Biological Sex Differences: Implications for Biomedical Research and Animal Use

There is increasing evidence that research on men is often not applicable to women. And while women are far less frequently used in clinical trials, results from women are also often not applicable to men. There are significant biological and health differences between the sexes. Women live longer than men, but have higher morbidity rates: they encounter more nonfatal conditions and disability, while men contract more life-threatening chronic diseases and die at a younger average age. With regard to mental health, rates of serious illnesses are similar, though the most common metal health problems show different prevalence. Women are more prone to: autoimmune and rheumatologic diseases; anemia; thyroid conditions; gall bladder conditions; migraines; arthritis; eczema; upper respiratory infections; gastroenteritis, and other short-term infectious diseases. Men have higher rates of: coronary heart disease; cancer; cerebrovascular disease; emphysema; liver cirrhosis; kidney disease, and atherosclerosis (1).

Here, we summarize some salient examples to illustrate how and why this is so, and what should be done to redress it. We also pose the question: *if extrapolation of knowledge between sexes of the same species is difficult, what are the implications for reliably extrapolating from one species to another?*

Cancer

Men and women are differently susceptible to various cancers. Women are much more likely to suffer from tobacco-related cancers, e.g. of the lung and mouth (2), but are less susceptible than men to cancers related to pesticide exposure, such as non-Hodgkin's lymphoma and soft-tissue sarcomas. There are differences in dioxin-related cancers: women are more prone to stomach and colon cancers; men to leukemia and esophageal and rectal cancers (3).

Women are up to 3.5-times less likely to suffer from brain tumors, regardless of age, tumor type, or race—a disparity that is not explicable solely by differences in sex hormones, as sex-related differences in prevalence also occur in childhood cases (4). This is true not just for primary brain tumors, but also secondary ones: brain metastases are also much less common in females, including those tumors that, in their primary form, are of equivalent prevalence in men and women, such as lung cancer and melanoma. These differences are thought to arise from differences in gene expression in males and females that are measurable well before birth. Other sex-related differences affecting cancer incidence are present during the development of the embryo: the expression of many genes, not just genes on the sex chromosomes, is evident, and major differences in various metabolic pathways that may affect cancer cell growth are present.

Prognosis and outcome, as well as incidence, of brain cancers, and even their metastases, are also different: females have a higher survival rate compared to men.

Depression

Women are almost twice as likely to be diagnosed as suffering a major depressive disorder during their lifetime as men. This is thought to be a result of a combination of dysregulated stress responses, the indirect effects of hormones (5) and different manifestations of symptoms of depression in men and women (6). Because depression is associated with immune function, which also differs markedly between the sexes (see "Immune Disorders" below), these two factors combined may contribute substantially to sex differences in health and disease (1).

Posttraumatic Stress Disorder (PTSD)

Women have a higher risk of PTSD. This is thought to be due to several factors, including the types of trauma experienced, differences in the prevalence of younger age of exposure, and how the traumas are perceived by those experiencing them. Importantly, sex-specific psychobiological reactions are also involved (7).

The biology underpinning these reactions is complex, but pivotal to stress responses and their regulation is the hypothalamic-pituitary-adrenal (HPA) axis. The HPA axis results in the production of "stress hormones"—glucocorticoids (GC) such as cortisol and corticosterone—that mediate many of the biological effects of stress. The HPA acts together with the sympathetic-adrenal-medullary system (SAM), which involves stress-activation of the autonomic nervous system, stimulating and inhibiting the sympathetic and parasympathetic nervous systems respectively to prepare the body for "fight or flight." As reviewed by Olff *et al.* (2007) (7): Gender differences in acute responses to trauma, in which the HPA axis and SAM are central, are believed to be a major contributor to elevated PTSD risk in women. Cortisol levels are more elevated in male motor vehicle accident survivors after one month, compared to females, and there is a greater likelihood of acute physiological symptoms in women after exposure to a disaster stressor. Women are more likely to experience dissociative responses (a state of limited or distorted awareness at the time of a traumatic event or in its immediate aftermath), making them more prone to PTSD—mediated by stress-induced glucocorticoids (i.e. the HPA axis), which itself is mediated by and the hormone dehydroepiandrosterone (DHEA) and its metabolite DHEA-S. Notably, there are sex differences in the levels of DHEA and DHEA-S in humans.

Women show great variability in the activity of their HPA axis, which is thought to contribute to their greater vulnerability to stress. Between puberty and menopause, activity is lower then in males, whereas at a certain stage of the menstrual cycle cortisol levels approach those of men. Women with PTSD have lower cortisol levels than those without, yet this is not the case for men. The hormone oxytocin also pays a role, as it—in conjunction with female reproductive hormones—may mediate a "tend and befriend" response in stressful situations, compared to more of a "fight or flight" response seen in males. Such a sex difference has evolutionary advantages: women would use this response to maximise their own and their offspring's survival, and to recover from adverse experiences in childbirth, for example. Finally, many of the major biological systems involved in PTSD, such as neurotransmitters and neurohormones, are affected by sex hormones.

Neural Differences

Sex differences in brain organization are thought to directly affect cognitive functions, such as verbal skills and visuospatial tasks; men and women react differently to visual stimulation, for instance (8). Further, they may be a factor in the incidence of neurological and psychiatric diseases (see below): for example, the incidence of anorexia nervosa and bulimia are much higher in women, but dyslexia, sleep apnea and Tourette syndrome are higher in males. Indeed, "Gender-dependent differentiation of the brain has been detected at every level of organization—morphological, neurochemical, and functional," controlled primarily by sex hormones (9).

When differences in brain size are taken into account, women have ten times more white matter, while men have almost 7 times more gray matter, which is related to cognitive skill versus higher affective functioning (10). The cerebral cortex—the outermost region of the brain involved in memory, thought, language and consciousness—shows significant sex differences in complexity, with consequences for functional cognitive and behavioral differences between men and women (11). In fact, sex differences in the central nervous system (CNS) have been identified at every level of brain organization: brain area and volume; number, structure and activity of cells; cell-cell connections and chemical composition (9).

Underlying such differences are biological mechanisms additional to those involving sex hormones and sex chromosomes. Small molecules called microRNAs (miRs) are involved in the regulation of around 70% of genes, and have been implicated in the development and adult function of all tissues, as well as (when dysregulated) in diseases including cancer, immune dysfunction, metabolic, and cardiovascular diseases (12). Sex differences have already been identified in the responses of miRs to cerebral ischemia and radiation, in some cancers, and in the differential development of the brain in men and women. The latter indicates that miRs, when disrupted, are a likely factor in the sex biases found in neurodevelopmental disorders.

Stroke

Males have a higher incidence of stroke across much of their lifespan, yet women have a higher incidence of stroke after the age of 80. The types of stroke differ between sexes: men have more atherosclerotic strokes, and women more cardioembolic strokes (10). Post-stroke disability and stroke-associated cognitive impairment are greater in women than in men (13). fMRI studies have shown for instance, that women rely on both sides of the brain for certain aspects of language, while men rely only on the left hemisphere (14).

Osteoarthritis (OA)

There are differences in the incidence and severity of OA between men and women, as well as between racial and ethnic groups. The burden is much greater in women for knee and hand OA, though not for hip OA, and women report greater pain and adverse consequences for quality of life (15). Menopause further increases OA severity, suggesting that estrogen levels cannot be at the root of these differences alone.

Cardiovascular Disease (CVD)

The prevalence and age-adjusted death rate of CVD are higher in men. However, more women eventually die of CVD due to their greater life expectancy, older age at onset, and the range of CVD risk factors associated with aging. Because more women live to an older age, women will live more years after the age of 65 with CVD than men.

There are sex differences in the presentation of acute coronary syndromes (ACS)—the clinical symptoms of obstruction of the coronary arteries—and in the presentation, course and outcomes of coronary heart disease (16).

Related to CVD, there are significant differences in total cholesterol levels in men and women. While levels rise with age in both sexes, they stagnate at around 50 in men but not until beyond 65-70 in women. After this stagnation, levels decline in both sexes. However, older women (55+ years) have significantly higher total cholesterol levels than men, but a lower risk of mortality (17).

There are also major sex differences in cardiac arrhythmias (irregular heartbeat): differences in arrhythmia initiation, sustenance and termination are sex-specific (18). Men and women have different basic electrical and physiological properties to their heartbeats to start with, and are affected by different types of arrhythmias. Both are thought to be due to sex-specific gene expression in the heart (19) and the effects of sex hormones (18).

Progression of heart failure, which is often different in men and women, is slower in women, in whom survival is more likely and development is at an older age (18).

Blood Pressure/Hypertension

Women have lower blood pressure (bp) than men, regardless of race and ethnicity, through much of life: it does not accelerate to approach average male values until the fifth or sixth decade, and whether or not it ever exceeds that of men is controversial (20). While the difference is not great (systolic differences rarely exceed 10mm Hg), this is significant, as such an increase doubles the risk of cardiovascular disease and increases risk of stroke. Higher bp can also damage the brain, heart, kidney and eyes, and lead to type II diabetes.

Interestingly, existing treatments for hypertension are less likely to be effective in women, which also indicates the presence of sex differences in its pathology (13). These differences have been explained by sex differences in nervous control of the blood circulatory system and in nervous system function, which also lead to differences in male and female incidence of migraine, Raynaud's disease, and postural orthostatic tachycardia syndrome (POTS).

Alzheimer's Disease (AD)

Women are at an increased risk of AD. The cumulative risk for 65 year-old women to develop AD by the age of 95 is almost two and a half times that of men (21). In the U.S., there are an estimated 3.4 million women to 1.8 million men aged 65 or greater with the disease, i.e. two-thirds of sufferers are female (22). It is difficult to know if the greater lifespan of women contributes to this, and if so by how much, but there

does seem to be a sex-related factor with regard to prevalence. Further, there are sex differences in severity: degree of dementia and decrease in cognitive performance may be greater in women. Loss of the protective effects of estrogen in menopause may be a factor.

Asthma and Other Airway Diseases

Sex differences in airway behavior and airway disease occur throughout life, and are related to biological as well as sociocultural factors. Atopy—a form of allergy in which a hypersensitivity reaction such as asthma occurs in a part of the body not in contact with the allergen—is considered the most important host risk factor for asthma and the most important determinant of host airway behavior. It is more prevalent in women in their reproductive years than men of the same age, though lower in females during childhood and post-menopause. A type of antibody known as IgE—central to allergic reactions—is present at different levels throughout life dependent on sex. Hormonal factors are believed to contribute greatly to these manifestations (23).

With specific regard to asthma, it is also more common in adult females, but less common in females during childhood. Women are also more likely to suffer from severe asthma. These sex differences appear to be due to a combination of biological, sociocultural and environmental differences, and it has been established that certain genetic polymorphisms are particularly related to asthma in females (24).

Immune Disorders and Response

Generally, women have a much higher incidence of immune disorders. For instance, female prevalence of autoimmune thyroid disorders, systemic lupus, and rheumatoid arthritis are 15, 9, and 3 times higher than that of males respectively (1), and female prevalence is also higher for multiple sclerosis (MS), primary biliary cirrhosis and Hashimoto's thyroiditis (25). With specific regard to MS, the ratio of affected women to men is up to 3:1, and this increases with age, and the peak incidence and prevalence are also higher in women (10).

Women typically generate more robust immune responses to infection and vaccination, producing significantly more antibodies. The greater immune response is not restricted to antibody-mediated immunity, however: the lower incidence of tumors in women, and their more rapid rejection of skin grafts, illustrate a greater cellular immune response, too (25).

Sex is also an important factor in morbidity and mortality resulting from sepsis (a severe, whole-body inflammatory response to infection): mediated largely by sex hormones, this results in a greatly decreased susceptibility for females (26).

Pain

Many studies point towards a lower pain threshold in women, in whom pain is more severe, frequent and long lasting—possibly due to sex differences in generic pain mechanisms (27).

Attention Deficit Hyperactivity Disorder (ADHD)

While ADHD is more prevalent in males, affected girls appear to show greater intellectual impairment, and lower levels of hyperactivity and other externalizing behavior (28).

Schizophrenia

Differences between men and women are so marked that some researchers have claimed they are almost distinct diseases. Men have an earlier onset, a poorer prognosis and inferior response to treatment. Other explanations for this include differences in stress responses and protective factors (29).

Response to Drugs

Women respond differently to many drugs compared to men—possibly to a degree much greater than previously believed (30). This has been known for many years: for example, a substantial list of drugs affecting men and women differently was published in 1985 (31). Women also report more adverse reactions than men, and can have different dosage requirements—such as with psychoactive drugs. Examples of differences in pharmaceutical efficacy and toxicity include anesthetics, HIV therapies and anti-arrhythmic drugs (30, 32). Nearly 50% of new drug applications do not include analysis of sex differences. Of those that do, an estimated 7% show at least a 40% difference in pharmacokinetics (how the body deals with a drug: i.e. how it is absorbed, distributed, metabolized, and excreted) between men and women, meaning that "…the impact could potentially be very significant for both drug development and for overall clinical outcomes" (32).

The oral bioavailability of drugs—a measure of what proportion of a drug taken by mouth reaches the circulation unmodified—, and drug distribution around the body, appear to be particularly different between men and women, due to differences in the activity of major metabolic enzymes in the intestine and liver. Such differences are sometimes due to differences in body composition and weight, and hormonal effects, but many are due at least in part to sex-specific expression of metabolic enzymes (33). Men and women show differential activities of the major enzymes responsible for drug metabolism, the cytochrome P450 (CYP) enzymes ((34) and reviewed extensively in (30)). For example, CYP1A2 and CYP2E1 are more active in men, while CYP2D6 is more active in women (35). Some CYPs are affected by sex hormones, while others are differentially inducible by various drugs, depending on sex (2). The CYP3A family of enzymes, one of the largest subgroups that accounts for the metabolism of over 50% of commercially available drugs, and which has been linked with the toxicity of many drugs, is much more active in women than men (32). Sex differences in drug transporter molecules (involved in absorption, excretion, and entry of drugs into target organs, for example) also are known (32). This is a major safety issue: such differences lead to different dosing requirements and inconsistent effects of a wide variety of drugs between men and women, in addition to differences caused by the other variables mentioned.

Pharmacokinetics and pharmacodynamics (how drugs affect the body) of antipsychotic drugs differ in men and women. Typically, women require much lower doses, which is at least in part due to gender differences such as body composition, diet, smoking, other medications, exercise, substance use, and hormonal transitions.

Women also suffer from some specific adverse effects, and adverse effects that are more common and more problematic (36).

Pain-relief medications also have different effects: opioids appear to be more effective in women than in men, and may also show differences in their unintended effects such as respiratory depression, nausea and vomiting (32, 37). Drug-induced arrhythmias (irregular heartbeat) also differ, due to sex hormones affecting different channels in the heart (14, 32).

These differential reactions—a result of failing to properly ascertain and account for gender differences in drug development—are not trivial, and can have very serious consequences. For example, women have a greatly increased propensity to drug-induced long QT syndrome, in which the heart's electrical activity, regulating its contraction, is altered. This may lead to anything from palpitations and fainting to cardiac arrest and sudden death. For the drug d-Sotalol, prescribed for disturbances of the heart's rhythm and hypertension, the risk of death in women was found to be 2.5 times greater than that in men. Further: women predominate among patients with drug-induced liver injury, which is estimated to account for approximately half of all acute liver failure.

Sex Differences in Animal Models

Sex differences are not, of course, unique to humans—very many are known to exist in many if not all non-human species too. While this is not the aim of this report, it is important to consider them as they underline the main thrust of it, and the question posed earlier: *if extrapolation of knowledge between sexes of the same species is difficult, how can one reliably extrapolate from one species to another?*

For example, significant differences are apparent in the behavior of different strains of mice, frequently used in transgenic research, including anxiety-like behavior, hyperactivity, coordination, and spatial and non-spatial task performance. These disparities led to the conclusion that the "…importance of the genetic background and sex of mice for the molecular biological and pharmacological studies" should be highlighted—an assertion supported by findings from previous research (38). Sex is also known to confound data obtained in mouse studies of kidney disease (39), and in rat studies of obesity and gastric bypass surgery (40), for example. Recent papers have been published that highlight sex differences in animal models of: CVD (41); various autoimmune disorders (42); the brain and behavior in general (43) including stress, depression and post-traumatic stress disorder (44-46); migraine (47); Parkinson's disease (48); drug dependence (49); neonatal injury (50), and toxic responses of the kidney (51).

Frequently, there are sex differences apparent in some species but not others—further amplifying the case against the extrapolation of data form one species to another. For example, Hashimoto thyroiditis is present in ten times more women than men, but mouse and rat models are not sex discrepant. Systemic lupus is up to nine times more prevalent in women than men, but is highly strain dependent in mouse models: in different strains, symptoms are more prevalent in males, females, or neither sex (14).

Animals are Poor Models for Research into Human health

These many, diverse and significant differences between sexes of the same species are writ large in the repeated manifestation of problems when attempts are made to apply data from one species to another, such as animal data to human diseases and drug responses. Many drugs that appear safe and effective in animals fail in humans, and often cause significant harm and death. Examples include: hormone replacement therapy for women, which is thought to have caused tens of thousands of cases of breast cancer and cardiovascular events; the development of HIV protease inhibitors, which was delayed by years; the anti-inflammatory drugs Vioxx and other COX-2 inhibitors, which may have cause a third of a million heart attacks and strokes, and 140,000 deaths globally; the harmful effects of smoking, and the development of numerous antibiotics, antivirals, antidepressants and cardiovascular medications. The most recent analysis reports that 94 per cent of drugs that enter clinical trials following animal testing fail to achieve marketing approval, and the failure rate is at least 95 per cent for cancer drugs. Of the remaining drugs that are approved, half are withdrawn or relabelled due to severe or lethal adverse effects that are not detected during animal testing.

All of the more than 85 preventive and therapeutic HIV/AIDS vaccines successful in non-human primates failed in human trials. More than 4,000 studies report the efficacy of more than 700 treatments in animal models of stroke, however none of the approximately 150 of these tested in humans has shown clinical benefit. Developmental toxicity studies in animals show considerable discordance between different species, and are not predictive of human response.

Summary

It has been clear for some years—and is now scientifically clearer than ever before—that men and women are significantly biologically different, over and above the obvious primary gender differences. Men and women often suffer differently from the same diseases; different symptoms; different incidence and prevalence; different severity, outcome and survival; different pathological processes. Men and women often process and respond differently to drugs, meaning different doses are required, and different efficacies and adverse effects must be considered. All of these differences are underscored by myriad, widespread and wide-ranging differences in biology: in genetics, biochemistry and physiology. It is therefore abundantly clear that sex variability should be an important facet of biomedical research, drug development and medical practice.

This was echoed by a major report by the U.S. Institute of Medicine (IOM) on sex differences in biomedicine in 2001 (14), which recommended a variety of major changes in attitudes to research and medical practice, based on the knowledge that sex differences are not simply due to hormonal influences. Their recommendations included: investigating sex differences at a cellular level (every male and female cell has a different complement of genes); “studying sex differences from womb to tomb” as differences occur at different, and all, stages of life;

In spite of this, science and medicine are not yet adequately responding to do what they must to account for the differences. Most toxicology studies focus only on males; most epidemiologic studies report only on males, and those studies that include both

sexes often ignore differences (2). “Biomedical science has shown a strong sex bias and blindness to sex,” in which males are studied much more than females, and medical texts reflect male, rather than male and female, biology (52). With specific regard to the development of new drugs, evidence appears to be damning: “Pharmaceutical studies likewise slight females” (2); “women subjects remain seriously under-represented in clinical cohorts…their [sex imbalances in research] cumulative effect is pernicious: medicine as it is currently applied to women is less evidence-based than that being applied to men” (53); “Drug development is based on research in males, even for diseases that are more prominent in females, and despite evidence that drug metabolism and efficacy differ in the two sexes”—leading to sex differences being underappreciated due to a lack of data and knowledge (52).

This is all despite reforms undertaken in the 1990s, following the recognition that sex bias in clinical trials was a problem. Women had been excluded from drug trials for years, due to Food and Drug Administration (FDA) concerns over the involvement of “women of childbearing potential”—i.e. most adult women (2), and a general belief that men and women would neither be significantly different in most situations, nor that female hormonal cycles would be a factor—despite female hormonal cycles being accepted as a confounding factor that would be desirable to keep out of new drug trials (14). While this has been redressed to some degree since a change in FDA recommendations in 1993, there remains an erroneous tendency to believe that most sex differences are due to body size and morphology (2).

There is still some distance to go to improve matters significantly and to a level that will benefit science, medicine and consequently human health, however. Ethical, financial, sociological, and scientific barriers still exist (14), and remedies include changes of attitude and priorities in scientific and medical arenas, and, importantly, “Speeding more women into the senior ranks of science, which they still struggle to reach” (53).

Crucially, we contend that there is one critical aspect that has been overlooked: the continued, and burgeoning, use of nonhuman species in research into human diseases, and for the development of human drugs. As stated previously: *if extrapolation of knowledge between sexes of the same species is difficult, how can one reliably extrapolate from one species to another?* The problem illuminated by this rhetorical question is further compounded—even encapsulated—by the knowledge that “The vast majority of drugs—more than 90%—only work in 30 or 50% of the people” (54). Major differences in drug efficacy and safety are therefore known not just between sexes of the same species, and even between ethnic groups (humans) or breeds and strains (e.g. dogs and rodents), but even between *individuals* of the same species—not only of the same sex, but even identical twins (55). When we live in an era in which it is widely acknowledged we are entering an age of personalized medicine, it is a folly to continue our use and dependency on other species as models for human beings. That science is not working and in the call for progress in combatting human disease, it should be abandoned in favour of in vitro non-animal methods that will lead to the breakthroughs long awaited and too often hindered not advanced by the animal model.

Gender, therefore, is a crucial, and as yet poorly understood and under-investigated factor affecting biomedical research, drug development and safety testing. In making

our case for the need for greater research into human gender differences, we are echoing the opinion of The U.S. Institute of Medicine (IOM), which asserts:

> "Because differences between the sexes are pervasive across all subdisciplines of biology, all research sponsors should encourage research initiatives on sex differences. Research sponsors and peer-review committees should recognize that research on sex differences may require additional resources."

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