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Commentary

Precision medicine requires understanding how both sex and gender influence health

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https://doi.org/10.1016/j.cell.2022.04.012

Progress in studying sex as a biological variable (SABV) is slow, and the influence of gendered effects of the social environment on biology is largely unknown. Yet incorporating these concepts into basic science research will enhance our understanding of human health and disease. We provide steps to move this process forward.

Introduction

Sex differences in the biology of health have not been a target of study to the detriment of both women and men. This stems from historically excluding females as subjects in most studies beyond investigations of reproductive biology. Such exclusion eliminated, or at least greatly reduced, the option of comparing the biology of males and females and created a knowledge gap on the health of women that has only begun to be remedied in relatively recent years.

There are a number of reasons women were excluded as subjects in human research. These included concern about exposing women of childbearing potential to experimental risk. Yet, this important consideration was generalized to include all women, and it did not account for the capability to control reproduction. A second rationale for exclusion of women asserted that female hormonal cycles could affect outcomes and thus confound the results of an experiment. However, this rationale presents a paradox. Namely, if female hormonal variation can change outcomes, how can the data derived on men be applied to women? A third rationale is drawn from biases that have permeated both society and science about whether there was a need to study women (Shansky, 2019). Such notions have guided our research, even without purposeful discrimination, and studying males has represented the norm or standard. As a consequence, it has been largely presumed that for any given measure, everyone can be evaluated relative to this determination.

Spurred by grass roots advocacy and subsequent changes in requirements from governmental research funding agencies, such as those in the United States, Canada, and the European Union (EU), women began to be included more systematically as research participants in the 1990s. The importance of sex as a biological variable (SABV) has been recognized by the Canadian Institutes of Health Research since 2010 and by the EU's research commission since 2014. It was in 2014 that comprehensive reviews and meta-analyses of rodent data demonstrated that hormonal and behavioral variation in males, due to factors such as varying testosterone levels and dominance status, was as great as in females (Shansky, 2019). Two years later, in 2016, the NIH updated its grant application requirements to include consideration of the influence of sex on outcomes or the examination of sex differences, which currently applies solely to human and vertebrate animal studies.

Although adequate representation of women as participants in clinical research remains a challenge, investigations of biological as well as social determinants of health for women and men across a wide variety of disorders demonstrate differences in prevalence, risk, presentation, disease physiology, and response to clinical interventions (Mauvais-Jarvis et al., 2020). For example, women are more likely to develop autoimmune disorders, Alzheimer's disease, and higher global lifetime rates of depression after puberty. Rates of COVID-19 infection are similar by sex, yet men are more likely to die from the disease. Men are more likely to smoke cigarettes yet, when smoking is comparable between women and men, women are more likely to develop lung cancer. Women incur greater primary protection from ischemic stroke with the use of aspirin, and standardized dosing of various common medications, such as ondansetron for treatment of nausea, result in higher blood levels for women than men. In addition, basic science research focusing on disorders with high morbidity and mortality ranging from cancers to heart disease have found that sex influences the biology of disease through genetic regulation (Mauvais-Jarvis et al., 2020). Sex chromosomes influence protein expression, receptors, and signaling and, consequently, cellular pathways are often regulated differently in male and female cells. Despite these findings of sex differences at the level of the cell and the organism and available blueprints for analyzing the influence of sex (Rich-Edwards et al., 2018), analyses of SABV in published findings are often absent (Woitowich et al., 2020). In this commentary, we discuss the value of studying SABV and considering gendered effects of social variables on biology to further expand our knowledge of human health and disease. We illustrate the benefit of this approach in examining the mechanisms underlying cardiovascular disease.

Studying SABV

Charged with conducting evidence-based reviews of pressing health questions affecting the nation, the Institute of Medicine (IOM, now integrated into the National Academies of Science, Engineering and Medicine [NASEM]) convened a committee to determine whether there was added value in studying the biology of females beyond their reproductive biology. In a 2001 report, Exploring the Biological Contributions to Human Health: Does Sex Matter?, the committee concluded that sex differences beyond those in reproduction influenced health and health outcomes and that these differences should be studied.

In addition to endorsing research on how biological sex influences health, the committee emphasized that existing data clearly show that social experience also plays a key role in determining health. To differentiate these areas of investigation, the committee suggested that the term "sex" be used when studying biology, and the term "gender" when investigating social experience (NIH Office of Research on Women's Health. https://orwh.od.nih.gov/sex-gender).

These important considerations regarding the concepts of sex and gender are again changing as binary distinctions are increasingly questioned. For example, research on conditions such as androgen insensitivity syndrome, 21-hydroxylase deficiency, and five alpha-reductase deficiency complicate the binary distinction of sex, and gender assigned at birth can transition to a spectrum of gender identities (Goetz et al., 2020). As empirical data on the relationships of nonbinary life and biology are very limited, the terms men and women will be used in this commentary while recognizing the need to understand the biology and social experience of a range of identities. Finally, new steps forward in science indicate that dividing inquiry into either a biological or social model overlooks the ways in which biology and experience intersect to influence mechanisms of disease and subsequent health outcomes (Mauvais-Jarvis et al., 2020). In studying SABV and social variables, and exploring how gendered effects of experience affect biology, we have the opportunity for greater precision in what we discover about biology and behavior.

Cardiovascular disease

To illustrate how the development of pathophysiology can vary by sex and how social factors can affect this process, we examine the leading cause of mortality for men and women throughout much of the world—cardiovascular disease (CVD). Drawing from this example, we highlight opportunities for advancing research on the mechanisms of CVD, which can provide insights into improved diagnosis and treatment.

Pathophysiology of CVD can vary by sex

Men and women both have high rates of ischemic heart disease and suffer from heart attacks, yet the pathophysiology can vary by sex. Most men with myocardial ischemia suffer symptoms or heart attacks from obstructive coronary artery disease (CAD) due to atherosclerosis associated with hyperlipidemia. However, the phenomena of myocardial infarction and no obstructive coronary artery disease (MINOCA) and ischemia and no obstructive coronary artery disease (INOCA) are common and largely occur in women. As a function of differences in the pathophysiology, symptom presentation for obstructive CAD and myocardial ischemia in the absence of CAD varies, which has led to underdiagnosis in women. Prominent symptoms of heart attacks caused by obstructive CAD, most frequent in men, include chest pain, shortness of breath, and pain in the jaw, neck, arm, or shoulder. The presentation of heart attacks in those with MINOCA can include these symptoms, yet it is also likely to include nausea and/or indigestion, light headedness, and unusual fatigue. Therapies for INOCA have been tested in small samples, yet many in this mostly female population are not receiving treatment or are under-treated while awaiting findings from ongoing large randomized controlled trials (Bairey Merz et al., 2020). However, recent guidelines for the evaluation and diagnosis of chest pain now recommend advanced testing for INOCA (Gulati et al., 2021). Contrasting with traditional techniques used to detect blockages in the coronary arteries, strategies for identifying INOCA focus on assessing attenuated coronary blood flow in response to vasodilatory agents. Non-invasive imaging techniques include use of positron emission tomography (PET) myocardial perfusion imaging, and invasive testing of blood flow, which can be performed during cardiac catheterization.

The observation of sex differences in symptoms of ischemic heart disease and heart attacks led to subsequent investigation and identification of divergent mechanisms of disease that differ largely on the basis of sex. These differences are now formally recognized in diagnostic guidelines, and treatment for MINOCA and INOCA will be evolving based on emerging results from large-scale treatment trials.

Sex differences in blood pressure regulation

Hypertension is a major risk factor for heart attack and stroke, and sex differences are found in its progression and severity. For example, men have an earlier onset of hypertension than women due to differences in regulation of the renin-angiotensin (RAS), bradykinin, and nitric oxide (NO) systems. Compared to men, hypertension develops more slowly in women, but women have a greater lifetime stroke risk, and stroke risk begins at a lower blood pressure threshold (Ji et al., 2022).

Sex differences in sympathetic neural regulation of blood pressure are apparent in healthy, reproductive-age women. In young women, β-adrenergic receptors blunt the vasoconstrictor effect of resting sympathetic nerve activity, such that no direct relationship is found between sympathetic activity and vasoconstrictor tone. This blunting of sympathetic activity is not present in young men or postmenopausal women, for whom sympathetic nerve activity is directly related to the level of vasoconstrictor tone in the peripheral vasculature (Hart et al., 2011).

Research on sympathetic regulation of blood pressure demonstrates that enhanced β-adrenergic receptor responsiveness acts to partially protect young women against the effects of high sympathetic nerve activity and contributes to the greater risk of developing hypertension in young men compared to young women. Thus, it is likely that female reproductive hormone exposure provides protection in women by lowering lipids and increasing β-adrenergic receptor

responsiveness, and this appears to be lost after menopause. An intervention with the β_2-adrenergic receptor agonist terbutaline increased vasodilation in young but not postmenopausal women confirming that β_2-adrenergic receptor responsiveness is blunted in postmenopausal women (Harvey et al., 2020).

These studies provide insight into the intersection of sex and age-related mechanisms controlling blood pressure, a key risk factor for CVD. Drawn from these data is the potential for therapeutic interventions that reduce sympathetic activity to improve vascular function in older women.

Implications at the cellular level

Traditionally, cellular mechanistic pathways have been studied in males with the data applied to males and females, or the sex of cells were undetermined based on the assumption that both sexes rely on the same regulatory pathways. However, this is not always the case. Male and female sex chromosomes differentially impact vascular smooth muscle and endothelial cells as well as cardiac muscle cells. Because the sex of cells under investigation may influence research findings, reporting the sex of cells is important in both laboratory and clinical cardiovascular studies. Further, studies of sex differences using permanent cell lines do not routinely reflect sex differences in humans because sex chromosomes can be altered in the creation of cell lines. Thus, either freshly isolated primary cells, or cell lines tested for sex chromosomes to determine the sex of cells, are optimal.

Social factors and gendered effects of social factors can affect biology in CVD

While sex differences in biological mechanisms regulating blood pressure appear less obvious as women approach menopause, socially constructed variables, such as race and ethnicity, are associated with subclinical indicators of CVD in postmenopausal women. For example, measures of arterial atherosclerosis with intimal medial thickness show more profound CVD in women in the U.S. grouped as Black compared to White or Chinese postmenopausal women (Barinas-Mitchell et al., 2020). In younger populations, non-Hispanic Black men and women are disproportionally affected by hypertension, with earlier onset of hypertension in Black relative to White women (Virani et al., 2020). The interaction of race and sex on blood pressure regulation has also been addressed in a recent comprehensive review (Brothers et al., 2020) showing that blood pressure and peripheral microvascular responsiveness are reduced in healthy young non-Hispanic Black men and women compared to young non-Hispanic White men and women.

Additionally, the biological mechanism for the poor microvascular responsiveness differs between Black men and women. In Black men, nicotinamide adenine dinucleotide phosphate oxidase and xanthine oxidase contribute to blunted NO-mediated cutaneous microvascular function, but Black women appear unaffected by this proposed superoxide mechanism. In showing sex differences in the causes for microvascular dysfunction within a young, Black population, data indicate elevated sympathetic neural outflow in men, but not in women. Rather, preliminary data suggest that young, Black women have increased vasoresponsiveness to sympathetic stimulation. Taken together, a potential mechanism for increased blood pressure in Black women compared to White women may be related to increased vasoconstrictor tone and/or responsiveness to sympathetic activation, likely associated with several variables including heightened sympathetic vascular transduction, impaired β2-adrenoreceptor-mediated vasodilation, endothelin-1-mediated vasoconstriction, and angiotensin II, among others (Brothers et al., 2020). While these recent studies have been revealing, they have not yet defined the pathway for these effects and have been limited to healthy, young Black men and women, requiring future studies in higher risk populations.

Importantly, "upstream" social mechanisms for increased hypertension and CVD include higher rates of mental stress-induced pathophysiological outcomes due to everyday discrimination experienced by Black populations. Moreover, a 2-fold increase in the adjusted odds of mental stress-induced myocardial infarction has been found in Black women compared to Black men (McKinnon et al., 2021). This example of the effect of sociocultural variables, namely race, stress, and gender, on biology illustrates the importance of understanding biology in a social context. It also highlights how socially defined factors, which determine individual and group behaviors and experiences, can affect health directly, e.g., in reports of greater stress in women than men, and indirectly, e.g., in greater risk for adverse health events (Lowe et al., 2021). These studies demonstrate that complex questions regarding the intersection of CVD, race, sex, and gendered effects of the environment can only be answered by intentionally studying females and males, social constructions such as race and gender, and contextual experience.

Opportunities for advancing research

The inclusion of SABV into basic science investigations on the mechanisms of disease offers the opportunity for refined methods of diagnosis and novel approaches to treatment. Moreover, consideration of the influence of social experience on biology recognizes an important source of variation within and across study groups. Three main hurdles present themselves in shifting research paradigms to the study of females as well as males, and to considering sex and gender effects on study outcomes.

The first hurdle revolves around how to proceed when there are no existing data in the literature for sex differences in an investigator's area of study. As Rich-Edwards et al. (2018) point out, this may not be surprising due to the exclusion of females as research subjects, yet "the absence of evidence for sex differences is not necessarily evidence for the absence of sex differences." Consequently, if no data are apparent, it is even more important to initiate an examination of trends by sex in all research studies and by gender in human investigations. If trends are found, opportunities exist to develop new hypotheses or conduct meta-analyses. Opportunities are also available to take advantage of administrative grant supplements to examine more fully the effects of sex and gender or diversity.

The second hurdle entails using experimental designs that allow examination of SABV, or effects of gender on biology in

human studies. This challenge often focuses on the need to increase sample size while meeting the demands of experimentation cost. However, initiating changes to experimental designs does not necessarily mean ensuring a sample size that allows detection of statistically significant sex differences. Other methodological options consistent with NIH guidelines (e.g., reporting main effects by sex, stratifying analyses, or providing sex-specific data for subsequent meta-analyses) provide important insights into the influence of sex on outcomes (Mauvais-Jarvis et al., 2020). Consultation with statistical and methodological experts prior to the initiation of a study, rather than after the data are collected, allows for the inclusion of important design adaptations to respond to this challenge. Roadmaps for analyzing the influence of sex also are available in the literature (Rich-Edwards et al., 2018), and resources for methodological options to aid researchers in considering SABV and provided by major funding sources (e.g., https://orwh.od.nih.gov/sex-gender).

The third hurdle is centered in the slow institutional acceptance of the influence of sex and gender on health and of the intersection of biological and social determinants of health. For example, many journals now require reporting the sex of subjects for *in vivo* animal and human studies, yet do not require reporting of sex and gender in analyses, results, and interpretation of findings. To address this challenge, it is incumbent upon journal editors to progressively endorse agreed-upon guidelines, such as the Sex and Gender Equity in Research (SAGER) Guidelines, that promote such examination and reporting. Similarly, it is as important for funding agencies to increasingly require investigations be designed to uncover the influence of sex and gendered experience on biological processes.

Surmounting the hurdles encountered in exploring SABV will enhance rigor and reproducibility and increase the precision of our discoveries. Incorporating the intersection of sex as well as gender and other social variables into our scientific inquiry will advance the relevance and practical benefit of research.

DECLARATION OF INTERESTS

The authors declare no competing interests.

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