The Importance of Female Inclusion in Clinical Trials

The Importance of Female Inclusion in Clinical Trials:

Towards a More Inclusive Future

By

Rinki Minakshi

Cambridge Scholars Publishing

The Importance of Female Inclusion in Clinical Trials:
Towards a More Inclusive Future

By Rinki Minakshi

This book first published 2024

Cambridge Scholars Publishing

Lady Stephenson Library, Newcastle upon Tyne, NE6 2PA, UK

British Library Cataloguing in Publication Data
A catalogue record for this book is available from the British Library

ISBN (10): 1-0364-0428-5
ISBN (13): 978-1-0364-0428-4

TABLE OF CONTENTS

CHAPTER 1

CLINICAL TRIALS IGNORING FEMALES

> "The dissemination of the right to live on this planet is equal for all life forms, be it the microbes, plants, animals or humans, nature has never drawn lines of discrimination."

In today's scientific world, humanity is enjoying the best of almost every aspect of living. We are witnessing unprecedented development in the field of technology that has led to unseen advancements in medical science. We now know, with accumulating research evidence, that human diseases affect the systems of females and males in different ways. The innate as well as adaptive immune response varies markedly in females and males. The spectrum of susceptibility towards infectious and autoimmune diseases is different in female and male systems. Female brains, hearts and hormones respond differently to various diagnosis tests and medications. Despite this knowledge, health research tends to ignore this distinction and only focuses on the reproductive aspect of female biology. The words sex and gender got their delineations: sex defined the biological characteristics under the influence of sex chromosomes and reproductive organs, whereas gender demarcated the social and cultural impact on the discernment and expression capability of a human being. Over time, gender-based medication popularized the term "bikini medicine," where the health science only focused on the body parts of females that would be covered by a bikini (Talesnik 2018).

The Historical Perspective

The background to this whole story germinated during World War II when Nazis started abusive experimentations on human subjects (Pauker 2002). In 1938, the synthetic hormone, Diethylstilbestrol (DES) was prescribed to approximately 200,000–400,000 females in Canada for preventing miscarriage. But the damaging effects of this drug only came to light after thirty years when the daughters of those mothers who had consumed DES showed vaginal adenocarcinoma and infertility issues (Fuller 2002). Between 1959 and 1962, Canadian data reported that the drug Thalidomide,

which was prescribed for the prevention of morning sickness in pregnant females, caused peripheral neuritis and severe limb malformation in newborns (Pauker 2002). These disastrous incidences provoked regulatory bodies to implement protective guidelines for females in their reproductive years (childbearing age). So, in the late 1970s, a policy was adopted by the US Food and Drug Administration (FDA) that recommended the exclusion of females in their reproductive years from participating in Phase I and Phase II clinical trials (US Food and Drug Administration 1993). To prioritize the safety of females in their reproductive ages, various medical procedures and research endeavors started excluding them from their trials. The policy excluded females in their reproductive years from the early phases of clinical trials but this trend of exclusion started to translate to the marginalization of all female subjects from any pharmaceutical research trials (Lippman 2006).

The intention behind the exclusion of females from clinical trials was good, but slowly this practice led to male physiology being the standard reference in the field of healthcare research. Assumptions were made by the researchers conducting drug trials that females would respond the same way as males. Strikingly, the physiological hormone fluctuation observed in females during their reproductive years gave the researchers a discerning view regarding females in clinical trials. Female participation was considered confusing and expensive (Wizemann and Pardue 2001). A plethora of incidences started pouring in that disclosed the pretentious nescience of healthcare practitioners towards female patients. Females suffer different symptoms of heart disease compared to males but studies on cardiovascular research didn't include females, which led to the underdiagnosis of females with heart complications (Mastroianni et al. 1994). The same followed in the cases of acquired immunodeficiency syndrome (AIDS), where the survival rates of females were lower owing to late diagnosis and less belligerent treatment approaches (Mastroianni et al. 1994).

In 1992, the US Government Accountability Office (GAO) performed an analysis on the distribution of all new drug applications that had been permitted from January 1988 to June 1991. This was to study the representation of females in the drug trials, which showed that there were adequate numbers of female participants for most of the new drugs, except for the drugs used in the treatment of cardiovascular diseases (female participants were less than 40 % of the total subjects). Awakened by the alarms raised by various reports, the National Institutes of Health (NIH) introduced the Revitalization Act, in 1993, which mandated the

participation of females in clinical trials funded by the NIH. However, this directive was not taken seriously by several investigators (Schiebinger et al. 2016). It was reported by the GAO that although female participation in the drug trials did occur, their participation percentages were variable with the stages of the trials: phase I studies had 22 %, whereas phase II and III included 56 % female subjects. It was noticed that the FDA, as well as the sponsors, didn't abide by the guidelines available for inclusion in all of the sex-specific parameters (US Government Accountability 2001). One study focused on the comparative analysis of the sex-specific participation vis-à-vis the frequency of females in the study groups for diseases in phase III trials between 2007 and 2009. The data showed that 64 % of diseases had equal, or higher, female participation but in the trials for hypertension, acute coronary syndrome and AIDS, which are more prevalent in female patients, the enrolment of female patients was lower (Poon et al. 2013).

Sex-Specific Drug Response

The difference in sex influences the expression of genes involved in the transportation and metabolism of drugs, which are collectively called drug-metabolizing enzymes and transporters (DMETs). The clinical response to several drugs varies between females and males. For instance, drugs (like erythromycin, cyclosporin, verapamil, diazepam) that are substrates for CYP3A4 (cytochrome P450, CYPs, family proteins, discussed later in this chapter) show greater clearance in females and, hence, are pharmacokinetically significant with respect to sex specificity (the hepatic expression and activity of CYP3A4 in females is higher) (Greenblatt and von Moltke 2008). One study analyzed the response to antiemetics, which was higher in males than in females. Also, the drug lovastatin, which is used to reduce the risk of stroke and heart attack, instigates more frequent adverse events in females (Gartlehner et al. 2010). Events due to aspirin induced bleeding in the gastrointestinal tract and peptic ulcer occur more commonly in females (Ridker et al. 2005). The sex-based response to drugs has been known to be caused not only by the expression levels of CYPs but also due to the interactions of several other drug-metabolizing enzymes and their transporters. All of them constitute various pathways involved in the metabolism of drugs.

Recent years have drawn our attention towards the very conspicuous sex-specific adverse drug reactions (ADRs). One report showed that approximately 5 % of hospitalization events were due to ADRs. They have specified that being "female" is one of the risk factors that accounts for

ADRs. The risk of ADR is 1.5–1.7 times higher in females (Rademaker 2001). Incidences of drug withdrawal were noticed and the GAO has uncovered the retraction of ten prescription drugs from the market between 1997 and 2001. The anomalous responses to these drugs were starkly inconstant: eight out of these ten prescription drugs posed a "higher health risk in females" than males. So, the NIH, in 2014, mandated drug research to consider sex difference as a biological variable (Clayton and Collins et al. 2014). The reason presented behind these drug withdrawals was mainly due to the differences in the pharmacodynamics of the drugs and higher drug exposure in females (US Government Accountability 2001). The higher occurrences of ADRs in females might be due to the exclusion of females from the phase I and phase II clinical trials. The optimal dose of the drugs was decided according to the response inferred from the male participants, which resulted in underexamined sex-specific complications.

The sex-specific disparity in drug response leading to ADRs is pervasive. Females above the age of nineteen years were reported to have 43 % to 69 % more occurrences of ADRs (Martin et al. 1998). Females also use more than one medicine concurrently, which results in drug interactions contributing to ADRs.

Understanding the Drug Chemistry in the Biological System

The efficiency and safety of a drug, whether it is prescription or over-the-counter, should be the top criteria of the government, the healthcare system and the pharmaceutical companies. The cost spent in making and testing a new drug or vaccine is a whooping amount. So, the interaction of a drug with the biological system with improved therapeutic value and minimum adverse events becomes the primary focus of clinical pharmacology. The science of pharmacology studies the interaction of drugs with the cellular architecture and the tissue organization in a biological system. The efficacy of the drug primarily pivots upon the understanding of the pharmacokinetics (PK) and the pharmacodynamics (PD) of a new drug in a wide variety of the population. This is the most important parameter. After the administration of a drug in a biological system, the response of the system towards the drug is termed the PK, whereas the effect of the drug on the system is called the PD. PK essentially centers around the rates of various chemical reactions in the biological system, pertaining to the absorption of the drug, its distribution, bioavailability, metabolism and, finally, excretion. PD studies the receptor binding of a drug, its effect on the signaling cascade and

crosstalk with other metabolic pathways. Thus, PK is the study of the relationship between drug concentration and time, whereas PD is the analysis of the effect of drug with time.

Pharmacokinetics: *Phases of Drug Disposition*

Nicholas and Barron were the first to report the sex-specific response in the pharmacological study on hexobarbital (sedative-hypnotic drug). They described that the hypnotic effect of the medication lasted longer in female rats (Nicholas and Barron 1932). This was followed by several research attempts aimed at understanding the causes behind such events. The primary step involved in deciphering the reason for sex-specific drug behavior is to fathom the way the drug is disposed of in the biological system. The principle of pharmacokinetics describes the disposition of a drug through the following phases.

Absorption. The routes of administration of drugs in the biological system are variable. The common routes that are discussed in the literature regarding the effect of sex-specific behavior are oral, intramuscular, transdermal and pulmonary. Apart from these, rectal, vaginal, intravenous, intrathecal, intra-arterial and intraperitoneal are also routes of drug administration to the biological system. Despite being a route that gives complications when it comes to the arrival of a drug at the calculated pharmacological recipient site or organ, the oral route is the most common among the above-mentioned routes of drug administration. The term bioavailability describes the portion of the drug administered in the system that goes into systemic circulation. The systemic circulation is the system of vasculature carrying oxygen (from the left ventricle of the heart) and nutrients to the cellular system. The fraction of the drug that reaches the central compartment, which comprises the plasma and the perfused tissues, like the liver and kidneys, is of utmost importance when we are discussing pharmacokinetics. The rate of drug absorption is not considered while studying bioavailability, rather, the factors that impact the absorption of a drug in the system are of primary importance to the researcher. Subsequent to the oral intake of a drug, it gets absorbed in the gut and, then, reaches the liver through portal circulation. So, the bioavailability of the drug taken orally is dependent on parameters like its metabolism in the liver, the status of its presystemic metabolism and the deterioration of the drug. This process leads to the loss of a fraction of the drug, which is different in the female and male systems. Propranolol, a beta blocker (beta-adrenergic blocking agent that blocks the binding of neurotransmitters norepinephrine and

epinephrine to their respective receptors), shows different behavior in the systems of males and females. In males, testosterone stimulates the metabolism of propranolol; however, the female hormones androgen and estrogen have no effect on the metabolism of this drug (Walle et al. 1994). The oral absorption of aspirin is faster in females than in males (16.4 min in females and 21.3 min in males) (Aarons et al. 1989). Males display higher gastric secretions and shorter gastric, as well as intestinal, clearing times (Franconi and Campesi 2014). Thus, the fact that females have longer gastric, as well as intestinal, clearing times proposes that they should wait longer to intake drugs meant to be taken on an empty stomach after a meal (Whitley and Lindsey 2009). The composition of bile is different in both the sexes: males have higher levels of cholic acid (it helps in the absorption of fat and the excretion of cholesterol) in the liver whereas females have more chenodeoxycholic acid (it dissolves cholesterol, inhibits its production in the liver and slows down its absorption in the intestine; thereby, preventing gallstone formation). This parameter impacts the drug solubility differences in both sexes (Nicolas et al. 2009).

The good vascularity of the muscle bulk favors the reach of intramuscularly injected drugs into the systemic circulation. Some of the sites of intramuscular drug injection are the gluteus maximus (located in the buttocks), the deltoid muscles (located in the shoulders) and the vastus lateralis (located in the thigh). The diversity of gene expression in the muscles of both sexes is highly significant. It has been reported that more than 3,000 genes express differentially in the skeletal muscles of females and males. Males show larger muscular mass, which is related to the pubertal rise in testosterone (Welle et al. 2008). The average thickness of subcutaneous fat in the gluteus of females is 24.90 mm while in males it is 15.92 mm (Joo and Sohng 2010). Thus, there should be consideration about the length of the injection needle used for females. The absorption of drug through the intramuscular route of administration is slower in females. It was studied that the absorption of aspirin (nonsteroid anti-inflammatory drug used as an antipyretic, analgesic and antithrombotic) through intramuscular route is slow in females (Aarons et al. 1989). In a case study of a cephradine (first-generation cephalosporin antibiotic) intramuscular injection in three regions: gluteus maximus, deltoid muscles and vastus lateralis, the absorption of the drug was found to be slower with lesser bioavailability in the gluteus maximus of females (Vukovich et al. 1975).

The technique of transdermal drug administration delivers the drug into the systemic circulation through skin penetration. The architecture of the skin and its barrier potential varies between females and males. The females have

more adipose tissue in their subcutaneous locations and they have smaller skin pores (Singh and Morris 2011). Thus, consideration of transdermal dose with respect to sex becomes important in order to prevent variation in drug absorption response. As was seen in the study of nitroglycerine (used to control hypertension during surgery), when a transdermal injection was administered to obese females, the female subjects displayed a nitroglycerine-induced rise in body temperature vis-à-vis male subjects (Haebisch 1995).

Drugs that have a pharmacological focus on the lungs employ principles of inhalation administration, aiming at maximizing the drug's effect at the location of action. This is the basis of the pulmonary route of drug administration. Though this portal of drug administration is non-invasive, having several advantages over other routes of medication, the route has lots of limitations (Ghadiri et al. 2019). The physiology of the respiratory system is, indeed, influenced by the sex of an individual. The contribution of sex hormones to the mechanisms of lung inflammation has been studied. The receptors of sex hormones are present in the lung tissues, which clearly indicates that the PK of a drug would not be the same for female and male systems (LoMauro and Aliverti 2021). The sex hormone progesterone has been studied to be a potent pulmonary vasodilator in both female and male rats (English et al. 2001). In human females, the rise in progesterone during the luteal phase of the menstrual cycle is positively linked with the peak expiratory flow rate (this is the volume of air in the expiratory flow achieved by forced effort), which is indicative of the fact that there is a sex-specific alteration in pulmonary function (LoMauro and Aliverti 2021).

Thus, the absorption of drugs through various routes of administration discussed, here, clearly indicate that this parameter is affected by sex-specific differences. There is certainly inadequacy in the data on this parameter because of the lack of research focus on the female subjects.

Distribution. The anatomical, as well as physiological, architecture of females and males is markedly different. There are a number of features that need to be mentioned here. Females have higher percentages of body fat (average reference for adult body fat: 16.5 kg in females and 13.5 kg in males, 19.8 kg in pregnant females at forty weeks gestation). The higher load of body fat in females, especially during pregnancy, exerts the body with the burden of lipid soluble, slow-metabolizing toxins. Strikingly, females have smaller-sized major blood vessels and hearts. The number of circulating red blood cells in the plasma of females is lower. Nevertheless, the lipid composition also differs in both sexes as females have higher levels

of high-density lipoprotein (HDL) and lower concentrations of triglycerides. This feature of the blood lipid profile is termed “antiathrogenic.” However, females become more athrogenic after menopause, which is associated with the rise in the incidences of heart disease in such subjects (Huxley 2007).

The case of the aminosteroid (neuromuscular blocking agent or muscle relaxant) rocuronium is worth discussing here. Rocuronium is good for intubation (the process of the insertion of a tube through the mouth or nose of a patient down into the trachea during surgical procedures) but it does show sex-specific PK and PD. As, compared to males, females require a nearly 30 % lower dose of rocuronium when it comes to attaining the same level of neuromuscular blockage (Adamus et al. 2008). Diazepam, a medication for anxiety management, muscle spasms adjunct therapy and refractory epilepsy management (when medications fail to control the seizers) has been shown to behave in a sex-specific manner. In a trial of the effect of diazepam, females presented impaired psychomotor skills compared to males (Palva 1985).

The composition of the biological system affects the distribution of the administered drugs in a sex-specific manner. The volume of total body water (intracellular, blood, plasma and extracellular water) is higher in males, a feature that must affect the distribution of drugs in the body, thereby, meddling with the concentration of the medication. We will discuss this, here, with some examples.

The first-pass metabolism is the phenomenon where the metabolism of a drug happens at a specific location in the biological system that results in the reduction ofdrug concentration by the time it actually reaches its site of action or enters into the systemic circulation. The major locations where first-pass metabolism has been documented are the liver, lungs, gastrointestinal tract and the vascular system. The concentration of plasma protein, the action of enzymes and the motility of the gastrointestinal tract are some of the major factors of the biological system composition (which is sex-specific) that play integral roles in the first-pass metabolism of a drug (Pond and Tozer 1984; Doherty and Pang 1997). Clinically, the issue of first-pass metabolism is quite significant because the pharmacological dosing of the drug would vary in a sex-specific manner, which would interrupt the therapeutic value of the medication. The blood concentrations of the drugs undergoing the first-pass metabolism are monitored by the healthcare worker in order to maintain the safety and efficacy of the drug dose (Wargin et al. 1982). The pharmacokinetic parameter used to describe the volume of drug distribution (Vd) relates the total quantity of administered drug to its

levels in the plasma. This parameter is important because it describes the potential of a biological system to either sequester the drug in the plasma or execute its distribution across the tissues (Smith et al. 2015). Let us understand the term Vd here: a drug having a higher value of Vd leaves the plasma and enters into the extravascular compartments converse to a drug with a lower Vd value that results in the sequestration of the drug in the plasma. Thus, drugs with higher Vds are required to be administered at higher doses whereas those with lower Vds require lower doses to achieve the required plasma concentration. The Vd is highly influenced by the body architectural differences displayed by the female and the male systems. The events of altered Vd are clearly observed during the premenstrual and luteal phase of the menstrual cycle due to hyponatremia (water retention at an accelerated pace due to lowering of blood sodium levels). The regulation of body fluid and the function of the cardiovascular system is impacted by the concentration of estrogen and progesterone, which affect the blood pressure response due to sodium levels (Ciccone and Holdcroft 1999).

The effect of fat mass significantly influences the Vd of a drug in a sex-specific manner. Females have higher fat mass, whereas lean mass is higher in males. However, this disparity tends to become inconspicuous with aging. The same dose of a water-soluble drug shows different responses in males due to their higher Vd values (higher lean mass and volume of water) while a lipid-soluble drug displays higher Vd values in females (higher fat mass). In the electrocardiogram of a female, the QT interval (discussed in figure 1) is inherently longer, so drugs that aim to prolong this interval during certain heart conditions would lead to lethal effects in females. For instance, drugs like amiodarone, procainamide, digoxin and lidocaine (used in the treatment of ventricular arrhythmia-abnormal heartbeats originating in the ventricles and various other heart conditions) involve a risk of ADR in females undergoing long-term therapy with these medicines. These are all lipid-soluble drugs that reach peak concentration (expressed as C_{max}: the highest drug concentration after the administration of a drug in the blood / cerebrospinal fluid / target organ) faster in females (Stolarz and Rusch 2015).

The effects, including the adverse effects, of alcohol in females are significantly different from the effects in males. The first-pass metabolism of ethanol is higher in males, whereas the distribution volume is smaller in females. This indicates that the concentration of ethanol in the blood would be higher in females. The activity of gastric alcohol dehydrogenase is lower in young females and estrogen influences the distribution volume of alcohol (Parlesak et al. 2002). Thus, the same dose of ethanol would reach higher

concentrations in the smaller-volume distribution landscape of a female than a male. Females have a lower threshold for alcohol toxicity resulting in rapid liver injury (Baraona et al. 2001).

The major plasma proteins binding the drug are albumin, alpha globulins and alpha-1 acid glycoprotein (AAG). AAG is the key plasma protein that has good drug affinity and it has been shown to behave in sex-specific manner. The glycosylation of AAG and its concentrations are affected by the action of estrogen. Females have lower mean plasma concentrations of AAG. Strikingly, the binding of AAG with the drug disopyramide (a medication for the treatment of abnormalities in heart rhythm) is significantly higher in females than males (Kishino et al. 2002).

Furthermore, the serum-binding globulins like the sex-hormone-binding globulin (SHBG) and thyroxine-binding globulin (TBG), produced mainly by the liver, increase in the plasma under the effect of estrogen. The SHBG binds all the three female and male sex hormones: estrogen, dihydrotestosterone (DHT) and testosterone, serving as a carrier for these hormones. The SHBG has strong affinity for androgens but lower affinity for estrogen. The expression level of SHBG in males increases until the age of forty-nine but decreases in the case of females (Park et al. 2020).

Metabolism. The first pass metabolism is also known as the presystemic metabolism because the administered drug is first metabolized mainly in the intestine or the liver, before reaching systemic circulation. After oral drug administration, the gastrointestinal physiology largely influences the fate of the drug. Here, the permeability glycoproteins (P-gp), which are important membrane efflux transporters expressed in the apical enterocytes and hepatocytes, play major role in the final status of the orally administered drug. They are termed as “housekeeping” proteins since they act as a natural barrier to xenobiotic absorption into the systemic circulation (Seelig 2020). The P-gp effluxes the drug substrates back into the gastrointestinal lumen from the enterocytes and into the bile from the hepatocytes. This phenomenon reduces the bioavailability of the administered drug.

Duo *et al*. studied the influence of food and substrates like ranitidine and ganciclovir on the expression pattern of P-gp in female and male Wistar rats. The male rats showed a significant decrease in the expression of P-gp across segments of their intestines, especially in the colon region, after food ingestion. However, the female rats exhibited the opposite effect, wherein all the regions of the intestine, except the colon, had significantly higher levels of P-gp expression after food intake. The study also reported that the

intestinal permeability to ranitidine (a medication used to reduce stomach acid) and ganciclovir (an antiviral medication) were sex-specific (Duo et al. 2018). The bioavailability of ranitidine (in preparations co-formulated with excipients, like polyethylene glycol) administered through the oral route was more prominent in male rats (Afonso-Pereira et al. 2016).

The liver plays an important function in the metabolism of drugs, resulting in their chemical alteration, which is termed biotransformation. Drug treatment imposes the condition of toxicity in the liver making it susceptible to drug-induced liver injury (DILI) (Navarro and Senior 2006). The biotransformation occurs by a series of mechanisms that are sorted into phase 1 modification and phase 2 conjugation. During phase 1 modification, there is an alteration in the chemical structure of an administered drug, involving the processes of oxidation, reduction, cyclization/de-cyclization or hydrolysis. Sometimes, this step can result in the conversion of a metabolically inactive prodrug into an active one. The concept is explained well in the familiar classical Ames test, which is an assay ascertaining the sensitivity of a chemical substance to mutagenicity in the presence of mouse liver extract (containing liver enzymes) (Ames 1971). The phase 2 conjugation step couples the administered drug with another molecule, which generally renders the drug into an inert, water soluble compound, facilitating its excretion. The various processes involved are acetylation, methylation, sulphation or glycine/glutathione conjugation. The enzymes involved in the various processes of phase 1 and phase 2 mechanisms are the critical factors that show sex-specific behavior.

The catalysis of the phase 1 mechanism is chiefly performed by the CYPs, cytochrome P450. The hemoprotein enzyme CYP family affects the chemistry of drug metabolism in a biological system. There are fifty-seven genes in the human genome that constitute the eighteen CYP families, which are categorized into forty-one protein-coding subfamilies. The predominant location of the CYP enzymes is the liver but they are also expressed in the brain, heart, lungs, adrenal glands, kidneys, small intestine, ovary and placenta (Zanger 2013). The family constitutes integral membrane proteins, primarily housed in the smooth endoplasmic reticulum (ER), that are essential enzymes in the pathways of steroid, cholesterol, bile acids, thromboxane A_2 and prostaglandins production (Nebert 2002). Additionally, these enzymes not only detoxify the xenobiotics but also metabolize the pharmacological drugs that we take in our system through oxidative biotransformation. These proteins are also found in the mitochondria as well as the cell surfaces. The number of proteins belonging to this family goes beyond fifty but the ones which mainly take part in the

metabolism of drugs are CYP1A2, CYP2C9, CYP2C19, CYP2D6, CYP3A4 and CYP3A5.

The genes of CYPs are distributed as gene clusters over autosomal chromosomes. These drug-metabolizing proteins are under the control of more than one gene; hence, they display polymorphism, which forms the basis of the variability in metabolism, and the efficacy and toxicity of drugs between individuals (Saha 2018). Although a few of the CYP enzymes are monogenic and show polymorphism leading to their functional variability, most of the enzymes are multifactorial. Apart from these genetic factors, other features that control the expression and regulation of the CYP genes include the sex, age and health status of the individual and the influences of hormones and diurnal rhythms (Zanger 2013).

An introduction to the nuclear receptors (these are transcription factors, which are activated by their respective ligands), the constitutive androstane receptor (CAR) and the pregnane X receptor (PXR) is worth including, here, because these play central roles in the biotransformation process and in the clearance of the administered drug from the biological system. The foregut is the origin of the liver during the early phases of embryonic development; hence, the influence of gut microbiota on hepatic activity is quite certain. We will be discussing this aspect in a subsequent chapter. The nuclear receptor transcription factors discern the concentrations of various nutrients as well as xenobiotics coming through the portal vein (the main vessel draining the blood out of gastrointestinal tract, pancreas, spleen and gallbladder into the liver) by modulating the metabolism in the liver through the regulation of the expression of various genes (Desvergne et al. 2006). Several genes of the phase 1 metabolism enzymes, like CYP3A4, CYP2B6 and CYP2C8/9, and the phase 2 conjugation enzymes, like UDP-glucoronysyltransferase, are regulated by CAR. The activity of CAR has been shown to be greater in female mice. It's interesting to note that estrogen activates CAR whereas androgens inhibit its activity. Research data on the sexual dimorphism related to the activity of CAR indicates that it shows greater activity in female mice, which maintain the basal expression level of various CYP genes (Hernandez et al. 2009). PXR is a relative of CAR that also impacts the metabolism of xenobiotics in the liver in a sex-specific manner. With its predominant expression in the liver, PXR is known to regulate hepatic genes that work in the clearance of drugs. PXR has been regarded as the "master xenobiotic sensor" because it induces the expression of several hepatic DMETs functioning towards the detoxification of the system (Wang et al. 2012). The prominent drug clearance genes regulated by PXR include CYP3A4 and UDP-glucuronosyltranferase 1

family polypeptide a1 (UGT1A1) (Wang et al. 2012). The downregulation of genes (due to the loss of PXR) like those regulating T cell activation, inflammatory response and the differentiation of osteoclasts (these are specialized cells that resorb bone) in the liver works in a sex-specific manner. Interestingly, the role of PXR in lipid and glucose metabolism has been shown to work in a sexually dimorphic manner. Also, PXR acts as a possible repressor of immune activity in the female liver (Barretto et al. 2021). In response to toxicants, CAR and PXR show crosstalk through their heterodimerization.

The metabolic signaling operating in the liver is dependent on the cardiac output and the flow of blood in the organ. It has been observed that both these parameters are at lower levels in females, which clearly plays an important role in the sex-specific dimorphism in pharmacokinetics (Tamargo et al. 2017).

Elimination. The kidneys are the primary organs concerned with the elimination of drugs. The glomerular filtration rate (GFR, a parameter that measures the filtration function of the kidneys), proximal tubular secretions and resorption are the important functions occurring in the kidneys. Studies have shown that these functions are slower in females (Soldin and Mattison 2009). Moreover, drugs whose elimination is exclusively dependent on the renal route purge more slowly out of the female system (Schwartz 2003). This is clearly seen for several medicines like gabapentin (an anticonvulsant, used in the treatment of partial seizure and pain due to neuropathy), pregabalin (an analgesic and anticonvulsant) and antibiotics like vancomycin, cephalosporin and fluoroquinolones. These medicines have been shown to undergo slow renal clearance in females (Anderson 2005; 2008). In another example, the drug digoxin, which is used in the treatment of several heart conditions, showed slower elimination through the renal route (Yukawa et al. 1997). Interestingly, one study group analyzed the reports of higher mortality in females undergoing digoxin therapy and gave strong conclusions regarding the sexual dimorphism in the PK of the drug. They noted that males received higher doses of digoxin than females (in accordance with their body mass index) but that the levels of the drug in the serum of the females was evaluated to be slightly higher (Rathore et al. 2002). This is clearly a case of sexual dimorphism in the PK of digoxin. Similar is the case with lepirudin, an anticoagulant medication functioning as thrombin inhibitor, which is also eliminated via the renal route. Intriguingly, the half-life of lepirudin is longer in females, as the drug remains for up to forty-eight hours in the female circulatory system, compared to only two hours in

males. The lower GFR in females has been reasoned to be behind this issue (Abdel-Rahman 2017).

Apart from the phases of drug disposition, discussed above, two very important aspects that influence the PK of a drug are the sex-specific factors, like the hormonal status, and the gut microbiota. An elaboration on these elements will be covered in further chapters. The effect of pharmaceutical excipients (molecules used along with the pharmacologically active drug) has also been questioned many times regarding their influence on the PK in a sex-specific manner. They aid in the permeability and solubility of a drug in the biological system. It is well established by now that the excipients not only affect the bioavailability of a drug but they can become the reason behind unacceptable toxicity. Citing a few cases, here, the excipient polyethylene glycol 400 was shown to enhance the bioavailability of ranitidine in male subjects (Ashiru et al. 2008). A study on rats by Mai *et al*. hypothesized that the difference in bioavailability of ranitidine and ampicillin between the sexes is due to the association between P-gp and polyethylene glycol (Mai et al. 2017). Analogously, the bioavailability of the drug cimetidine (which is used to treat peptic ulcers and heartburn) was shown to be significantly enhanced in male volunteers due to the effect of polyethylene glycol 400. Here, also, the authors have proposed that the sex-specific modulation of drug bioavailability is due to the sexual dimorphism in the expression pattern of P-gp. Polyethylene glycol 400 boosts the bioavailability of cimetidine only in males (Mai et al. 2020). Mai *et al*. also showed the similar effects of other excipients (with polyoxyethylated structures), like polyethylene glycol 2000, Tween 80, Poloxamer 188 and Cremophor RH 40, in the escalation of the bioavailability of ranitidine in male rats (Mai et al. 2019). Cumulatively, the excipients that are substrates for P-gp down-regulate the intestinal expression of P-gp in male rats only. The excipients also elevate endogenous compounds like testosterone in male rats that, in turn, regulate the intestinal membrane efflux transporters (Mai et al. 2022).

Another very crucial factor that can affect the PK of a drug is stress. The influence of stress on the biological system shows strong sexual disparity. The effect of stress covers many aspects of the human physiology and there are numerous episodes where stress interacts with the sex of an individual. Various processes of the gastrointestinal tract, metabolism, renal function, distribution of lipids, flow of blood and albumin binding ability are modified by stress. Since females are more prone to be subjected to stress than males, the PK of a drug is naturally impacted by these factors (Kokras et al. 2019; Kessler et al. 1985).

All of these evidences regarding the PK of drugs in the biological systems of females and males must be strongly considered when it comes to prescribing drugs to female patients. As we have witnessed in the above discussion, the physiological differences in females are “not only” restricted to the differences in their reproductive systems to males. The cellular, tissue and organ architectural disparity between the female and the male systems is an area of vast research. We have started to gather information on this aspect lately through studies in mice but still there are gaps when it comes to human data.

Pharmacodynamics

The PD shows the physiological, molecular, and biochemical impacts of a drug on the biological system. After a drug is administered to the system, the effects are translated through a plethora of interactive sessions that the drug conducts with various biological structures at the molecular level. This interaction results in an induction of changes in the function of the target molecule that is dissipated across the molecules interacting with the drug target. In pharmacology, the transduction of the effect of a drug is caused by PK, whereas the consequence by PD. We will understand this with the examples of certain drugs: the aggregation of platelets is inhibited post administration of aspirin; hence, it should be prescribed to lower the risks arising during a cerebrovascular event and not to target the inhibition of platelet aggregation (Cirillo et al. 2020). In another example, insulin should be recommended at a concentration that addresses the risk of complications in the kidneys and eyes, but not to lower the concentration of blood glucose post insulin loading (Meng et al. 2020). The relationship between the dose of the drug and its receptor in the biological system is the basis of PD. Thus, PD insists on standardizing the minimum required dose of a drug that yields the maximum effect of the therapy and, at the same time, minimizes the adverse events.

The literature of pharmacology has a dearth of valid data on the sexual dimorphism in the PD. This is attributed to the comparatively lower participation of females in animal studies, as well as in human clinical trials (Mauvais-Jarvis et al. 2021). Here, we will be discussing some of the important paradigms where sexual dimorphism affects the PD.

Electrophysiology of Heart

The electrical impulses produced by the specialized muscular cells of the heart show a pattern that is predictable in a healthy subject, which is represented in an electrocardiogram (ECG). However, during the conditions of any cardiac event, these signals don't spread to the cardiac muscles as they do normally, giving rise to variability in the parameters of an ECG. The pattern represented on an ECG is a waveform that gets repeated with each cardiac cycle (Figure 1).

For instance, the QT interval (representation of ventricular action potential duration) (Figure 1) is longer when ventricular arrhythmia or cardiac events arise (Wang et al. 2012).

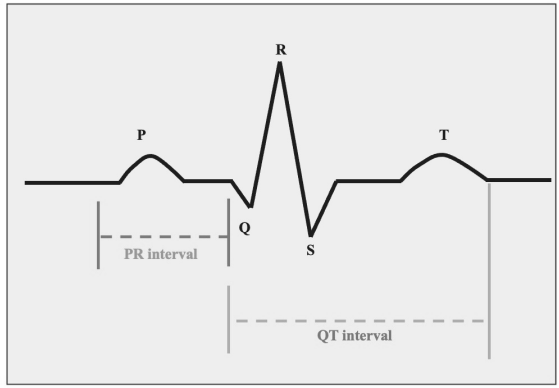

Figure 1: QT interval.

The duration of the QT interval depends upon the closing and opening of ion channels (an influx of positive ions like sodium and calcium cause depolarization and an efflux of potassium causes repolarization). An excessive intracellular accumulation of positive ions becomes the reason for the longer action potential that shows as a prolonged QT interval. Sexual dimorphism has been well documented in cardiac electrophysiology. Females show higher resting heart rates and longer QT intervals than males (Regitz-Zagrosek and Kararigas 2017). A life-threatening condition arising due to QT prolongation, termed torsades de pointes, is a form of polymorphic ventricular tachycardia. Torsades de pointes could be due to either congenital or acquired reasons. Most often, acquired QT prolongation is drug-induced and is witnessed frequently in females. A wide array of

drugs (blocking potassium currents) have been discussed in various literatures that review the higher incidences of torsades de pointes in females, such as antibiotics (erythromycin), antiarrhythmics (d-sotalol), antimalarials (halofantrine) and antihistamines (astemizole). FDA-funded studies have shown that several drugs used in different therapeutic classes have the potential to induce torsades de pointes. Rabbit models have been researched to study that androgens tend to shorten the QT interval in males, thereby protecting them from ADR (Ebert et al. 1998). Antibiotics like levofloxacin and moxifloxacin have been shown to exert sexual dimorphic effects via prolongation of the QT interval (Alizadeh Ghamsari et al. 2016). Paradoxically, the administration of antiarrhythmic drugs (used to overwhelm faster rhythms under abnormal conditions) in females predispose them to conditions of new arrhythmia (termed as proarrhythmia), vis-à-vis males. The class III antiarrhythmic drug, sotalol, has been associated with the increased risk of prolonged QT interval in females (Lehmann et al. 1996). The risk of proarrhythmia in females undergoing treatment with class III antiarrhythmic drugs varies with the phases of the menstrual cycle. The extent of the prolongation of the QT interval induced by the drug ibutilide was shown to have an inverse relationship to the level of progesterone. The drug increases the risk of arrhythmia during menstruation and the ovulatory phase of the menstrual cycle (Rodriguez et al. 2001). Atrial fibrillation is a condition when arrhythmia results in the formation of blood clots in the heart. This further increases the complications of thromboembolic stroke (formation of clots inside vascular lumen that get propagated to proximal extensions). There is a higher risk of the demonstration of thromboembolic stroke in females than males (Andersson et al. 2014).

Figure 2 (next page): **The renin-angiotensin-aldosterone system**. The liver produces angiotensinogen, which is constantly circulating in the plasma. In response to a decrease in the blood pressure/ sodium level/ fluid volume, the kidneys secret renin (hormone). Renin reaches blood stream to cleave its target, angiotensinogen into angiotensin I. The lungs secrete angiotensin-converting enzyme (ACE) that converts angiotensin I into angiotensin II. Angiotensin II imparts its broad spectrum effect on the brain, adrenal cortex, kidneys and arterioles, after binding to its cognate receptors. The effect of angiotensin II is multifarious- in hypothalamus, it stimulates thirst that results into increased intake of water; in posterior pituitary, it induces the secretion of antidiuretic hormone (ADH or vasopressin) that further encourages the kidneys to increase the water reabsorption; and it decreases the baroreceptor reflex (it applies control on arterial pressure) sensitivity. Due to the effect of angiotensin II in the kidneys, there is an increase in the reabsorption of sodium, resulting into a rise in the blood osmolarity that causes fluid flow into the blood as well as the extracellular space. Angiotensin II stimulates the adrenal glands leading to the release of aldosterone, which successively affect the reabsorption of water and salt in the kidneys. These events subsequently raise the arterial pressure. The angiotensin II causes vasoconstriction of systemic arterioles, again leading to escalation in blood pressure.

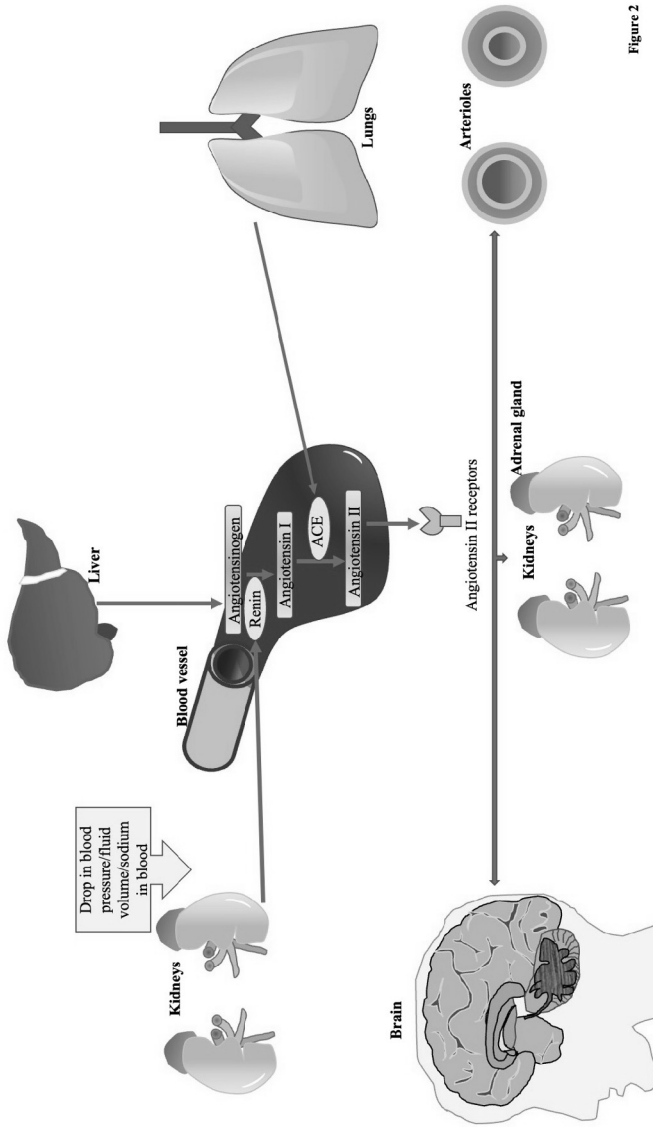
Lungs
Arterioles
Liver
Angiotensinogen
Renin
Angiotensin I
ACE
Angiotensin II
Blood vessel
Angiotensin II receptors
Adrenal gland
Kidneys
Drop in blood pressure/fluid volume/sodium in blood
Kidneys
Brain

Figure 2

The Renin-Angiotensin-Aldosterone System (RAAS)

The critical regulation of blood volume and blood pressure is upheld by the RAAS (Figure 2). The major compounds involved in the system are renin (a hormone controlling blood pressure, also called angiotensinogenase), angiotensin II (functions in the regulation of blood pressure and the homeostasis of electrolytes, it is a vasoconstrictor) and aldosterone (a hormone influencing water and salt regulation in the biological system). The coherent action of all three molecules increases arterial pressure when there is a decrease in renal blood pressure, which maintains the blood pressure in the system (Drummond et al. 2019). The key organs where the RAAS plays are the brain, lungs, systemic vasculature and the kidneys (Santos et al. 2019). Thus, the RAAS plays a central role in the maintenance of fluid homeostasis because its components are frequently targeted in the treatment protocol of hypertension (specifically angiotensin-converting enzyme, ACE, and angiotensin I receptor, AT1R). The influence of sex hormones is reasonably pronounced in the functioning of RAAS. The expression levels of the various components and receptors of the RAAS show sexual dimorphism (Ahmed et al. 2019). Studies compiling data on human, as well as rat, females show that their concentrations of angiotensinogen are higher than in males. In female rats, alterations in angiotensin II concentration cause little changes in blood pressure. The expression of receptor AT1R is higher in males while the expression of angiotensin II (AT2R) is lower in female rats (Leete et al. 2018). There is an increase in the cases of renal injury with the elevation of RAS activation in males. However, the elevation in angiotensin II doesn't predispose females to renal injury. Irrespective of the sex of an individual, RAS inhibitors are usually prescribed during the therapy of chronic renal disease (Sullivan et al. 2008). It has been shown, in the case of rats, that alterations in the angiotensin II concentrations cause feeble changes in female blood pressure (Sandberg and Ji 2012). The ACE inhibitors (ACEI) have been reported to work well in reducing the blood pressure of male spontaneously hypertensive rats (Reckelhoff et al. 2000), whereas, a human population-based study showed that congestive heart failure was better addressed in females by using the angiotensin receptor blocker (ARB) (Hudson et al. 2007). A bioequivalence study revealed that females in the follicular phase of their menstrual cycle have significantly lower ACE activity than males (Zapater et al. 2004).

Thus, the RAAS-related sexual dimorphism can easily affect the drug therapy. So, the treatment of both sexes with the same dose of drug may lead to ADR.

Nitric oxide (NO). Nitric oxide has been regarded as a vasoprotective agent. The contribution of NO to the regulation of hemodynamics and vascular tone is important. NO also controls water and sodium homeostasis. Ample clinical literature has helped in understanding that hypertension and many other cardiovascular diseases are associated with a deficiency of NO (Zimmerman and Sullivan et al. 2013). The bioavailability of NO is higher in females than males (Zimmerman and Sullivan et al. 2013). The enzyme nitric oxide synthase (NOS) synthesizes NO from the precursor amino acid, arginine. It has been studied in rats that the inhibition of NOS results in hypertension, whereas with increasing NO concentration there was a lowering in the blood pressure of the hypertensive male rats (Baylis 2012). It was shown, in one study on mice, that premenopausal female mice were protected from the injury caused by cardiovascular events. It was assessed that this protection was mediated through estrogen-activated endothelial NOS (Cross et al. 2002). We have research data on the significance of the oral microbiome in the production of NO, which shows sexual dimorphism. Females have been studied to exhibit greater action of their oral microbiota in the reduction of nitrate, resulting in higher nitrite concentrations (Kapil et al. 2018). The phases of the menstrual cycle influence the production of nitric oxide. The production of NO was shown to be at a maximum during the mid-stage of the menstrual cycle, which explains the lower incidences of cardiovascular events in premenopausal females (Kharitonov et al. 1994). The function and phenotype of platelets show variation between the sexes. The reactivity of platelets in females is not the same as in males. NO plays an inhibitory role on the activation of platelets only in females (platelets get activated with lower levels of NO production in the platelets and vice-versa) (Godwin et al. 2021). Thus, the production of NO in the platelet shows sexual dimorphism, which is a substantial factor that should be considered during the studies on antiplatelet medications. The amount of exhaled NO is a marker for the inflammation of airways (a characteristic of asthma). This level varied markedly during the menstrual cycle (Mandhane et al. 2009).

Adrenoreceptors or Adrenergic Receptors

The effects of neurotransmitters/hormones, epinephrine and norepinephrine are mediated by the adrenoreceptors, which are an assemblage of seven transmembrane components (these belong to the G protein-coupled receptor, GPCR, class). These receptors have a wide distribution in the biological system because they are involved in various processes of physiology. The binding of their cognate ligands on the specific adrenoreceptors

offers a plethora of effects such as bronchodilation and a rise in the heart rate modulation of the immune cell response. These receptors are either alpha (subtypes alpha-1 and alpha-2) or beta (subtypes beta-1, beta-2 and beta-3) type. There is a clear sexual dimorphism in the effect of vessel vasodilation and vasoconstriction. It has been shown in rat models that norepinephrine causes lesser constriction of vessels in females than males. Beta-adrenoreceptors are the crucial GPCRs that are widely expressed in cardiovascular cells: functioning in the regulation of the vascular and cardiac systems. A derangement in the responsiveness of beta-adrenoreceptors becomes the pathophysiological reason behind several cardiac events like hypertension, stroke and heart failure. Consequently, the drugs used to block these receptors become the main tools for the therapeutic approach. The selective stimulation of beta adrenoreceptors triggers greater relaxation of vessels in female rats than in males (Riedel et al. 2019). Similar reports have been gathered from human studies. The selective stimulation of beta-adrenoreceptors in females increases the flow of blood in the forearm while in males the same stimulation decreases in response to norepinephrine. This effect in females was prominent during the middle phase of their menstrual cycles (Kneale et al. 2000). Furthermore, the effect of adrenoreceptor-mediated constriction is lesser in the mammary artery in females. However, this difference retracts in aged subjects, indicating the significance of the menstrual cycle (respective sex hormones) on the functioning of adrenoreceptors (aged females are menopausal) (Riedel et al. 2019). In fact, there is an acquaintance between endothelial NOS and beta-adrenoreceptors. The stimulation of these receptors elicits the production of NO. Strikingly, the efficacy of beta-adrenoreceptor blockers is a debatable issue as there are no set guidelines for female and male subjects. An analysis has shown that the therapy for hypertension using beta-blockers predisposed female subjects to a higher risk of heart failure that resulted in a spike in mortality (Bugiardini et al. 2020). The beta blockers metoprolol (slows down the heart rate and relaxes the blood vessels) and propranolol are metabolized in the liver by the activity of CYP2D6, which is lower in females than in males (Tanaka and Hisawa 1999).

Blockers of Calcium Channels

Calcium-channel blockers, or calcium antagonists, are represented by a cluster of organic compounds that inhibit the entry of calcium ions to an excitable cell. Calcium-channel blockers act as dilators of smooth muscle cells (Katz 1986). Due to the action of calcium-channel blockers, blood

vessels relax resulting in a higher supply of oxygenated blood to the heart muscles. Consequentially, this helps in lowering blood pressure.

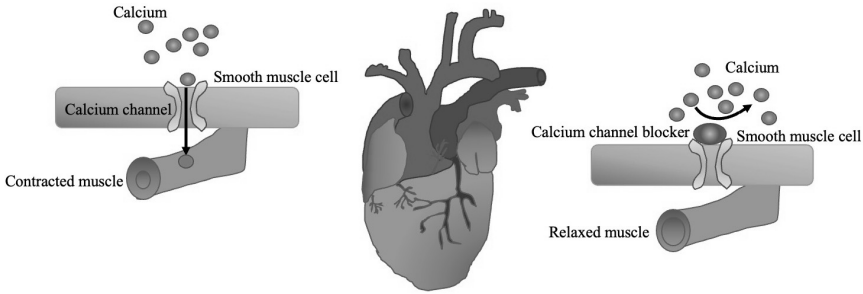

Figure 3: The action of calcium-channel blocker: The calcium-channels execute the influx of calcium ions resulting in the contraction of the smooth muscle cells. In the presence of an antagonist, a calcium-channel blocker, the result is relaxation of the smooth muscle cells.

The common calcium-channel blocker, verapamil, showed a greater decline in the blood pressure of elderly females (Jochmann et al. 2005). Similarly, verapamil induced a higher heart rate in hypertensive females (Krecic-Shepard et al. 2000). Another calcium antagonist, amplodipine, led to a larger decline in blood pressure and the induction of oedema in females undergoing hormone replacement therapy (Kloner et al. 1996). Higher events of ADR were noticed in females taking the nifedipine calcium antagonist (Fan et al. 2008).

Antagonists of Endothelin-1 Receptor

Endothelin (ET) is a peptide that is produced largely by the vascular endothelium and, to a smaller magnitude, by cells like the smooth muscle cells of the pulmonary artery and lung fibroblasts (Dupuis and Hoeper 2008). There are three isoforms of ET: ET-1, ET-2 and ET-3. The predominant isoform that holds pathophysiological significance is ET-1. The receptors for ET, called the endothelin receptors (ET_A and ET_B), also belong to the GPCR family. These receptors mediate the vasoconstricting effect of ET. The ET system regulates the control of blood pressure and kidney function. Any distortion in the system, either due to overactivation or dysfunction, becomes a reason for hypertension and kidney disorder. Therefore, the ET receptor antagonists are of pharmacological importance with regard to therapeutic interventions. Recent investigations have

revealed the presence of sexual disparity in the ET system. Studies on animal models have shown that males display more renal mRNA expression of ET-1 and, thereby, higher ET_A-mediated activity, which causes increased events of renal injury. However, females have a higher ET_B function that gives them relative protection from the risk of high blood pressure and kidney injury. Though, the genetic polymorphism of the ET system exhibited in females has been correlated with higher occurrences of renal injury and hypertension (Kittikulsuth et al. 2013). In the cases of pulmonary arterial hypertension (PAH), which leads to the debilitating condition of heart failure, the concentration of ET-1 intensifies. The most common approach to treat the condition is the use of endothelin receptor antagonists where females show a superior treatment response in comparison to males (Gabler et al. 2012). There is a variation in the levels of ET-1 during the menstrual cycle because at the time of the follicular and luteal phases, the plasma ET-1 levels go down (Lekontseva et al. 2010). The intradermal microdialysis administration of BQ-788, an ET_B receptor antagonist, helped in deciphering that the ET_B receptors induce vasodilation in females but vasoconstriction in males (Kellogg et al. 2001). The cerebral artery of females is less reactive to ET-1; however, the level of mRNA for the expression of the ET_B receptor is higher (Ahnstedt et al. 2013).

Statins

Statins are also documented as hydroxymethylglutaryl-CoA (HMG-CoA) reductase inhibitors (preventing the action of the enzyme, HMG-CoA reductase that leads to a reduction in the synthesis of intracellular cholesterol), which are used in the treatment of hypercholesterolemia in adjunction to maintaining healthy lifestyle. Hence, they are used in therapeutic interventions for cardiovascular diseases. Females have been reported to present side effects related to their muscles, like myalgia and myopathy, after the administration of statins (Baigent et al. 2010). Because of the associated side effects, females more commonly discontinue statin therapy than males (Rosenbaum et al. 2013). In fact, being a female is one of the risk factors for statin-associated muscle symptoms (SAMSs) (Ward et al. 2019).

Platelets and Aspirin

Arachidonic acid, an omega-6-polyunsaturated fatty acid (PUFA), and its metabolites constitute a cascade, which plays a very crucial role in the functioning of the cardiovascular system. The interesting biochemistry of