Let’s Talk About Sex: Differences in Drug Therapy in Males and Females

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Abstract

Professor Henry Higgins in *My Fair Lady* said, ‘Why can’t a woman be more like a man?’. Perhaps unintended, such narration extends to the reality of current drug development. A clear sex-gap exists in pharmaceutical research spanning from preclinical studies, clinical trials to post-marketing surveillance with a bias towards males. Consequently, women experience adverse drug reactions from approved drug products more often than men. Distinct differences in pharmaceutical response across drug classes and the lack of understanding of disease pathophysiology also exists between the sexes, often leading to suboptimal drug therapy in women. This review explores the influence of sex as a biological variable in drug delivery, pharmacokinetic response and overall efficacy in the context of pharmaceutical research and practice in the clinic. Prospective recommendations are provided to guide researchers towards the consideration of sex differences in methodologies and analyses. The promotion of disaggregating data according to sex to strengthen scientific rigour, encouraging innovation through the personalisation of medicines and adopting machine learning algorithms is vital for optimised drug development in the sexes and population health equity.

Keywords
Sex and gender differences; Gastrointestinal pharmacokinetics and pharmacodynamics; Drug response and side effects; Personalized pharmaceuticals and medicines; Artificial intelligence and machine learning; 3D printing drug delivery systems; In silico and PBPK modeling; Cell lines; Pharmaceutical drug product design and development; Health equity
1.0 Introduction

In general, women are prescribed more drugs than men, require increased access to health care services but suffer from more adverse drug reactions (ADRs) and are hospitalised more often due to ADRs than men (even when adjusted for age-related differences) [1]. The lack of consideration of potential sex differences exists in nearly all areas of research and development and has seeped into the mainstay of society. For example, research from van Hoof et al. observed that office building thermostats are based on male metabolic rates with temperatures set too low for many women [2]. Some consequences, however, can be life threatening. In engineering, many devices and machines have been designed to fit male bodies; military and commercial cockpits were traditionally based on male anthropometry. As a consequence, it was potentially dangerous for some women or small men to become pilots [3]. Appropriate female representation has vastly been ignored in scientific research including immunology, pharmacology and neuroscience. Interestingly, in the preclinical field of behaviour and reproduction, sex as a biological variable is of particular interest [4].

Our cells are innately infused with sex differences that cannot be ignored. The phrase “every cell has a sex” captures the essence of how fundamentally different men and women are when it comes to health and disease (Figure 1a). In fact, every nucleated cell has a sex containing the sex chromosomes (in its simplest form, XX in females or XY in males [5]). A female-predominance to chronic disease is seen in epidemiology, pathology, clinical course and diagnosis of Alzheimer’s disease, influenza and pneumonia to name a few (Figure 1b). The onset and development of heart disease, specific cancers and chronic pulmonary disease, however, are leading causes of death in men [6]. In light of the COVID-19 pandemic, the global number of confirmed cases, severe symptoms, differing immune response and mortality rate due to the disease are higher among men [7-10]. However, recent epidemiology data from the COVID Symptom Study application revealed that women were more likely to develop “long COVID” where symptoms persist for longer than 12 weeks [11].

In biomedical research, women and non-human female mammals have often been under-represented. Although there is some recognition today of the need for appropriate female representation in clinical trials, in previous decades, the consideration and inclusion of males overshadowed females in clinical research design and conduct. Figure 1c demonstrates a male
bias in articles involving interdisciplinary research including biology, neuroscience, physiology, pharmacology and behaviour. Females, however, were the sex of interest in subject areas spanning reproduction, endocrinology and behavioural physiology [4]. Although Figure 1c outlines the employment of both sexes, it is clear that results are seldom disaggregated according to sex which can potentially skew or even conceal sex-specific differences in biomedical research (Figure 4) [4]. The leading assumption is that i) results from male studies appropriately apply to females or ii) hormonal cycles decrease the homogeneity of study populations and complicate experimental designs to such an extent that it may not be worth studying females from the outset [4]. In addition, the risk of adverse effects such as teratogenicity outweighed other considerations and thus, females of child-bearing potential were largely excluded from clinical trials [12]. In some cases, little evidence exists for the safety profiles of drugs in pregnant or breastfeeding females, therefore such females and their healthcare professionals are advised to consider the risk-benefit ratio of therapeutic use. The COVID-19 vaccination programme is such an example [13]. In addition, when studying diseases prevalent in both sexes, Caucasian males were considered to be the typical study population [14] highlighting the lack of consideration of potential ethnic differences too [15].

To address the historical overrepresentation of male subjects in biomedical research, a 10-year follow-up study of sex inclusion across interdisciplinary research was conducted by Woitowich et al. [16]. The work identified that there was a significant increase in the number of studies that included both sexes across general biology, immunology, neuroscience, physiology, pharmacology, endocrinology, reproduction, behavioural physiology and behaviour. However, in all subject areas bar pharmacology, there was no change in the proportion of studies that included data specifically analysed by sex. In addition, the studies failed to provide rationale for single-sex studies or the lack of sex-bases analyses outlining a clear sex gap in biological research disciplines [16].
Figure 1. Sex as a biological variable as modifiers of health, disease and research outcomes. A) Inter-relation between biological sex and societal gender in health, disease pathophysiology and clinical manifestations. B) Distribution of the ten leading causes of death disaggregated by sex in the US in 2017. (CPD = chronic pulmonary disease). C) Percentage of articles that used male subjects, female subjects, both male and female subjects or did not specify sex of sample population. D) Percentage of articles disaggregating pre-clinical animal results according to sex in different scientific disciplines. Adapted with permission from [4] and [6].
To clarify nomenclature, this review will go forward with ‘sex’ and not ‘gender’ in its terminology. Sex refers to the biology of living things, i.e. as male or female according to reproductive organs of functions based on the chromosomal complement [5]. Gender, however, refers to sociocultural attributes, behaviours or personal identification [17]. As such, this review will comprehensively report on how the lack of pharmaceutical analyses considering sex differences in systems biology, sex-specific needs and behaviour, regulatory affairs and post-marketing surveillance has led to a disparity in optimum drug development. As peroral administration is the main route of drug delivery due to convenience and consequently patient medicine adherence [18], a key focus is dedicated towards sex differences in the gastrointestinal (GI) tract and drug absorption in male and female human adults or equivalent preclinical models. Recommendations will also be proposed on how scientists should rethink standards and reference models across the drug delivery pipeline with the aim to integrate sex analyses into research and innovation.

2.0 Sex bias in pharmaceutical research

Government reports from 1980s to 90s indicated that women had lower representation in federally funded studies investigating diseases that affected both sexes [19-22]. In 1992, the United States of America’s Food and Drug Agency (FDA) and Food and Drug Law Institute concluded that young women needed to be included in clinical trials in order to understand female response to pharmaceuticals [12]. The regulation and guidance published by the FDA on female participation in industry-sponsored clinical trials has transformed over the half century, instigated by the thalidomide tragedy in pregnant women [23]. Thalidomide was developed by the Swiss company CIBA in 1953 and introduced to the pharmaceutical market in 1956 by German pharmaceutical company, Chemie Grunenthal [24]. Initially marketed with the brand name Contergan, thalidomide was prescribed as a non-barbiturate sedative to induce deep sleep. Pre-clinical testing, however, failed to establish a median toxic dose and the drug was believed to be non-toxic to humans [25]. In that era, testing for harmful teratogenic effects were not considered. The drug was used as a sedative but soon became popular for its anti-emetic effects in pregnant women suffering with morning sickness [26]. In 1961, observations linked thalidomide use in pregnancy to congenital malformations in multiple cases worldwide [25, 27], and thalidomide was ultimately withdrawn from the pharmaceutical market in the same year.
In 1977, a guidance document from the FDA advised that women of child-bearing potential (females capable of becoming pregnant, including pre-menopausal single abstinent women, women using contraceptives or women with sterile partners) should be excluded from Phase I and early-Phase II trials. If a drug was deemed to have a positive risk-benefit ratio, women could then be included in late-Phase II and Phase III trials providing animal teratogenicity and fertility studies were completed [20]. In 1993, however, the FDA reversed the 1977 FDA guidance which lifted the ban on women of child-bearing potential to be excluded from early clinical trials research. The guidance further specified that clinical trial participants should be representative of the patient population likely to be prescribed the drug once regulated for market approval [20]. In the same year, the National Institutes of Health (NIH) formalised an NIH Revitalisation Act entitled Women and Minorities as Subjects in Clinical Research where four main issues were addressed; 1) Women and minorities are to be included in all clinical research, 2) Numbers in Phase III clinical trials be sufficient to allow for valid analyses of potential sex and ethnic differences, 3) Such groups should not be excluded due to trial costs and, 4) Programmes and support outreach efforts should be created to enrol women and minorities in clinical trials [28]. Although progress has been made towards the appropriate representation of females across the whole clinical arena in the last decade (Figure 2), sex differences in drug response are still demonstrated following regulatory approval and entry of a drug into market [29].
**Figure 2.** Significant events in the history of female participation in clinical trials in the United States in line with guidelines from the US FDA. Adapted from [30, 31].
A 2018 review of 107 NIH funded randomised control trials that enrolled both men and women found that only 26% reported even one outcome disaggregated by sex or included both sexes as a co-variate [32]. 72% simply did not include sex as a factor in their analyses. NIH policies mandated over a quarter century ago have yet to yield the intended increases in reporting by sex. A consequence of this sex inequality hides in plain sight today: most drugs are prescribed to women and men at the same dose. Many currently prescribed drugs were approved by FDA prior to 1993, with inadequate inclusion of female animals in preclinical research and of women in clinical trials [33]. The existing knowledge base on sex-aware prescribing lacks information on sex differences for one-third of all drugs [34, 35]. Pharmaceutical companies responsible for generating pre-approval data often fail to include information on sex differences in New Drug Applications (NDA) documents, and the FDA has previously failed to enforce its own requirements before approving new drugs [36]. Consequently, potential sex differences in pharmacokinetics, pharmacodynamics and their relation to adverse side effects often remain unknown. Most of the data submitted to the FDA by drug companies are not publicly available and not subject to peer-review by the broader scientific community [35]. Regulatory agencies have historically paid insufficient attention to differences between women and men in terms of both sex and gender which perpetuates inequalities by neglecting drug safety problems that are sex-specific. In addition, this disparity allows for misleading drug marketing [36]. For example, irritable bowel syndrome (IBS) is disproportionately diagnosed in females, despite recent evidence that males equally both suffer and access health advice for IBS symptoms [37, 38]. Tegaserod was approved for IBS in females first, followed by a FDA-approved extension to males for chronic constipation based on two clinical trials with over 85% females participants [39, 40]. Tegaserod was later removed from market following a meta-analysis of 29 trials reporting an increased risk of cardiac adverse events [41].

In the preclinical arena, routine in vitro models, namely cell lines, are not sufficient to study and understand sex differences in early drug development as the cells are often derived from a single animal or human subject to reflect a specific organ. The sex of cell lines is often not reported, failing to acknowledge potential sex differences in the in vitro mechanisms. To overcome these shortcomings, scientists should state the sex of their in vitro models in their publications. Cvitanovic Tomaš et al. have created a computational model LiverSex [42], taking sex differences in the liver into account, adapted from SteatoNet in silico model [43]. Data from oestrogen and androgen receptor responses are included which includes sex-related effects on growth hormone release. Currently, the model has been validated in mice but not in
humans. A step further, Thiele et al., have created two validated, sex-specific, whole body metabolic models called Harvey and Harvetta [44]. Here, the male and female physiologies have been represented with 20 organs, 6 sex organs, 6 types of blood cells, the systemic blood circulation, the blood-brain barrier and the GI lumen, including the microbiome. These sex-specific models represent systems biological approaches to precision medicine.

3.0 Sex differences in human physiology

Men Are From Mars, Women Are From Venus by John Gray in 1992 outlined that differences in communication tactics between males and females stem from fundamental differences in psychological processes between the sexes. Indeed, such sex differences are not limited to psychology but extend to the complete physiological system and anatomy itself. For many years - except for studies related to the physiology of reproduction - physiological principles contained in classical physiological and medical textbooks have been based on the androcentric model of 70 kg healthy Caucasian males between 18 to 40 years of age [45]. In addition, thousands of genes differ in their expression between males and females in the liver, adipose tissue and muscle with the brain being less sexually dimorphic [42]. The appreciation of this led to the US Institute of Medicines declaring in 2001 that biological sex will considerably affect the course and prevention of disease [46].

Significant physiological differences exist between men and women such as percentage of body fat, body water volume, plasma volume and organ blood flow, in addition to body weight (Figure 3). As such, women are not small men. These parameters, however, are often overlooked in the drug development process and can consequently lead to differing response to medicines [15]. These have been reviewed elsewhere [47, 48], however herein, focus will be invested towards sex differences in the processes involved following solid oral drug administration, i.e. the GI tract.
Figure 3. Physiological differences in males and females that can affect drug processing. Adapted with permission from [49].

3.1 Sex differences in gastrointestinal physiology and the influence on oral drug performance

There are significant sex-specific differences in terms of drug bioavailability and pharmacokinetics which can, in turn, differentially affect drug efficacy and safety. Underlying reasons for sex-related variations in drug performance include obvious differences in physiological parameters such as body fat content and hormonal control [50]. Fundamental differences at the level of the GI tract, liver and kidneys can further influence drug absorption, distribution, metabolism and elimination, and consequently lead to variability in drug therapy and potential toxicity [51]. It is difficult to envisage that differences in drug performance and adverse effects are linked to a single pharmacokinetic parameter and governed by a single
organ. Instead, it is much more likely that sex differences may be a result of the interplay of the complete system following oral drug administration. Herein, focus on the GI tract, potential differences between the sexes and its influence on oral drug variability will be discussed.

Tissue exposure of orally administered medication is affected by variability in gastric fluid pH and volumes, gastric emptying time (GET) and intestinal transit time (ITT), competition and/or regulation of intestinal transporters and drug metabolising enzymes, and the potential interactions of sex steroids on drug PK [52] (Figure 4). In terms of gastric and small intestinal fluid volumes, males have been reported to have higher volumes than females [53] which may affect the extent of drug dissolution. Average fasted gastric pH is significantly higher in females (2.79 ± 0.18) than in males (2.16 ± 0.09) (p < 0.05) which may be attributed to reduced acid secretion due to the smaller stomach size seen in females [54]. The basal acid output in the fasted state was nearly half in females than in males, 2.1 ± 0.2 and 4.0 ± 0.2 mmol/h, respectively [55]. Lowered gastric acid secretion may influence drug ionisation, particularly of weak bases, the solubility of pH-sensitive drugs and the degradation of acid-labile drugs, thereby affecting absorption and consequently, oral drug bioavailability. Sex differences have also been reported in bile acid composition with higher concentrations of cholic acid being reported in males and higher concentrations of chenodeoxycholic acid reported in females [56].
Figure 4. Key sex differences at the level of the gastrointestinal tract that impact oral drug delivery and bioavailability, (M = Male; F = Female). Adapted with permission from [57].

*Denotes variable transporter expression in the regions of the small intestine between males and females.

Females have a significantly longer GET for solids and calorific liquids (118.0 ± 8.1 min) compared with males (91.4 ± 7.5 min), however, GET decreases in post-menopausal women (97.9 ± 7.6 min) becoming similar to that in men of the same age [58]. Variabilities in drug pharmacokinetics can be attributed to differences in GET; for example, peak plasma concentration of orally administered carbidopa was achieved 22 min later in women than men.
due to longer GET [59]. Sex differences in the oral bioavailability of a gastro-resistant ketoprofen formulation has also been demonstrated. Males showed a higher $c_{\text{max}}$/AUC than females ($0.468 \pm 0.094$ versus $0.361 \pm 0.087 \text{ h}^{-1}$) and a significantly lower $t_{\text{max}}$ ($3 – 5 \text{ h}$ versus $5 – 10 \text{ h}$) respectively. Such differences were attributed to the faster SITT in males which allowed for ketoprofen to reach the appropriate site of absorption in the intestinal environment for absorption to occur more rapidly [60], therefore leading to $t_{\text{max}}$ to occur at an earlier time point. Females have longer transverse and descending CTT, but shorter rectosigmoid transit time compared to males [61]. The longer GET and CTT and so the overall GI residence of sustained-release dosage forms may facilitate enhanced drug absorption in women, as demonstrated with diltiazem which is sensitive to GI transit time [62]. This, however, may be further affected by the regulation of intestinal membrane transporters and metabolising enzymes located in the GI mucosa.

Distinct sex differences in drug performance have been further demonstrated in treatments for GI syndromes. For example, alosetron, a 5-hydroxytrptamine (5-HT) receptor 3 antagonist, is a drug that is effective in females but has low performance in males [63]. At identical plasma concentrations, alosetron achieves therapeutic levels only in females. Sex differences may partially contribute towards variability in the activity of serotonergic receptors in the colon. Serotonergic type III receptors are involved in postprandial colonic responses in health and diarrhoea and findings reported that with alosetron treatment, females displayed a significantly greater overall colonic transit compared to males (a colonic geometric centre mean at 24h of -1.45 and -0.32, respectively) [64]. Viramontes et al. proposed that the pharmacogenomics of 5-HT$_3$ may be a factor. Although, additional studies into serotonin synthesis and genotypic serotonin synthesis and metabolism were suggested to further understand the sex difference [64]. Alosetron, however, was withdrawn from the pharmaceutical market in 2000 due to significant side effects but was reintroduced in 2002 in the US under restrictive conditions of use only for females suffering from severe diarrhoea-related IBS [65].

The gut microbiota adds further to the complexity to GI physiology and varying drug response in males and females. For example, levodopa, a treatment for Parkinson’s disease, has been subject to increased metabolism in the presence of Helicobacter pylori, which consequently reduced the drug bioavailability. The eradication of Helicobacter pylori infection, however, improved levodopa action and clinical symptoms. The prevalence of Helicobacter pylori
infection, however, is more prevalent in male than female individuals [66] and as such, may lead to differences in levodopa pharmacokinetics between the sexes.

Research in the understanding of differences between the sexes and the clinical performance of drugs continues to be limited. It is clear that males and females respond differently to medicines due to the dynamic interplay of GI physiology, drug pharmacokinetics and contributions from other associated organs. A single pharmacokinetic parameter cannot be considered as the rate-limiting step as this may occur in a drug-by-drug basis. For a better understanding of the basic mechanisms of sex differences, future studies should be designed with this primary focus in mind to determine the extent that these differences may have on clinical management [67].

4.0 Sex differences in pharmacokinetics

Many drugs show distinct pharmacokinetic differences between the sexes in humans (Table 2) and preclinical animal models (Table 3). A hallmark example displaying significant sex differences in drug response is the sedative zolpidem. It was approved by the FDA one year before the NIH Revitalisation Act and marketed under several names including Ambien, Edluar and Zolpimist, where males and females were prescribed the same dose of 10 mg and 12.5 mg for immediate-release and extended-release products, respectively [68]. During decades of post-marketing drug surveillance, women were found to be more susceptible to next-day effects due to a slower rate of drug elimination, with emergency department visits from exclusively females with cognitive defects [69]. The FDA subsequently recommended that the dose of zolpidem be reduced by half for women [70]. Many other drugs administered in equal doses to males and females likely require re-evaluation for sex-specific dose adjustment. An analysis of 10 prescription drugs that were withdrawn from the market from 1997 – 2001 found that eight posed “greater health risks for women”, mainly because of adverse drug events due to known pharmacodynamic differences (e.g. 3 drugs withdrawn due to risk of Torsades de Pointes) or because women are more prone to polypharmacy [71].

Apart from the innate differences in physiology, chemical and biological processes, pharmacokinetic and pharmacodynamic processes add further to the complications of varying drug response. In addition, endogenous steroid hormone exposure (from peripubertal to adulthood) and sex differences in exogenously administered steroids, the higher rates of
polypharmacy in women and sex differences in reporting rates contribute to the manifestation of sex differences in drug response [72]. Up to 6 – 7% of new drug applications that include sex analysis report at least a 40% difference in pharmacokinetics between males and females [73]. In general, drug disposition occurs through separate phases including absorption, distribution, metabolism and elimination. Sex differences have been demonstrated for each phase [50, 74].

4.1 Absorption

Absorption of drug products across the gut epithelium depends on a number of highly complex mechanisms [75]. Absorption can be a rate-limiting step for an orally administered drug to reach its target site of action and is drug- and mechanism-dependent. Sex differences in absorption can be seen for a number of drugs. In addition, sex hormones were recently found to affect the passive diffusion and active transport of drugs to different extents in males and females [76].

A key case that illustrates sex differences in drug absorption is aspirin. For example, one study showed that oral administration of aspirin in young healthy adult males (n = 9) and females (n = 9) resulted in faster oral absorption in females than in males (statistically significant terminal $t_{1/2}$ [16.2 and 20.6 mins] and mean residence times [33.5 and 39.9 mins], respectively) [77]. Whilst the females were lighter in weight than the males, which resulted in different dose per kilogram body weight, weight was not considered a major factor in absorption as the pharmacokinetics of aspirin was reported to be independent of dose [78]. A further study on aspirin disposition in seven young females, six young males, six elderly females and six elderly males found statistically significant higher plasma levels ($C_{max}$) in young and elderly females compared with the male counterparts. Age, on the other hand, did not show a statistically significant effect on the pharmacokinetics of aspirin [79, 80].

A population pharmacokinetic analysis (n = 449 for learning and n = 247 for validation with similar clinical and biological characteristics except for weight) showed a longer time of absorption ($t_{max}$) in males, with medians of over 3 hrs for men, compared with 40 mins in females, for the antihistamine mizolastine [81]. The absorption of copper was reported to be significantly ($p = 0.02$) higher in females (71%) than in males (64%) aged 20 – 59 years (n = 127) [82]. Interestingly, the permeability of lactulose and sucralose was reported to decline
with ageing in females ($p = 0.05$, $r^2 = 0.24$ and $p = 0.01$, $r^2 = 0.41$, respectively) but not in males in healthy adults ($n = 17$) and children ($n = 15$), with a suggestion that the age- and sex-related deterioration was mediated by glucocorticoid hormones [83].

Drugs may compete for intestinal membrane transporters into cells that affect the downstream metabolism or availability of the drug at its target site. The influx pump OATP1B1, encoded by the gene *SLCO1B1*, transports oestrogens including estrone-3-sulfate and oestradiol 17β-D-glucuronide, as well as statins. Competition for the transporter may occur when multiple substrates are present [84], which may limit the efficacy of statin treatment [85]. Sex-specific effects of *SLCO1B1* genetic variants (*SLCO1B1* rs4149056 (T > C) polymorphism) were reported in a pilot study, which showed homozygous males displayed the lowest decrease ($\Delta - 21.2 \pm 7.2\%$) of total cholesterol, compared with females where the same genotype was associated with the highest ($\Delta -33.5 \pm 7.6 \%$) decrease ($P = 0.04$) [86]. Females of older age were associated with an increased risk of statin-related myopathy (relative risk of 2.0 (95% confidence interval, 1.0 to 3.9)), especially amongst carriers of the *SLCO1B1* c.521C allele with impaired renal function and diabetes and those who take amiodarone [87].

In addition, there is an increasing body of literature evidence that report the inherent sex-specific expression of a number of efflux transporters (Table 1) [88, 89] that result in differential treatment outcomes (Figure 5a). P-glycoprotein (P-gp) is the most studied drug efflux pump, encoded by the *MDR1* gene. Polymorphisms in the *MDR1* gene is linked with higher levels of neutropenia with docetaxel [90]. Sex hormones are believed to modulate P-gp expression and inhibit drug absorption by P-gp-mediated efflux at the intestinal epithelia [91, 92]. In a similar manner, multidrug-resistant protein transporters (MRPs), display sex differences in their expression, modulated by sex hormones [93].

<table>
<thead>
<tr>
<th>Tissue and Transporter mRNA</th>
<th>Efflux Membrane Protein</th>
<th>Model</th>
<th>Sex difference</th>
<th>References</th>
</tr>
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<tr>
<td>Kidneys</td>
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<td>Equilibrative nucleoside</td>
<td>Mice</td>
<td>F &gt; M</td>
</tr>
</tbody>
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Table 1. Sex differences in efflux transporter mRNA expression between male and female research models in the kidneys, liver, lung, brain and intestinal tract. Adapted from [94].
<table>
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<td>Mdr1b</td>
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<tr>
<td>Bcrp</td>
<td>Breast cancer resistance protein</td>
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<tr>
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<td>Multidrug and toxin extrusion 1</td>
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**Liver**

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<td>Mate1</td>
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**Lungs**

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**Intestine**

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### 4.2 Distribution
Most drugs will bind to plasma proteins in the systemic circulation that are specific to the drug. The distribution of a drug is affected by several body composition parameters (Figure 4). Sex differences in these parameters may account for differences in the concentration of a drug at the target site and result in varying responses.

It has been reported that males have higher content of total body water (i.e. extracellular water and intracellular water), total blood volume and plasma volume than females. The higher percentage of body fat in females, especially in pregnant people, may also alter the distribution of lipid-soluble, slowly metabolised or toxic substances in the body. For example, differences in increased organ blood flow and body fat in females accelerated the onset of action but prolonged the duration of vecuronium and rocuronium bromide in females (e.g.) [103, 104] (Figure 5b). Differences in body fat content and in protein binding are responsible for sex-related pharmacokinetic differences in the distribution of diazepam, where females have been shown to have larger volumes of distribution than males due to higher free fraction [105]. The degree of plasma protein binding is affected by sex hormones with wider variation seen during the time of menstruation [106].

4.3 Metabolism

The majority of drug metabolism occurs in the liver, but biotransformation can also occur in the intestinal tract, lung, kidney and skin. Despite intra-individual variations in drug metabolism following normalisation for height, bodyweight and body surface area, differences in drug metabolism can be dependent on the sex of the individual due to transporters and enzymes expressed in hepatocytes. Drugs metabolised by Phase I and Phase II are cleared faster in males when compared with females [107, 108]. For example, the activity of gastric alcohol dehydrogenase (ADH) is lower in females than in males [109], distinct in younger age (20 – 40 years). As age increases (41 – 60 years), the opposite is found, females show higher gastric ADH activity than males. In older age (61 – 80 years) [110], no sex differences are found. These differences in ADH are believed to be cause by the lower first pass metabolism of alcohol in females, leading to higher blood levels in females than male.

Cytochrome (CYP) enzymes are responsible for the metabolism of a number of drug substrates of which CYP2C and 3A are most commonly expressed in the small intestine. Significant sex
differences are observed in the expression of hepatic drug metabolising enzymes which contribute variabilities in clinical drug performance [111]. Numerous studies have shown that females have higher rates of CYP3A substrate metabolism compared with men [112-114]. A large retrospective analysis into sex dimorphic drug pharmacokinetics found statistically significant sex differences; an average of 20 – 30% higher clearance for drugs that are CYP3A substrates, compared with males [115]. Endogenous and oral exogenous oestrogen are shown to alter hepatic enzymes [116]. The nuclear receptor transporters pregnane X receptor (PXR) and constitutive androstane receptor (CAR) regulate the expression of cytochrome P450 enzymes, including CYP3A4. These receptors are activated by a variety of compounds including steroid hormones [117]. The nuclear hormone receptor ERα has been shown to modulate CYP1B1 expression directly which could affect its drug substrate levels [118].

Interestingly, dose-related sex differences were found in some drug metabolisms. Using zolmitriptan as an example, the bioavailability of zolmitriptan was significantly higher in women than in men after both 5 mg oral dosing and intravenous dosing. However, there were no reported sex differences in oral bioavailability with a dose of 2.5 mg [119]. This sex-related variation was smaller than the finding in the previous report which also demonstrated sex difference in the bioavailability of zolmitriptan after 10 mg oral administration [120] (Figure 5c). It therefore stands a dose-related manner in the bioavailability of zolmitriptan. The reason for this sex-dependent difference was assumed to be most likely explained by a difference in first-pass metabolism [121], as the plasma concentrations of zolmitriptan in women were higher than in men with relatively higher levels of the active metabolite in men.

4.4 Elimination

The kidney is the main site of excretion of waste products following metabolism, xenobiotics, parent drug compounds and their metabolites. In addition, the kidneys are responsible for the maintenance of the water/electrolyte balance and of the synthesis, metabolism and secretion of hormones. There are known sex differences in all three major renal functions (namely glomerular filtration, tubular secretion and tubular reabsorption) resulting in generally higher renal clearance in men than in women [122-124].

A number of transporters present in the kidney show sex-bias in their expression. From investigations into the mRNA expression in human kidneys Joseph et al., found 21 genes with
male dominance and 2 transporter genes with female dominance [125]. Sex differences in drug transporters expression has been suggested for the differential induction of renal diseases via sex-specific toxicities in the kidney. Sex hormones may mediate these differences through alterations in the renin-angiotensin system [126]. Renal sex differences are also seen in the subunits of glutathione-S-transferase (GST) isoenzyme [127]. GST plays a role in cellular detoxification [128] and polymorphisms and sex differences may influence its activity [129]. Female rats showed greater levels of subunits 3 and 4, whereas subunits 1 and 2 showed greater levels in male rats [130].

Aspirin is more rapidly cleared from women and its metabolite, salicylate, has an increased rate of absorption in women. On the other hand, the clearance of acetaminophen, gemcitabine and heparin is slower in females than in males [50, 131], with 71% of patients admitted to hospital for acetaminophen overdose being women [131, 132]. It may be due to the increase in renal blood flow and glomerular filtration in men, which increase the elimination rate of drugs cleared by the kidneys. Renal blood flow, glomerular filtration, tubular secretion and tubular reabsorption are all greater in men than in non-pregnant women, however, changes in renal blood flow, the glomerular filtration rate, hepatic blood flow, bile flow and pulmonary function may alter elimination of a drug in women during gestation.

Such differences have already resulted in sex-specific dosing. Desmopressin (Figure 5d), which activates vasopressin receptors in the kidneys to regulate water homeostasis, is such an example. Women have been found to be more sensitive to the antidiuretic effects of desmopressin than men due to the gene coding for the arginine vasopressin receptor. This gene is found on the X chromosome and in humans, several other genes involved in water homeostasis are located on the X chromosome [133]. As males only have one X chromosome, only one copy of the vasopressin receptor gene is likely to escape X chromosome related-inactivation, unlike in females, having two copies of the gene [134]. It has been reported that older females taking desmopressin are more likely to have lowered sodium concentration leading to unwanted side effects such as weakness, dizziness and fainting. To prevent adverse reactions, both the EU and Canadian medical agencies have recommended lower dosages of desmopressin be used by women [49]. Lower doses of desmopressin have also elicited good response in female paediatric patients [135], consistent with research in adults [134]. The drug is consequently marketed with different recommended doses on the labelling package for men.
and women. A comprehensive portfolio of sex-specific differences in the pharmacokinetics is outlined in Table 2.

![Sex differences in drug pharmacokinetics](image)

**Figure 5.** Hallmark examples of sex differences in drug pharmacokinetics. **A)** Sex differences in drug absorption have been observed with the co-formulation of different doses of PEG 400 with ranitidine in the human volunteers. Adapted with permission from [136]. **B)** Dose-response curves of rocuronium in male and female anaesthetised patients. Adapted with permission from [137]. **C)** Mean AUC$_{0-\infty}$ after 2.5 mg, 5 mg and 10 mg zolmitriptan in males and females. Adapted with permission from [119, 138]. **D)** Mean desmopressin concentration profiles by dose (120 µg and 240 µg) and sex. Reproduced with permission from [134].
Table 2. Sex-specific differences in the drug pharmacokinetics in humans and pre-clinical rat models following oral administration.

<table>
<thead>
<tr>
<th>Drug</th>
<th>Sex differences in pharmacokinetic effect</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Human data</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Aspirin</td>
<td>Higher clearance in females than males and shorter t$_{1/2}$ in females</td>
<td>[77]</td>
</tr>
<tr>
<td>Chlor-</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Diazepox-</td>
<td>Higher AUC in males than females</td>
<td>[139]</td>
</tr>
<tr>
<td>Cefotaxime</td>
<td>Longer duration in females than males</td>
<td>[140]</td>
</tr>
<tr>
<td>Daidzein</td>
<td>Higher AUC$<em>{0-480}$ in females than in males; no differences reported between pre- vs. post-menopausal females in t$</em>{1/2}$, t$_{max}$ and AUC due to influence from gut microflora</td>
<td>[141]</td>
</tr>
<tr>
<td>Diazepam</td>
<td>Higher free fraction and larger distribution in females than males</td>
<td>[105]</td>
</tr>
<tr>
<td>Fentanyl</td>
<td>Lower urinary excretion in females than males due to a higher glomerular filtration rate in males</td>
<td>[123]</td>
</tr>
<tr>
<td>Fluconazole</td>
<td>Higher plasma levels in females than males due to sex differences reported in CYP3A4, CYP2C9, CYP2C19 expression</td>
<td>[142]</td>
</tr>
<tr>
<td>Flurazepam</td>
<td>Higher AUC of its major metabolites (N-1-hydroxyethylfurazepam and N-1-desalkylflurazepam) in females than males</td>
<td>[143]</td>
</tr>
<tr>
<td>Glucose</td>
<td>Fluctuating absorption levels in females than males; lower absorption is reported during the first hour but increased in absorption in the last hour of a three-hour oral glucose tolerance test in females Early glucose absorption lower in females with impaired glucose intolerance</td>
<td>[144] [145]</td>
</tr>
<tr>
<td>Heparin</td>
<td>Longer duration in drug distribution in females than males</td>
<td>[146]</td>
</tr>
<tr>
<td><strong>Levofloxacin</strong></td>
<td>Higher $C_{\text{max}}$ in females but larger $\text{AUC}_{0-480}$ in males</td>
<td>[147]</td>
</tr>
<tr>
<td><strong>Lignocaine</strong></td>
<td>Higher distribution in females than males</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Plasma binding of lignocaine was almost completely attributed to changes in $\alpha_1$-acid glycoprotein concentration which is reduced by oestrogens</td>
<td>[148, 149]</td>
</tr>
<tr>
<td><strong>Losartan</strong></td>
<td>Larger AUC in females than males</td>
<td>[150]</td>
</tr>
<tr>
<td></td>
<td>Lower total body clearance and volume of distribution in girls as higher content of water in men which can influence water-soluble drugs such as losartan</td>
<td></td>
</tr>
<tr>
<td><strong>Methylicapnisolone</strong></td>
<td>Lower distribution, higher clearance and shorter t$_{1/2}$ in males than females</td>
<td>[151]</td>
</tr>
<tr>
<td></td>
<td>Differences in $\text{IC}_{50}$ values for cortisol secretion, basophil and helper T-lymphocyte trafficking are sensitive to methylprednisolone suppressive effects</td>
<td></td>
</tr>
<tr>
<td><strong>Metoprolol</strong></td>
<td>Lower distribution, higher clearance and shorter t$_{1/2}$ in males than females</td>
<td>[152]</td>
</tr>
<tr>
<td></td>
<td>Stereoselectivity in oral clearance appeared to be greater in subjects with higher clearance (i.e. males) thus increasing metoprolol exposure in females</td>
<td></td>
</tr>
<tr>
<td><strong>Metronidazole</strong></td>
<td>Higher distribution in males than females</td>
<td>[153]</td>
</tr>
<tr>
<td><strong>Midazolam</strong></td>
<td>Lower AUC in females than males due to lower level of CYP3A expression in males</td>
<td>[154]</td>
</tr>
<tr>
<td><strong>Ofloxacin</strong></td>
<td>Higher AUC and $C_{\text{max}}$ but lower total body clearance and volume of distribution in young females</td>
<td>[155]</td>
</tr>
<tr>
<td></td>
<td>Significantly higher $\text{AUC}_{0-480}$ in younger females than younger males</td>
<td></td>
</tr>
<tr>
<td><strong>Quinine</strong></td>
<td>Lower distribution, higher clearance and shorter t$_{1/2}$ in males than females</td>
<td>[140]</td>
</tr>
<tr>
<td><strong>Rocuronium</strong></td>
<td>Rocuronium is a lipid-soluble drug and a longer duration of rocuronium distribution was reported in females than males due to a higher content of fat</td>
<td>[104]</td>
</tr>
</tbody>
</table>
Torasemide  Lower clearance in females with higher AUC and $C_{\text{max}}$ due to a higher glomerular filtration rate in males  [156]
Vecuronium  Longer $C_{\text{max}}$ in females than males  [104]
Verapamil  Clearance of oral verapamil was accelerated in females, $t_{1/2}$ and mean residence times were significantly shorter in females than males  [157-159]
   Sex difference not evident when administered intravenously suggesting that intestinal processes likely influence sex-specific differences in drug clearance
Zolmitriptan  Higher bioavailability in females after both 5 mg oral dosing and intravenous dosing  [119]

**Table 3.** Sex-specific differences in the drug pharmacokinetics in pre-clinical rat models following oral administration.

**In pre-clinical rat models**

<table>
<thead>
<tr>
<th>Drug</th>
<th>Sex differences in pharmacokinetic effect</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>Celastrol</td>
<td>Higher oral bioavailability in female rats due to altered mechanism in absorption and metabolism</td>
<td>[160]</td>
</tr>
<tr>
<td>Clindamycin</td>
<td>Higher plasma levels in female rats due to sex differences in CYP3A4, CYP2C9, CYP2C19 expression</td>
<td>[161]</td>
</tr>
<tr>
<td>Diltiazem</td>
<td>Higher $C_{\text{max}}$ and longer $t_{\text{max}}$ in female rats than males</td>
<td>[162]</td>
</tr>
<tr>
<td>Letrozole</td>
<td>Higher $C_{\text{max}}$ and AUC$<em>{0-480}$ in female rats; AUC and $t</em>{1/2}$ in females were 3-fold and 4-fold higher than in males</td>
<td>[163]</td>
</tr>
<tr>
<td></td>
<td>Tissue/plasma drug concentration in female rats 24 h after dosing was significantly higher in female rats than in males in the heart, spleen, brain and genital glands</td>
<td></td>
</tr>
<tr>
<td>Letrozole</td>
<td>Letrozole metabolism more extensive in male rats</td>
<td>[163]</td>
</tr>
<tr>
<td>Compound</td>
<td>Description</td>
<td>Reference</td>
</tr>
<tr>
<td>-------------</td>
<td>-----------------------------------------------------------------------------</td>
<td>-----------</td>
</tr>
<tr>
<td>Nimodipine</td>
<td>Higher AUC(<em>{0-480}) and C(</em>{max}) in female rats</td>
<td>[164]</td>
</tr>
<tr>
<td>Ranolazine</td>
<td>Significantly higher plasma concentrations in female rats than male rats; C(_{max}) and AUC in female rats were roughly 2-to 3-fold greater</td>
<td>[165]</td>
</tr>
<tr>
<td>Ranolazine</td>
<td>t(_{1/2}) in male rats were shorter than in female rats</td>
<td>[165]</td>
</tr>
<tr>
<td>Schizandrin</td>
<td>Higher C(<em>{max}) and AUC(</em>{0-480}) in female rats</td>
<td>[166]</td>
</tr>
<tr>
<td>Taurocholate</td>
<td>Lower renal clearance in males</td>
<td>[167]</td>
</tr>
</tbody>
</table>
5.0 Adverse drug reactions in women

The occurrence of ADRs is 50 – 75% higher in females than men [168] and 60% of all patients hospitalised for adverse drug effects were women [169, 170]. This may be due to the interplay of differences in physiology, sex hormones, pharmacodynamics and pharmacokinetic response in the processing drugs. In addition, women may be more frequently overdosed and more commonly polymedicated than men [171]. Males and females also display different non-adherence behaviours. A cross-sectional questionnaire in the Swedish population did not find sex differences in the reporting of non-adherence. However, males were more likely to forget to take their dose or change their dose. Whereas in contrast, females were reported to collect their prescription medicine and not take it and omit their medication due to ADRs [172]. An extensive table of sex-specific differences in ADRs is outlined in Table 4.

Males and females appear to respond differently to pain and opioid analgesics. Women are reported to experience more severe postoperative pain and need a greater dose (+11%) of morphine than men postoperatively [173]. Greater analgesic effects were reported with opioid analgesic in females compared with males, with more adverse side effects than males [174]. In addition, pain response are more variable in females and more painful diseases are more commonly reported among females. Sex hormones and different density and modulation of the endogenous opioid system may contribute towards these sex differences [175]. Cepeda et al., showed that women had a 60% higher risk of nausea and vomiting than men following opiates use, though response did not differ between the sexes [176].

Largely ignoring female participants or not powering for sex in clinical trials has resulted in a distinct female-bias in ADRs [68], even to the point that pharmaceuticals have been withdrawn from the market due to a greater risk of side effects in women [71]. For example, Posicor (mibebradil dihydrochloride) approved for hypertension and angina, lowered the heart rate of elderly women and interacted with 26 other drugs [71]. Although the FDA outlined that both sexes should be represented in all phases in clinical trials to avoid undetected sex differences in drug efficacy and side effects [50, 177, 178], there is still a long way to go. Labots et al. conducted a cross-sectional, structured research into the publicly available registration dossiers of the FDA-approved drugs that are frequently prescribed. For 38 of the drugs where sufficient data was publicly available, a clear disparity in male and female representation between the phases of clinical trials was identified. For example, only 22% of female participation was
demonstrated in Phase I in comparison to 48% and 49% in Phase II and III trials respectively [179].
Table 4. Drugs with adverse drug reactions experienced more commonly by females (risk factors and statistical differences provided in brackets). Adapted from [68]. The symbols F refers to females, M to males and ↑ to greater or higher.

<table>
<thead>
<tr>
<th>Drug</th>
<th>Indication</th>
<th>Adverse drug reactions</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Cardiovascular system</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Aliskiren</td>
<td>Hypertension</td>
<td>Diarrhoea (rates ↑ in F at a dose of 150 mg daily, whereas rates only ↑ in M at dose of 300 mg)</td>
<td>[180]</td>
</tr>
<tr>
<td>Amlodipine</td>
<td>Angina and hypertension</td>
<td>Oedema (F 14.6 % and M 5.6 %), flushing (F 4.5 % and M 1.5 %), palpitations (F 3.3 % and M 1.4 %) and somnolence (F 1.6 % and M 1.3 %)</td>
<td>[181, 182]</td>
</tr>
<tr>
<td>Clopidogrel</td>
<td>Atherothrombotic and thromboembolic events</td>
<td>Fracture (rates ↑ in F at low doses for all fractures and hip fractures, whereas rates only ↑ in M at higher doses), bleeding (risk ↑ in F [relative risk 1.40; 95 % CI, 1.00-1.96]), GI symptoms with IBD (control 60%, F 70 %, p = 0.0003 and M 61%, p = 0.8312)</td>
<td>[183, 184]</td>
</tr>
<tr>
<td>Dabigatran</td>
<td>Prophylaxis of thromboembolic events</td>
<td>Bleeding (↑ in F elderly, confounded by decreased renal function, low body weights &amp; more drug-drug interactions)</td>
<td>[185, 186]</td>
</tr>
<tr>
<td>Digoxin</td>
<td>Atrial fibrillation and heart failure</td>
<td>Mortality from any cause (5.8% ↑ in F)</td>
<td>[187-189]</td>
</tr>
<tr>
<td>Dofetilide</td>
<td>Arrhythmia</td>
<td>Torsades de Pointes (risk was 3 times ↑ in F)</td>
<td>[190]</td>
</tr>
<tr>
<td>Pravastatin</td>
<td>Hypercholesterolaemia</td>
<td>Coronary heart disease (incidence ↑ in older F)</td>
<td>[191]</td>
</tr>
<tr>
<td>Drug</td>
<td>Condition</td>
<td>Side Effect</td>
<td>Reference(s)</td>
</tr>
<tr>
<td>------------</td>
<td>------------------------------------------------</td>
<td>------------------------------------------------------------------------------</td>
<td>--------------</td>
</tr>
<tr>
<td>Propranolol</td>
<td>Thyrotoxicosis, migraine prophylaxis, arrhythmias, hypertension, angina and anxiety</td>
<td>Dizziness, muscle pain, headache and dry mouth (incidence substantially ↑ in F)</td>
<td>[192]</td>
</tr>
<tr>
<td>Torasemide</td>
<td>Oedema and hypertension</td>
<td>Hospitalisation (66% of cases occurred in F)</td>
<td>[193]</td>
</tr>
<tr>
<td>Warfarin</td>
<td>Anticoagulant</td>
<td>Major bleed (3.35 times more likely in F than M)</td>
<td>[194, 195]</td>
</tr>
</tbody>
</table>

### Nervous system

<table>
<thead>
<tr>
<th>Drug</th>
<th>Condition</th>
<th>Side Effect</th>
<th>Reference(s)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aripiprazole</td>
<td>Schizophrenia</td>
<td>Heart rate (significantly ↑ bpm in F), elongated QTc (significantly ↑ in F than M) and nausea and vomiting (F 42.6% M 20.2%, p = 0.037)</td>
<td>[196]</td>
</tr>
<tr>
<td>Buprenorphine</td>
<td>Severe pain and treatment of opioid dependence</td>
<td>Sleep disturbance (M significantly less likely to report sleep disturbances than F)</td>
<td>[197]</td>
</tr>
<tr>
<td>Bupropion</td>
<td>Smoking cessation</td>
<td>EEG abnormalities (EEG sharp waves in F ↑ by a factor of 2.53 compared to M), seizure (F 1.5-fold ↑ likelihood)</td>
<td>[198, 199]</td>
</tr>
<tr>
<td>Carbamazepine</td>
<td>Epilepsy</td>
<td>Cognitive impairment (reaction time significantly more impaired in F), elevated LDL/HDL (significantly ↑ in F than M and control)</td>
<td>[200, 201]</td>
</tr>
<tr>
<td>Citalopram</td>
<td>Depressive illness, panic disorder</td>
<td>Elevated antidiuretic hormone (F is a risk factor)</td>
<td>[202]</td>
</tr>
<tr>
<td>Clozapine</td>
<td>Schizophrenia and psychosis in Parkinson’s disease</td>
<td>Increase in blood glucose, type II diabetes (fasting blood glucose ≤6.0 mmol/L M 88%, F 41%, p &lt; 0.0001), laxative use (F 49.1% and M 29.1%, p &lt; 0.01), ileus (F odds ratio (OR): 1.60 confidence interval (CI): 1.10–2.31), neutropenia (F↑ OR 1.45 95% CI 1.28 to 1.67),</td>
<td>[203-212]</td>
</tr>
</tbody>
</table>
leukopenia (F ↑, \( p = 0.026 \)), obesity, weight gain (F +5.5\% and M +1.3\%, \( p = 0.01 \))

**Diazepam**
- Muscle spasm, anxiety, dystonic reactions, sedation and panic attacks
- Psychomotor impairment (F reportedly felt clumsier) \( [213] \)

**Eszopiclone**
- Insomnia
- Dysgeusia (lasted longer and more intense in F [66\%] than M [53\%]) \( [214] \)

**Fluoxetine**
- Major depression
- Hypercortisolemia (F 98\% and M 68\%), elevated albumin (F only 23\%, \( p<0.05 \)), elevated tryptophan (F 83\% and M 32\%), suicidal ideation (F risk factor) \( [215-218] \)

**Gabapentin**
- Epilepsy and neuropathic pain
- Dizziness, somnolence, nausea (probability F 0.6 and M 0.4, and F 1.9 times more likely to report ADRs) \( [219] \)

**Imipramine**
- Depressive illness and nocturnal enuresis
- Dry mouth, constipation, sweating, tremor, treatment discontinuation (F 27.8\% and M 11.5\%) \( [220] \)

**Methylphenidate**
- Attention deficit hyperactivity disorder
- Anxiety disorder (F 20.8\% and M 5.9\%) \( [221] \)

**Morphine**
- Pain
- Respiratory depression (F 52\% and M 32\%), emesis (F 18\% and M 0\%), nausea (F 35\% and M 3\%) \( [173, 222] \)

**Nortriptyline**
- Depressive illness and neuropathic pain
- Dry mouth (In a 6-week clinical trial, self-rated dry mouth was present for 6 weeks in F and 2 weeks in M) \( [223] \)

**Oxycodone**
- Pain
- Nausea (F 24\% and M 12\%), pruritus (F 9\% and M 5\%), functional impairment (F \( \tau[584] = 3.02, p < 0.01 \)), psychiatric severity (F \( \tau[636] = 3.99, p < 0.001 \)) \( [224-226] \)
<table>
<thead>
<tr>
<th>Drug</th>
<th>Indication</th>
<th>Side Effects</th>
</tr>
</thead>
<tbody>
<tr>
<td>Perampanel</td>
<td>Epilepsy</td>
<td>Dizziness, headache, treatment discontinuation (F 10.9% and M 6.8%)</td>
</tr>
<tr>
<td>Pramipexole</td>
<td>Nausea, fatigue</td>
<td>Nausea (F 20.8% M 6.7%), fatigue (F 10.5% and M 7.3%)</td>
</tr>
<tr>
<td>Risperidone</td>
<td>Schizophrenia, psychosis and mania</td>
<td>Hyperprolactinemia (F 127 ng/ml and M 54 ng/ml), headache (F 31% M 11%), hypotension (F 17% and M 0%)</td>
</tr>
<tr>
<td>Sertraline</td>
<td>Depressive illness, obsessive compulsive disorder, panic disorder</td>
<td>Cholesterol (F $\chi(2)(1) = 7.15, \ p = 0.008$), nausea (F 36.7% and M 21%), dizziness (F 19.3% and M 10.5%), delusions (F $t(257) = -2.10, \ p = .04$)</td>
</tr>
</tbody>
</table>

**Respiratory system**

<table>
<thead>
<tr>
<th>Drug</th>
<th>Indication</th>
<th>Side Effects</th>
</tr>
</thead>
<tbody>
<tr>
<td>Terfenadine</td>
<td>Antihistamine</td>
<td>Torsades de Pointes (F ↑ susceptibility)</td>
</tr>
</tbody>
</table>

**Infection**

<table>
<thead>
<tr>
<th>Drug</th>
<th>Indication</th>
<th>Side Effects</th>
</tr>
</thead>
<tbody>
<tr>
<td>Erythromycin</td>
<td>Antibiotic</td>
<td>Torsades de Pointes (F 58% and M 32%)</td>
</tr>
<tr>
<td>Primaquine</td>
<td>Malaria</td>
<td>Nausea (F 35% and M 12%)</td>
</tr>
</tbody>
</table>

**Endocrine system**

<table>
<thead>
<tr>
<th>Drug</th>
<th>Indication</th>
<th>Side Effects</th>
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<tbody>
<tr>
<td>Liraglutide</td>
<td>Diabetes</td>
<td>Headache, vomiting, nausea (F 44% and M 6.3%, $p = 0.02$)</td>
</tr>
<tr>
<td>Prednisone</td>
<td>Steroid – inflammatory conditions</td>
<td>Depression (F 24.4% and M 16.1%, $p = 0.09$), fatigue (F 34.4% and M 29.2%, $p = 0.6$), hair loss (F 28.1% and M</td>
</tr>
</tbody>
</table>
3.6%, \(p < 0.0001\), mood swings (F 43.1% and M 30.7%, \(p = 0.03\)), weight gain (F 68.8% and M 56.2%, \(p = 0.03\))

### Genito-urinary system

<table>
<thead>
<tr>
<th>Medication</th>
<th>Condition</th>
<th>Adverse event</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Trospium</td>
<td>Urinary frequency, urgency and incontinence</td>
<td>Cognitive impairments (F 2 times more likely)</td>
<td>[240]</td>
</tr>
</tbody>
</table>

### Malignant disease

<table>
<thead>
<tr>
<th>Medication</th>
<th>Condition</th>
<th>Adverse event</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Capecitabine</td>
<td>Cancer</td>
<td>Dose-limiting toxicity (F 68% and M 52%)</td>
<td>[241]</td>
</tr>
<tr>
<td>Fluorouracil</td>
<td>Cancer</td>
<td>Stomatitis, leukopenia, alopecia, diarrhoea, mucositis</td>
<td>[242, 243]</td>
</tr>
<tr>
<td>Paclitaxel</td>
<td>Cancer</td>
<td>Lower lesion revascularization (F 11.5% and M 22.6%, (p &lt; 0.001))</td>
<td>[244, 245]</td>
</tr>
</tbody>
</table>

### Musculoskeletal system

<table>
<thead>
<tr>
<th>Medication</th>
<th>Condition</th>
<th>Adverse event</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Infliximab</td>
<td>Inflammatory diseases</td>
<td>Allergic reactions (F 38% and M 22%, (p = 0.009); OR 2.2, 95% CI 1.2-4.1)</td>
<td>[246]</td>
</tr>
</tbody>
</table>
6.0 Sex differences in pharmacodynamics

Pharmacodynamic differences based on sex have not been reported as extensively as pharmacokinetic differences due to difficulties in the quantification of such effects. Pharmacodynamic differences occur when the same plasma concentration of a drug in both sexes does not cause the same pharmacological response between the sexes [247]. Signalling pathways are believed to be similar in structure between the sexes, but differently expressed and regulated by sex hormones [248]. Soldin and colleagues proposed that women may be more pharmacodynamically sensitive than men [171], with sex differences in the binding affinity and number of receptors and differences in signal transduction pathway following receptor binding.

A significant pharmacodynamic sex difference is the increased prevalence of QT interval prolongation in women which is reported for many drugs (Table 4), leading to an increased incidence of ventricular tachyarrhythmias, syncope and increased risk of the cardiac arrhythmia Torsades de Pointes. An early study investigated 32 cases of arrhythmia induced Torsades de Pointes and found 70% of all cases occurred in women [249]. The antiarrhythmic agent, quinidine, shows greater QT prolongation in females than men for the same plasma concentration [250]. Several drugs were removed from the market due to this sex-specific pharmacodynamic effect which includes terfenadine, astemizole and cisapride [71]. The ICH E14 guidance [251] requires all new compounds to be tested for effects on the QT interval according to the Thorough QT (TQT) protocol, with standardised approaches for males and females. In mice, testosterone appears to be the main influence for lower risk of Torsades de Pointes which increases the rapid repolarisation of potassium channels [252].

Dofetilide, another antiarrhythmic agent, can increase the risk of QT prolongation in males and showed a 14 – 22% higher exposure in females compared with males, after adjustments for weight and creatinine clearance [250]. Higher dofetilide exposure in females may be due to lower creatinine clearance with higher sensitivity and longer QT interval at baseline. Sex differences exist in cortisol pharmacodynamics with women showing increased sensitivity to cortisol suppression and may therefore be more sensitive to basophils and helper T lymphocytes [253]. Sex differences were also found for the pharmacodynamics of prednisolone with a significantly smaller 50% inhibitory concentration (IC$_{50}$) value, which may be mediated
by endogenous oestrogens with increased sensitivity found at higher oestradiol concentrations [151].

Sex differences in pharmacodynamics have been investigated in anaesthetic agents response with males showing a 30 – 40% greater sensitivity to the effects of propofol than females [254]. Females showed an increase pharmacodynamic sensitivity to diazepam [213]. Women were reported to require a smaller dose of olanzapine to achieve a 70% binding of the dopamine D₂ receptor for therapeutic efficacy [255]. Adjusting for weight, height, age or concomitantly administered medicines did not affect olanzapine clearance and testosterone and/or oestrogen may modulate the pharmacodynamics of olanzapine [256].

7.0 Female-specific states that affect oral drug performance

Fluctuations in endogenous steroid sex hormones naturally occur throughout the menstrual cycle, pregnancy and in the transition towards menopause; such continuous variation in biological females has the potential to manipulate drug efficacy and ADRs [257]. Women can also take exogenous hormones for use as contraceptives and for the symptoms of the menopause. A bi-directional relationship can be observed; exogenous hormones can influence other drug products by altering metabolism whilst at the same time, drug metabolism pathways may also impact exogenous hormones used for therapy. Ritz et al., suggest adding a single sex hormone, oestrogen or testosterone for example, to in vitro cell cultures to investigate the effect on a particular outcome. The action of sex steroid hormones can exert epigenetic changes such as DNA acetylation and methylation on cell behaviour which can cause sex differences in human physiology and consequently on the mechanism of drug action [258].

7.1 Menstrual cycle

The menstrual cycle is a 28-day process and can be divided into three distinct phases; the follicular, ovular and luteal phases whereby plasma hormone concentration widely varies due to fluctuations in oestradiol and progesterone concentrations. High levels of both oestradiol and progesterone can encourage water retention which may affect body composition through the menstrual cycles [259, 260]. An overview of the known physiological variations that occur
during the menstrual cycle which may impact drug pharmacokinetics is shown in (Figure 6), although several findings need further research.

Figure 6. A) Endogenous hormone changes during the menstrual cycle. B) Physiological variations during the menstrual cycle and its potential influence on drug pharmacokinetics. Adapted with permission from [261].

Females taking ibutilide were reported to be more susceptible to QT prolongation particularly during the ovulatory phase of the menstrual cycle, compared with the luteal phase [262]; testosterone has been proposed to have protective effects by shortening the action potential duration and diminishing the QT response as seen in the luteal phase [263]. The effect of hormones is suggested for pharmacokinetics, pharmacodynamics and ADRs sex differences, although contrary to expectations, the activity of many drugs is not influenced by the menstrual cycle [264, 265].

7.2 Pregnancy

Evidently, several physiological changes can occur during the gestation which affect drug pharmacokinetics including i) volume of distribution: increased plasma volume, extracellular fluid space and total body water; ii) cardiovascular systems: plasma volume expansion, increased in carbon monoxide, altered regional blood flow (i.e. increased uterine, renal, skin and mammary blood flow but decreased skeletal blood flow), increase in stroke volume and
heart rate; iii) respiratory changes: compensated respiratory alkalosis; iv) decreased plasma albumin and; v) GI absorption: altered activity in uridine diphosphate glucuronosyltransferase (UGT) isoenzyme [116]. Changes in endogenous hormones associated with pregnancy are known to modify drug efficacy and with some drug products, may have adverse effects of foetal development. 64% of pregnant women take medication and is expected that two thirds of which may not have been tested in pregnant women [266, 267].

Pregnancy is able to modify the distribution and clearance of drugs due to the increase of blood and extracellular fluid volume. Hormonal changes, however, are further capable of influencing enzyme activity; for example, both CYP2C19 and CYP1A2 activity is decreased during pregnancy with the latter affecting the drug metabolism of caffeine and theophylline [268]. An increase in CYP2C9, CYP3A4 and UGT1A4 activity, however, is observed in the second and third trimester of pregnancy which will affect the drug processing of such CYP substrates. The effect of hormonal changes with pregnancy on drug transporter genes, however, is not fully understood but may involve the activity of oestrogen and androgen receptors [269].

7.3 Oral contraceptives
Oral contraceptives are the most common form of contraception between the ages of 15 – 49 years old, with 28% using its as their main method of contraception [270]. Most oral contraceptives contain a combination of oestrogen and progesterone to suppress ovulation and luteinising hormone secretion, respectively [271]. These exogenous hormones can impact the metabolism of a multitude of other medications through the inhibition of multiple cytochrome P450 enzymes, with moderate inhibition of CYP1A2 and CYP2C19 and weak inhibition of CYP3A4 [272-275]. Decreased CYP activity as a result of oral contraceptives is believed to be due to competitive inhibition. Although there is some evidence that oestradiol may downregulate CYP2C19 expression by the interaction of oestrogen receptor-α with a binding site in the CYP2C19 promoter [269]. A study found that oral contraceptive-induced CYP2B6 inhibition led to higher plasma concentration of bupropion, partly metabolised by CYP2B6 [276]. For the commonly administered drug product ibuprofen, the phase of the menstrual cycle did not affect the pharmacokinetics of S-ibuprofen or R-ibuprofen, but women treated with oral contraceptives had lower AUC and higher clearance than women not taking oral contraceptives [277]. Significantly, the use of oral contraceptives is not often considered in the prescribing of concomitant medicines.
7.4 Menopause

Menopause is the natural permanent cessation of menstruation, after the loss of ovarian follicular development [278]. With menopause, circulating oestrogen can decrease by up to 90% [279]. Women use exogenous hormones, such as combined oestrogen and progestogen tablets, skin patches and gels to control the symptoms of menopause which include hot flashes, night sweats, sleep disturbances and vaginal dryness. Adipose tissue and skin become the predominant source of oestrogen, where androgens are converted to oestrogen by aromatase, encoded by \textit{CYP19A1} [279]. Changes to other drug metabolising enzymes are reported, such as a 20% reduction in the activity of intestinal CYP3A4 [280]. Conflicting results are found in the literature on pharmacokinetic changes in women relating to menopausal status. Several studies on the pharmacokinetics of erythromycin and prednisolone in pre- and postmenopausal women, considering changes in intestinal or hepatic CYP3A4 activity, found no significant differences in drug metabolism according to menopausal status [281].

Reports suggest that postmenopausal women respond differently to antidepressants compared with premenopausal women [232], for example, the response of postmenopausal women to antidepressant treatment was in general worse than those in premenopausal women showing an association with high basal levels of follicle-stimulating hormone (FSH) in the postmenopausal women [282]. Sex hormones are known to interact with serotonergic, noradrenergic and dopaminergic systems [283].

7.5 Transgender people

Transgender is a term for those whose gender identity differs from the sex assigned at birth [284]. Long-term testosterone or oestrogen treatments are standard practice for transgender people, taken to align secondary sex characteristics with gender identity [285]. Due to potential physiological differences related to the XX or XY chromosomes and the various effects of administering endogenous and exogenous hormones, it can be difficult to predict the drug response in transgender people [267]. There are few studies on hormone-drug interactions in transgender patients [286]. Clinicians may use drug-drug interaction data from the general adult population. However, this does not consider the pharmacodynamics effects of hormone therapy [287].
The limited investigations have however found that in transmen with XX chromosomes who followed testosterone treatment, increases in serum triglycerides and low-density lipoprotein cholesterol were found which caused an increased risk of venous thromboembolism [288, 289]. For transwomen with XY chromosomes who used exogenous oestrogen treatment, there was a reported increase in the risk of stroke and myocardial infarction [290, 291]. Trans patients who receive sickle cell disease treatment combined with hormone therapy poses a challenge as the symptoms of sickle cell disease and the side effects of hormonal therapy can both cause cardiovascular complications [286]. In transgender women with human immunodeficiency virus (HIV), antiretroviral therapy can interact with oestradiol, which may result in lower rates of virologic suppression and higher HIV-related mortality [292]. In addition, trans patients may require adjustments prior to starting steroid hormones [267]. The authors direct the reader towards a recent comprehensive review article on the clinical pharmacology in transgender people, which includes pharmacokinetic and pharmacodynamics considerations associated with hormonal treatments [287].

8.0 Sex differences in Bioequivalence Studies

Generic formulations are by far the most prescribed drugs [293]. Before entry into the pharmaceutical market, the manufacturer of the generic drug is expected to prove bioequivalence (BE) to the marketed, reference drug [293]. This is achieved by comparing the systemic AUC of the generic formulation to that of the reference drug in a crossover clinical trials design with individuals acting as their own control. To achieve BE, the AUC and peak concentrations of the generic drug need to be within 80 – 120% of the reference drug [294]. In BE studies, the US FDA guidelines states that ‘if the drug product is intended for use in both sexes, the sponsor should attempt to include similar proportions of males and females in the study.’ [295]. Unsurprisingly, BE studies, however, are typically conducted in healthy, young adult male volunteers [296]. The argument in favour for carrying out BE studies exclusively in males is that though the sexes may pharmacokinetically respond differently, there is an assumption that intra-individual variabilities in BE are similar between males and females.

Research from the FDA Center for Drug Evaluation and Research reviewed 26 BE studies submitted to the FDA which compared original and generic drug formulations in men and women. The study found that five generic drug products (22%) were statistically different
between the sexes with respect to variability in AUC and in four (18%) variability in peak plasma concentration (Table 5) [297]. The incorporation of sex as a biological variable will have major implications for the management and interpretation of BE studies. The BE results of alprazolam in men show negligible intrasubject variability, i.e. a small number of individuals will only be needed to show BE. However in women, intra-subject variability in alprazolam was 6-fold. This consequently means that a larger number of individuals will need to be studied in order to power for BE. This distinct drug example is one of many to show that drug variability is much larger in women, precluding the ability to generalise results from men to women.

The fluctuating hormonal status of females along the menstrual cycle may also affect drug pharmacokinetics. Ranitidine, for example, is subjected to varying pharmacokinetic response according to menstrual period. In the follicular phase, AUC was 7312 ng/ml/h although was 29% lower in the luteal phase at 5195.83 ng/ml/h. In men, AUC was 11,471.94 ng/ml/h [298]. This highlights that studies of BE of drugs targeting women must compare the reference and generic drug formulation during similar stages in the menstrual cycle.

Table 5. Drugs with statistically significant sex differences in bioequivalence. Adapted with permission from [297].

<table>
<thead>
<tr>
<th>Drug</th>
<th>Variability in AUC (%CV)</th>
<th>Variability in C&lt;sub&gt;max&lt;/sub&gt; (%CV)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Males</td>
<td>Females</td>
</tr>
<tr>
<td>Alprazolam</td>
<td>4.9</td>
<td>29.4</td>
</tr>
<tr>
<td>NAPA</td>
<td>9.0</td>
<td>4.4</td>
</tr>
<tr>
<td>Nitroglycerin</td>
<td>21.3</td>
<td>39.5</td>
</tr>
<tr>
<td>Phenylacetate</td>
<td>4.3</td>
<td>9.9</td>
</tr>
<tr>
<td>Cimetidine</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Ketoprofen</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

AUC, area under the concentration-time curve; C<sub>max</sub>, peak plasma concentration; CV, coefficient of variation; NAPA, N-acetylprocainamide

8.1 Sex differences in excipient effects

In generic drug manufacturing, not only is the drug synthesis process likely to be different to that of the reference drug product, but the formulation itself. As such, a generic drug may differ
in concentrations or nature of pharmaceutical excipients from the reference formulation. Studying only men in BE studies requires the hypothesis that both males and females respond to excipients similarly. Prior to the 1990s, many excipients were generally regarded as inert with the majority comprising molecules that were structurally simple, biologically inactive and of natural origin such as wheat, minerals and sugars. However, in the present day, the number of excipients has substantially increased with over 1000 excipient types from 40 functional categories being used in commercialised drug products [299]. Excipients are considered a reliable source of safe chemical matter, co-formulated only to carry out their intended function in a dosage form (i.e. disintegrants, glidants, lubricants, binders, taste masking and colouring agents). However, an exponentially growing body of research and clinical reports contest their biologically inert character [300]. In addition, it is also being found that excipients affect males and females differently which further complicates BE studies and suggests that certain populations may experience adverse reactions to pharmaceutical excipients more commonly [299].

For example, polyethylene glycol (PEG) 400, a commonly used solubility enhancer, is osmotically active at pharmaceutically relevant concentrations. Following a 10 g dose, PEG 400 reduced GI transit time by 35% [301] and when co-formulated with ranitidine, accelerated small intestinal transit which consequently reduced drug absorption by 31% [302]. A further study evaluated the effect of different PEG 400 concentrations (1 g, 2.5 g and 5 g) on liquid GI transit and ranitidine bioavailability and found ranitidine bioavailability to be reduced by 38% at higher PEG concentrations, which was thought to be due to PEG 400 stimulating GI motility [302]. Conversely, at the lowest concentration (1 g), drug bioavailability was significantly increased by 41% possibly due to the modulation of intestinal permeability. These studies, however, were conducted in male participants.

The activity of PEG 400 was then investigated in a human study conducted in both males and females at pharmaceutically relevant doses. In male volunteers, the co-formulation of PEG 400 at 0.5 g, 0.75 g, 1.0 g, 1.25 g or 1.5 g increased bioavailability by 34%, 63%, 49%, 43% and 6% in comparison to the control treatment of ranitidine alone. At equivalent drug-excipient formulations administered to female subjects, however, ranitidine bioavailability decreased by 24%, 8%, 13%, 13% and 13% against the control. As such, all doses of PEG 400 enhanced the bioavailability of ranitidine in male subjects but not in females. The most pronounced effect was noted with the 0.75 g dose of PEG 400 attributed to a 63% increase in bioavailability in
males [136]. Recent research has shown that the bioavailability-modifying effect of PEG 400 is not only seen in the presence of ranitidine but extends to another BCS Class III drug, cimetidine. Cimetidine co-formulated with PEG 400 at 0.5 g, 0.75 g, 1.0 g and 1.5 g significantly increased cimetidine bioavailability in male participants by 34%, 58% and 41% respectively. No such enhancement, however, was seen in female participants, similar to what was observed in the presence of ranitidine. At 1.5 g PEG 400, however, both sexes displayed a reduction in ranitidine bioavailability [303] (Figure 7).

![Figure 7](image.png)

**Figure 7.** Percentage difference in cimetidine bioavailability when co-formulated with different doses of PEG 400 against the control (cimetidine alone) in male and female human volunteers (mean ± S.D., n = 6). *Denotes statistical significance against the control (p < 0.05). Adapted with permission from [303].

As such, if a ranitidine and cimetidine BE study were to be conducted in men only, the dose of the generic product if using PEG 400 in its generic drug formulation would be significantly different when compared with the reference drug. The above examples illustrate very clearly
that, for any drug product, BE in women cannot be extrapolated from BE studies conducted in males only, i.e. if a generic formulation is to be taken by women, it must be tested with sufficient power in women as well as men [304].

8.2 Sex differences in food effects

Sex differences exist in the processing of food. For example, female bodies tend to take longer to digest food. As aforementioned, the concomitant intake of food and drugs may affect the oral drug performance between the sexes. In preclinical and clinical studies, diets affect weight, metabolism, hormone and immune function, therefore diet formulation should be stated [304, 305]. Following a food intake, the secretion of acid is significantly higher in males than females [306].

Animal models such as the rat are used in preclinical studies, although potential sex differences are often not considered by pooling males and females or using male animals due to ease of handling and faster elimination of some drug products. Dou and colleagues recently reported sex-differences in the P-gp expression in both prandial states in rats. In the fasted state, male rats exhibited a significantly higher P-gp expression than females. In the fed state, however, the P-gp expression was significantly higher in females, 77% higher in the jejunum than the male counterparts [307]. Sex differences were also identified in male and female human jejunal and ileal tissues via mRNA and protein quantification via real-time polymerase chain reaction (RT-PCR), Western blot and liquid chromatography-tandem mass spectrometry (LC-MS/MS) respectively. Small intestinal P-gp was higher in human males than females with an increasing trend from the proximal to distal regions which was closely reflected in a pharmaceutically common preclinical model, Wistar rats [89]. Additionally, an ex vivo Ussing chamber found that the P-gp substrates ganciclovir and ranitidine demonstrated sex differences in their intestinal permeability [308]. Sex differences in the bioavailability of cyclosporine A, a P-gp substrate, was reported after a fat-rich meal; decreased bioavailability in females and increased bioavailability in male humans [309]. Diets rich in phytoestrogens, a component in soy, which is often included in rodent diet, may have sex-specific effects of cardiac health. In male humans, soy-based diets significantly decreased cardiac function and associated heart failure, observed to a lesser extent in females [310].
9.0 Suggestions for sex-informed scientific approaches

Historically, drug development has followed a ‘one size fits all’ approach. The incorporation of sex-informed perspectives, however, increases rigor, promotes drug discovery and expands the relevance of biomedical research. Thoughtful and deliberate methodology can improve study design and progress towards identifying potential sex differences in research. Promoting sex-as-a-biological-variable approaches in drug prescribing can start with relatively simple yet powerful steps with the use of female and male cells, tissues and organisms throughout the preclinical and clinical drug development, powering for any sex-related influences to be determined. Drug development should also consider the physiological nuances between males and females for effective drug delivery and the active inclusion of women of childbearing age and of pregnant women in drug clinical trials and diagnostic tools (Figure 8).
9.1 Sex-specific recommendations towards pharmaceutical research

If female cells and animals are not included in early phases of drug development, sex-specific differences in efficacy and toxicity will not be detected. Female and male cells are affected by their sex chromosomes and influenced by hormones in their environment. Certain types of cells such as those found in the liver produce different amounts of metabolic enzymes (Table 6). If sex differences are not considered in preclinical trials, experimental results can be irreproducible. As such, analysing the cellular response to medicines in a sex-specific manner...
can offer early indications of potential differences that could influence the subsequent processes of drug development [312, 313]. If women are not included in early clinical trials, real-world effects of a medicine, such as adverse side effects, will subsequently not be detected before its release into market. It is known that women experience more unwanted side effects than men and the magnitude of the problem is difficult to assess as many countries have not included sex information in their statutory reports of side effects [314]. New reporting guidelines such as Prisma-Equity Extension [315] and Consort Equity 2017 [314] advocate for the disaggregation of data by sex in large comparative studies and meta-analyses aimed at predicting unwanted side effects better.

**Table 6.** Most commonly used cell lines in the pharmaceutical preclinical arena for drugs and formulations intended for oral administration. Adapted from [316].

<table>
<thead>
<tr>
<th>Cell line</th>
<th>Sex</th>
<th>Description</th>
<th>Species</th>
<th>Year derived</th>
<th>Origin</th>
</tr>
</thead>
<tbody>
<tr>
<td>A6</td>
<td>Male</td>
<td>Kidney epithelial</td>
<td>Xenopus</td>
<td>1965</td>
<td>Non-cancerous tissue</td>
</tr>
<tr>
<td>AGS</td>
<td>Female</td>
<td>Stomach epithelial</td>
<td>Human</td>
<td>1979</td>
<td>Cancerous tissue</td>
</tr>
<tr>
<td>AML-12</td>
<td>Male</td>
<td>Liver epithelial</td>
<td>Mouse</td>
<td>1994</td>
<td>Non-cancerous tissue</td>
</tr>
<tr>
<td>C2BBel</td>
<td>Male</td>
<td>Colonic epithelial cell</td>
<td>Human</td>
<td>1988</td>
<td>Cancerous tissue</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(a Caco-2 subclone)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Caco-2</td>
<td>Male</td>
<td>Colonic epithelial</td>
<td>Human</td>
<td>1977</td>
<td>Cancerous tissue</td>
</tr>
<tr>
<td>Capan-1</td>
<td>Male</td>
<td>Pancreatic epithelial</td>
<td>Human</td>
<td>1N/A</td>
<td>N/A</td>
</tr>
<tr>
<td>CFPAC-1</td>
<td>Male</td>
<td>Pancreatic epithelial</td>
<td>Human</td>
<td>1990</td>
<td>Cancerous tissue</td>
</tr>
<tr>
<td>CV-1</td>
<td>Male</td>
<td>Kidney fibroblast</td>
<td>African green monkey</td>
<td>1964</td>
<td>Non-cancerous tissue</td>
</tr>
<tr>
<td>Cell Line</td>
<td>Sex</td>
<td>Tissue Type</td>
<td>Species</td>
<td>Year</td>
<td>Tissue Type</td>
</tr>
<tr>
<td>-----------</td>
<td>------</td>
<td>----------------------</td>
<td>---------</td>
<td>------</td>
<td>-------------------</td>
</tr>
<tr>
<td>H4TG</td>
<td>Male</td>
<td>Liver epithelial</td>
<td>Rat</td>
<td>1964</td>
<td>Cancerous tissue</td>
</tr>
<tr>
<td>HEP 3B</td>
<td>Female</td>
<td>Liver epithelial</td>
<td>Human</td>
<td>1983</td>
<td>Cancerous tissue</td>
</tr>
<tr>
<td>HEP G2</td>
<td>Male</td>
<td>Liver epithelial</td>
<td>Human</td>
<td>1994</td>
<td>Cancerous tissue</td>
</tr>
<tr>
<td>HK2</td>
<td>Male</td>
<td>Kidney epithelial</td>
<td>Human</td>
<td>1994</td>
<td>Non-cancerous</td>
</tr>
<tr>
<td>HPAF-II</td>
<td>Male</td>
<td>Pancreatic epithelial</td>
<td>Human</td>
<td>1982</td>
<td>Cancerous tissue</td>
</tr>
<tr>
<td>HT-29</td>
<td>Female</td>
<td>Colonic epithelial</td>
<td>Human</td>
<td>1964</td>
<td>Cancerous tissue</td>
</tr>
<tr>
<td>HuTu80</td>
<td>Male</td>
<td>Duodenal epithelial</td>
<td>Human</td>
<td>N/A</td>
<td>Cancerous tissue</td>
</tr>
<tr>
<td>IEC-6</td>
<td>Male</td>
<td>Small intestinal epithelial</td>
<td>Rat</td>
<td>1978</td>
<td>Non-cancerous tissue</td>
</tr>
<tr>
<td>KATO III</td>
<td>Male</td>
<td>Gastric carcinoma mixed</td>
<td>Human</td>
<td>1978</td>
<td>Cancerous tissue</td>
</tr>
<tr>
<td>LLC-PK1</td>
<td>Male</td>
<td>Kidney epithelial</td>
<td>Human</td>
<td>1977</td>
<td>Cancerous tissue</td>
</tr>
<tr>
<td>LS 174T</td>
<td>Female</td>
<td>Colonic epithelial</td>
<td>Human</td>
<td>1976</td>
<td>Cancerous tissue</td>
</tr>
<tr>
<td>MDCK</td>
<td>Female</td>
<td>Kidney epithelial</td>
<td>Dog</td>
<td>1958</td>
<td>Non-cancerous tissue</td>
</tr>
<tr>
<td>MIA-PaCa-2</td>
<td>Male</td>
<td>Pancreatic epithelial</td>
<td>Human</td>
<td>1975</td>
<td>Cancerous tissue</td>
</tr>
<tr>
<td>MKN45</td>
<td>Female</td>
<td>Gastric carcinoma</td>
<td>Human</td>
<td>N/A</td>
<td>Cancerous tissue</td>
</tr>
</tbody>
</table>

9.2 Sex-specific recommendations towards the application of physiologically based pharmacokinetic (PBPK) modelling and simulation
Analyses have found that the majority of attrition in drug candidate is due to unfavourable pharmacokinetic behaviours [317]. Therefore, prediction of the absorption, distribution, metabolism, and excretion properties could help to de-risk the drug development pathway. Physiologically based pharmacokinetic (PBPK) models can be used to predict inter-individual variability in the pharmacokinetic profile of drugs between the sexes. Here, female or male-specific physiological and pharmacokinetic differences as well as drug-specific physicochemical data can be inputted from in vitro and in vivo preclinical and clinical data. These physiological sex differences can be calibrated in the PBPK models by adjusting absorption values (fraction absorbed and intrinsic clearance), rate constants, scaling factors and enzyme or transporter activity coefficients. Parameter sensitivity analyses can be tested to understand if sex-specific parameters are required to accurately predict drug response between the sexes. The population simulator feature of PBPK software can be harnessed to alter the variance associated with input parameters to show the sex differences [317, 318]. A goal of the model construction would be to provide insight into the right dose for the patient. Since the 1990s regulatory agencies such as the FDA and the EMA have encouraged pharmaceutical companies to use PBPK modelling to understand drug response. Model parameters are chosen to reflect the inter-individual variability in the physiology of patient groups, in this case, male and females.

The development of sex-specific PBPK models requires quantitative data for the physiological sex differences. However, in the literature, sex differences are often presented as a relative comparison (for example, males > females, males < females, males = females) [319]. The successes of machine learning (ML) stem from its capability to discern patterns from complex and large volume data sets [320, 321]. In the drug discovery arena, artificial intelligence (AI) is facilitating research and development in drug candidate selection in larger pharmaceutical companies [322] as well as start-ups such as Google’s DeepMind [323]. However, within drug product development and the field of prediction of pharmacokinetics, pharmaceutical companies are yet to realise the potential of AI.

Personalised medicines require an understanding of inter-individual differences in drug response [324]. AI has been applied to assess patient response to oncology therapeutics [325], drug-drug interactions [326] and ADRs [68, 327]. However, the majority of ML models fail to account for sex and its influence on disease and therapeutic outcomes. Therefore, the results may be discriminatory and be sex bias. For example, the US Department of Veterans Affairs
healthcare system was used to assess the risk of acute kidney injury [328]. Female patients comprised of 6.37% of patients in the dataset and therefore algorithm performance was lower in the females, compared with the males.

Drug product development is increasingly being guided by Big Data, with large data sets computationally analysed to reveal patterns, trends and associations [329]. Clinical and pharmaceutical Big Data has the potential to provide untapped insights into health and disease, as well as to explore sex differences [324]. However, the majority of genome-wide association studies concentrate on white male subjects, ignoring potential sex differences in diseases [330]. Biomarkers are increasingly being explored to facilitate detection and diagnoses. The FDA has recently approved the use of a number of digital biomarker devices that monitoring symptoms and measurables in clinical trials [331]. Ramsey et al., found that the concentration of 56% of biomarkers varied between males and females, concluding that sex and female hormonal status should be reported when collect biomarker-related data. AI models should incorporate sex and gender differences, so effective personalised medicines and tailored treatment plans can be recommended [332]. Furthermore, algorithm validation, regulation, explanation and interpretation must be ensured as much as possible [333].

9.3 Sex-specific recommendations towards personalised medicines

Reductionism in the biomedical research has resulted to the identification and mutations in the human genome. Omics-sciences can uncover the intricacies of healthy and disease pathways, particularly of defective molecules or specific cellular phenotype responsible for the latter. Sex-specific research is a promising field in which genomics, epigenomics, transcriptomics, proteomics and metabolomics can be applied to investigate the mechanistic reasons responsible for sex-related differences in complex multi-factorial disease pathophysiology and even drug response [334]. By using multi-omics approaches, information on sex differences in gene expression and protein levels can be understood, and successfully applied towards the development of sex-specific pharmaceutical therapeutics and medical devices.

Typically based on a ‘one size fits all’ concept, traditional manufacturing processes are unsuitable for the production of personalised drug delivery therapies involving labour-intensive, dose-inflexible and time-consuming processes [335, 336]. Due to recent manufacturing innovations and technologies, however, the number of drugs and treatments
available for individualised therapies has increased nearly 10-fold from 13 to 113 [337, 338]. Continuous manufacturing and additive manufacturing, for example, has transformed the healthcare industry towards tailored medicines development [339]. Specifically, three-dimensional (3D) printing, an additive manufacturing technique, is set to be a major disruptive technology in healthcare by the formation of bespoke intricate objects of virtually any shape and size, layer by layer [340, 341]. Structures can be created from a digital 3D file using a computer-aided design (CAD) software to readily manufacture objects individualised to each patient [342]. Since its introduction nearly three decades ago, 3D printing has transformed manufacturing abounding all fields and applications. 3D printing, however, is set to become a revolutionary technology within the healthcare space [343]; from its capability to create individualised objects, personalised medical prosthetics, implants and devices can be tailored to the individual needs of the patient. In the arena of drug delivery, various constructs have already been prepared using 3D printing [344-346] from drug-eluting implants and personalised solid oral dosage forms with characteristics such as increased patient acceptability, orally dissolving tablets, modified drug release, polypills and novel therapeutics for orphan diseases [347-352]. This technology has been explored as a viable method for personalising medicines at the point of use with a view to expand into rapid throughout screening of new drug candidates on 3D printed-biological tissues to identify intra-individual therapeutic responses [353]. Majority of the research of 3D printing pharmaceuticals or medical devices have focused on formulation characteristics and drug performance. Less efforts, however, have been invested in larger variables of personalisation such as sex-specific formulations in terms of the wider patient population. Due to its innate unique proposition in delivering personalised medicine, 3D printing could be employed to manufacture sex- and dose-specific oral drug products to limit side effects whilst providing optimum therapeutic effect according to the individual.

9.4 Sex-specific recommendations towards regulatory agencies

Bridging the sex-gap between drug development and patient care is not possible without approval from regulatory agencies. As aforementioned, a number of guidance documents published by the FDA and NIH amongst others have formalised the inclusion of women and minority groups to be included in all clinical research. The British Journal of Pharmacology also recommends that all future studies either include both sexes in experimental designs or provide explanation as to why sex or gender perspectives are not relevant for their research.
methodology [312]. Other journals and funding agencies have followed suit and adopted similar policies to promote sex analyses in drug development [76]. Despite this, sex inequality still remains. As such, regulatory agencies should *require* and not simply *recommend* sex-disaggregated data reporting of all drug trial results submitted by the pharmaceutical industry. Regulatory bodies should also ensure that sex-specific information is available to prescribers and patients on drug websites and labels. In addition, as many pharmaceuticals in the market were tested and approved in years when women were not appropriately included in clinical trials, post-market surveillance is the only avenue to obtain data on sex differences in efficacy and toxicity. Post-pharmaceutical surveillance should also disaggregate side effect reporting between males and females to identify sex-specific drug responses.

10.0 Conclusion

Recent governmental policies mandate that researchers across the drug development pipeline should collect and analyse data by sex. It is clear, however, that the onus to incorporate the study of sex differences is on investigators to address these perspectives adequately and accountably at all levels of basic, clinical and population research. In human clinical trials, it should be an imperative to investigate and aggregate data according to sex. A focus of sex differences in the innovation process will further illuminate fundamental, modifiable causes of disease and highlight potentially significant findings in optimum drug efficacy and importantly, toxicity. If sex as a biological variable is skilfully addressed and powered in experimental designs and analyses, this will decrease the prevalence of patients experiencing adverse drug reactions, better treatment options and may give rise to new insights for men and women that will be critical for next generation scientific and therapeutic discoveries in the age of precision medicine.

**Declaration of Interest**

The Authors declare no conflict of interest.

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Sex differences
Cellular function and physiology

Post-market surveillance
Adverse drug reactions in women

Sex-bias in pharmaceutical research

Pre-clinical studies
76% male cell-based
75% animal studies

Phase I and II
67% male human studies

Clinical care
80% female health care consumers