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COMMENTARY

Considering Sex as a Biological Variable in Biomedical Research

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Abstract

In June 2015, the National Institutes of Health (NIH) announced a new policy highlighting the expectation that sex as a biological variable (SABV) be factored into research designs, analyses, and reporting of vertebrate animal and human studies. NIH-funded research grants and career-development grants are now under this new policy and the first scientific reviews are complete. Since implementation of this policy, the research community has voiced concern about exactly how to study males and females, particularly in basic research. Investigators are asking: ''What does it mean to consider SABV?'' This commentary serves to provide some perspective.

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Purpose and Background

The Sex as a Biological Variable (SABV) policy is part of the National Institutes of Health's (NIH's) re-energized focus on the importance of rigor and transparency to reproducibility, including appropriate accounting for the potential influence of sex on experimental outcomes in preclinical research.[1–3] Just like randomization, blinding, sample-size calculations, and other basic design elements, consideration of sex is a critical component of experimental design.[2,4] Specifically:

- The NIH expects investigators to explain how relevant biological variables, such as sex, age, weight, and underlying health condition, are factored into research designs and analyses of studies in vertebrate animals and humans.
- This applies to basic, preclinical, and clinical research.
- Studies proposing to use only one sex should provide strong justification from the scientific literature or preliminary data to support this decision.
- Cost alone and absence of known sex differences are inadequate justifications for not addressing SABV.[5,6]

These are not new expectations. The NIH has always sought the most rigorous science—strict application of the scientific method to ensure robust and unbiased experimental design, methodology, analysis, interpretation, and reporting of results[5]—and assessment of these factors has always been implicit in peer review. However, the NIH has now formalized these expectations for grant applications and peer review.

It is important to point out that the language of the policy is purposefully broad and not prescriptive. The intrinsic goal is to encourage—never limit—the creative thinking and innovative ideas within each investigator. How researchers consider SABV must be driven by the science and context of the individual research question.[6]

Sex is a fundamental biological variable with profound consequences.[7] Underrepresenting female cells and animals in preclinical research has resulted in a poorer understanding of the biological, physiological, and pathophysiological mechanisms in the female compared with the male. Without data from females, it is impossible to determine whether results obtained in male cells and animals also apply to female cells and animals. Historical reliance on male vertebrate animals (e.g., rats, mice) in preclinical research has resulted in the generation of incomplete data available to inform translation to clinical trials enrolling both men and women.[8,9] And, these issues are not limited to the basic biological fields. A report of studies from the surgical literature revealed that for female-prevalent diseases, of those studies that stated the sex of the animals, only 12% studied female animals.[10]

We are asking investigators to consider the potential influence of sex and to address sex in the design and analysis of biomedical research. We would like to ensure that, from the very first idea about a biomedical research area, sex influences are examined. This will lead to a stronger foundation on which to build clinical research and trials and inform the community as to whether such influences will

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need to be factored into the design and power calculations in clinical trials.

Sex Is a Basic Biological Variable

Since the landmark Institute of Medicine report entitled *Exploring the Biological Contributions to Human Health: Does Sex Matter?*,[11] there has been an exponential expansion of basic science evidence and clinical data to support the concept that sex is a basic biological variable and that every cell has a sex. There are distinct differences in global gene expression patterns between male and female animals. In mice, the majority (50%–75%) of genes have been shown to be sex-biased (i.e., expressed at a different level in the two sexes) in tissues such as liver, fat, and muscle.[12] Seventy-two percent of active genes in the liver had sexually dimorphic expression, 68% in adipose tissue, 55% in muscle tissue, and 14% in brain tissue. While sexually dimorphic genes only displayed a mean difference of 8%–9% in expression level between males and females, this sex difference in the majority of genes could reflect fundamental sex differences in physiology.[13]

In humans, DNA methylation patterns in whole blood specimens are different for men and women for genes implicated in metabolic and cardiovascular disease.[14] *CYP1A1*, known to play a prominent role in metabolism of polycyclic aromatic hydrocarbons, is more active in women[15]; this may have bearing on the fact that female smokers have been shown to have higher levels of aromatic/hydrophobic DNA adducts in lung tissue than do male smokers. Epidemiological studies suggest that women may be at greater risk for developing lung cancer than men. Also, human female liver cells have more cytochrome *CYP3A* compared to male liver cells.[16–18] This is particularly significant as *CYP3A* actions account for metabolism of half of pharmacopeia drugs.[19]

Clinically, we have learned that, even though men and women should pay attention to the risk factors that affect heart health, the cardiovascular risks and responses to intervention differ between women and men. Low-dose aspirin has different preventive effects in men and women. In women, aspirin reduces risk of ischemic stroke, whereas, in men, low-dose aspirin therapy reduces risk of heart attack.[20–23] Cholesterol plaque in women might not build up into major artery blockages, but instead would spread evenly throughout artery walls.[24] Consequently, artery blockages can be more difficult to detect on coronary angiography in women, who may also present with subtle symptoms of ischemia but are still at high risk for myocardial infarction.[25,26] And, women experience higher rates of adverse drug reactions than men.[27,28]

Although many sex differences likely stem from a differential of X and Y genes, sex hormones act directly on genes throughout the genome.[12,13] Testosterone can cause significant brain sexual dimorphism.[29] Widespread areas of the cortical mantle are significantly thicker in women than in men, ratios of gray:white matter also differ, and there are sex differences in every brain lobe.[30–33] Cell-culture studies have demonstrated that male (XY) and female (XX) neurons respond differently to various stimuli. Male neurons are more sensitive to stress from reactive oxygen species and excitatory neurotransmitters, and do not have the capacity to maintain intracellular levels of reduced glutathione.[34] Female neurons are more sensitive to some stimuli that prompt apoptosis.[34] These data have potential implications in treatments for stroke, traumatic brain injury, neurodegenerative diseases, cerebral ischemia, and other sex-skewed neurological conditions, such as Parkinson's disease and schizophrenia.[32,34] In addition, genomic imprinting affects the development of several mental disorders in a sexually dimorphic manner, and there is accumulating evidence for effects of other inherited epigenetic mechanisms including DNA methylation, histone modifications, nucleosome repositioning, higher-order chromatin remodeling, noncoding RNA mechanisms, and RNA and DNA editing.[29,35–37]

What Does It Mean to Consider SABV?

Appropriate strategies that consider SABV depend on the context of the research question, existing knowledge about male and female biology and behavior in a given area of research, available methodology, and other factors.[6] For this reason, the language of the policy is intentionally broad.

We would like investigators to consider how sex plays a role in the work that they are doing—not to adhere to a policy just to adhere to a policy. We would like investigators to truly consider, to rethink, how (and if at all) sex may be playing a role in their work, in their observations, in their experimental materials, in their study designs, in the data, in how the data are analyzed, in how the data are interpreted, and, of course, in how the data are reported.

Accounting for SABV in applications for NIH-funded research could be reflected in the ways discussed in the following sections.[4,38]

Consider the influence of sex in study design

Research findings may be influenced by sex and/or gender, as women and men are characterized by both. Factors that contribute to biological sex differences include biological and physiological characteristics encoded in DNA.[11] These effects may include chromosomal or biochemical interactions, hormonal cycles and reproductive stages, and pathways and clinical presentations in health. Consider the role of sex chromosomes.[3] Consider the role of sex hormones.[3]

Review available literature for the influence of biological sex

Add search terms, such as sex, male, and female, to literature searches on the research topic of interest.[3,38,39]

Consider the influence of sex when formulating the research questions

Sex-skewed disease prevalence may suggest underlying sex-based influences on physiological or pathological processes.[4,38]

Incorporate both males and females into studies

Include both females and males in test groups (factorial, randomized block designs, etc.) Stratify randomization of males and females into experimental conditions. Conduct pilot studies, such as adding a steroid hormone treatment to tissue cultures.[38] When little or no sex-specific data are available, observation of measures in both males and females could be appropriate; in contrast, previously observed sex differences may prompt sex-specific hypotheses.[4]

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Researchers working with animal models should consider whether the female estrous cycle is a relevant factor for the design and analysis. It might be relevant for some research questions and not others. Investigators examining variability among female and male rodents found that females were not more variable than males for any endpoint, that males were substantially more variable for several traits, and concluded that the estrous cycle was not a reason to exclude females.[40]

Analyze data and report data disaggregated by sex

Characterize study results for males and females. Examine treatment or toxicity effects for each sex separately. Report sex-based data and any identified sex-based influences. Report when sex differences are or are not detected in analyses. For studies using both sexes, prospectively develop a methodological plan that includes, at a minimum, reporting of data disaggregated by sex (whether significant in effect or not), which may be valuable for future research and meta-analysis.[4] In exploratory or early mechanistic studies, or in research areas where SABV has not previously been considered, an appropriate first step could be to include both sexes, disaggregate data by sex, and discuss appropriate generalizations that can be drawn from findings.[6]

For studies designed to examine sex differences, the experimental design should include consideration of effect size and power calculations to determine the number of samples/subjects in the study, if applicable.[4]

Consider the influence of sex in the interpretation of study results

Were there trends in study results that may be due to an influence of sex? Considering SABV does not mean designing all studies to examine sex differences,[41] or powering all studies to discern a small sex difference. It is not expected that every study will be designed to detect sex differences at some level of statistical power.[6] The science is still developing in many areas, which means there is value in reporting subset analyses. Similar trends identified across multiple studies would inform the design of future definitive sex-differences studies.

Articulate strong justification for a single-sex study

Strong justification should be provided 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., nonhuman primates), or literature/findings that indicate that SABV is not relevant to research in the area under question. Recognize that the absence of data regarding sex differences in an area of research does not, by itself, constitute strong justification to study only one sex.[4]

Appropriately generalize research findings

Acknowledge limited 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.[4] Researchers should be mindful that sex-specific influences might change with age or any other biological variable. Hence, sex-specific data in young adult animals, might not generalize to juvenile or aging animals.[6]

Additional Notes

Please note that this is not an exclusive list. Additional resources regarding SABV in biomedical research are located on the NIH Office of Research on Women's Health (ORWH) website,[42] which includes research and training materials, such as online courses, resources on methods and techniques, and recent reports. A SABV decision tree for reviewers is available and is also useful for applicants.[43] The ORWH website also includes research summaries[44] on specific topical areas with known sex differences. Finally a validated search tool for sex- and gender-based literature may be found on the Sex and Gender Specific Health website.[39,45]

What about gender? Gender refers to social, cultural, and psychological traits linked to human males and females through social context.[11] This commentary addressed the consideration of sex in biomedical research because the NIH policy specifies that SABV be factored into research designs, analyses, and reporting of vertebrate animal and human studies. In the context of human subjects research, the SABV policy is complementary to the NIH inclusion policy,[46] mandating the inclusion of women in NIH-supported clinical research with additional requirements for valid analysis for outcomes differences in women and men for phase-3 clinical trials. We encourage investigators to consider how gender may affect their observations, given that gender can play an important role in human health and disease, and to realize that sex and gender are interrelated and are not necessarily mutually exclusive. For example, both sex and gender and their interactions may drive epigenetic influences, as in stress responses and resultant physiological reactions. The Canadian Institutes of Health Research website offers modules on strategies to integrate sex and gender in health research.[47,48] As the science evolves and more sex-specific data are available, we will understand further how to study the interaction of sex with gender.

Supporting optimal and rigorous research designs is the best investment we can make. Considering the possible role of sex early in the research continuum may save resources by revealing differences or similarities that need to be taken into consideration in subsequent phases of study. Moreover, by making more sex-specific data available, investigators can build on a stronger body of knowledge more readily, which will likely enhance the efficiency of future research.[6]

Conclusion

We are asking investigators to examine their research questions and scientific hypotheses; consider the potential influence of sex; and address these in design, analyses, and publications. It is our sincere hope that, from the very first idea about a biomedical research area to the bedside and beyond that, sex influences are considered, collected, and reported.

Author Disclosure Statement

No conflicts of interest exist.

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