Why we need to talk about sex and clinical trials

In this article, which was originally published in *The Pharmaceutical Journal*, Rachel Brazil examines the consequences of the under-representation of women in clinical trials.

The under-representation of women in clinical trials is slowly being addressed, but we cannot move towards truly personalised healthcare unless we have an equal balance of the sexes in all new medicine trials.

Not only do men and women respond differently to disease — a fact first noticed by Hippocrates during an influenza outbreak more than 2,000 years ago — they also respond differently to the pharmaceuticals used to treat it.

However, despite these differences, many current therapies will have only been tested on men in their preregistration clinical trials, leading to questions over whether women are receiving optimal treatment[1].

The under-representation of women in clinical trials stems from the long-held assumption that the male perspective represents the norm. Medical

education textbooks typically default to the male in case studies and anatomical drawings, while women are represented only in matters specific to reproductive biology.

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"[Given] that women have fluctuating hormone levels month to month, it was thought that including women in clinical trials [would introduce] a lot of complicating factors," explains Natalie DiPietro Mager, professor of pharmacy practice at Ohio Northern University.

The thalidomide disaster in the early 1960s highlighted the potential damage pharmaceuticals could do during pregnancy and led to the sentiment that women of reproductive age should be protected from any similar exposure in clinical trials. This led the US Food and Drug Administration (FDA) to issue guidelines in 1977 that essentially excluded women from all trials[2].

However, it has become increasingly apparent that the male response to medicines does not represent both sexes. Rather than protecting women, evidence shows their exclusion from trials has led to an unrepresentative assessment of drug efficacy and side effects, potentially leaving them at risk of serious harm[1].

The difference between men and women

Anna Dorothea Wagner, an oncologist at Lausanne University Hospital in Switzerland, started her work in the area of sex diversity after noticing differences in her chemotherapy patients. "I had the impression that women [experienced] a higher toxicity, more fatigue, more nausea and more often need hospitalisation, and I just thought 'there is something wrong'," she explains.

In 2018, Wagner and her colleagues collated data from four chemotherapy trials for oesophagogastric cancer and found that 16.7% of 326 female patients experienced serious side effects compared with 9.5% of the 1,328 male patients[3].

Results from other therapeutic areas show similar effects. A 2019 study looked at adverse drug reactions (ADRs) reported by healthcare professionals and patients between 2003 and 2016 to the Netherlands pharmacovigilance centre, Lareb — an independent foundation that identifies risk associated with the use of medicines in daily practice.

The study found potentially relevant sex differences in 363 (15%) of the 2,483 drug–ADR combinations reported, with most of these reported for women (322 combinations; 88.7%)[4].

The impact of these differences has become more visible in recent years. Between 1997 and 2001, eight out of ten FDA-approved medicines pulled from the market showed higher levels of harm to women than men; four of these were taken more frequently by women, but the remaining four had equal use[1]. One example is troglitazone, a medicine used to treat diabetes that was withdrawn by the FDA in 2000 owing to the associated risk of liver failure; in a 2002 study, 58 (67%) of 87 cases of acute liver failure associated with troglitazone were in women[5].

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The sleeping pill zolpidem "was an extremely important example of a situation that really opened the eyes of researchers and clinicians", says Alyson McGregor, director for the division of sex and gender in emergency medicine at Brown University, Rhode Island. Following its approval in 1993, it became apparent from subsequent trials and FDA monitoring that blood zolpidem levels were 25–33% higher in women than in men; higher zolpidem levels can increase the risk of next-day impairment of driving[6].

These higher levels arise owing to differences in alcohol and aldehyde dehydrogenase expression in men and women, resulting in slower zolpidem clearance in women. Based on an accumulation of evidence from studies — including driving simulations — the FDA halved the recommended dose for women only in 2013. Despite this, the European Medicines Agency did not follow suit, considering the data insufficient to make a conclusion[7].

Another disparity exists in the risk of drug-induced arrhythmias after taking combinations of antihistamines (terfenadine and astemizole), antibiotics (erythromycin), antimalarials (halofantrine) and antiarrhythmics (quinidine and d-sotalol). When given in certain combinations, these medicines trigger the life-threatening ventricular arrhythmia, torsades de pointes (TdP), more often in women than in men8. For example, after the approval of terfenadine in 1997, reports began to arise of significant hepatic dysfunction or TdP in women treated with the drug along with certain antibiotics[9].

Even flibanserin, the treatment for low libido (or 'female viagra' as it came to be known), had sex-based disparities. Initially tested on 23 men and only 2 women, it was only after its 2015 release that it became apparent that women who took flibanserin while drinking alcohol were at an increased risk of losing consciousness.

The differences between the male and female responses to medicines are not simply related to average body size, but fundamental metabolic and hormonal differences that cause changes in both the pharmacokinetic and pharmacodynamic drug profiles.

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which affect the metabolism of that drug," says McGregor.

Female hormones allow women to convert food into fat more easily, causing increased deposition of fat: "Women have about 15% more body fat for a person of equal weight and height, which has an impact on drug metabolism," adds Wagner.

Women also have longer QT intervals (the time taken for ventricular depolarisation and repolarisation) than men, and medicines that block potassium currents can prolong this even further, increasing the risk of arrhythmia.

In addition, the differences in male and female genetics have a much broader footprint than was once thought.

"There are differences in gene expression patterns in cancers arising in men and women," says Wagner.

"I think this is something we have to take into account and we haven't until now."

The problem is not isolated to the effect of the active pharmaceutical ingredient — it is thought the inactive ingredients in drug formulations could also impact the bioavailability of a medicine differently in women and men *(see Box)*.

Including more women in clinical trials

In 1993, the FDA lifted its ban on women participating in clinical research and an act of Congress called on the US National Institutes of Health (NIH) to ensure women's inclusion in trials. The European Union's Clinical Trials Directive now expects that confirmatory phase III trials reflect the population to be treated once the medicine is on the market.

However, we are still some way from equal representation in trials.

Excipients

Evidence from animal studies shows that excipients – the inactive ingredients in drug formulations – could impact the bioavailability of a medicine differently in women and men, with the potential to reduce efficacy.

Abdul Basit, professor of pharmaceutics at University College London (UCL), looked at the influence of polyoxyethylated solubilising excipients on ranitidine in rats and found bioavailability increased in males, but not females10. It is thought this is owing to differences in the levels of P-glycoproteins – plasma membrane proteins that act as a localised drug transport mechanism – between the sexes.

Yang Mai, a researcher who carried out the study with Basit and is now at Sun Yat-sen University, Guangzhou, China, says there is currently little information on which excipients show sex-dependent effects.

"More work should be done to set up a bigger database, and this is what we are doing now," she adds.

Basit's group at UCL and Mai's group in Sun Yat-sen University are carrying out a project focusing on the sex-based effect of excipients on membrane transporters and the mechanisms behind this.

They are also looking at whether medicines formulated with excipients using 3D printing technologies will have sex differences and whether food alters membrane transporters in a sex-related manner.

According to a 2017 FDA report, women represent 43% of clinical trial participants globally[11]. A Dutch study published in 2018, looking at 38 drug trials, concluded that, overall, women are studied in adequate proportions, but the number of female participants was only 22% in phase I trials[12]. The number of women in more than 25% of trials did not match the proportion of women affected by the diseases being studied.

DiPietro Mager says she thinks that women are now close to being around half of all clinical trial subjects, but that the statistics may be skewed by trials for conditions that only affect women.

"[In] trials that impact both men and women, some reports indicate that women are not always being enrolled at a level proportional to the levels at which they experience that disease state."

Even though heart disease affects men and women equally and is the leading cause of death for both globally, a study of 36 pivotal cardiovascular disease trials from 2005 to 2015 showed women made up only 34% of participants — with particularly low levels in trials related to heart failure, coronary artery disease and acute coronary syndrome[13].

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"Things are moving in the right direction," says McGregor, "but the difficulty is changing the system because there are so many different parts."

This includes academic trials, trials run by pharmaceutical companies, and issues such as patient enrolment and how trials are funded and subsequently published.

Achieving a sex balance

There are still plenty of loopholes in trials. Current regulations from the NIH and other funders do not cover research funded by drug companies, and sometimes the way the trials are set up can inadvertently exclude women, says DiPietro Mager. For example, women often present with cardiovascular disease at a later age than men, so if a trial selects participants using an age profile that fits the disease in men, women are likely to be excluded.

Generic drugs, McGregor says, are still almost exclusively tested on men. Bioequivalence studies are the only in vivo evaluation that a generic drug must overcome to reach the market, and the results are not analysed to determine sex differences[14]. However, they can differ in the way they are formulated and these differences can mean that bioavailability data for

a generic may not be equally valid for both sexes.

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"We don't even know whether the [original] brand-name drug was tested in both men and women," says McGregor.

"Now we're taking that medication and just assuming that if the generic formulation is bioequivalent to the branded drug in men, that it would also be bioequivalent for women."

Drawing on her own interactions with patients, she adds: "Many patients feel differently when they're [switched to] a generic drug, especially women".

A way to go

Equity in trial registration numbers is only half of the battle. To be able to understand and act on differences, trial data need to be broken down by sex difference, which is often not reported. A 2011 review examining 86 studies from nine high impact journals showed that two-thirds of the trials did not report results by sex[15].

In 2014, the NIH announced it would require sex and gender inclusion plans in preclinical research and, in 2016, the FDA added requirements to study both sexes in preclinical vertebrate trials and provide disaggregated data.

But there is still some resistance to the idea. A 2014 op-ed in the magazine Scientific American by neuroscientist R. Douglas Fields complained that including both sexes in experiments was a waste of time. And, in 2015, a group of US women and gender studies academics argued that the NIH mandate on preclinical trials would reduce the resources available for studying areas more relevant to sex disparities in humans.

UK funders have been slow to act on the issue in both clinical and preclinical trials. The Wellcome Trust has no specific guidelines, although it expects researchers to have appropriate sex balance, and the Medical Research Council also has no guidelines on clinical trials or the inclusion of female animals.

For many in the field of women's health, the current enthusiasm for personalised medicine is ironic given that the effects of one of the most fundamental genetic variables has, for years, been neglected in clinical trials.

"I think [we need to] back up and first appreciate the importance of sex as a biological variable," says McGregor.

References available on request. This article was originally published in The Pharmaceutical Journal; 304 online (2020) , doi: 10.1211/PJ.2020.20207976, and is republished courtesy of the Royal Pharmaceutical Society.