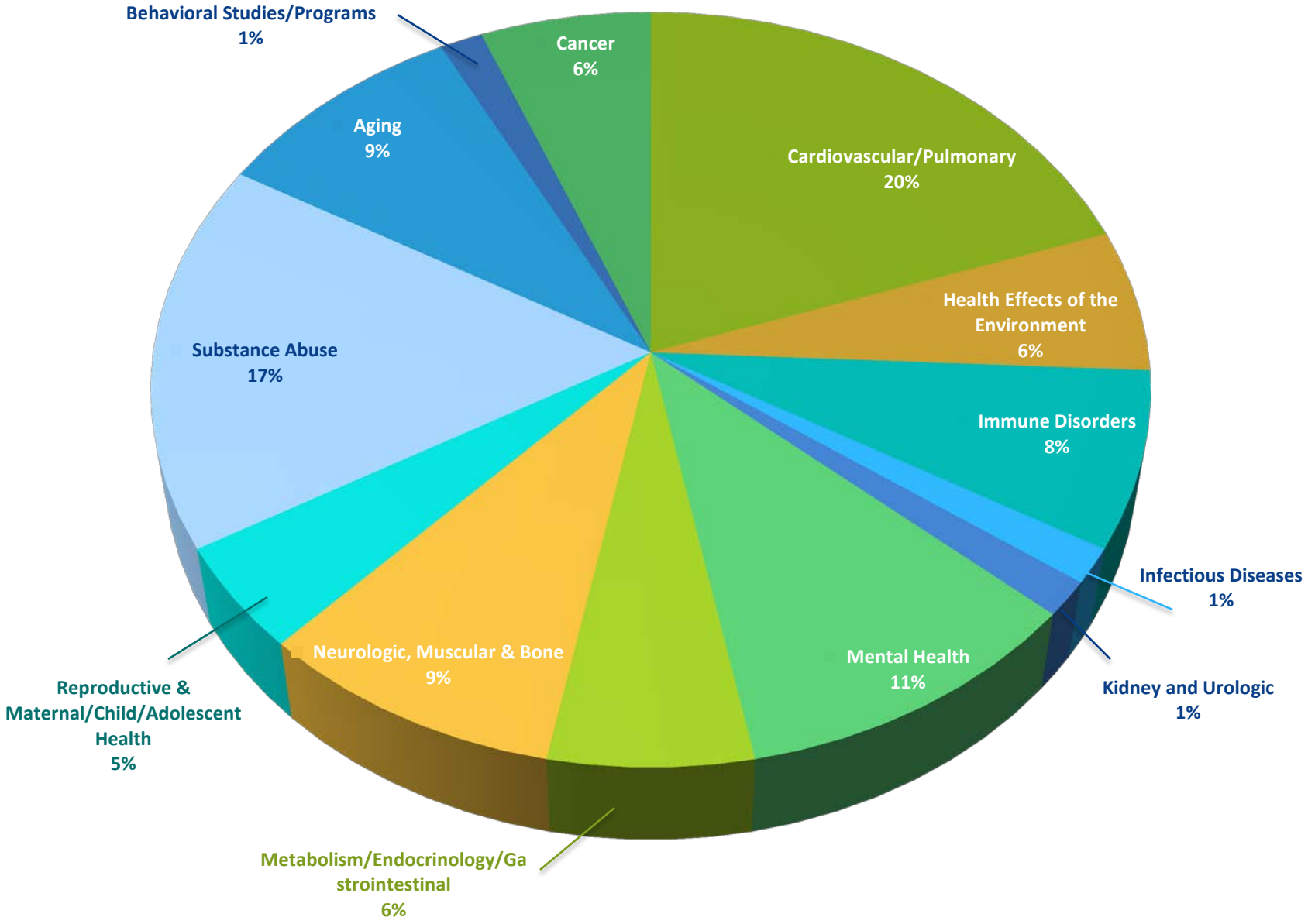
Selected Topics

- Molecular and Functional Mechanisms of Pediatric Heart Failure
- Alzheimer's Disease Neuroimaging Initiative
- Characterizing a Cue-Vulnerable Pharmacoresponsive Endophenotype in Smokers
- Enhancing Neonatal Immunity to *Streptococcus pneumonia*
- Innovative Efficacy Measures of Lupus Nephritis Therapies
- Preventing Transition of Acute to Chronic Neuropathic Pain: Models, Mechanisms, Mediators
- PAAR-Pharmacogenomics of Anticancer Agents Research Group
- The Role of MeCP2 in Rett Syndrome

Selected Approaches

"… add a second group of animals of the opposite sex for comparative analyses"
"… leverage already existing samples/technologies to identify gender-specific differences in biomarkers"
"… characterize the effects of sex in pharmacogenomics phenotypes"
"… test methodological issues for understanding sex differences"
"… test for differences in epigenetic marks in males and females"
"… estimate sex-related differences in disease outcomes in computational models of risk prediction"

Administrative Supplements: Health Topics (FY 2014)



National Institutes of Health
Office of Research on Women's Health

U.S. Food and Drug Administration
Protecting and Promoting *Your* Health

The Science of Sex and Gender in Human Health: Online Course Site

The Basic Science and the Biological Basis for Sex- and Gender-Related Differences

- Understanding the Importance of Sex and Gender in Biomedical Research
- Legislative Process Framework
- Cell Physiology
- Developmental Biology
- Pharmacodynamics and Pharmacokinetics
- Clinical Applications of Genomics

Sex and Gender Differences in Health and Behavior

https://sexandgendercourse.od.nih.gov/

- Clinical Research Methodology
- Endocrine Effects on Immunity
- Drug Therapeutics During Pregnancy
- Understanding Sex and Gender in Mental Health
- Autoimmunity, Autoimmune Disease, and Sex Bias
- Sex and Gender Differences in Irritable Bowel Syndrome

The Influence of Sex and Gender on Disease Expression and Treatment

- **Sexual Dimorphism in Metabolic Bone Disorders**
- **Cardiovascular Disease in Women: A Focus on Heart Failure**
- **Sex and Gender Differences in Pulmonary Function and Health**
- **The Neural Basis of Sex Differences in Pain**
- **Sex Differences in Substance Abuse and Treatment**

CME or CPE credit can be awarded to eligible candidates who successfully complete these courses

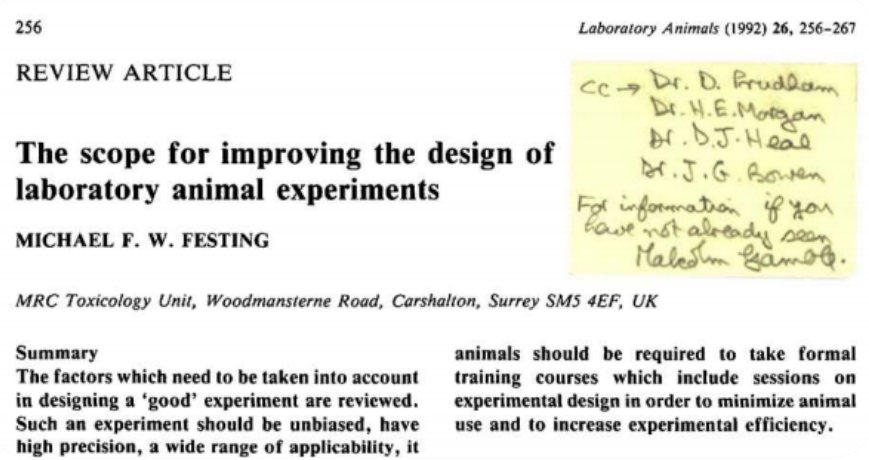
Good Experimental Design (and Reporting) Underlies Rigor and Reproducibility of Findings

Five requirements for a "good" experimental design:

- Be unbiased
- Have high precision
- Have a wide range of applicability
- Be simple
- Have the ability to calculate uncertainty

COX, D.R. *Planning Experiments*, John Wiley and Sons, New York, 1958.

Factorial Design: Addressing Sex as a Second Independent Variable



256 *Laboratory Animals* (1992) 26, 256–267

REVIEW ARTICLE

The scope for improving the design of laboratory animal experiments

cc → Dr. D. Prudham
Dr. H.E. Morgan
Dr. D.J. Head
Dr. J.G. Bowen
For information if you have not already seen Malcolm Gamble.

MICHAEL F. W. FESTING

MRC Toxicology Unit, Woodmansterne Road, Carshalton, Surrey SM5 4EF, UK

Summary
The factors which need to be taken into account in designing a 'good' experiment are reviewed. Such an experiment should be unbiased, have high precision, a wide range of applicability, it animals should be required to take formal training courses which include sessions on experimental design in order to minimize animal use and to increase experimental efficiency.

Review *TRENDS in Pharmacological Sciences* Vol.24 No.7 July 2003 341

ELSEVIER

Principles: The need for better experimental design

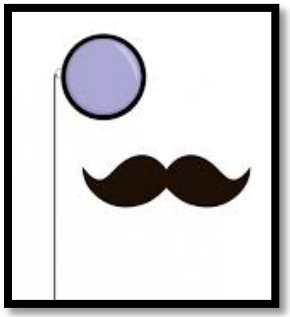
Michael F.W. Festing

c/o FRAME (Fund for the Replacement of Animals in Medical Experiments), Russell and Burch House, 96–98 North Sherwood Street, Nottingham NG1 4EE, UK

"The importance of variables can often be evaluated efficiently using factorial experimental designs, without any substantial increase in the overall number of animals."

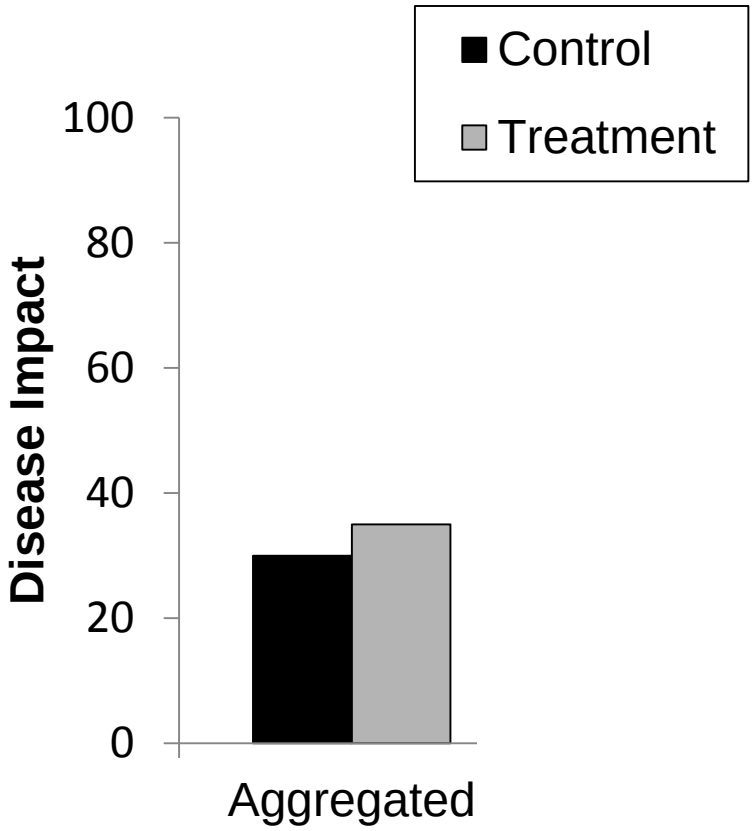
Lab Anim. 1992 Oct;26(4):256-68.; *Exp Gerontol*. 1997 Jan-Apr;32(1-2):39-47.; *Trends Pharmacol Sci*. 2003 Jul;24(7):341-5

Factorial Experimental Design

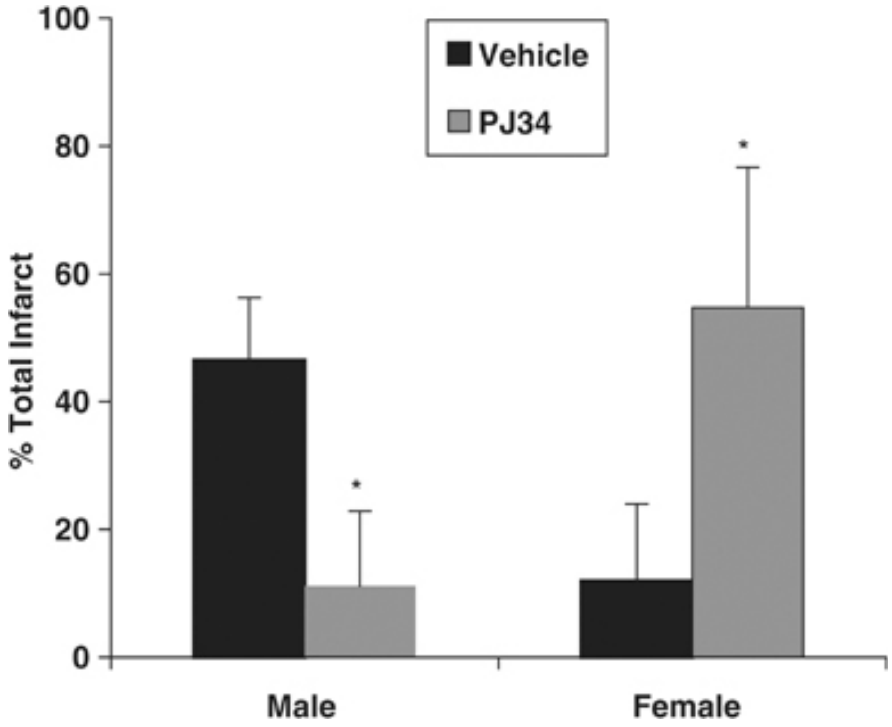


Control	--
Treatment	++

Biological/Disease Impact of Experimental Design



Real Life



The effects of the selective poly-ADP ribose polymerase (PARP-1) inhibitor PJ-34 in wild-type (WT) mice of both genders. Treatment with PJ-34 at ischemic onset reduced total infarction in male mice compared with saline-treated controls ($P < 0.001$). A significant increase in ischemic damage was seen in PJ-34-treated females compared with control ($P < 0.001$).

McCullough LD, et al. *Journal of Cerebral Blood Flow & Metabolism* (2005) **25,** 502–512.

Males and Females in Preclinical Research: The Landscape

- Congressional interest and inquiries
  - Research for All Act (Jim Cooper, D-TN, Cynthia Lummis, R-WY)
  - Rosa DeLauro (D-CT), Nita Lowey (D-NY) report language
  - Briefing to enhance understanding (July 2014)
- Developing NIH policies for deliberate approach to considering sex in preclinical research:
  - Trans-NIH Sex Differences in Research Working Group
  - RFI released (Sept 2014)
- Journal editors and publishers guidelines
  - Increasingly reference requirements to report sex

Accounting for Sex in Animals

nature biotechnology

A mouse knockout library for secreted and transmembrane proteins

Tracy Tang[1], Li Li[2], Jerry Tang[2], Yun Li[2], Wei Yu Lin[3], Flavius Martin[3], Deanna Grant[1], Mark Solloway[1], Leon Parker[4], Weilan Ye[4], William Forrest[5], Nico Ghilardi[1], Tamas Oravecz[6], Kenneth A Platt[6], Dennis S Rice[6], Gwenn M Hansen[6], Alejandro Abuin[6], Derek E Eberhart[6], Paul Godowski[3], Kathleen H Holt[6], Andrew Peterson[1], Brian P Zambrowicz[6] & Frederic J de Sauvage[1]

UCDAVIS KOMP Repository KNOCKOUT MOUSE PROJECT

NIH The Common Fund

- Reproducibility, rigor, generalizability, and utility
  - Broad phenotypic screen of 472 knock-out lines
    - Validated assays, relevant to therapeutic areas
    - All animals screened at the same age, same order of assays
    - Results reported for M/F aggregated, M alone, and F alone
- One of many approaches to account for sex as a biological variable

Tang T, et al. A mouse knockout library for secreted and transmembrane proteins. *Nat Biotechnol.* 2010;28:749-55.

NIA Interventions Testing Program

- Standardized preclinical evaluation of health-span prolonging interventions ("anti-aging" treatments)
- Test subjects = male and female genetically heterogeneous mice, bred as 4-way cross
- Compares multiple experimental agents to two control groups
- Sufficient numbers of male and female mice tested:
  - 80% chance of detecting an increase/decrease in lifespan of about 10%

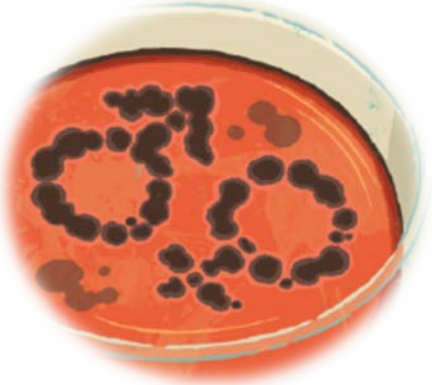
17αEstradiol: Extended lifespan in males but not females

Rapamycin: Extended lifespan in both sexes

NDGA, Aspirin: significant lifespan extension in males

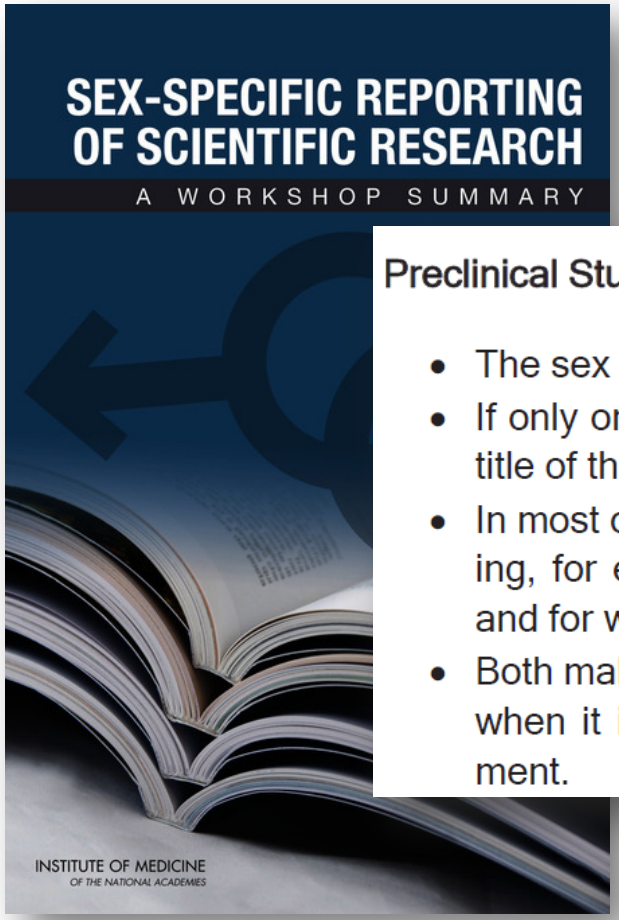
Miller et al. An Aging Interventions Testing Program: study design and interim report. *Aging Cell*. 2007;6:565-75.

How Important Is the Sex of Cells to Cell Function and Disease?



- Sex of origin knowable for cells derived from tissues, primary cells/cultures
  - *Usually* easy to record, report but not always straightforward to ID sex
- Incorporating consideration of sex using cells has unique challenges
  - Authentication of cell lines, genomic instability…
- *If* sex-related information is available and reliable, benefits include:
  - Better understanding of sex-affected mechanisms, processes
  - Present new questions for other investigators

Reproducibility Also Requires Reporting

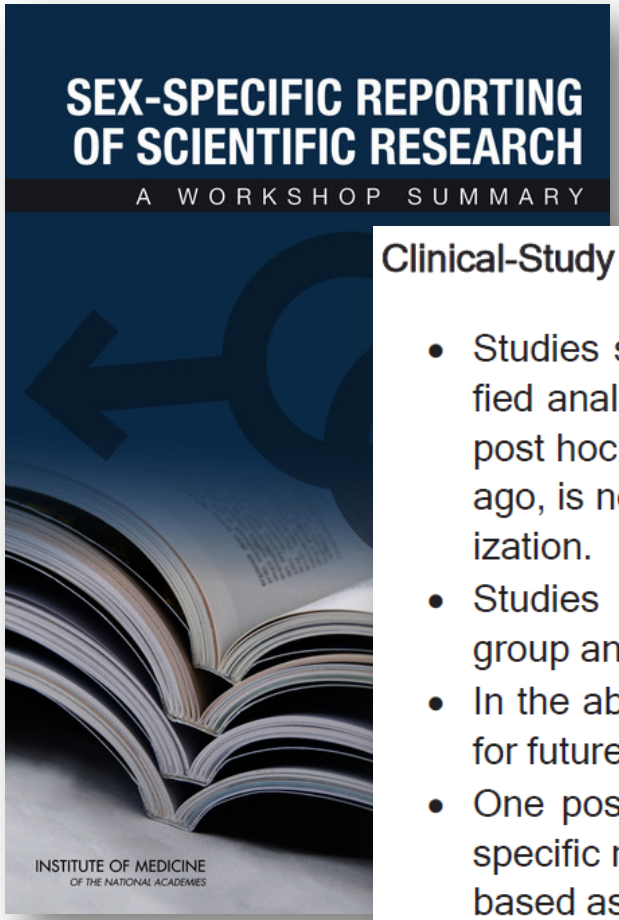


Preclinical Studies

- The sex of the animals studied should be reported.
- If only one sex of an animal was studied, this should be indicated in the title of the article.
- In most cases, the sex of origin of cells used should be reported (excluding, for example, immortalized cell lines, which are highly transformed and for which the sex of the original cells may not be relevant).
- Both male and female animals should be studied when appropriate; and, when it is possible, both sexes should be studied in the same experiment.

http://www.iom.edu/Reports/2012/Sex-Specific-Reporting-of-Scientific-Research.aspx

Reproducibility Also Requires Reporting

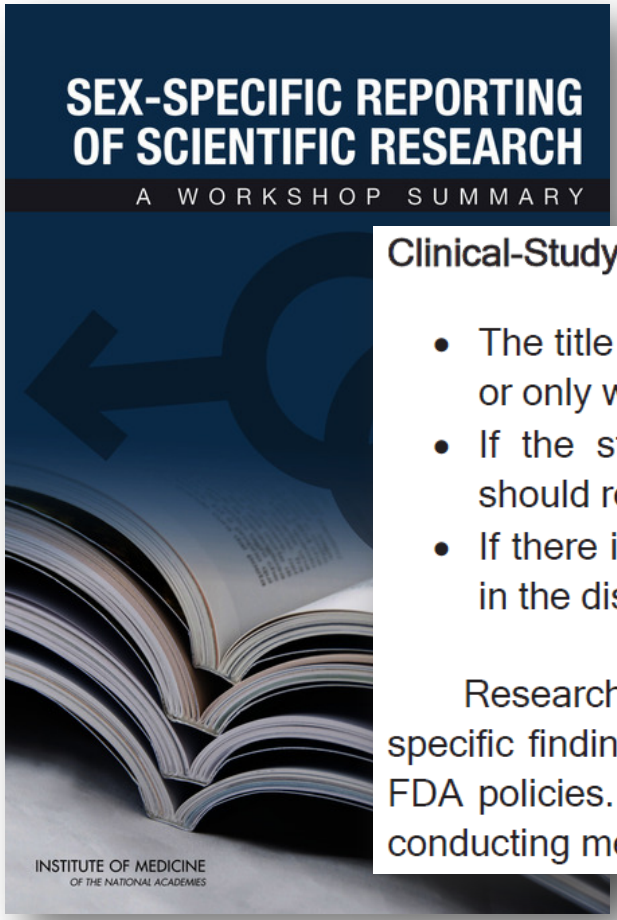


Clinical-Study Design

- Studies should be designed with stratified randomization by sex; stratified analyses should not just be conducted post hoc. Simply mandating post hoc subgroup analyses today, on a study that was started 10 years ago, is not necessarily valid, because it will probably violate the randomization.
- Studies should be designed with adequate statistical power for subgroup analyses and to test for interactions.
- In the absence of adequate power, raw data should be archived by sex for future pooling and meta-analysis.
- One possible criterion for requiring the analysis and reporting of sex-specific results should include an a priori reasonable likelihood that sex-based associations might exist.

http://www.iom.edu/Reports/2012/Sex-Specific-Reporting-of-Scientific-Research.aspx

Reproducibility Also Requires Reporting



Clinical-Study Reporting

- The title and abstract should indicate whether a study involved only men or only women.
- If the study design allows identification of sex differences, journals should require authors to present these results.
- If there is an inability to identify sex differences, this should be reported in the discussion of the limitations of the study.

Researchers should be allowed to report inconclusive or descriptive sex-specific findings as raw data in electronic-only appendixes to meet NIH and FDA policies. As above, this will make the data available to researchers for conducting meta-analyses.

http://www.iom.edu/Reports/2012/Sex-Specific-Reporting-of-Scientific-Research.aspx

Does animal experimentation inform human healthcare? Observations from a systematic review of international animal experiments on fluid resuscitation

Ian Roberts, Irene Kwan, Phillip Evans, Steven Haig

- New drugs and procedures are usually tested in animals before conducting clinical trials
- Validity of animal experiments is essential for human health care and fundamental to animal welfare
- A systematic review of animal experiments on fluid resuscitation found that most studies were underpowered and provided little information on possible bias
- Systematic reviews of animal experiments allow a more objective appraisal of the evidence and reduce the chance of false negative results
- Systematic reviews across species would help determine whether the results are generalizable to humans

BMJ. 2002 Feb 23;324(7335):474-6

Research with Animals: Implications for Human Health

- Animal experiments inform human health care only if their results are valid and generalizable
- Issues: bias, small sample size
- Options:
  - Systematic reviews to avoid duplication of experiments
  - Prospective registration
- Consider the 3 R's of animal research first suggested by William Russell:
  - Replacement
  - Reduction
  - Refinement

BMJ. 2002 Feb 23;324(7335):474-6

Let's Talk

McCullough *et al. Biology of Sex Differences* 2014, **5**:15
http://www.bsd-journal.com/content/5/1/15

BIOLOGY OF SEX DIFFERENCES

REVIEW Open Access

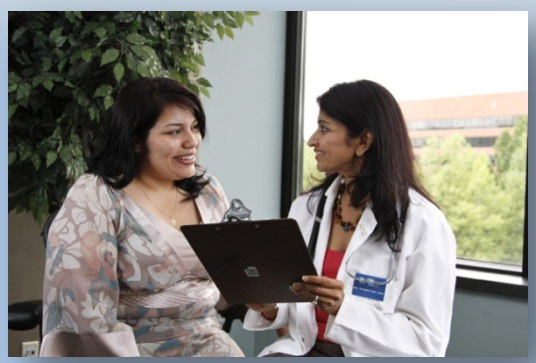
NIH initiative to balance sex of animals in preclinical studies: generative questions to guide policy, implementation, and metrics

Louise D McCullough[1], Geert J de Vries[2], Virginia M Miller[3,4*], Jill B Becker[5], Kathryn Sandberg[6] and Margaret M McCarthy[7]

The FASEB Journal • Life Sciences Forum

First steps for integrating sex and gender considerations into basic experimental biomedical research

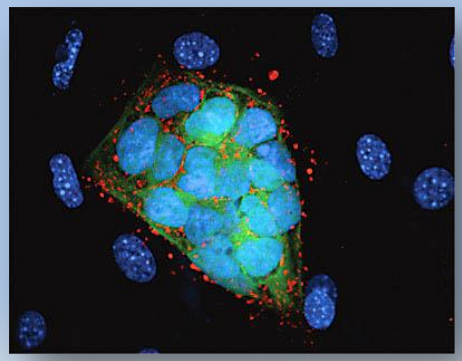
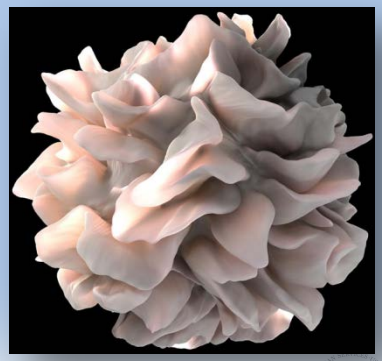
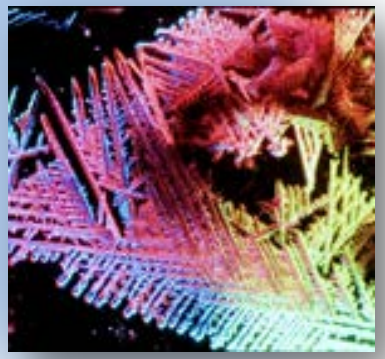
Stacey A. Ritz,[*,†,1] David M. Antle,[§] Julie Côté,[†,§] Kathy Deroy,[‖] Nya Fraleigh,[¶] Karen Messing,[†,‡] Lise Parent,[†,#] Joey St-Pierre,[‖] Cathy Vaillancourt,[†,‖] and Donna Mergler[†,‡]

NIH...

Lawrence.Tabak@nih.gov

Turning Discovery Into Health

NIH National Institutes of Health
Office of Research on Women's Health