Cell Metabolism
Essay

Sex and Gender: Critical Variables in Pre-Clinical and Clinical Medical Research

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In this Essay, we discuss the critical need to incorporate sex and gender in pre-clinical and clinical research to enhance our understanding of the mechanisms by which metabolic processes differ by sex and gender. This knowledge will allow for development of personalized medicine which will optimize therapies specific for individuals.

Most pre-clinical and clinical medical research, both animal and human, has been biased with respect to sex. There has been a tendency to treat the sexes as equivalent and not consider how fluctuations of sex hormones in experimental settings impact outcomes, thereby limiting our understanding of the molecular mechanisms that drive sexual dimorphisms. The National Institutes of Health (NIH) has recently recognized this gap in scientific knowledge and now mandates that studies be conducted in both sexes.

NIH Mandates Focusing on Sex Hormones in Research Sex and Gender

With few exceptions, basic science—pre-clinical (Beery and Zucker, 2011; Woodruff et al., 2014) and clinical (Institute of Medicine Board on Population and Public Health, 2012; Institute of Medicine Forum on Neuroscience and Nervous System Disorders, 2011)—research has predominately used male subjects, consequently ignoring the contribution of sex in outcome measurements. Failure to include both sexes in experiments, and/or insufficient analysis of data by sex, in fact generates data not biologically relevant to either sex. The Congressional Caucus for Women's Issues, women's health advocacy groups, and the NIH collectively realized that excluding women from clinical research was bad for women and bad for science. As a result, in 1993 the NIH Revitalization Act was passed to begin to address these inequities by requiring the inclusion of both sexes in NIH-funded clinical research. This issue has become sufficiently important that the NIH more recently established the Office of Research on Women's Health (ORWH); nonetheless, despite these efforts, many publications continue to neglect sex-based considerations, contributions, and analyses in pre-clinical and clinical studies.

The NIH is now implementing policies that require grant applicants to explicitly detail plans for the use and inclusion of male and female cells and animals in pre-clinical studies, unless sex-specific exclusion is warranted based on rigorously defined exceptions. Furthermore, several relevant organizations have taken steps to increase awareness of, and address unconscious bias about, the importance and difference between sex and gender in biomedical research, and several journals now require authors to specify sex- and gender-related information.

With the growing emphasis on inclusion of both sexes in research, it is critical to define and use the terms "sex" and "gender" appropriately. Sex and gender are different constructs. Sex, according to the Canadian Institutes for Health Research Panel on Sex and Gender (Canadian Institutes of Health Research, 2015), "refers to a set of biological attributes in humans and animals. It is primarily associated with physical and physiological features including chromosomes, gene expression, hormone levels, and reproductive/sexual anatomy." In contrast, gender refers to how people perceive themselves and others, as well as how they act and interact. Gender additionally refers to social behaviors, expectations, expressions, and identities of girls, women, boys, men, and gender-diverse people. Assessments of health and disease risk need to take both sex and gender into account (Phillips, 2005). Below are considerations pertaining to how sex and gender should be applied to pre-clinical and clinical research.

Gender

Cultural norms pertaining to gender roles and sex-related behaviors fluctuate and change over time, as well as across cultures. The berdache (a French term used by Native Americans to refer to younger partners in male homosexual relationships), the fa'afafine (Samoan for "the way of a woman") in the Pacific, and the kathoey in Thailand are all examples of cultures that differ from the traditional Western classification of people into "males" and "females," demonstrating that sex and gender are not always neatly or in fact easily divided along binary lines. As an example, among some native North American communities, gender is considered a continuum, enabling acknowledgment of individuals who have both masculine and feminine qualities and characteristics.

Gender impacts disease risk, diagnosis, and treatment. As one example, men are often thought to be at an increased risk for cardiovascular disease (CVD), in part due to their gender-based propensity to engage in risk-taking behaviors such as smoking or alcohol consumption. Importantly, women who have

taken on societal roles associated with the male gender have an increased disease prevalence linked with the pressures associated with these gender-defined roles (Bekhouche et al., 2015; Hausmann et al., 2012; Izadnegahdar et al., 2014; Kawase et al., 2013; Sozzi et al., 2007). Lastly, using the term "gender" when referring to in vitro assays or animal studies in basic science research is incorrect, and instead the term "sex" should be used.

Sex

As indicated above, the role of sex in scientific discovery, disease detection, diagnosis, and treatment is often underappreciated. For while the sex of the subject in clinical studies is obviously important, so too is the sex of the cell, or the hormonal context in which in vitro studies incorporate sex in the model. For while many intrinsic properties of cells can appear hormone independent, cells may also exhibit differential variations upon exposure to sex hormones. Female and male cells respond differently to chemical and microbial stressors, and yet the sex of cell lines studied in vitro is mostly ignored and rarely reported.

Hormonal Impact on Disease Risk: Estrogens

When sex is factored into disease risk, it is well established that premenopausal women are relatively protected from diseases associated with the metabolic syndrome, including cardiovascular disease (CVD), when compared to age-matched men (Collins et al., 2002; Ren and Kelley, 2009; Skafar et al., 1997; Yanes and Reckelhoff, 2011). This "sex advantage" disappears after menopause, leading to the generally accepted conclusion that sex hormones, and in particular estrogens, protect against the metabolic syndrome. Compelling data suggest the protective effect of estrogens are a continuum such that low levels of estrogens are associated with increased CVD risk, with several lines of evidence linking hypoestrogenemia (HypoE) in young women to increased CVD. Additionally, early menopause, ($\leq$45 years) is associated with accelerated atherosclerosis and a 2.6-fold increase in the risk of CVD (95% CI 2.05–3.35) (Kannel and Wilson, 1995), as well as increased CVD mortality (Cooper and Sandler, 1998; Jacobsen et al., 1997) compared to women experiencing later menopause. Conditions resulting in severe HypoE, including Turner's syndrome (TS) and primary ovarian insufficiency (POI), are also associated with elevated rates of CVD in young women (Swerdlow et al., 2001).

There is a large amount of data published with a variety of findings focusing on the role of female hormones in CVD. Here, we briefly summarize the literature with respect to the protective role for estrogens in CVD. Initial observations suggested that postmenopausal women receiving hormone replacement therapy (HRT) are relatively protected from the metabolic syndrome compared to women not receiving HRT. However, these early observations have since been challenged by two randomized prospective primary and secondary prevention trials, both of which found a significantly increased CVD risk in postmenopausal women on HRT. Data on the benefits or determinants of estrogen replacement therapy in postmenopausal women have more recently given rise to the "timing hypothesis," which suggests that hormonal replacement prior to menopause is associated with reduced cardiometabolic risk, whereas hormone replacement following menopause is associated with increased cardiometabolic risk. A recent article in the New England Journal of Medicine supports the "timing hypothesis" reporting that estradiol replacement therapy in early menopause is associated with vascular benefit, whereas estradiol replacement therapy late in menopause is less advantageous or even contraindicated (Hodis et al., 2016). *When, at what level, and at what age are estrogens protective in females?*

In men, testosterone (T) can be aromatized to estrogens (E), with more than 80% of circulating E in men being derived from aromatization of T (Carani et al., 1997). Finkelstein et al. found that blocking the aromatization of T results in increased adiposity and reduced sexual function in men (Finkelstein et al., 2013), further supporting the concept that E deficiency is largely responsible for some of the key consequences of male hypogonadism. As serum levels of T decline with aging, there is a concomitant decline in serum levels of E. In a related report, Jankowska et al. demonstrated that men with the lowest quintile of estradiol (E2) (lowest 20%, <12.90 pg/mL) were found to have the highest death rates from congestive heart failure over a 3-year period, while men with E2 in the range of 20–30 pg/mL had the lowest rates (Jankowska et al., 2009). However, men with the highest E2 levels ($\geq$37.40 pg/mL) also had a greater incidence of atherosclerosis (heart disease), diabetes, obesity, stroke, enlarged prostate, breast tissue growth, breast cancer, and other problems. *Is there an optimal level of estrogens for men that provides disease protection, and how does this relate to age?*

Hormonal Impact on Disease Risk: Testosterone

Testosterone (T) has also been investigated with respect to modulating disease risk, and the results are conflicting. Lower T levels in middle-aged and older men are associated with insulin resistance, the metabolic syndrome, and diabetes. Furthermore, lower T in older men predicts cardiovascular events, including stroke and transient ischemic attack, and is associated with higher CVD and overall mortality (Schwarcz and Frishman, 2010). One interventional study using T therapy in men with CVD found beneficial effects on exercise-induced myocardial ischemia (Bhasin et al., 2006). However, in another trial of older men who were randomized to receive a substantial dose of T, the authors reported cardiovascular *adverse* effects (Snyder et al., 2016). Importantly, these effects were not observed in a comparable trial where men received a more conservative dose of T, suggesting that optimal dosing of T in older men with existing CVD is critical. In the Cardiovascular Risk in Young Finns Study, higher levels of T in younger men (24–45 years old) were associated with favorable cardiovascular risk profiles characterized by lower levels of triglycerides, insulin, and systolic blood pressure, and higher levels of high-density lipoprotein cholesterol (HDL-c) (Firtser et al., 2012). For women, elevations in T production, as seen with polycystic ovarian syndrome (PCOS), are associated with insulin resistance and CVD risk (Dokras, 2013). However, Barrett-Connor et al. followed Caucasian men (40–79 years old) for 12 years and found that none of the sex hormones measured (testosterone, androstenedione, estrone, or estradiol) was significantly associated with CVD risk at baseline or with subsequent cardiovascular mortality or ischemic heart disease