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

Request for Information

The NIH has formed a trans-NIH working group to inform the development of these policies. This Request for Information (RFI) seeks input from the research community and other interested stakeholders on the following topics regarding the consideration of sex as a biological variable in biomedical research. Public comment is sought for but not limited to the following:

For more information, see NIH Guide Notice, NOT-OD-14-128.

Comments Deadline: October 13, 2014 11:59:59 PM EDT


Comment 1:

Whether consideration of sex as a biological variable is an issue affecting the reproducibility, rigor, and/or generalizability of research findings.

We would argue that this is indeed the case, i.e. that a lack of systematic focus on designing and then analyzing and reporting studies by sex is a major impediment to reproducibility, rigor, and/or generalizability of research findings if the study refers to the whole population. That is, we understand that studies conducted in males (accounting for hormonal status) can be conducted with rigor and be designed to be reproducible in males, and the same is true for studies in females alone. Not accounting for hormonal status in the context of age in either sex might be considered less rigorous. HOWEVER, studies conducted in only one sex cannot be generalized to males and females which can have major implications for clinical outcomes, drug efficacy, and other study outcomes. There could be a potential “down-side” to a blanket statement from NIH to consider sex as a biological variable, without clear guidelines, in that the use of female animals by an uninitiated or uniformed investigator could lead to large variability and/or loss of rigor or even interpretability. Thus, it is critical to educate the field in general about the methods necessary to design and interpret studies of sex differences. “Sex” is a complicated variable, and just like any other complex variable, necessitates proper study design that reflects that complexity. See our comments in #3, below, for cells/tissues.

Clinical examples include, among others, the following. Recent discoveries in neuroscience illustrate the importance of evaluating and analyzing sex differences. Neurobiology clearly demonstrates sex differences in the brain that are outside of those related to reproductive function. Variability in brain development and functioning across the lifespan has significant impact on a number of functional domains, including stress response, emotions, short and long-term memory, and “appetitive-behaviors” such as drinking, smoking, and drug use and dependence. In fact, preclinical and clinical work has demonstrated that sex differences in these brain circuits relate to behavior and to sex differences in the incidence of multiple psychiatric and neurologic disorders. This knowledge has not been used systematically to develop new therapeutics, understand variability in response to treatments, or enhance treatment efficacy.

Failure to consider the impact of sex differences can lead to substantial clinical impact and harm. Ambien (zolpidem) provides a recent example for which differential metabolism and target effects differ by sex, leading to different efficacy and side effects in women compared to men. Women and men metabolize the drug differently, and prior research demonstrates sex differences (in preclinical and clinical studies) in the impact of the neurotransmitter, gamma aminobutyric acid (GABA), on the brain (and zolpidem “works on” “GABA-pathways). Thus, sex-
specific mechanisms may contribute to a drug’s attenuated or enhanced efficacy. Failure to use sex-specific or sex-dependent knowledge to develop CNS therapeutics will miss therapeutic opportunities. Likewise, failure to analyze treatment effects by sex may lead to adverse consequences for the patient.

Other examples of failures to consider sex as a biological variable that may limit rigor and scientific discovery by missing biologic associations that differ by sex, include the following (among others).
1) greater incidence and prevalence of preserved ejection fraction (pEF) heart failure, compared to reduced ejection fraction (rEF) heart failure in women than men, and the limited progress in preventing/treating pEF compared to rEF heart failure in recent decades;
2) greater susceptibility of women to develop diabetes/glucose intolerance with statin therapy than for men;
3) disproportionate impact of diabetes on CVD risk in women than in men, and again the lagging research on mitigating these risks;
4) higher risk of autoimmune disorders, osteoporosis, depression, and many other conditions in women than men, with few new insights to help close these gaps.

Due to the lack of inclusion of female animals and cells, and specific study designs and analyses by sex, the science is underdeveloped and today we are only in the early stages of explaining the underlying physiology of observed sex differences across many chronic diseases.

Comment 2:
Areas of science (e.g., cancer, neuroscience) or phases of research (e.g., basic, translational) conducted with animals that have the greatest opportunity or need for considering sex as a biological variable.

Preclinical research, at the basis of translation, has contributed to almost every medical advance of the last century. Without it, we would not have insulin for diabetes, statins for cardiovascular risk, or chemotherapy for leukemia (1). Thus, in order to understand the myriad of findings on sex differences in the incidence of almost every chronic disease, we must insure that adequate research on sex differences at the preclinical level is supported. Since all cells have a sex, incorporating the analysis and reporting of results by sex is crucial to extending our knowledge of how sex affects disease. However, at the preclinical level, we also understand that it is not only the inclusion of female and male animals, but also a question of study design and when in the course of the hypothesis development is it practical, useful, and heuristic.

The following focuses on four diseases that have high incidence and substantial sex differences clinically that are critical to investigate.

Cardiovascular Disease (CVD): Although we know more about CVD than most other diseases, there is much we do not understand about the physiologic mechanisms that underlie its sex differences. While there have been advances in the study of sex differences in vascular function, this has not connected directly to understanding sex differences observed in CVD (2). Most animals used in research in the areas of physiology, pharmacology, and endocrinology—the basic sciences most closely aligned with CVD research—are male or unspecified (3). Stroke is the third leading cause of death in women; each year, approximately 55,000 more women than men experience a stroke (4). Animal studies have shown that the molecular pathways that affect ischemic outcomes in stroke differ in male and female mice, which may have implications for sex-specific treatments (5). Unfortunately, most animals used in research in the areas of physiology, pharmacology, and endocrinology associated with CVD are male or unspecified (3).
Disorders of the Brain afford multiple examples of significant sex differences in incidence (depression, schizophrenia, autism, multiple sclerosis, and Alzheimer's disease). Below, we use two examples of brain disorders to make our point, given that for each of these, women have an almost two-fold increased risk.

**Depression:** Depression is the leading cause of disease burden worldwide, and twice as many women than men suffer from depression with direct costs exceeding $20 billion annually (6-8). In fact, by 2020, the comorbidity of depression and CVD will be the number one cause of disability worldwide (9). Research now clearly demonstrates that sex hormones play a role in the development of brain regions that regulate mood and response to stress (6, 10). Major endocrine changes throughout a woman’s life, including puberty, pregnancy, and menopause, have been directly linked to increased risk of depression. In adulthood, sex hormones interact with stress hormones to regulate brain activity under stressful conditions (11, 12). Importantly, women with depression show disruptions in the relationship between estradiol, stress hormones and brain activity. Understanding how sex hormones change the way our brain deals with stress will help elucidate sex-dependent pathways that lead to depression, which will, in turn, help researchers design clinical trials.

Yet basic neuroscience, pharmacology, and physiology—all related to depression—continue to have strong sex biases. In neuroscience, despite substantial sex differences in risk for most brain disorders, animal studies that rely exclusively on males outnumber studies in females 5.5 to 1. Fewer than 45 percent of animal studies on anxiety and depression use female lab animals, despite the fact that these disorders are twice as common in women (13). A recent example is a study in Cell 2014 (14) describing potential mechanisms to contribute to explaining how exercise may attenuate depressive-like behaviors. Again, in spite of the increased prevalence of depression in women, this study was performed only in male mice.

**Alzheimer’s Disease:** Two-thirds of the 5.1 million people currently suffering from Alzheimer’s disease (AD) are women (15, 16). Women are also the primary caregivers of adult loved ones with AD, meaning they shoulder both the risks and the burdens of the illness. Even though a woman’s overall lifetime risk of developing AD is almost twice that of a man, the prevailing thinking in the field is that this is simply because women live longer. However, although there have been some preclinical and clinical findings suggesting sex-dependent factors other than age itself, this has never been systematically investigated nor have potential treatments been evaluated systematically in the context of sex. This is critical given that, left untreated, AD will cost the U.S. up to a trillion dollars by 2025 and bankrupt Medicare.

**Autoimmune Diseases:** The largest female:male ratio in incidence of diseases in medicine is in the field of autoimmune disorders, such as multiple sclerosis, rheumatoid arthritis and systemic lupus erythematosus, for which the ratios begin at 2:1. In addition, there is emerging data indicating that sex affects disease course as well as the underlying pathophysiology of these diseases, which is particularly manifest during key endocrine transitions throughout the lifecycle, such as puberty, pregnancy and menopause/andropause. However, the causes of these sex-dependent discrepancies are still unknown. Sex-dependent and sex-specific therapies are now being explored, such as estriol and testosterone treatments in MS, and there is increasing evidence that basic immune mechanisms differ between males and females with these diseases. Sex differences in autoimmune disease remain a major unmet need and area for potential therapeutic development.

**Comment 3:**
Areas of science or phases of research conducted with cells and/or tissues that have the greatest opportunity or need for considering sex as a biological variable.

As the 2001 Institute of Medicine reported, “Every cell has a sex”, and we must understand the implications of this for health and disease.

The consideration of genetics, evolving ideas of epigenetics, gonads, and hormonal stimulation combine to make this a complicated question. That is, one can consider cells and/or tissues that compare XX versus XY genotypes, but those are not the only configurations found in humans (e.g., XX with different degrees of X inactivation, XO, XXY, etc.). Further, cellular history of exposure to gonadal steroid hormones changes future responses. It is critical to assess, at what point in the exposure process are comparisons by sex critical (17). These are some of the complex issues that must be considered in the design of studies of sex differences at the cellular and/or tissue levels.

Clinical examples of areas of science to pursue at the cellular level include (among others) the following.

**Cardiac Research:** Stem cell investigator, Dr. Doris Taylor, (who leads regenerative medicine at University of Texas in Houston) has shown that female and male stem cells behave differently when used to populate a cardiac scaffold, with female stem cells leading to greater stiffness in the heart compared with those created with male stem cells (18).

Delay in the onset of coronary disease in women is related to a loss of certain bone marrow progenitor cells. In animal studies when female progenitor cells are injected into male mice, there is a delay in progression of disease.

**Lung Cancer:** Lung cancer takes the lives of more U.S. women than breast, ovarian, and uterine cancers combined. Women have a higher incidence than men of adenocarcinoma (i.e., a type of cancer that begins in the glandular cells), the most common type of non-small cell lung cancer and the one that accounts for over 80 percent of lung cancers. Differences that increase women’s susceptibility to lung cancer may start at the molecular level. Genetic mutations associated with certain cancers occur at a higher frequency in women, Asians, and people who never smoked (19, 20). Researchers have also found that sex hormones, particularly estradiol, influence lung cancer development and mortality (19). A possible link between estradiol and lung cancer growth has raised questions about the impact of menopausal combination hormone therapy (MHT) in women with lung cancer, with at least one large clinical trial demonstrating an increased risk of dying when MHT is taken after the lung cancer develops (21). Estrogens are also thought to make women metabolize nicotine faster than men, a finding that may explain smoking behavior and decreased efficacy of nicotine replacement therapy in women (19, 22). These findings demonstrate the importance of understanding sex differences in lung cancer at the biological level to prevent cancer, improve treatment, and increase survival rates for the disease (19). One of the most significant advancements in lung cancer therapy in the last several decades (for both women and men) is that of personalized medicine, where unique molecular and genetic mutations guide specific drug therapies. Interestingly, sex has been found to play a dominant role in the incidence of specific genetic mutations. For example, an adenocarcinoma of the lung in a woman is far more likely to express specific genetic mutations in proteins found on the surface of cells (i.e., epidermal growth factor receptors, or EGFR) than a similar tumor present in a man, and these mutations are predictive of a marked therapeutic response to specific targeted therapies (i.e., tyrosine kinase inhibitors, gefitinib and erlotinib) (19,23,24).
Depression
Depression is often accompanied by an impairment in biologic pathways that regulate hormone production (i.e. endocrine dysregulation), meaning that the production and levels of sex and "stress" steroid hormones are abnormal (6). Women are at a higher risk for these conditions. We also know that, when certain adverse prenatal conditions occur, adult female mice express “depressive-like” behaviors more than male mice (9, 25, 26). Animal studies in genetically engineered mice have demonstrated that this sex difference may vary depending on the genotype (9, 26-29). Clinical and basic science research have led to the discovery of genetic abnormalities in regions of the brain associated with the regulation of mood, allowing links to be made between genes, brain, and behavior (9, 29).

Studies of sex differences must start at the cellular and animal-research levels. We suggest that NIH base funding and regulatory approval on research should include design plans of sex differences hypothesis-testing, including adequate numbers of female subjects, or provide a sound rationale for why the research focuses on only one sex. Moreover, results should be reported by sex so that potential sex differences can be evaluated.

Comment 4:
Main impediments (e.g. scientific, technical, and other) to considering sex as a biological variable in research.

The requirement for preclinical and clinical studies to include females in the design of the study is not a simple request. Barriers include the following challenges at the level of:
- Study design and analysis (scientists need to be educated about how to study sex differences for their question of interest)
- NIH review committee expertise
- Adequate funding in an economically challenging time

These requirements will demand larger budgets, which includes increased study time and design complexities, and recruitment of experts that are experienced in designing studies of sex differences (which is not simply “adding females” or “separating one’s data by sex”). Basic science and drug research are often marred by a desire to avoid “inconveniences” associated with potential variations across the menstrual cycle in female animals. However, designing studies to investigate these sex differences can also uncover valid similarities between the sexes when factoring in sources of variability.

Specific training for research investigators in the importance of including sex as a biological variable, including hormonal and genetic aspects that may change over the lifecourse, and how to incorporate these into new and existing research is needed, and could be co-sponsored by the NIH.

Comment 5:
Ways in which NIH can facilitate the consideration of sex as a biological variable in NIH-supported research

NIH Policies
Ultimately, as a research community we strive to create knowledge that will inform more individualized treatments for diseases and a more responsive healthcare system by applying a sex-dependent and sex-specific lens to the creation of this knowledge base. Having NIH motivate consistent and pervasive work on sex differences in health and disease in preclinical
and clinical modeling is a critical step to operationalize this goal. It will be imperative for NIH to couple the requirement of sex differences analyses with increased funding to meet that requirement. However, increased funding is not the sole answer. Given limited resources, it is essential to map out a comprehensive plan of action.

The plan might include:
- Review of currently available data on which new studies could capitalize;
- Develop database of knowledge as sex differences studies are implemented;
- Catalyze and fund models that include public-private collaborations;
- Increase expertise of sex differences on study review panels and among the scientists applying for these funds;
- Add “Plan for Consideration of Sex Differences” in the Human Subjects section of NIH grants;
- Require reporting of all data from NIH-funded studies to include a report of sex-specific results.

In summary we recommend having biomedical research, where applicable, include adequate numbers of female research animals, include specific designs to address the sex differences questions (e.g., including hormonal status, genes, age, etc), and report the sex of the animals in the study. Studies of sex differences must start at the cellular and animal-research levels. This is true even for stem cell research, and thus we support the need for cell lines that are differentiated by sex. NIH could base funding and regulatory approval on research plans that either include study designs of sex differences, including adequate numbers of female subjects, and the reporting of sex-stratified findings or provide a sound rationale for why the research focuses on only one sex. The current policies at NIH must be more actively enforced and strengthened. Proposals that include adequate numbers of women and men, and include a robust design plan and analysis, publication, and distribution of findings could receive higher scores and priority for funding. New mechanisms for research opportunities in sex differences should be developed and funded.

**Medical and Scientific Journals**
The NIH should mandate that researchers receiving NIH funding report the sex of lab animals and human subjects in publications in medical and scientific journals as well as sex-specific or sex-dependent results.

**Online Gateways**
We recommend the establishment of an online gateway in which investigators could access sex-stratified analyses derived from research that is conducted and supported by NIH. NIH already requires the inclusion of adequate numbers of women and of underrepresented groups in clinical trials, as well as the reporting of such inclusion. These data, however, are not available to other researchers and clinicians, which could accelerate our understanding of sex differences and similarities, and why they matter.

**IRBs**
NIH should educate applicants and their organizations’ IRBs on the importance of design and inclusion of female subjects in research as well as the reporting of sex-specific and sex-dependent results.

**Comment 6:**
The history of sex differences in medicine began in reproduction. However over the course of many years, it was evident that sex differences are pervasive in medicine, including most chronic diseases. Thus, an understanding of the causes of sex differences in diseases is at the heart of the development of so-called “personalized” or “precision” medicine. In order to build and extend the strengths of women's health and sex differences research, we must integrate clinical and population-level studies with preclinical studies to advance our knowledge of sex-specific disease biomarkers. There are some shared mechanisms for understanding sex differences in biology and pathology across organs and tissues (e.g., heart and brain). These shared mechanisms may involve hormones, genes, inflammatory pathways, growth factor signaling, vasculature factors, cell cycle and proliferation, and other peptides, mechanisms that will require preclinical work.

Although clinical translation is critical, basic science provides the foundation for translation. That is, an understanding of the basic mechanisms through which disease emerges or resilience is maintained is critical for guiding development of efficacious treatments. Although there is a substantial history to preclinical studies of sex differences in the regulation of development and functioning of brain and body tissues, there has been relatively little funding to maintain and grow this arena of work. This is, in part, a function of the fact that the prevailing ideology in medicine was that sex differences and women’s health research was primarily relegated to reproduction. However, we now know that even the liver expresses numerous sexually dimorphic genes, a finding discovered in mice. We now know that sex chromosome genes act in non-gonadal tissues to cause sex differences in behavior and traits, and that hormonal regulation of genes is the primary driver of sex differences, again findings that were critically discovered in preclinical studies.

The recent call for requiring preclinical studies in NIH-funded work is a long-time coming and necessary to change the prevailing ideology. A brief listing of the history of this underscores the importance of associating funding and supportive national policies with the requirement, since the call for this dates back to 1991.

- 1991: NIH supported a conference to initiate a research agenda on women’s health which became the foundation of the creation of the Office for Research in Women’s Health (ORWH).
- 1997: NIH published the “Agenda for Research on Women’s Health for the 21st Century”, under Ruth Kirschstein’s leadership as Deputy Director of NIH. Although this was critical in raising awareness as to the importance of sex differences in medical disorders, there was relatively little funding to support this arena of work.
- 2001: Institute of Medicine (IOM) reports on “every cell has a sex” in Exploring the Biological Contributions to Human Health: Does Sex Matter? Again, IOM recommends the promotion of research at the cellular level. However, there little funding followed the IOM call.
- 2010: IOM supported another workshop, specifically in neuroscience, calling together scientists, industry, and NIMH to raise the issue again with regard to instituting sex differences in neuroscience research specifically, including preclinical studies.
- 2010: This was underscored and extended more broadly to medicine in general by the Office for Research on Women’s Health (ORWH) 10-Year Strategic Plan.
2011-2012: Report of the Advisory committee on Research on Women’s Health detailing ORWH and other NIH support of women’s health research showing only approximately ~$32 million spent on sex differences research in years 2011 and 2012, out of the $30 billion invested by NIH yearly.

Thus, although there have been multiple calls over almost 25 years to change the prevailing ideology about sex differences in medicine and research in this arena, the research dollars have not followed the insights, even though the field has made important gains with support from NIH over the last 23 years.

We strongly support incorporating and requiring sex as a biologic variable in study designs, ranging from cell culture, to animal studies, to clinical research and trials. The failure to do so risks missing new discoveries and mechanisms that could lead to clinical benefit for both men and women.

These comments are from the Connors-Brigham Research Institute (BRI) Center for Research on Women’s Health and Gender Biology. Dr. Jill Goldstein (Director of Research) and Research Advisory Committee members: Drs. Julie Buring, Tanuja Chitnis, Hadine Joffe, Paula Johnson (Exec. Dir. Connors Center), Ursula Kaiser, Meryl LeBoff, JoAnn Manson, Cynthia Morton, Page Pennell, Kathryn Rexrode, Emily Stern, and Drs. Therese Fitzgerald (Director of Policy & Advocacy, Connors) and Jacqueline Slavik (Executive Director, BRI)