Sex and gender matter to biology

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Flip through a few TV channels or browse the Internet for a bit and you will be quickly reminded that, in our day and age, everyone is thinking about ‘sex’. Biologists think about sex too – albeit more in the biological sense than the act itself. The problem is they don’t think enough about it. Indeed, though most animals display marked differences in sexual anatomy and reproductive function, sex is regularly overlooked in biomedical research at both the clinical and basic science levels. Over 25 years ago, The National Institutes of Health recognized this as problematic; exclusion of women from large clinical trials blunted their ability to detect sex differences in the safety and efficacy of therapeutic drugs. In 2001, the Institute of Medicine echoed these concerns, calling for expansion of research into sex differences at the biochemical and cellular levels. Despite this, investigators still regularly ignore the sex of cell lines studied in vitro, as well as failing to include both sexes in animal studies. In this article, we briefly discuss the nature of sex differences and highlight their importance to future basic and translational research.

Sex hormones

Many studies of sex differences have focused on how sex hormones directly affect health and metabolism. For instance, when sex is factored into estimates of disease risk, it is well established that premenopausal women are relatively protected from diseases associated with the Metabolic syndrome, such as cardiovascular disease (CVD), compared with similarly aged men. After menopause, however, the prevalence of CVD in women increases to levels comparable to or even greater than similarly aged men. Reproductive-aged women with low oestrogen, as well as women who experience early menopause, are also at increased risk of CVD. These observations led to the generally accepted conclusion that the action of sex hormones and oestrogens in particular, protect against Metabolic syndrome and confer a ‘sex advantage’ to women.

Testosterone has also been investigated with respect to modulating CVD risk, but with conflicting results. Low testosterone levels in middle-aged men are associated with insulin resistance and metabolic syndrome, and also predict cardiovascular events and mortality. However, studies of testosterone therapy in men and women report both beneficial and adverse consequences in terms of CVD events. For example, in the Cardiovascular Risk in Young Finns Study, higher levels of testosterone in younger men (24–45 years old) were associated with lower levels of triglycerides, insulin and systolic blood pressure, as well as higher levels of high-density lipoprotein cholesterol. For women, however, elevations in testosterone, as seen in polycystic ovarian syndrome, are associated with insulin resistance and CVD risk.

Testosterone can be converted to oestrogens by the enzyme aromatase, and the majority of circulating oestrogens in men are derived through ‘aromatization’. Finkelstein et al. found that blocking the aromatization of testosterone to oestrogens actually increased adiposity and reduced sexual function in men. A related study demonstrated that men with the lowest plasma oestradiol concentrations had the highest death rates from CVD over a 3-year period. Conversely, men in the mid-range of oestradiol had the lowest rates, and men with the highest oestradiol levels had a greater incidence of atherosclerosis, diabetes, obesity and stroke. Clearly, oestrogens exerts important metabolic effects in men; however, the weight of the evidence suggests that the exact dosing and mechanisms by which oestrogens promote optimal health differ between men and women, and points to an underlying sex difference in how non-reproductive tissues respond to sex hormones. This notion is relevant to both animal and in vitro studies insofar as exposure to sex...
hormones or sex hormone mimetics (for example, oestrogenic compounds in animal feed or cell culture media) could substantially impact research questions that are not expressly designed to detect sex-based effects. Strict characterization and control of the hormonal milieu is, therefore, essential in all experimental designs.

**Sex chromosomes**

While sex hormones are established players in the study of sex-based differences, the contribution of sex chromosomes to gene regulation and disease risk is a less well-studied area of interest. The X and Y sex chromosomes initially evolved from a pair of similarly sized autosomes. Over time, the Y chromosome lost the ability to exchange genetic information with the X chromosome and began to evolve independently. Today, the human Y chromosome contains only 3% of the genes it once shared with the X chromosome. It is present exclusively in men and was once thought to solely govern the expression of male reproductive traits. However, genes conserved on the Y chromosome are also expressed in cells throughout the body and are involved in autosomal gene regulation. For example, single-nucleotide polymorphisms on the Y chromosome are correlated with risk factors associated with CVD, independently of sex hormones. Still, the Y chromosome has mostly been excluded from the larger genome-wide association studies (GWAS) due to the enduring belief that it is a 'genetic wasteland'.

Women, on the other hand, have two X chromosomes and, therefore, possess two copies of every X-linked gene. To compensate for the fact that men have only one X chromosome, one female X chromosome is randomly 'inactivated,' allowing for adjustments in the dosage of X-linked genes between the sexes. X-inactivation occurs early in female development, making females more vulnerable than males to genetic or environmental perturbations during embryonic development. Moreover, different different cell types silence X chromosomes in different patterns, providing a mechanism of natural variation at the cellular and tissue levels. Despite this, the X chromosome has also been 'ignored' in the analyses of GWAS data, with only 33% of the reported studies from 2010 to 2011 factoring in the X chromosome.

**Sex hormones and chromosomes in the transgender population**

Integral to the study of sex in humans is an understanding of how biological 'sex' differs from the closely related, but distinct, concept of 'gender.' 'Sex' comprises biological traits encoded in DNA, such as chromosomes, while 'gender' refers to the social behaviours, expectations, and expressions of men and women. While sex informs gender, it does not dictate it. For approximately 700,000 people in the United States, gender does not match biological sex. This estimate of the 'transgender' population might actually be low; one account claims a prevalence of over ~1.4 million. Despite this sizable number, the transgender community represents one of the most underserved and understudied populations in healthcare.

Transgender patients may opt for interventions designed to bring their biological sex into congruence with their gender identity. Cross sex hormone therapy (CSHT) and sex re-assignment surgery (SRS) represent two established therapeutic approaches. In CSHT, patients receive exogenous sex hormones in order to induce the appearance of sexual characteristics consistent with their gender identity while suppressing endogenous hormone levels and secondary sex characteristics associated with their biological sex. Within the first 6 months of CSHT,
changes in men transitioning to women (transwomen) include breast growth, decreased testicular volume and decreased spontaneous erections. Women transitioning to men (transmen) experience changes in body fat distribution, muscle mass and hair growth. Critically, since the chromosomal configurations remain unchanged despite CSHT or SRS, studies of transgender populations provide unique opportunities to investigate which metabolic responses are irreversibly sex-differentiated at the sex chromosomal level, which are determined by the prevailing milieu of sex steroids and how chromosomal sex interacts with sex steroids to affect sexually dimorphic biological processes. To this end, we have recently investigated the role of sex hormones and their influence on insulin sensitivity and hepatic steatosis in a population of transwomen with and without testes (Nelson et al., in press, *Transgender Health*, 2016). Despite receiving similar oestrogen therapies, markers of metabolic health improved in transwomen who elected to have their testes removed compared with transwomen with testes. Furthermore, transwomen with the highest plasma testosterone concentrations also had the highest incidence of hepatic steatosis and insulin resistance (Nelson et al., in press, *Transgender Health*, 2016). These data suggest that suppression of naturally produced testosterone in transwomen improves insulin sensitivity and reduces hepatic steatosis. These are important considerations not only for future studies of the influence of sex hormones and chromosomes on metabolism, but also possible future transgender care guidelines.

How sex hormones and sex chromosomes impact metabolic phenotypes and disease risk is an area of much-needed research. Future investigations will require integration of endocrinology with molecular genetics methods to alter hormone action in a cell type-specific manner and manipulate the copy number and expression of X and Y genes to probe the constitutive genetic differences in the complete genome of XX and XY cells, tissues and whole organisms. In doing so, investigators will gain a deeper understanding of how sex fundamentally influences biology.

**Future directions**

Here, we have discussed the importance of sex and gender in biology research. In order to increase the fidelity of experimental models, clinical and basic researchers must ensure that both sexes are adequately represented in their experimental designs, and that their analyses account for sex-based and, if appropriate, gender-based effects. Sex hormones play a key role in the manifestation of sex differences and must be rigorously characterized and controlled during experimental design. Furthermore, the ability of sex chromosomes to influence metabolic gene regulation needs to be further explored. Statements such as ‘there are no sex differences’ will need to be strongly defended following rigorous characterization of the impact of sex hormones and chromosomal sex.

In conclusion, understanding how sex and gender impact biology is critical for the development of more powerful biological models, and ultimately, to the development of truly personalized medicine.
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