

Opinion: Focus on preclinical sex differences will not address women's and men's health disparities

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Last spring, the US National Institutes of Health (NIH) announced a new policy calling for the use of both male and female materials—animals, tissues, cells, and cell lines—in preclinical research (1). Canada and the European Union have recently instituted similar policies. Advocates argue that requiring analysis of sex in preclinical research will advance scientific understanding of sex differences in human health outcomes, such as higher rates of adverse drug events (ADE) in women compared with men (2). We disagree.

To be useful in addressing health disparities, sex-linked variables in preclinical materials must effectively model differences

between human men and women. In the absence of evidence that this is so, the addition of sex as a variable in all preclinical studies is likely to introduce conceptual and empirical errors into research. Biomedical research institutions and funders can better remedy sex differences in health outcomes by focusing on the scientific study of the interaction of sex and gender variables in health outcomes in human populations.

Sex differences in rates of ADE may be a result of biological factors, gender-related social factors, or a combination of sex- and gender-related variables. “Sex” refers to chromosomal complement, reproductive organs, or specific hormones related to sexual

reproduction. “Gender” refers to sociocultural norms, expectations, and practices ascribed to males and females (3). Gendered factors, such as women’s propensity to take multiple pharmaceuticals simultaneously (polypharmacy) compared with men, and their greater likelihood to see medical doctors than men, play a well-documented role in sex differences in health outcomes (4–6).

Take the case of zolpidem (Ambien). In 2013, the Food and Drug Administration issued an unprecedented advisory reducing the recommended zolpidem dosage for women, following reports of higher numbers of ADE in women compared to men (7). Since then, researchers have sought the biological basis for this sex difference in reports of zolpidem-related ADE. Surprisingly, experimental studies of sex differences in the pharmacokinetics and pharmacodynamics of zolpidem in human men and women found that body weight, not sex, is the culprit. Women clear zolpidem from their system more slowly than men, but body weight eliminates the statistical significance of sex as a variable in clearance of zolpidem (8). Because body weight, not sex, is the independent biological variable, sex-based preclinical research protocols would likely not have predicted sex differences in rates of ADE with zolpidem.

The zolpidem case provides an example of the need for studies aimed at uncovering the embodied interaction of human sex- and gender-related variables in sex differences in ADE. Weight is distributed differentially across male and female bodies. In present-day American populations, weight may interact with gender-related variables. For example, higher rates of zolpidem use and polypharmacy in women compared to men, as well as biopsychosocial factors, such as women’s greater sensitivity to and reporting



The authors contend that requiring sex as a variable in preclinical studies will do little to mitigate human health disparities. Image courtesy of Dave Cutler.

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of the experience of being impaired, could increase reports of ADE among women taking zolpidem. If lower body weight, greater tendency to use pharmaceuticals, and higher likelihood of reporting adverse events are major factors in the higher rates of ADE among women, policies mandating the study of sex-related variables in cells, tissues, and animal models are an impoverished approach to this issue.

Proponents of policies mandating consideration of sex in preclinical research are concerned about the impact of underrepresentation of female materials on biomedical research (9–12). But from a human health perspective, unequal numbers of male and female animals, or of tissue samples derived from males and females, are only of concern if male- and female-derived cellular and nonhuman animal materials offer informative biological models of human sex differences.

Each form of preclinical biological material—cell and tissues, cell lines, and animals—raises distinctive empirical issues for justifying policies mandating consideration of sex in preclinical materials. In cells or tissues, sex refers to the presence of XX or XY chromosomes, but chromosomal complement is only one biological component of sex determination and differentiation. XX and XY cells are not a widely accepted model for studying sex differences in human bodies. Beyond the sex chromosomes, endogenous hormones and diverse genetic factors interact with gendered environmental cues, such as use of hormone replacement therapy or differential health exposures resulting from the gendered stratification of paid labor, to create sex differences in health outcomes.

Studying sex in cell lines is also far from straightforward. Cell lines are unique lineages with significant chromosomal mutations, intraspecies contamination, and other irregularities compared to primary cells (13). Whether cell lines can be said to have a sex is conceptually unclear (14). Many cell lines derived from male donor cells have been shown to have lost Y-chromosomal gene expression, eliminating chromosomal sex as a meaningful variable (see table 1 in ref. 15). Some have even proposed that cell lines are distinct species. Developing a well-validated cell line is no easy task. Cell lines are odd-acting lineages and no two paired cell lines are likely to have enough similarities to be valid models of sex differences (14).

Animal models are no less complex. Many in the scientific community have critically discussed the practicalities of a mandate that requires the study of sex-related variables in

laboratory animals. For example, some suggest that studying females as well as males in adequately powered numbers will be expensive and space-intensive. Thus, it is particularly important that this element of institutional policies be strongly evidence-based.

Animal models are regularly used to study aspects of sex, but sex dimorphism, sexual behavior, and their developmental pathways vary considerably among common laboratory species. Just like a cell in vitro, a laboratory animal at different stages in its own ontogeny may not effectively model—both for hormonal-milieu and gender-contextual reasons—the “environment” of the adult human male or female. Important human life-cycle traits, such as extended life span and menopause, are poorly represented in animal models yet contribute to health outcome differences between human men and women.

Sociality in rodents illustrates the potential problems with modeling human sex differences in nonhuman model organisms. Prendergast et al. (16) reviewed whether female mice are more variable than males and hence require testing at each stage of the estrus cycle. Contrary to a common presumption, they found that males are more variable than females for several endpoints and that estrus-cycle-related variability does not need to be controlled in female mice. An equally striking result of their study, however, was the incidental identification of a profound, non-sex-related source of variation in mice. Group vs. individual caging appears to be a major and unexpected variable mediating outcomes in mouse studies: group housing increased variability in both males and females by 37% (16). This finding highlights the need for rigorous validation of animal

models for the study of any particular human biological sex-difference pathway. Giving special salience to the variable of sex without attending to interacting species-specific variables, such as animals per cage, may produce findings of questionable relevance to human health.

We support basic research on sex in preclinical materials and affirm the need for better research on sex differences in ADE. But we are concerned that mandates, such as that of the NIH, dedicate institutional resources to the study of basic sex differences in preclinical materials at an opportunity cost to sex-gender-based lines of research more relevant to understanding women's and men's health disparities. Diverse gender- and sex-linked variables, including body weight/body mass index, hormones, rates of polypharmacy, and age play a role in human sex differences in health outcomes, such as overall rates of ADE.

Proponents acknowledge that analysis of sex in basic clinical materials cannot model these variables, but view policies such as the new NIH mandate as an imperfect step in the right direction. But the new policy's focus on nonhypothesis-driven documentation of sex differences in basic laboratory research is more likely to introduce conceptual and empirical problems in research on sex and gender than bring new clarity to differences in men's and women's health outcomes. If the goal is to advance human health, we see a stronger empirical basis for directed funding initiatives in two areas: scientific validation of preclinical models for studying human sex differences, and human studies of the interaction of sex- and gender-related variables in producing health outcomes that vary by sex.

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