Pervasive Neglect of Sex Differences in Biomedical Research

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Females have long been underrepresented in preclinical research and clinical drug trials. Directives by the U.S. National Institutes of Health have increased female participation in research protocols, although analysis of outcomes by sex remains infrequent. The long-held view that traits of female rats and mice are more variable than those of males is discredited, supporting equal representation of both sexes in most studies. Drug pharmacokinetic analysis reveals that, among subjects administered a standard drug dose, women are exposed to higher blood drug concentrations and longer drug elimination times. This contributes to increased adverse drug reactions in women and suggests that women are routinely overmedicated and should be administered lower drug doses than men. The past decade has seen progress in female inclusion, but key subsequent steps such as sex-based analysis and sex-specific drug dosing remain to be implemented.

In the not too distant past, men were considered representative of the human species; differences from the male norm were viewed as atypical or abnormal, just one aspect of a broader sexism that ranks among the most pervasive human prejudices (Perry and Albee 1998). The notion that women and girls were inferior to men and boys was in play when agriculture and sedentary cultures emerged (Ananthaswamy and Douglas 2018), with the status of women lower than that of men from the dawn of recorded history (Rosen 1971; Morsink 1979). Sex bias persists today in virtually all walks of life, creating an environment that disadvantages women.

Women and nonhuman female mammals have been given short shrift in biomedical research. Until recently, the research community labored under the misguided assumption that information garnered from studies of males could be generalized without modification to females. Since then, sex differences in mechanisms underlying basic biological processes, from pain signaling (e.g., Mogil et al. 2003; Sorge et al. 2015) to synaptic inhibition (Huang and Woolley 2012), to drug metabolism (described

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below), have been detailed with important consequences for women's health (Heinrich 2001; Klein et al. 2015).

The belief that hormonal variations associated with estrous and menstrual cycles renders females more variable than males—now discredited (Mogil and Chanda 2005; Prendergast et al. 2014; Becker et al. 2016)—discouraged inclusion of women and female rodents in experimental protocols, negatively impacting the quality of medical care for women. Mazure and Jones (2015) provide a detailed chronology of changes instituted by the U.S. National Institutes of Health (NIH) to address sex bias in human medical research, and provide a comprehensive list of actions needed to remove remaining barriers to ensure appropriate consideration of sex as a biological variable (SABV) in biomedical studies.

Here we review historical and current trends of female inclusion in preclinical and clinical research, evolving policies aimed at increasing participation of females in biomedical research, and ongoing deficits in analysis with sex or gender as a factor. The assumption that periodic fluctuations in hormone secretion compromise female participation in scientific studies is discredited, and the pharmacological basis of human sex differences in adverse drug reactions (ADRs) is explored.

HISTORICAL ANALYSIS

Inclusion of both sexes in research studies has been consistently low (∼15%) over a 100-year interval (Fig. 1A; Beery and Zucker 2011). In the early to mid-twentieth century, the majority (60%–80%) of articles dealing with nonhuman mammals failed to report the sex of research subjects used in biological research. Since then, the percent of articles omitting subject sex has markedly decreased, but has been accompanied by a concomitant increase in the percent of articles reporting the exclusive use of males.

Inclusion of women in clinically relevant research has been marginally to substantially better over the past half century (Beery and Zucker 2011). Between 1949 and 1989, 32%–45% of studies incorporated both male and female subjects, with a marked increase to >60% in 1999 and 2009. Articles with sex unspecified declined from over 20% between 1949 and 1979, to ∼7% between 1989 and 2009.

CHANGES IN THE PAST DECADE: SEX BIAS

In 2009, we documented extensive male bias in research on humans and nonhuman mammals in eight of ten surveyed biological subdisciplines (Beery and Zucker 2011). The ratio of articles reporting on only males versus only females was most skewed in the fields of neuroscience (5.5:1) and pharmacology (5:1)—two research domains with strong preclinical relevance. A female skew was present only in studies of reproduction (1:1.6), and in immunology (1:2.2). Subject sex was often not reported in publications in 2009. Sex was omitted in 22%–42% of articles in neuroscience and physiology; at least 92% of articles in the behavior, endocrinology, and pharmacology categories specified sex of experimental animals or tissues (Beery and Zucker 2011).

In 2016, a U.S. National Institutes of Health policy change (NOT-OD-15-102; NIH) required investigators to consider SABV in grant applications. Woitowich and Woodruff (2019) assessed the short-term impact of this directive by surveying attitudes about the 2016 policy and perceptions regarding its implementation among NIH study section members in 2016 and 2017. A majority of respondents considered it important for NIH-funded research to consider SABV and thought it would improve rigor and reproducibility of findings. The percentage of grant applications that successfully addressed and incorporated the policy increased over this span.

A follow-up study analyzed articles in nine biological disciplines in 2019 to compare with a similar survey a decade earlier, and to assess the extent of incorporation of SABV in the years after the 2016 NIH directive (Woitowich et al. 2020). The percent of studies that included both sexes increased across pooled subdisciplines (Fig. 1B), with significant gains in many but not all specific fields (e.g., neuroscience but not pharmacology). Overall, sex-inclusive research practices have increased since 2009, although male bias remains in

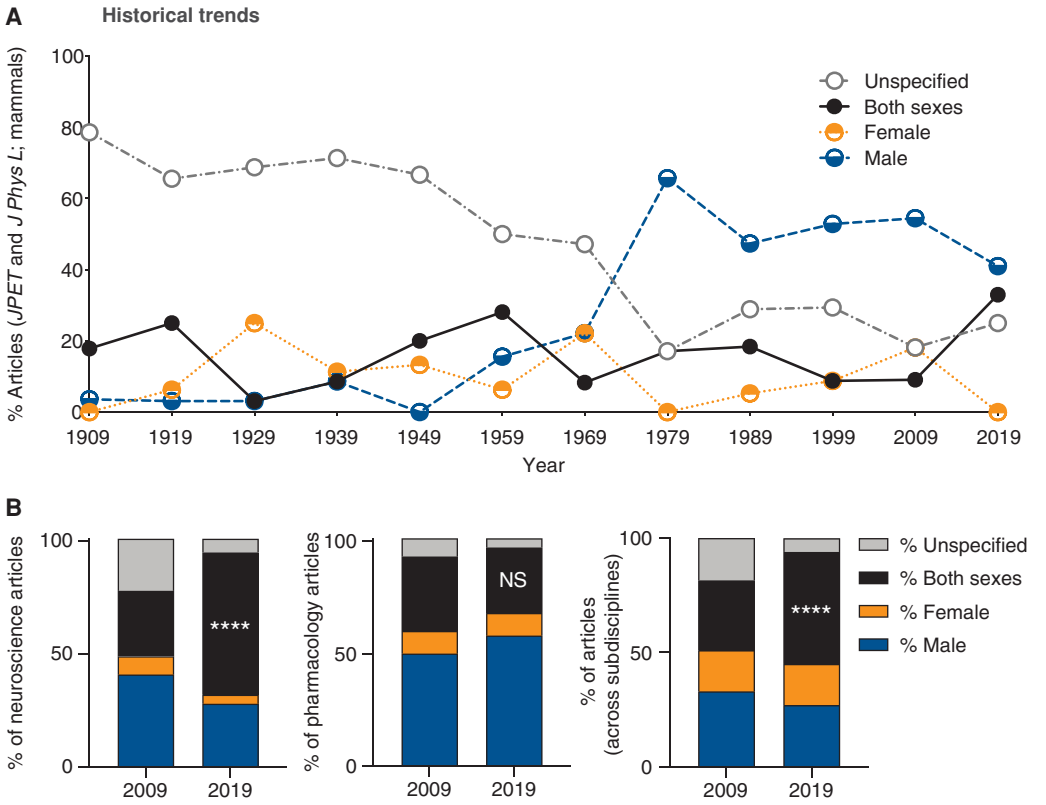

Figure 1. Sex bias in research subjects tested over time. (*A*) Sampling of mammalian studies published in two physiology journals over the past century reveals that including both sexes is not the norm. Decreased omission of the sexes of research subjects ("unspecified") over time coincides with an increase in reports of male-only studies. Historical data through 2009 are from Beery and Zucker (2011) (reprinted with permission from the authors). 2019 data from the *Journal of Physiology* (London) were obtained from the supplementary data file in Woitowich et al. (2020) (reprinted with permission from the authors) (note the *Journal of Pharmacology and Experimental Therapeutics* was not sampled for this year). (*B*) Across biological science subdisciplines surveyed, more 2009 studies examined only males than examined females or both sexes. By 2019, studies involving both sexes in at least one part of the study were significantly more common. This pattern was evident in some subdisciplines and not others. For example, there was an increase in sex-inclusive articles in neuroscience, but no such increase in pharmacology. $^{****}p < 0.0001$; NS = not significant.

several biological disciplines (Woitowich et al. 2020), and has increased in some fields (e.g., cardiovascular research [Ramirez et al. 2017]). In neuroscience, these gaps appear more prevalent for research performed in some species (e.g., rats, ferrets), but do not currently directly reflect funding source (Mamlouk et al. 2020). Most reports of single-sex male studies did not provide a rationale for excluding females (Woitowich et al. 2020), but justification for female exclusion still sometimes invoked the belief that females exhibit cyclicity-driven variability (now discredited, see below).

LACK OF CHANGE IN THE PAST DECADE: SEX-BASED ANALYSIS

Whereas inclusion of females has improved over the past decade, there has been no concomitant increase in the percentage of studies on both sexes that analyze results with sex or gender as a factor in analyses, and fewer than half of sex-inclusive studies make reference to any such analysis (Fig. 2; Mamlouk et al. 2020; Woitowich et al. 2020). Thus, improved female inclusion has not yet translated into increased reporting of female biology.

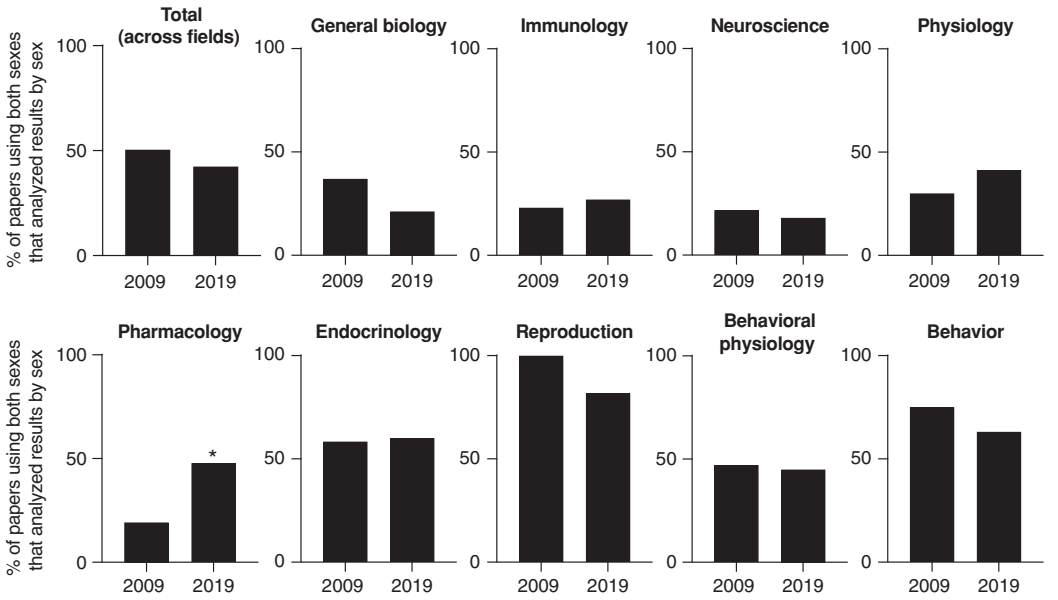

Figure 2. Lack of progress in analysis of findings by sex. While inclusion of both sexes increased from 2009 to 2019, there was no change in sex-specific analysis of the data: across fields, less than half of the studies that used both sexes reported any analysis by sex. Separate analysis of nine biological subdisciplines showed only the field of pharmacology increased in percentage of sex-based analyses, but this needs to be understood in the context of a field that has not increased inclusion of females. In other words, when that minority of papers in this field do include females (see Fig. 1B), they are likely to look for sex differences. (Data are from Beery and Zucker 2011 and Woitowich et al. 2020; reprinted, with permission, from the respective authors.)

The lack of sex-based analyses in sex-inclusive studies is potentially problematic. Males and females should not be pooled in analysis without—at a minimum—screening for sex differences (and reporting this screen); otherwise, experimental power may be lost (Beery 2018; Buch et al. 2019). When sex differences are present, inclusion of sex as a factor in analysis allows authors to identify these differences, as well as to increase the power to detect main effects of manipulations or treatments in the presence of sex differences in mean values (Beery 2018). Exploratory analysis of sex effects using factorial designs can assess the main effects of treatment and subject sex with effectively the same power as pairwise tests, without increased sample size (Collins et al. 2014; Buch et al. 2019). In the case where there is an interaction between sex and the main variable of interest, additional samples may be needed, and testing designed to capture sex differences will be biologically meaningful (Becker et al. 2005).

Fears of the need for doubled sample sizes (or more) in the presence of sex differences are thus unfounded. When sex differences are absent or when they are present without a sex × treatment interaction, no sample size increase is needed (Beery 2018; Buch et al. 2019). When both sex differences and a sex × treatment interaction are present (i.e., the situation requiring the largest sample size increases), a recent model revealed necessary increases of only 14%–33% under different conditions to include both sexes, even after statistical correction for the use of multiple factors (Buch et al. 2019).

The actual prevalence of sex differences in biology remains unknown, in part because there have been so few systematic surveys of the topic. This gap in reporting suggests that known sex differences are just the tip of the iceberg. To improve the validity of preclinical and clinical research, it is not enough simply to include females in research studies; one must also examine whether there are effects of sex/gender. Several

guides for researchers to analyze sex differences now exist (Becker et al. 2005; Beltz et al. 2019; Buch et al. 2019).

FEMALE RODENTS ARE NOT A LIABILITY IN PRECLINICAL RESEARCH

The long-standing assumption that the estrous cycle renders females intrinsically more variable than males may be the single greatest contributor to the sex biases cataloged in the preceding section (Mogil and Chanda 2005). The underrepresentation of female animals in biomedical research is based on the assumption that, for any given trait, females exhibit more variability than males, and therefore must be tested at each of four stages of the estrous cycle to generate reliable data (Wald and Wu 2010). Satisfying this requirement would quadruple the number of females compared to studies employing males, thereby increasing experimental effort and cost. However, the question of whether such sex differences in variability even exist had not been addressed until recently.

In an assessment of more than 8000 individual measurements collected on 40 different mouse strains in three different laboratories, researchers found that females tested at random points in their estrous cycles were no more variable than males on an acute thermal pain test; no sex differences in variability measures were observed in acute and tonic chemical nociception tests (Mogil and Chanda 2005). This inference transcended measures of pain, evidenced by assessment of variability between male and female mice on ∼10,000 morphological, physiological, and behavioral traits (Prendergast et al. 2014). This analysis focused on measures obtained without regard for the stage of the estrous cycle, thus maximizing any supposed female variability. Across this diverse array of traits, female variability was shown to be no greater than that of males even when estrous cycles were not monitored, thereby eliminating the barrier that fostered underrepresentation of female rodents in biomedical research (Fig. 3A).

The analysis also examined coefficients of variation (CVs) sorted into 30 broad trait categories but found no systematic pattern of sex-biased CVs—a similar conclusion was reached from an analysis of a set of >2 million data points, across 218 traits measured from >26,000 mice (International Mouse Phenotyping Consortium; Zajitschek et al. 2020). An evaluation of variability in gene expression, performed on 293 microarray data sets from mice and humans, found gene expression in males to be slightly more variable than that of females, although the difference was small (Fig. 3B; Itoh and Arnold 2015). The most common tissue source for this analysis was brain, which showed either a slight male bias in CV or no difference. Distinct patterns of sex differences in variance were identified in individual organs: in most cases CVs were higher in males (kidney, adrenal, skeletal muscle), but variance was greater in females in one tissue—spleen (Itoh and Arnold 2015). Overall, the report concluded that variability in gene expression was no greater in females than males.

An analogous meta-analysis, using similar methods, was performed focused on neuroscience-relevant measures in male and female rats (Becker et al. 2016). Evaluating over 6000 trait measures, the authors found no overall differences in variability (Fig. 3C). As in prior reports, the analysis identified individual trait categories in which CVs were greater in one sex; and again, this occurred more often in males. Indeed, the authors found that even in the subset of ∼300 traits that were measured at known phases of the estrous cycle, segregating data by cycle day did not yield a reduction in variability of female data (Fig. 3D). Taken together, results from meta-analyses of data sets in multiple species and across diverse research domains are characterized by a consistent failure to support the hypothesis that females are intrinsically more variable than males.

Changes in physiology and behavior across the estrous cycle are, however, well-documented in female rodents (Krzych et al. 1978; Barthelemy et al. 2004); indeed, these observations presumably fueled the notion that females must be more variable (and thus more difficult to study). How, then, do these well-established estrous fluctuations fail to result in increased variability in females? A possible answer lies in closer ex-

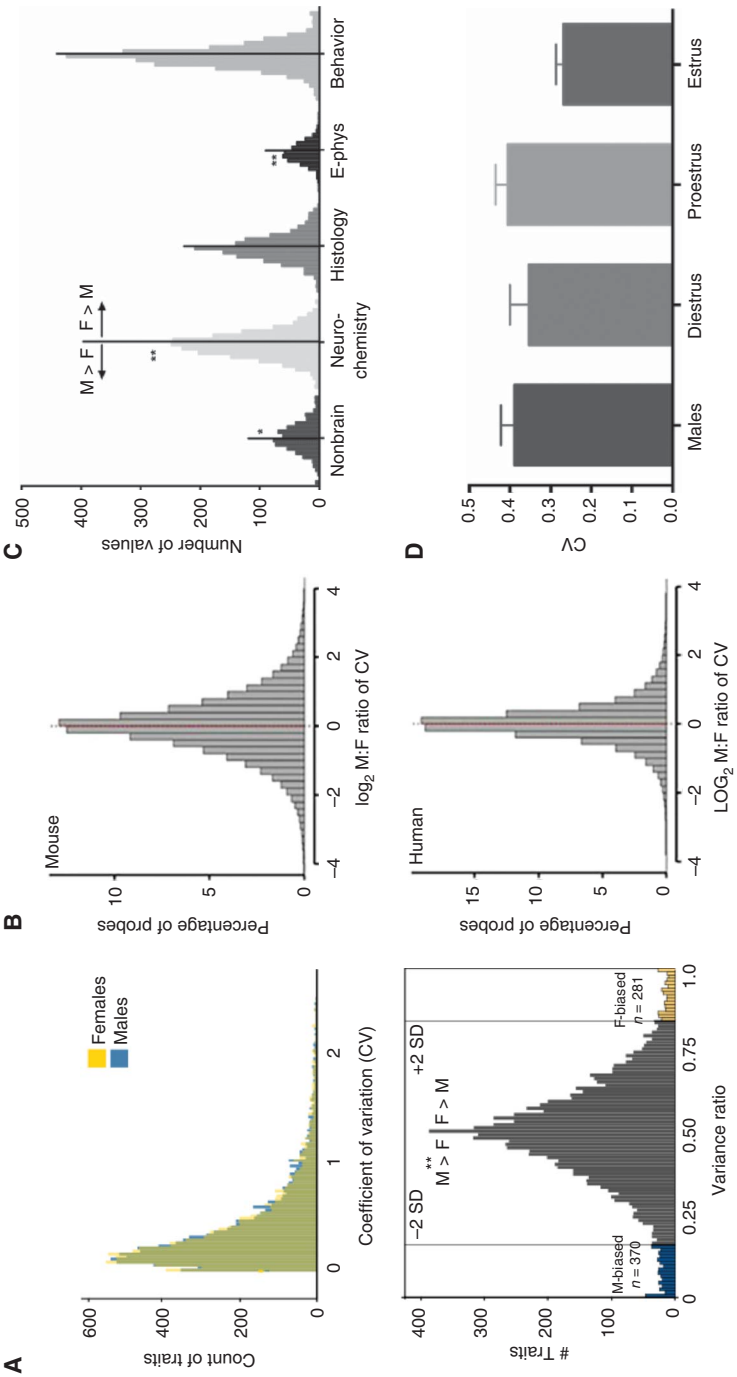

Figure 3. Trait variability is no greater in female than male mice. (*A*) Trait variability comparisons in mice. *Top* panel indicates coefficients of variation (CVs; standard deviation [SD]/mean) for phenotypic traits ($n = 9932$) of male (blue) and female (yellow) wild-type mice as reported in 293 peer-reviewed articles published between 2009 and 2012 (Prendergast et al. 2014). Green shading indicates overlapping areas of yellow and blue histograms. *Bottom* panel depicts CV ratios ($CV_{female}/[CV_{female} + CV_{male}]$) for traits in the *top* panel. The CV ratio distribution ranges from 0.0 to 1.0, with CV ratios >0.5 indicating greater trait-specific variance in females, and CV ratios <0.5 indicating greater variance in males. (*B*) CV comparisons in microarray data sets. Histograms depict $\log_2$ transformed male-to-female CV ratios across ~2.7 million human and ~2.4 million mouse microarray probes. In most bins, slightly more probes showed $CV_{male} > CV_{female}$ (Itoh and Arnold 2015). (*C*) CV comparisons in laboratory rats. Histogram depicting CV ratios for 6252 traits in laboratory rats, parsed into five broad trait categories. CV ratios were male biased for electrophysiology and neurochemistry measures, and female biased for nonbrain measures. (*D*) Estrous cycle stage did not affect trait variability. Among rats examined in panel C, even when data were aggregated by stage of the estrous cycle, CV_{female} did not differ from CV_{male} (Becker et al. 2016). (Data used under Creative Commons Attribution 4.0 International [CC BY 4.0: https://creativecommons.org/licenses/by/4.0].)

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amination of the sources of variance within each sex. Whereas a potent source of female variability may be found in hormonal fluctuations over the ovulatory cycle (Quinlan et al. 2010; Datta et al. 2016), the sources and patterns of male variability have not been systematically evaluated, let alone characterized. Accounting for the overall absence of sex differences in variability is warranted. The magnitude of variance associated with the estrous cycle may be sufficiently small as to have little impact; alternatively, trait variability of males over several consecutive days may simply be as great as that of females over the estrous cycle. Potential sources of this "hidden" male variance were investigated by performing time-series analyses of locomotor activity and body temperature recorded continuously over the estrous cycle of female mice and in yoked males (Smarr et al. 2017). Overall variability in circadian power was comparable between the sexes for both traits. Remarkably, infradian variability (variance across days) was greater in fe-

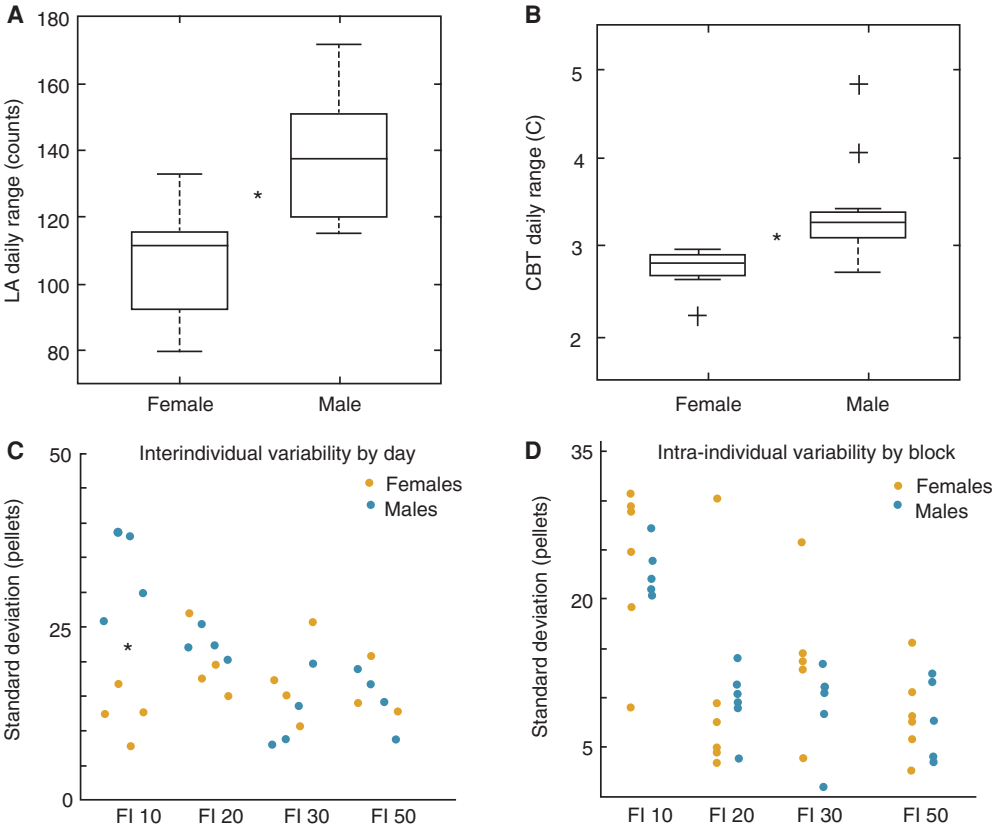

Figure 4. Female mice do not exceed males in variability of activity, body temperature, or food intake. (*A*) Box plots of the daily variability in locomotor activity (LA), and (*B*) core body temperature (CBT) of female and male mice housed in a 12L:12D light:dark cycle indicate that males have a higher intra-animal daily range in LA and CBT. (*C* and *D*) Food intake data within and between male (blue symbols) and female (yellow symbols) mice subjected to schedules of food availability and reward over 4-day blocks (corresponding to the estrous cycle, which was not monitored). Under increasing cost of feeding, females exceeded males in intra-individual variability, (*D*) but not (*C*) interindividual variability. The overall variance did not differ by sex under any of the food reward schedules. Food intake (FI) designations represent the delay in seconds after which responses delivered a 20 mg food pellet. (Data in *A* and *B* are from Smarr et al. 2019; reprinted, with permission, from the authors. Data used under Creative Commons Attribution 4.0 International [CC BY 4.0: https://creativecommons.org/licenses/by/4.0].)

males and ultradian variability (variance within days) was greater in males (Fig. 4A). The results also indicated that, for any given trait, depending on how data are collected (e.g., across multiple days, in a single day, during a fixed time span within a single day), measures will capture variance from different sources. Exclusion of female mice from studies may thus increase variance in investigations in which measures are collected over a span of several hours. Ultimately, this factor may limit generalization of findings from males to females. Smarr and colleagues (2019) also detailed sex differences in variance structure within ingestive behavior. Mice of both sexes were subjected to schedules of food availability and reward over 4-day blocks. Females exceeded males in intra-individual variability, but not interindividual variability (Fig. 4B). Overall, variance did not differ by sex under any of the food reward schedules.

Thus, convergent evidence from multiple species and across diverse traits fails to support the hypothesis that females are more variable than males. Mogil and Chanda (2005) speculated that "male mice [may] feature their own sex-specific variability." Now that the default assumption of greater female variability has been assessed and rejected, a productive new direction may lie in efforts to identify the sources of variance that render males as variable as females, and in many cases, more variable than females.

NEGLECT OF WOMEN IN BIOMEDICAL RESEARCH HAS NEGATIVE HEALTH IMPLICATIONS

Many currently prescribed drugs were approved by the U.S. Food and Drug Administration (FDA) with inadequate enrollment of female animals in preclinical research, and women in clinical trials. For example, the popular sedative-hypnotic drug zolpidem (Ambien), was first approved in 1992 with just 19 women and 49 men (FDA NDA 19908) as the sole pre-approval assessment of the effect of sex on pharmacokinetics (PK), which revealed marked elevation of drug concentrations in women and longer elimination times. Only after decades of post-marketing reports of cognitive deficits in women were sex-based dose adjustments recommended, at which point women were advised to receive half the zolpidem dose given men. Many other drugs administered in equal doses to women and men require reevaluation for sex-specific dose adjustments, but relevant data are lacking for almost all currently prescribed pharmaceuticals. Zolpidem is but one of many drugs not administered on an mg/kg of body mass basis; women are given the same dose as men, despite lower body weights and sex differences in drug absorption, distribution, bioavailability, metabolism, and excretion. Consequently, women may be overmedicated.

Across all drug categories, ADRs are substantially more common in women than men (de Vries et al. 2019); more female ADR reports were submitted in all regions of the world and all age groups from 12 to 17 years and older (Watson et al. 2019). It is conceivable that some of the increase in ADR reports for women might be caused by the possible overmedication discussed above. Women are also significantly more likely to be hospitalized secondary to an ADR (Tharpe 2011; Nakagawa and Kajiwara 2015; Damien et al. 2016). This disparity is pervasive: 46% of a large sample of drugs manifests significant sex/gender differences in ADRs (Yu et al. 2016). Women over the age of 19 were 43%–69% more likely than men to have an ADR recorded by their general practitioner (Martin et al. 1998). ADRs also peak 20 years earlier among women than men (Martin et al. 1998). Frequently reported ADRs included nausea, headache, drowsiness, depression, excessive weight gain, cognitive deficits, seizures, hallucinations, agitation, and cardiac rhythm anomalies. Women are more likely than men to use two or more medications concurrently (polypharmacy), and more unique medications per year, which may also contribute to increased female ADRs (Manteuffel et al. 2014).

Biological, psychological, and cultural factors contribute to the greater prevalence of ADRs in women, including sex differences in PK and pharmacodynamics (PD), endogenous sex-specific organizational and activational steroid hormone exposure, sex differences in exogenous steroid administration, higher rates of polyphar-

macy in women, sex differences in the expression of somatoform disorders, and sex differences in reporting rates (Kando et al. 1995).

PK differs in men and women for many drugs (Harris et al. 1995; Meibohm et al. 2002; Schwartz 2003, 2007; Gandhi et al. 2004; Soldin and Mattison 2009; Franconi and Campesi 2014, 2017), which affects drug efficacy and toxicity (Institute of Medicine (US) Committee on Understanding the Biology of Sex and Gender Differences 2001; Amacher 2014). Significant sex differences have been noted in PK in ~28% of bioequivalence studies (Chen 2000). But PK information is included in only a small minority of approved drug labels (Fadiran and Zhang 2015); fewer than 4% of drugs in the *Physicians' Desk Reference* list population PK information in labeling (Duan 2007).

To examine whether sex differences in PK, specifically elevated drug exposure and longer elimination times in women than men, are associated with clinically significant sex differences in ADRs, a literature survey was performed to identify drugs for which sex differences in both PK and ADRs had been examined (Zucker and Prendergast 2020). The analysis identified hundreds of FDA-approved drugs with sex differences in PK, and 86 drugs with statistically significant sex differences in PK, which also reported data on ADRs that were analyzed by sex (Fig. 5). PK differences commonly manifested in measures of the maximum drug concentration in the blood (C_{max}) and area under the curve (AUC), but also included distribution volume and measures of drug elimination rate (e.g., circulating half-life). Many drugs with pronounced sex differences in ADRs were excluded from the analysis because PK data were not available, typically because the drug was approved prior to the year 2000, or because sex-specific analyses were not available in the FDA database. The analysis also indicated that many sex differences in ADRs persisted even after corrections for body weight were considered.

The correspondence of sex differences in PK with sex differences in ADRs was striking. In 88% of instances, a sex difference in PK was linked to a similar sex difference in ADRs; PK-ADR concordance is summarized in Table 1. The sex difference in ADRs among these drugs is substantially higher than the 46% sex difference in ADRs across all drugs assessed without regard to PK differences (reported by Yu et al. 2016). Thus, including PK data in the consideration of ADRs and stratifying analyses of drugs by the presence and direction of PK differences greatly clarifies patterns of sex differences in ADRs. Nearly all (96%) drugs with higher PK values in women were associated with a higher incidence of ADRs in women than men, whereas only 29% of drugs for which PK values of males exceeded those of females did this sex difference positively predict male-biased ADRs (Fig. 5). Indeed, even in this small fraction of drugs with male-biased PK, ADRs were more prevalent or severe in women. These data show an alarming pattern: elevated drug concentrations and decreased elimination times are far more common in women than men. For drugs with female-biased PK, the clear mapping of PK onto ADRs suggests that drugs that exhibit female-biased PK present a major health risk for women that is not prioritized by the medical profession. An even stronger potential for risk lies in that far larger number of drugs for which no data on PK sex differences exist.

The lack of attention to female subjects during the early stages of drug development may have pervasive, unintended effects that contribute to the disproportionate occurrence of ADRs in women. Drug development pipelines often begin with preclinical modeling, in vitro experiments in human tissues and in vivo experiments in laboratory animal models (usually mice). Inattention to sex and gender in the early stages of drug development can create founder effects that may bias drug efficacy toward one sex. If, during early stages of preclinical development, a drug is optimized and titrated specifically in cells or mice of one sex, then any sex biases inherent in such model systems may be propagated into later stages of drug development. For example, three of the four human cell lines currently being used for SARS-CoV-2 (COVID-19) research are male (Takayama 2020). Disproportionate female-biased PK and ADRs that are likely to emerge may reflect echoes of sex-biased research decisions early in the scientific process.

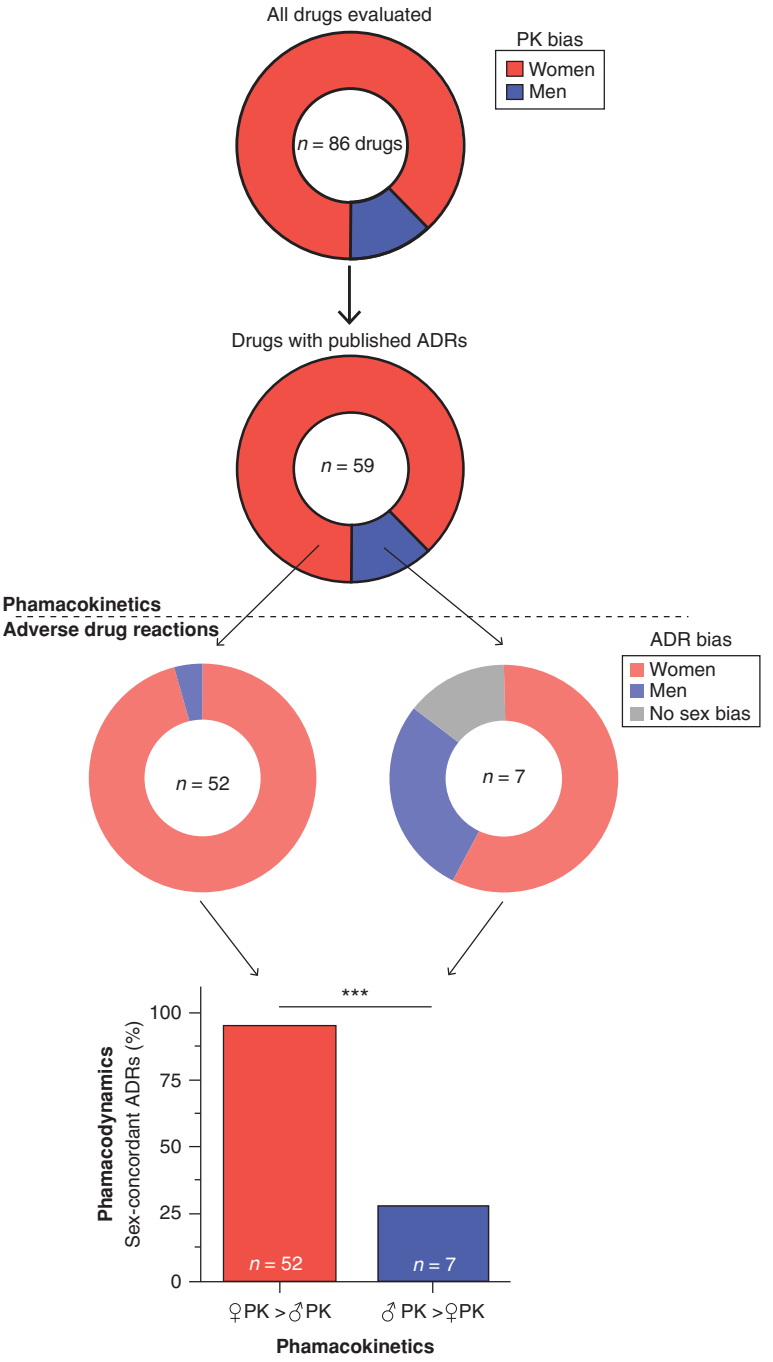

Figure 5. Relations between pharmacokinetics (PK) and adverse drug responses in humans. Sex differences in PK were identified in 86 U.S. Food and Drug Administration (FDA)-approved drugs, 59 of which also yielded data on sex differences in adverse drug reactions (ADRs). PK analyses identified greater drug exposure in women (a female PK bias) for 52 (88%) of these drugs. Among these 52 drugs with female-biased PK, 50 also had female-biased ADRs (96% concordance between PK and ADR biases). In contrast, only two of seven drugs with male-biased PK exhibited male-biased ADRs (29% concordance). (Data from Zucker and Prendergast 2020; reprinted, with permission, from the authors.)

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Table 1. Summary of sex-biased pharmacokinetic (PK) measures and concordance with clinical adverse drug reactions (ADRs)

World Health Organization anatomical therapeutic chemical category	Drug[a]	PK measure[b]	Female-biased ADRs	Male-biased ADRs	PK-ADR relation
Alimentary tract, metabolism	Liraglutide	CL_T	Headache, vomiting, nausea	-	Concordant
	Ranitidine	***AUC*, T_{max}**	-	**Duodenal damage**	Concordant
	Rosiglitazone	CL_T	Fracture	-	Concordant
Blood and blood-forming organs	Heparin	CL_T	Blood disorders, lymphatic disorders, bleeding (60 yoa)	-	Concordant
	Aspirin	AUC, CL_T, $t_{1/2}$	Elevated fibrinogen cardiovascular disease in type II diabetics	**Bleeding**	Concordant
	Warfarin	CL_T	Bleeding	-	Concordant
	Clopidogrel	AUC, C_{max}	Fracture, bleeding, gastrointestinal symptoms, inflammatory bowel disease	-	Concordant
	Dabigatran	AUC	Bleeding	-	Concordant
Cardiovascular system	Torasemide	AUC, C_{max}, CL_T, $t_{1/2}$	Hospitalization	-	Concordant
	Pravastatin	AUC, C_{max}	Congenital heart disease	-	Concordant
	Amlodipine	**CL_T**	Edema, flushing, palpitations	-	Discordant
	Digoxin	CL_T	Mortality	-	Concordant
	Verapamil	CL_T	Constipation, edema, fatigue, headache	-	Concordant
	Aliskiren	AUC, C_{max}	Diarrhea	-	Concordant
	Losartan	AUC, C_{max}	-	**Angina, mortality**	Discordant
	Propranolol	AUC, C_{max}, CL_T	Dizziness, muscle pain, headache, dry mouth	-	Concordant
	Dofetilide	SysExp	TdP	-	Concordant
Genito-urinary system, sex hormones	Mirabegron	AUC, C_{max}, SysExp	Treatment discontinuation (CAN), treatment discontinuation (CZE)	**Treatment discontinuation (UK)**	Concordant
	Darifenacin	AUC, C_{max}, CL_T	F > M, ADRs not specified	-	Concordant
	Trospium	AUC, C_{max}	Cognitive impairments	-	Concordant

Continued

Table 1. *Continued*

World Health Organization anatomical therapeutic chemical category	Drug[a]	PK measure[b]	Female-biased ADRs	Male-biased ADRs	PK-ADR relation
Systemic hormonal preparations	Prednisone	AUC, CL_T	Depression, fatigue, hair loss, mood swings, weight gain	-	Concordant
Anti-infectives	Levofloxacin	CL_T, SysExp	Fluoroquinolone toxicity	-	Concordant
	Erythromycin	**CL_T**	TdP	-	Discordant
	Voriconazole	AUC, C_{max}	Cardiac QTc symptoms	-	Concordant
Antineoplastics, immunomodulators	**Cyclosporin**	**CL_T**	-	-	Discordant[c]
	Flurouracil	AUC, CL_T	Stomatitis, leukopenia, alopecia, diarrhea, mucositis	-	Concordant
	Paclitaxel	CL_T	Myocardial infarction, death, lesion, revascularization	-	Concordant
	Capecitabine	AUC	Dose-limiting toxicity	-	Concordant
	Infliximab	CL_T	Allergic reactions	-	Concordant
	Adalimumab	CL_T	Allergic reactions	-	Concordant
Anesthetics, analgesics	Morphine	CL_T	Respiratory depression, hypoxic sensitivity, emesis, nausea	-	Concordant
	Oxycodone	CL_T	Nausea, pruritus, negative affect	-	Concordant
	Buprenorphine	AUC, C_{max}	Sleep disturbance	-	Concordant
	Tramadol	AUC, C_{max}	Treatment discontinuation	-	Concordant
	Zolmitriptan	AUC, C_{max}	Face and neck pressure	-	Concordant
	Ketamine	**CL_T**	-	**Verbal learning, subjective memory**	Concordant
Antiepileptics, anti-Parkinson's	**Carbamazepine**	**CL_T, $t_{1/2}$**	Cognitive impairment, elevated LDL/HDL	-	Discordant
	Gabapentin	C_{max}	Dizziness, somnolescence, nausea	-	Concordant
	Perampanel	AUC, CL_T	Dizziness, headache, treatment, discontinuation	-	Concordant
	Pramipexole	AUC, C_{max}	Nausea, fatigue	-	Concordant

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Psycholeptics	Olanzapine	CL_T	Severe weight gain	-		Concordant
	Clozapine	CL_T	Blood glucose, laxative use, obesity, type II diabetes, neutropenia, leukopenia, weight gain	**Hypertension, increases in BMI, elevated homocysteine, increased basal metabolic rate, increased plasma lipids, QTc symptoms, blood dyscrasias, myocarditis, cardiomyopathy**		Discordant
	Risperidone	CL_T	Hyperprolactinemia, neurological effects, headache hypotension	-		Concordant
	Aripiprazol	AUC, C_{max}	Blood pressure, heart rate, elongated QTc	-		Concordant
	Diazepam	$t_{1/2}$	Psychomotor impairment	-		Concordant
	Zolpidem	AUC, C_{max}, CL_T	Cognitive impairment, driving impairment	-		Concordant
	Eszopiclone	AUC	Dysgeusia	-		Concordant
Psychoanaleptics	Imipramine	CL_T	Dry mouth, constipation, sweating, tremor, treatment discontinuation	-		Concordant
	Nortriptyline	CL_T	Dry mouth	-		Concordant
	Fluoxetine	CL_T	Hypercortisolemia, elevated albumin, elevated tryptophan, suicidal ideation	-		Concordant
	Citalopram	CL_T	Elevated ADH	-		Concordant
	Sertraline	AUC, CL_T, $t_{1/2}$	Cholesterol, nausea, dizziness, delusions	**Dyspepsia, sexual dysfunction, urinary frequency**		Concordant
	Bupropion	AUC, C_{max}, $t_{1/2}$	EEG abnormalities, seizures	-		Concordant
	Methylphenidate	**AUC**	Anxiety disorder	-		Discordant

Continued

Table 1. *Continued*

World Health Organization anatomical therapeutic chemical category	Drug[a]	PK measure[b]	Female-biased ADRs	Male-biased ADRs	PK-ADR relation
Antiparasitics	Primaquine	AUC, C_{max}	Nausea	-	Concordant
Respiratory	Terfenadine	C_{max}	TdP	-	Concordant
	Fexofenadine	AUC, C_{max}	Visual attention deficits, reaction time deficits, driving impairments	-	Concordant
Miscellaneous	MDMA	CL_T	Elevated copeptin, hyponatremia	-	Concordant
	Cannabis	CL_T	Increased subjective emotional responses, problematic cannabis use, introvertive anhedonia	-	Concordant

(AUC) Area under the curve, (C_{max}) maximum circulating concentration, (T_{max}) time required to reach C_{max} value, ($t_{1/2}$) elimination half-life, (CL_T) clearance time, exposure, or plasma concentration after a fixed time interval, (SysExp) systemic exposure.

[a]Font style of drug name indicates direction of PK drug exposure bias: (normal font: F > M; **bold font**: M > F).

[b]Font style of PK measure indicates direction of greater drug exposure (normal font: F > M; **bold font**: M > F).

[c]No evidence of sex differences in ADRs.

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A number of steps might remediate this sex disparity in health and well-being that stems from female-biased ADRs. The high correlation between elevated PK in women and increased female ADRs suggests that for drugs with higher female PK, the initial dose should be lower for women than men and increased only if the lower dose fails to achieve the desired therapeutic effect.

Establishing sex parity in subject enrollment during the drug approval process should be explicitly identified as a long-term goal of the Department of Health and Human Services. The decades-long pattern of neglect of female animals in preclinical research and underrepresentation of women in clinical trials and research must be corrected; recent NIH oversight and vigilance is an important step in the right direction that needs to be maintained.

CONCLUDING REMARKS

Until relatively recently, female animals and women were woefully underrepresented in biomedical research. Beginning in the 1990s, spurred by the NIH revitalization act and continuing in subsequent decades, a remedial effort has increased inclusion of women and female rodents in clinical and preclinical studies. An increased emphasis on enrolling both sexes emerged in the past decade, but a majority of such studies still fail to consider sex or gender as factors in their analyses; this continued omission represents a missed opportunity and is a serious shortcoming. An increasing number of studies has established that average trait variability is no greater in females than males, thereby removing the long-held, unsubstantiated bias against inclusion of female rodents in research protocols. In many instances, sex inclusion does not require increases in sample size, but when such increases are necessary, they are smaller (e.g., 25% increase) than generally assumed.

The well-established increased susceptibility of women to adverse drug reactions has now been strongly correlated with substantial sex differences in drug PK; women given the same drug dose as men routinely generate higher blood concentrations and longer drug elimination times and thus may be chronically overmedicated. For drugs with known sex differences in PK, women should be administered lower drug doses.

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