

Consideration of Sex as a Biological Variable in NIH-funded Research¹

Purpose and Background

NIH's mission is to seek fundamental knowledge about the nature and behavior of living systems and the application of that knowledge to enhance health, lengthen life, and reduce illness and disability. There is growing recognition that the quality and generalizability of biomedical research depends on the consideration of key biological variables, such as sex. NIH has long appreciated the importance of enrolling men and women in clinical research so as to provide a basis for application of results and to identify factors that affect disease course and treatment outcome. Women now account for roughly half of all participants in NIH-supported clinical research, which is subject to NIH's Policy on the Inclusion of Women in Clinical Research.² However, preclinical research studies continue to rely heavily on male animals and/or omit reporting of the sex of animal subjects; this is particularly problematic in those studies intended to inform understanding of diseases and conditions affecting both sexes. Just like randomization, blinding, sample-size calculations, and other basic design elements, consideration of sex is a critical component of rigorous experimental design.³ Failure to account for sex as a biological variable may undermine the rigor, transparency and generalizability of research findings. The NIH expects researchers to study both male and female vertebrate animals and humans, where applicable, thereby improving our understanding of health and disease in men and women.

NIH policy on the consideration of sex as a biological variable. Anticipated changes to FY2016 research grant applications, to be in effect for FY2017 funding, will include accounting for sex as a biological variable in the Research Strategy section. In this section, applicants are asked to “explain how relevant biological variables, such as sex, are factored into research designs and analyses for studies in vertebrate animals and humans.” Furthermore, “strong justification from the scientific literature, preliminary data, or other relevant considerations, must be provided for applications proposing to study only one sex.” Anticipated changes to review criteria will include evaluation of the adequacy of the research plan with regard to consideration of sex as a biological variable.

Considering Sex as a Biological Variable in Research

Definitions of sex and gender. Research findings may be influenced by sex and/or gender, and it is important to use these terms appropriately. Women and men are characterized by both sex and gender, as highlighted in the Institute of Medicine report entitled Exploring the Biological Contributions to Human Health: Does Sex Matter? (2001).⁴ Sex is a biological variable defined by characteristics encoded in DNA, such as reproductive organs and other physiological and functional characteristics. Gender refers to social, cultural, and psychological traits linked to human males and females through social context. In most cases, the term “sex” should be used when referring to animals. Both sex and gender and their interactions can influence molecular and cellular processes, clinical characteristics, as well as health and disease outcomes.

Literature review. Consider and describe how sex and gender may influence the research question(s) at hand. Conduct a review of the human clinical literature and any relevant preclinical literature.⁵ If there are differences between males and females in previous preclinical or clinical studies, this would provide

¹ This document serves as a companion reference to NIH Guide Notice [NOT-OD-15-102](#).

² <https://grants.nih.gov/policy/inclusion/women-and-minorities/guidelines.htm>

³ Collins FS, Tabak LA. Policy: NIH plans to enhance reproducibility. Nature. 2014 Jan 30;505 (7485): 612-613.

<https://www.nature.com/news/policy-nih-plans-to-enhance-reproducibility-1.14586>

⁴ <https://www.iom.edu/Reports/2001/Exploring-the-Biological-Contributions-to-Human-Health-Does-Sex-Matter.aspx>

⁵ For literature review tools and resources, see references below for Jenkins & Wilson (2012) and Oertelt-Prigione, Gohlke, Dunkel, Preissner, & Regitz-Zagrosek (2014).

a strong rationale for building consideration of sex into the research design and analyses of data. The absence of previous study data in an area of research does not, by itself, constitute strong justification to study only one sex.

Research design, data analysis, and reporting. Choice of research design depends on a variety of considerations, including preliminary data, past studies, scientific literature review, scope of the work, and specific questions and hypotheses to be addressed. Appropriate strategies that consider sex as a biological variable depend on the context of the research question, existing knowledge about male and female biology and behavior in a given area of research, as well as available methodology. Where little or no sex-specific data is available, sex-specific hypotheses may not be possible, whereas previously observed sex differences may prompt sex-specific hypotheses.

Experimental design should include consideration of effect size and power calculations to determine the number of samples/subjects in the study, if applicable. Researchers working with animal models should consider if and how the female estrous cycle is relevant for experimental design and analysis; it may be relevant for some research questions and not others.⁶ For studies using both sexes, develop a data analysis plan prospectively that, at a minimum, provides for the collection of data disaggregated by sex. Acknowledge limitations in the applicability of findings that may arise from the samples, methods, and analyses used, in the research plan as well as in progress reports and publications.

In progress reports and publications, provide the sex of research subjects and/or materials, when possible. Report when sex differences are, or are not, detected in analyses, which may be valuable for future research and meta-analysis.

Single-sex studies. Applicants must provide strong justification for applications proposing to study only one sex. Such justification may include the study of sex-specific conditions or phenomena (e.g., ovarian or prostate cancer), acutely scarce resources (e.g., non-human primates), or investigations in which the study of one sex is scientifically appropriate. The absence of evidence regarding sex differences in an area of research does not constitute strong justification to study only one sex.

Further Resources

Additional resources that may provide a starting point regarding sex as a biological variable in biomedical research are located on the NIH Office of Research on Women's Health web site, Studying Sex to Strengthen Science⁷; these include research and training materials⁸ such as online courses, resources on methods and techniques, and scientific reports. The site also includes research summaries⁹ on specific topical areas with known sex/gender differences.

The following articles and web site may provide useful resources related to the consideration of sex as a biological variable:

1. Becker JB, Arnold AP, Berkley KJ, Blaustein JD, et al. Strategies and methods for research on sex differences in brain and behavior. *Endocrinology*. 2005; 146:1650-73.
2. Holdcroft A. Integrating the dimensions of sex and gender into basic life sciences research: methodologic and ethical issues. *Gend Med*. 2007; 4 Suppl B:S64-74.

⁶ Prendergast, BJ, Onishi KO, Zucker I. Female mice liberated for inclusion in neuroscience and biomedical research. *Neurosci Biobehav Rev*. 2014;40:1–5. 3

⁷ <http://orwh.od.nih.gov/sexinscience/index.asp>

⁸ <http://orwh.od.nih.gov/sexinscience/researchtrainingresources/index.asp>

⁹ <http://orwh.od.nih.gov/resources/sexgenderhealth/>

3. Hughes, RN. Sex does matter: comments on the prevalence of male-only investigations of drug effects on rodent behavior. *Behav Pharmacol.* 2007;18: 583-589.
4. McCarthy MM, Arnold AP, Ball GF, Blaustein JD, et al. Sex differences in the brain: the not so inconvenient truth. *J Neurosci.* 2012; 32:2241-7.
5. Nieuwenhoven L, & Klinge I. Scientific excellence in applying sex- and gender-sensitive methods in biomedical and health research. *J Women's Health.* 2010; 19: 313-321.
6. Prendergast, BJ, Onishi KO, Zucker I. Female mice liberated for inclusion in neuroscience and biomedical research. *Neurosci Biobehav Rev.* 2014;40:1–5.
7. Ritz SA, Antle DM, Côté J, Deroy K, et al. First steps for integrating sex and gender considerations into basic experimental biomedical research. *FASEB J.* 2014; 28:4-13.
8. Gendered Innovations web resources: <http://genderedinnovations.stanford.edu/methods-sex-and-gender-analysis.html> Accessed May 15, 2015.

The following may provide useful literature review tools and resources related to the consideration of sex as a biological variable:

1. Jenkins M & Wilson J. (2012). Finding the Evidence: A Sex-and Gender-Specific Medicine (SGSM) PubMed Search Engine Tool. Lubbock: Texas Tech University School of Medicine Health Sciences Center. (Instructions for accessing the database can be found here: http://genderedinnovations.stanford.edu/methods/Workshop_B_LWBSHI_Search_Engine.pdf).
2. Oertelt-Prigione S, Gohlke BO, Dunkel M, Preissner R, Regitz-Zagrosek V. GenderMedDB: an interactive database of sex and gender-specific medical literature. *Biol Sex Differ.* 2014; 5:7.
3. What a Difference Sex and Gender Make: A Gender, Sex and Health Research Casebook, (Canadian Institutes of Health Research, Institute of Gender and Health) <http://www.cihr-irsc.gc.ca/e/44734.html>